Biomedical Basis of Elite Performance 2022
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Abstracts

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SA01

Cardiac adaptations to endurance training in men and women

Benjamin Levine[1]

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The presence of testosterone (along with functional androgen receptors), especially during growth and development or periods of enhanced training leads to fundamentally different cardiovascular adaptations to endurance training in males compared to females. Males have larger hearts, both in absolute terms, and when scaled to lean body mass. In longitudinal studies, even when matched heart beat x heart beat for a full year of progressive training, young men evolve larger hearts, and greater increases in aerobic power. In this talk, I will review the cardinal features of the cardiovascular adaptation to endurance training in competitive athletes, and then review the data in longitudinal studies. The implications of these differences in cardiovascular adaptation for endurance performance will be discussed as part of the biomedical basis for elite performance differences between males and females.

SA02

Exercise thermoregulation in men and women: sex as a biological variable

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There are both physical and physiological differences between men and women, which have led researchers to wonder whether women are at an inherent “advantage” or “disadvantage” relative to men with regard to thermoregulatory responses in athletic events, particularly those of long duration. Women, on average, are physically smaller than men, which results in larger surface area-to-mass ratio (i.e., heat is generated based on body mass and dissipated based on surface area); however, it is unclear whether these size differences are quantitatively relevant across “human-relevant” sizes. Reproductive hormones have influences on thermoregulatory and volume regulatory responses. For example, estradiol promotes heat dissipation via both central effects on hypothalamic warm-sensitive neurons and peripheral effects on blood flow and sweating responses.

In previous decades, research in human thermoregulation in which sex was considered as a biological variable was minimal to absent. Those historical reports that did mention sex differences often started with the assumption (based on anecdotal or poorly controlled data) that women are at an inherent disadvantage with regard to exercise thermoregulation in hot environments. More recent, well-controlled studies indicate minimal differences between the sexes in terms of thermoregulatory mechanisms in scenarios that are relevant to the majority of athletic, military and industrial scenarios. This talk will review the evidence for sex differences in thermoregulation during exercise, and discuss practical implications for athletic endeavors.

SA03

Training to improve skeletal muscle capillarization

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Skeletal muscle capillarization is a central determinant of oxygen and nutrient delivery and removal of metabolites in skeletal muscle and a well-established capillary network has important implications exercise performance. Since the pioneering work from the 1970's, that revealed that physical activity promotes growth of new capillaries, numerous studies have shown that just a few weeks of regular training result in a 10-30% increase in number of capillaries per muscle fiber in previously untrained subjects and that capillarization can be more than 200% higher in elite athletes compared to untrained counterparts. However, comprehensive information about what is the optimal training intensity, duration and volume are lacking. While high intensity interval training has proven very effective in improving all sorts of health and performance parameters, indications from recent studies are that high intensity training is, at best, not very effective in promoting capillary growth.

The growth of new capillaries, angiogenesis, is governed by a large number of pro- and anti-angiogenic factors and the physiological stimuli regulating angiogenesis are the frictional force of flowing blood, known as shear stress, local metabolism and vessel stretch. These factors are stimulated during muscle activity but the magnitude of each may vary according to the intensity and duration of given training session. Ultimately, the stimulated release of pro- and antiangiogenic factors and the growth of new capillaries are dependent on training modality. While we are awaiting the perfect study that will reveal how to train to improve skeletal muscle capillarization, meta-analysis of training studies published over the past ~50 years can give indications as to what training modality is most effective.

This talk will cover the physiological stimuli that leads to growth of capillaries in skeletal muscle, the underlying mechanisms and how different training modalities may impose different angiogenic stimuli. In addition, meta-analysis of data on training induced capillarization from more than 50 trails are presented to provide recommendations for training as well as for directions of future research.

SA04

Hematological adaptations to endurance training

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Plasma volume increases rapidly with the initiation of endurance training and is elevated after just one exercise bout (Gillen et al., 1991). An increase in plasma albumin content and plasma osmolality following exercise is likely the main mechanism behind this expansion (Convertino et al., 1980). The augmented plasma volume results in an increased total blood volume and a concomitant transient reduction in hematocrit, often referred to as sports anemia. Only after months of endurance training, hematocrit normalizes as a consequence of the delayed expansion in red blood cell volume. In fact, one mechanism underlying the augmented red blood cell production includes a lowered hematocrit since the reduced arterial oxygen content results in a lower kidney tissue oxygen pressure that stimulates erythropoietin synthesis in the renal peritubular fibroblast-like cells (Montero and Lundby, 2018). However, mechanisms other than renal tissue hypoxia have been related to erythropoietin synthesis and include for instance plasma volume-regulating hormones such as angiotensin II and vasopressin, alterations in central venous pressure or estrogen and testosterone concentrations (Montero and Lundby, 2018). The relevance of these hematological changes for elite performance is highlighted in the fact that the increased plasma and red blood cell volume are major determinants of the improved exercise capacity with endurance training as these lead to greater maximal cardiac output and oxygen transport capacity (Montero and Lundby, 2018). In support of this, elite endurance athletes have greatly expanded blood volumes. Specifically, we found 25% higher total blood volumes in endurance champions than in untrained individuals (Oberholzer et al., unpublished). A large proportion of the volumetric variations was explained by differences in lean body mass which was strongly related to total blood volume (R^2=0.81, n=671) (Oberholzer et al., unpublished). The highly vascularized nature of skeletal muscle may justify this relationship, yet the exact mechanisms that explain the association between lean body mass and blood volume are so far unclear. Thus, while a vast amount of research is conducted on the short-term regulation of blood volume with endurance training, the mechanisms underlying the lifelong hematological adaptations in elite endurance athletes are still to be elucidated. In this presentation, current research on the regulation of plasma and red blood cell volume expansion with endurance training is discussed, while influencing factors such as sex differences and age are considered. Furthermore, new cross-sectional

data on the core factors that determine the hematological differences between endurance athletes and untrained individuals are presented.

Reference 1 :- Gillen CM et al. (1991). J Appl Physiol 71, 1914-20.

Reference 2 :- Convertino et al. (1980). J Appl Physiol Respir Environ Exerc Physiol 48, 657-64

Reference 3 :- Montero D & Lundby C (2018). Compr Physiol 9, 149-164.

Reference 4 :- Oberholzer L et al. (2022). Unpublished data

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SA05

Sex Differences in Neuromuscular Performance and Fatigability

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Every cell in the human body has a sex. Consequently, males and females differ in anatomy and physiology resulting in marked sex differences in neuromuscular performance and fatigability. Typically, males outperform females in athletic performance after puberty across all age groups due to hormonal effects, primarily testosterone which is ~15-20 times greater in males from ~18 years of age (Handelsman et al., 2018). Sex differences in neuromuscular performance such a muscle strength and power range from 10-30% depending on the demands of the task and the muscle groups involved. Males have larger, stronger, and more powerful muscles particularly in the upper limb due to differences within the size and compositional area of the skeletal muscle fibers. In contrast, there are minimal sex differences in the ability to activate the available skeletal muscle during voluntary contractions prior to fatiguing tasks (Hunter, 2016).

While males are stronger and more powerful than females, the relative decrement in force or power during a fatiguing task of males is usually larger than for females, primarily during isometric and slow-velocity fatiguing contractions. This sex difference in fatigability (relative, exercise-induced reduction in force or power) is not typically observed during fast-velocity contractions (Hunter, 2016; Senefeld et al., 2018). Multiple mechanisms are responsible for fatigability in both males and females that can include activation of the motoneuron pool from cortical and subcortical regions, synaptic inputs to the motor neuron pool via activation of metabolically-sensitive small afferent fibres in the muscle, muscle perfusion, skeletal muscle metabolism and altered crossbridge dynamics in the muscle fibres (Hunter, 2018). The greater fatigue resistance in females compared with males during isometric and slow velocity contractions however, is related to the sex differences in the fibre type proportional areas within the skeletal muscle and muscle perfusion (Hunter, 2016). Understanding

the sex difference in neuromuscular fatigability has important implications for training and rehabilitation in males and females.

Despite more concerted efforts in the last ~15-20 years to include females in mechanistic studies, there is still inadequate inclusion of females and knowledge on the mechanisms for sex differences of fatigability and athletic performance. The sex bias of studying more males than females in both human and animal experiments in physiology and fatigability (Beery & Zucker, 2011; Hunter, 2016) has led to the false assumption that males and females respond similarly to interventions including fatiguing exercise. The field is ripe with opportunities to clarify and understand the sex differences in neuromuscular fatigability, athletic performance, and the underlying mechanisms during different tasks.

Reference 1 :- Beery AK & Zucker I. (2011). Sex bias in neuroscience and biomedical research. *Neurosci Biobehav Rev* **35,** 565-572.

Reference 2 :- Handelsman DJ, Hirschberg AL & Bermon S. (2018). Circulating Testosterone as the Hormonal Basis of Sex Differences in Athletic Performance. *Endocr Rev* **39,** 803-829.

Reference 3 :- Hunter SK. (2016). The Relevance of Sex Differences in Performance Fatigability. *Med Sci Sports Exerc* **48,** 2247-2256.

Reference 4 :- Hunter SK. (2018). Performance Fatigability: Mechanisms and Task Specificity. *Cold Spring Harb Perspect Med* **8**.

Reference 5 :- Senefeld J, Pereira HM, Elliott N, Yoon T & Hunter SK. (2018). Sex Differences in echanisms of Recovery after Isometric and Dynamic Fatiguing Tasks. *Med Sci Sports Exerc* **50,** 1070-1083.

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SA06

The Neuromuscular Junction with Aging and Exercise

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The neuromuscular junction is a well-characterized point of vulnerability in aging skeletal muscle. The first study documenting neuromuscular junction morphological alterations in aged rodents was published in 1966 [1] with dozens of rodent studies since, and this has been corroborated in aging humans [2]. Although much of the morphological alterations to the neuromuscular junction with aging reflect a remarkable plasticity of this structure in helping to preserve the fidelity of the motoneuron:muscle fiber communication for most of the adult lifespan, evidence suggests that this plasticity is finite. Specifically, there is evidence for failed reinnervation in advanced age that leads to an accumulation of persistently denervated muscle fibers that are a major driver of accelerated muscle atrophy and weakness. Underscoring this point, our previous studies highlight that denervated muscle fibers in aging muscle are markedly atrophied, whereas innervated muscle fibers in aged muscle are only mildly atrophied compared to those from young adult muscle. On the other hand, a maintenance of reinnervation is characteristic of atrophy-resistance with aging, as is seen when comparing muscles with varying atrophy susceptibility within the same organism. In addition, superior reinnervation capacity is a feature of aging humans who are better able to maintain muscle mass (e.g., Masters track and field athletes). In this latter respect, although exercise itself likely contributes to better preservation of muscle innervation in advanced age (e.g., by preserving mitochondrial function), muscle proteomics analysis finds that at least some features of skeletal muscle in highly functioning elderly have not been previously identified as exercise-responsive, implicating a genetic and/or gene x environment interaction. Greater understanding of the mechanisms responsible for modulating reinnervation capacity and severity of aging muscle atrophy will be an important key to identifying strategies for better maintaining mobility with aging.

Reference 1 :- Gutmann, E. and V. Hanzlikova, *Motor unit in old age.* Nature, 1966. **209**(5026): p. 921-2.

Reference 2 :- Arizono, N., et al., *Morphometric analysis of human neuromuscular junction in different ages.* Acta Pathol Jpn, 1984. **34**(6): p. 1243-9.

SA07

Neural-specific responses to fatiguing muscle contractions: New insights with high-density surface electromyography decomposition techniques

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The mechanisms responsible for task failure during fatiguing submaximal contractions have not been clearly elucidated. Previous investigations have reported that muscle fatigue starts progressively from the beginning of the contraction due to linear reductions in motor unit discharge rate (Enoka & Duchateau, 2008). Nevertheless, other authors have reported non-linear variations in motor unit discharge rate (Mettler & Griffin, 2016), revealing the existence of two distinct phases of motor unit firing behaviour (Martinez-Valdes et al., 2020). By employing techniques of high-density surface electromyography (HDsEMG) motor unit decomposition, in combination with transcranial magnetic stimulation (TMS) we aimed to assess mechanisms responsible for this bi-phasic response in motor

unit discharge rate during submaximal contractions. In addition, potential exercise-induced adjustments in discharge rate were also investigated. We performed three independent studies where we assessed motor unit responses to fatigue during 1) Isometric knee extension at 30% of the maximum voluntary contraction (MVC) until task failure, 2) Isometric dorsiflexion contraction at 25% of the MVC until task failure in combination with TMS and 3) Isometric knee extension until task failure (30% MVC) before and after 2 weeks of endurance training (cycling). In these studies, we observed that during fatigue 1) motor unit firing rate varied in a non-linear fashion, showing an initial reduction in discharge rate followed by a later increase in discharge rate. These variations in discharge rate were mirrored by opposite changes in motor unit twitch force (increase followed by decrease), 2) variations in discharge rate were related to non-linear variations in cortico-spinal excitability/inhibition 3) endurance training delayed the time in which these later increases in discharge rate occurred and this was associated with an increase in the time to task failure. These results show that during the development of fatigue there are diverse adjustments in motor unit firing properties, which are the result of the interplay between both motor unit contractile responses and changes in cortico-spinal excitability/inhibition. Interestingly, increases in task failure following endurance training seem to be related to a delayed increase in firing rate during fatiguing contractions. Taken together, these findings show that the development of fatigue during submaximal contractions is not characterized by a progressive mechanism that occurs over the entire duration of the contraction but rather by a discrete time instant that marks the need to substantially increase the drive to the motor neuron pool, determining a reversal in motor unit firing behaviour.

Reference 1 :- Enoka RM & Duchateau J. (2008). Muscle fatigue: what, why and how it influences muscle function. *J Physiol* **586,** 11-23.

Reference 2 :- Martinez-Valdes E, Negro F, Falla D, Dideriksen JL, Heckman CJ & Farina D. (2020). Inability to increase the neural drive to muscle is associated with task failure during submaximal contractions. *J Neurophysiol* **124,** 1110-1121.

Reference 3 :- Mettler JA & Griffin L. (2016). Muscular endurance training and motor unit firing patterns during fatigue. *Exp Brain Res* **234,** 267-276.

SA08

Motor Unit Function in Muscle Fatigue and Damage

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Muscle fatigue is characterised by a reduction in force output. Increases in force production are partly mediated by adjustments in motor unit (MU) firing properties however, conflicting research has found MU firing rate to both increase and decrease following fatiguing exercise. This disparity

may be influenced by contraction type, as concentric (CON) and eccentric (ECC) contractions are known to require different levels of neural input and result in different responses to fatigue. As a result, other MU properties may also be differentially affected by fatigue depending on contraction type. Acute muscle damage is often more severe following ECC when compared CON contractions, resulting in localised structural, metabolic and functional changes of the muscle. MU firing rate has been demonstrated to increase after damage resulting from ECC exercise, yet the effects of ECC exercise on peripheral MU properties as a result of damage remains unknown. A better understanding of the contraction specific MU responses is important when considering training protocols and injury risk, particularly in populations with musculoskeletal impairments. This talk will address the effects of CON and ECC muscle fatigue and acute muscle damage on MU function using a combination of intramuscular and high-density surface electromyography methods.

SA09

MicroRNA: Redox interplay in skeletal muscle homeostasis

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Skeletal muscle ability to adapt to intra- and extracellular changes diminishes during ageing and disease; this can lead to muscle wasting. The molecular mechanisms underlying muscle wasting are not fully understood, however redox and epigenetic factors play key regulatory roles in regulating muscle adaptation and its loss.

MicroRNAs control muscle homeostasis through posttranscriptional gene expression regulation. MicroRNAs regulate redox responses in skeletal muscle and equally, redox imbalance can result in changes in microRNA function. Specifically, during conditions characterised by increased ROS, oxidised microRNAs are detected in muscle and serum. Oxidative modification of microRNAs can result in the regulation of non-native targets leading to their disrupted specificity for regulating protein content within muscle and muscle wasting. Our data demonstrate that oxidation of specific microRNAs, which play a role in maintaining muscle homeostasis, such as miR-378 or miR-133, can promote muscle wasting through affecting mitochondrial dynamics, as well as hypertrophy/atrophy pathways. Moreover, our data show that inhibiting oxidised miR-378 positively affects muscle strength. Together, these data indicate a pathological role of oxidised microRNAs in muscle wasting and reveal oxidised microRNAs as potential therapeutic targets.

SA10

Cell and extracellular matrix interactions of skeletal muscle fibres

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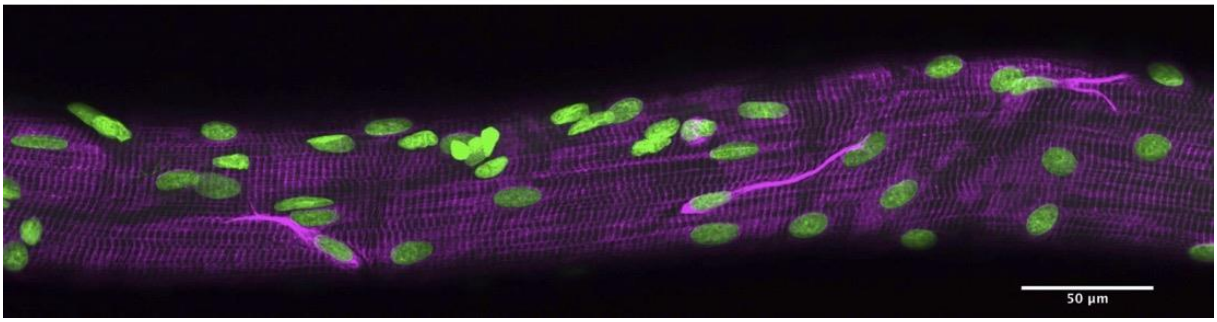
Human skeletal muscle has the potential to regenerate completely after injury induced under controlled experimental conditions. Examples of models suitable for humans include voluntary eccentric contractions or contractions induced by neuromuscular electrical stimulation (NMES). The major difference between these two models appears to be the proportion of muscle fibres damaged to the point of necrosis, with voluntary contractions rarely leading to muscle fibre necrosis in prime mover muscles (Crameri *et al.*, 2007). In studies where NMES has been employed, the proportion of necrotic fibres appears to range from approximately 15-40% (Crameri *et al.*, 2007; Mackey *et al.*, 2016; Karlsen *et al.*, 2020). While the events inside the myofibers as they undergo necrosis, followed closely by satellite cell-mediated myogenesis, have been described in detail (Mackey & Kjaer, 2017), much less is known about the involvement of the key mononuclear cell populations such as fibroblasts, immune cells and vessel associated cells. Furthermore, while the adaptation of the connective tissue structures surrounding the myofibres throughout the degeneration and regeneration processes is gaining interest, the role of individual muscle matrix components and their spatial interaction during repair is poorly understood – and may provide insight into the optimal timing of rest vs. return to activity after muscle injury. This not only relates to restoration of the insults to the muscle mid-portion, but also to the interface between the muscle fibres and the tendon – the myotendinous junction (MTJ). In general, it appears that the muscle connective tissue takes longer to recover after contraction induced damage than the muscle fibres themselves, which may explain the high rate or re-injury recorded after an initial strain injury. While progress is being made in determining the composition of the intact human MTJ (Karlsen *et al.*, 2022), many questions remain regarding the protein and cellular composition with injury and during repair.

Figure shows an almost fully regenerated human skeletal muscle fibre (desmin, magenta; nuclei, green). Note the three desmin-positive satellite cells on the myofibre surface.



Reference 1 :- Crameri RM, Aagaard P, Qvortrup K, Langberg H, Olesen J and Kjaer M (2007). Myofibre damage in human skeletal muscle: effects of electrical stimulation vs voluntary contraction. Journal of Physiology 583, 365-380.

Reference 2 :- Karlsen A, Gonzalez-Franquesa A, Jakobsen JR, Krogsgaard MR, Koch M, Kjaer M, Schiaffino S, Mackey AL and Deshmukh AS (2022). The proteomic profile of the human myotendinous junction. iScience 25, 2, 103836

Reference 3 :- Karlsen A, Soendenbroe C, Malmgaard-Clausen NM, Wagener F, Moeller CE, Senhaji Z, Damberg K, Andersen JL, Schjerling P, Kjaer M and Mackey AL (2020). Preserved capacity for satellite cell proliferation, regeneration, and hypertrophy in the skeletal muscle of healthy elderly men. FASEB J 34, 6418-6436.

Reference 4 :- Mackey AL and Kjaer M (2017). The breaking and making of healthy adult human skeletal muscle in vivo. Skeletal Muscle 7, 24.

Reference 5 :- Mackey AL, Rasmussen LK, Kadi F, Schjerling P, Helmark IC, Ponsot E, Aagaard P, Durigan JL and Kjaer M (2016). Activation of satellite cells and the regeneration of human skeletal muscle are expedited by ingestion of nonsteroidal anti-inflammatory medication. FASEB J 30, 2266-2281.

SA11

Epigenetics of muscle adaptation in response to exercise

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Epigenetics (loosely meaning ‘above genetics’) is the study of transcript behaviour and activity, without underlying modifications to the DNA code. This ‘programme’ of control involves a wide range of regulatory events, from chemical modifications to genomic DNA through to the production of small non-coding RNAs. The underlying commonality of these regulatory events is that they all have the capacity to regulate the behaviour of gene transcription and/or translation. It is this regulatory mechanism that has seen epigenetics become a popular field of study within exercise physiology. Indeed, since seminal work published around a decade ago, we have seen a surge in research that is involved in investigating skeletal muscle epigenetics, helping to characterise a whole new paradigm of molecular events that may, in part, help to orchestrate the post exercise adaptive response.

I will discuss some of the most important papers that have shaped our understanding of skeletal muscle epigenetics during homeostatic conditions, describe how these become dysregulated following various exercise modalities and identify some of the most exciting avenues of scientific research that could create some major breakthroughs in the field of physiology.

SA12

Gene expression and DNA methylation after mechanical loading in vitro

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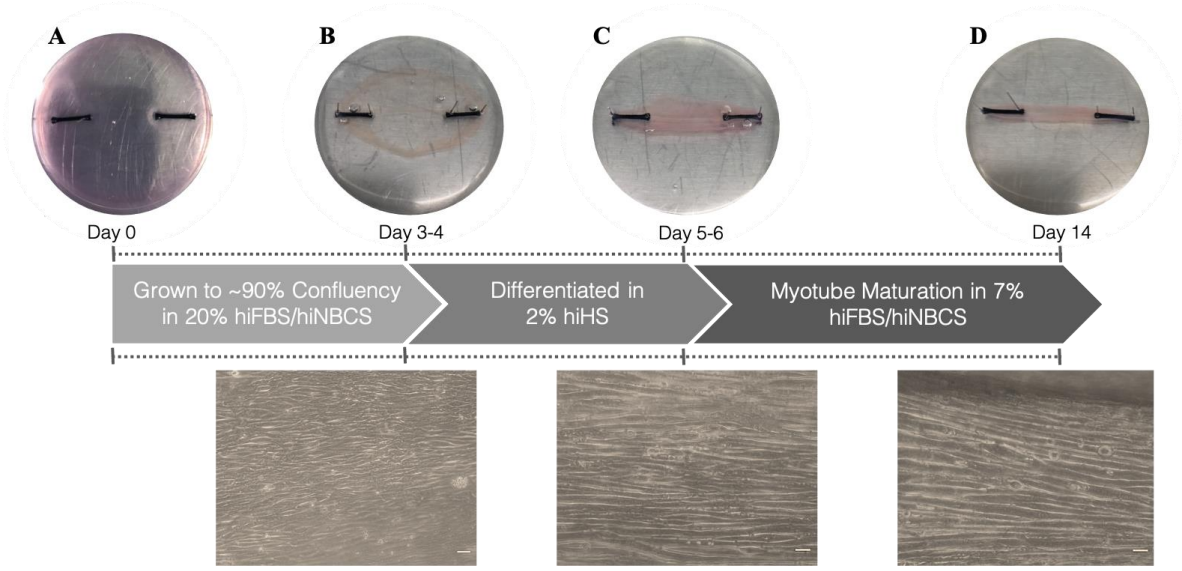
[1]Norwegian School of Sport Sciences, Oslo, Norway [2]University of Copenhagen, Copenhagen, Denmark [3]University of Florida, Florida, The United States of America [4]Liverpool John Moores University, Liverpool , United Kingdom [5]Loughborough University , Loughborough , United Kingdom

Bioengineered skeletal muscle (SkM) provides an effective model for investigating the molecular mechanisms of mechanical load-induced anabolism and hypertrophy in vitro. However, it is previously unknown whether mechanical loading of bioengineered SkM in vitro adequately recapitulates the molecular responses observed after resistance exercise (RE) in vivo. Furthermore, the epigenetic response to exercise-like stimuli in bioengineered SkM has not previously been determined. To address this, the transcriptional (assessed via RT-PCR) and epigenetic (DNA methylation assessed via targeted next generation bisulfite sequencing, tNGBS) responses were compared after mechanical loading in mouse C_2C_{12} bioengineered SkM (*n* = 4-5 replicate cultures) in vitro and after RE in vivo (Turner *et al.,* 2021). Specifically, genes known to be upregulated/hypomethylated after RE in humans (*n* = 8 healthy young men, Seaborne *et al.,* 2018a, 2018b) were analyzed. One-way ANOVA with post hoc analysis (Tukey HSD) showed that 93% (14/15 genes) of these genes demonstrated similar changes in gene expression post-loading in the bioengineered muscle when compared to acute RE in humans. Furthermore, similar differences in gene expression of the same genes were observed between loaded bioengineered SkM and after 4 weeks of programmed resistance training (RT) in adult (6 months old) Wistar rats (*n* = 5 biological replicates, Schmoll *et al.,* 2018). Following unpaired t-test statistical analysis, hypomethylation occurred for only one of the genes analysed (GRIK2) post-loading in bioengineered SkM. To further validate these findings, DNA methylation and mRNA expression of known hypomethylated and upregulated genes across pooled transcriptomic datasets (110 biopsies, 37 pre and 57 post after outlier removal) after acute RE in humans (Turner *et al.,* 2019) were also analyzed at 0.5-, 3-, and 24-hours post-loading in bioengineered muscle via a two-way mixed ANOVA analysis. The largest changes in gene expression occurred at 3-hours, whereby 82% (18/22 genes) and 91% (20/22 genes) of genes responded similarly when compared to human and rodent SkM, respectively. DNA methylation of only a small proportion of genes analyzed (TRAF1, MSN and CTTN) significantly increased post-loading in bioengineered SkM alone. Overall, mechanical loading of bioengineered SkM in vitro recapitulates the gene expression profile of human and rodent SkM after RE in vivo. Although some genes demonstrated differential DNA methylation post-loading in bioengineered SkM, such changes across the majority of genes analyzed did not closely mimic the epigenetic response to acute-RE in humans.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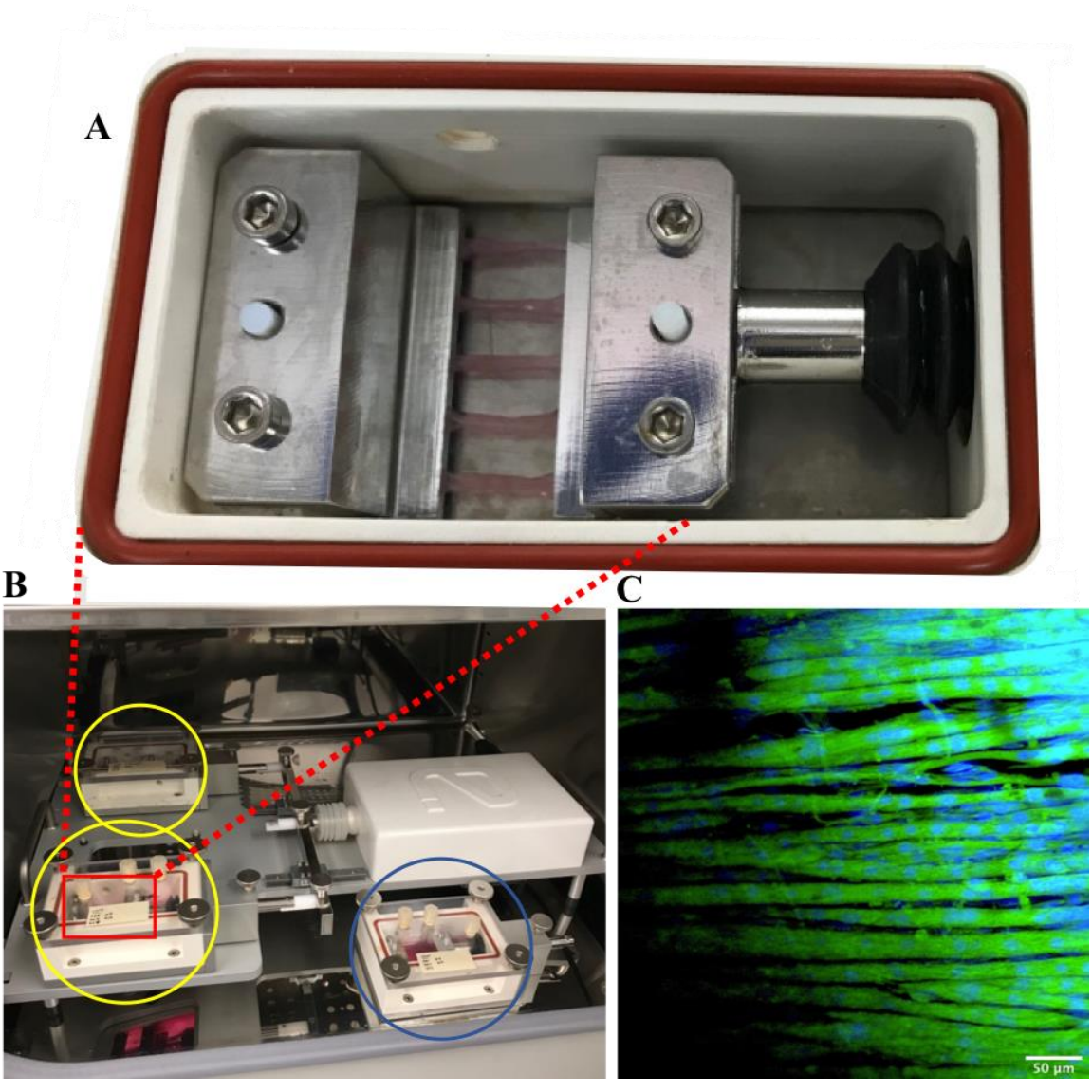
Grown to ~90% Confluency in 20% hiFBS/hiNBCS
Differentiated in 2% hiHS
Myotube Maturation in 7% hiFBS/hiNBCS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Reference 1 :- Turner DC et al. (2021). J Cell Physiol 236, 6534-6547.

Reference 2 :- Seaborne RA et al. (2018a). Scientific Reports 8, 1-7.

Reference 3 :- Seaborne RA et al. (2018b). Scientific Data 5, 1-9.

Seaborne RA et al. (2018b). Scientific Data 5, 1-9.

Reference 4 :- Schmoll M et al. (2018). PLoS One 13, 1-19.

Reference 5 :- Turner DC et al. (2019). Scientific Reports 9, 1-12.

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SA13

Human exercise response variability: a lifelong pursuit

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Over the last 50 years, we have focused most of our research on the quantification of human variability in response to acute and chronic exposure to aerobic exercise as well as alterations in energy balance conditions, and on the potential causes of the observed human heterogeneity. In the investigation of an exercise biology trait, it is essential to distinguish between the sedentary state and the changes in the same trait in response to an exercise program or exercise training. Using maximal oxygen uptake (VO_2max) adjusted for body mass and composition as an example, we have found that even among sedentary adults of the same sex and ethnic background, and within a limited age range, there is at least a two-fold range between very low and very high VO_2max individuals. This variability is not random as about 50% of the variance is accounted for by genetic differences as shown in twin and family studies. Cardiac output, blood volume and hemoglobin content, skeletal muscle oxidative capacity and transcriptomic profile, genomic variants, plasma secreted proteins and metabolites have been associated with VO_2max in the sedentary state but much remains to be learned about the nature of individual differences in the sedentary state. In a series of experiments, we studied the VO_2max response (trainability) to a number of exercise regimens in biologically unrelated adults and in pairs of identical twins and nuclear families. First, we repeatedly found that there is no correlation between VO_2max in the sedentary state and its trainability. Second, there is at least a 3-fold range between the low and high VO_2max gainers in response to a standardized endurance-training program. This is true in young and middle-aged adults, men or women, and in people of African or European ancestry. Third, the heritability of VO_2max training response reaches about 50% of the remaining variance after adjustment for relevant concomitants. Fourth, genome-

wide screens, *vastus lateralis* muscle transcripts abundance profiling, extensive proteomics and metabolomics exploration, and comprehensive bioinformatics pipelines have allowed to identify multiple molecular makers and pathways associated with human variation in VO_2max trainability. Fifth, DNA variants and protein levels significantly associated with VO_2max in the sedentary state and its response to exercise training are almost entirely different supporting the notion that these two traits are truly independent from one another. Sixth, the molecular and pathway profiles of VO_2max in the sedentary state are well aligned with its recognized physiological determinants (cardiac output, blood volume and hematopoiesis, skeletal muscle oxidative capacity, etc.). In contrast, they differ markedly for VO_2max trainability as they are dominated by developmental and embryonic, regulation of gene expression, angiogenesis, skeletal and cardiac muscle growth, extracellular matrix, regulation of apoptosis and autophagy, immunity, calcium signaling and energy metabolism pathways. Even though the notion of human variability of exercise-related traits is now almost universally accepted, we have a long way to go before a coherent description of the molecular and physiological basis for this variability is achieved.

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SA14

Carbohydrate - the highs and lows in elite endurance performance

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Success in elite endurance events is underpinned by the athlete's ability to produce muscular power rapidly and economically throughout the event or at critical moments. The importance of body carbohydrate (CHO) stores as a key muscle substate was established a century ago and formed the basis of the first expert sports nutrition guidelines in the 1990s. The evolution of these guidelines has involved several landmarks and different roles for CHO in the athlete's training and performance nutrition plans. An initial change involved an understanding of "CHO availability" – a dynamic evaluation of body CHO stores in relation to the fuel requirements of each training session - rather than total dietary CHO intake per se. This concept explains differences in the CHO intake of individual athletes, and changes within the same athlete across different days and phases of their training program according to the fuel needs of each session. Contemporary sports nutrition guidelines for competition performance promote a range of strategies before, during and between events to ensure that endogenous and exogenous sources of muscle CHO meet the fuel needs of the event. Optimal strategies for CHO loading and CHO intake during the event have evolved in terms of the timing, amount and source of CHO involved in the plan. Advances in the molecular approach to exercise provided insight that deliberate manipulation of low CHO availability during and in the recovery from an exercise session may upregulate cellular signalling and increase the adaptive response to endurance exercise. Although there is evidence of cellular benefits with a possible transfer to performance advantages in sub-elite athletes, the integration of "train low" strategies in

the diets of elite athletes may be less effective. A separate strategy which involves chronic implementation of a low CHO diet aims to retool the endurance-trained muscle to dramatically increase its capacity for fat oxidation as a replacement for its reliance on the finite body CHO stores. Despite popular interest and hype, adaptation to ketogenic and non-ketogenic versions of low CHO high fat diets has between shown to impair rather than enhance performance of elite endurance athletes, potentially due to the lower economy (higher oxygen cost) of fat oxidation compared with CHO oxidation, and the impaired utilisation of CHO stores that may be reintroduced. A final thread involves the recognition of the central benefits of CHO availability, particularly the benefits of mouth-sensing CHO to activate reward and pacing centres in the brain. This may be implemented in terms of timing of intake of CHO during competitive events as well as the use of CHO mouth rinses during fatiguing "train low" training sessions.

SA15

Skeletal muscle responses to intermittent vs continuous exercise in humans

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A hallmark of skeletal muscle remodeling elicited by exercise training in an increased oxidative capacity as measured using various biomarkers of mitochondrial content. This response is influenced by many factors that fundamentally include the type, intensity, duration, and volume of exercise performed. It was shown almost 50 years ago that a two-month program of intermittent (INT) or continuous (CONT) cycling exercise, matched for total work, elicited similar increases in succinate dehydrogenase activity in mixed human skeletal muscle (1). The response to each type of training differed between type I and type II fibres, however, an effect that was attributed to the pattern of fibre recruitment during exercise. Studies on rodents at the time that characterized the influence of intensity and duration on skeletal muscle responses showed that brief INT exercise increased mitochondrial content like CONT exercise despite a lower training volume, and these effects were influenced by fibre type (2). It was concluded, "the typical endurance training response of a biochemical change in mitochondrial content can be achieved at relatively intense exercise (i.e. exceeding VO2max) maintained for relatively short durations… (and) there seems to be a point where exercise duration becomes unimportant and the magnitude of the adaptive change is established by the intensity of exercise" (2). Despite many studies over the ensuing decades on human skeletal muscle responses to INT and CONT exercise, a vigorous debate endures regarding the most important factors to promote training-induced increases in mitochondrial content and the regulatory mechanisms involved (3,4). Recent work includes the finding based on single-leg cycling exercise (which facilitates a within-subject comparison of responses) that mixed skeletal muscle mitochondrial content increased to greater extent after short-term INT compared to CONT training matched for total work (5). This suggests that exercise intensity per se, and/or the pattern of contraction, is an important determinant of exercise-induced skeletal muscle remodelling in humans. Acute INT compared to CONT exercise has been shown to elicit greater activation of molecular signaling cascades linked to mitochondrial biogenesis, including in type II fibres, which could be

important for exercise-type specific responses (6). Other work has revealed that while “sprint”-type INT training increases mixed skeletal muscle mitochondrial content like CONT training despite a lower training volume, there are differential responses in some fibre types (7). It has also been shown in trained individuals with an already well-developed oxidative capacity that adding sprint INT to CONT exercise training augments the increase in mitochondrial proteins in mixed skeletal muscle and type II fibers despite matched total work (8). A considerate recent review by Bishop and colleagues (9) examines current controversies in the field including the role of INT and CONT exercise for promoting mitochondrial biogenesis and discusses methodological issues that need to be addressed to resolve existing conflicts. From an integrative perspective, there are of course many cellular processes that ultimately determine the skeletal muscle response to different training modalities including peripheral vascular remodeling (10).

Reference 1 :- Henriksson J, Reitman JS. (1976). Acta Physiol Scand 97(3): 392-397.

Reference 2 :- Dudley G et al. (1982). J Appl Physiol Respir Environ Exerc Physiol 53(4): 844-850.

Reference 3 :- MacInnis MJ et al. (2019). J Physiol 597(16): 4111-4113.

Reference 4 :- Bishop et al. (2019). J Physiol 597(16): 4115-4118.

Reference 5 :- MacInnis MJ et al. (2017). J Physiol 595(9): 2955-2968.

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SA16

Short-chain fatty acids as potential regulators of skeletal muscle metabolism and function

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A key metabolic activity of the gut microbiota is the fermentation of dietary fibre, which generates short-chain fatty acids (SCFAs) as the principal end products. SCFAs are absorbed from the gut lumen and can contribute to energy homeostasis by serving as an available substrate at different organ sites, including skeletal muscle. Furthermore, a widespread receptor system exists for SCFAs. These G protein-coupled receptors, free fatty acid receptor 2 (FFAR2) and 3 (FFAR3) are expressed in numerous tissues and regulate metabolic responses in the gut epithelium, adipose tissue and liver. This talk will evaluate the evidence demonstrating that SCFAs mediate metabolic cross-talk between the gut microbiota and skeletal muscle. It will discuss the direct and indirect mechanisms through which raising gut-derived SCFAs modules skeletal muscle metabolism. Finally, it will highlight the potential roles of these gut-derived metabolites in exercise performance and adaptations to exercise training.

SA17

Muscle Insulin resistance and fuel metabolism in response to inactivity.

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Introduction

Bed-rest (BR) reduces whole-body insulin-stimulated glucose disposal (GD) and alters muscle fuel metabolism(1). However, little is known about metabolic adaptation from acute to chronic BR, particularly when volunteers are maintained in energy balance. We therefore determined whole body GD, carbohydrate (CHO) and lipid oxidation and intramyocellular lipid (IMCL) content during acute and chronic BR whilst maintaining energy balance.

Method

Healthy males (n=10, 24±1.25 years, body mass index (BMI) 22.7±0.60) maintained in energy balance underwent 3 days of BR (acute BR; ABR). A second cohort matched for gender and body mass index (n=20, 34±1.8 years, 23.8±0.41) underwent 56 days of BR (chronic BR; CBR). An isoenergetic diet was maintained throughout both studies (30% fat,15% protein,55% CHO). A hyperinsulinaemic euglycaemic clamp (60 mU/kg body mass/min)was performed before and after BR. Indirect calorimetry was performed before and during the clamp steady-state to calculate rates of whole-body fuel oxidation. Vastus Lateralis muscle biopsies were taken before and after each clamp to quantify muscle glycogen content. Intramyocellular lipid (IMCL) content and expression levels of 191 mRNA targets were also determined in biopsy samples obtained before each clamp. Two-way repeated measures ANOVA was used to detect differences in end-point measures and Ingenuity

Pathway Analysis was used to interrogate mRNA expression changes. All values presented are mean ± SEM.

Results

ABR reduced insulin-mediated glucose disposal (GD, normalised to DEXA determined lean body mass; LBM) by 17% (11.5 ± 0.7 vs 9.3 ± 0.6 mg/kg LBM/min; $p<0.001$). CBR reduced GD by 22% (10.2 ± 0.4 vs 7.9 ± 0.3 mg/kg LBM/min, $p<0.05$). This reduction in GD following both ABR and CBR was paralleled by the elimination of a 35% increase in insulin-stimulated muscle glycogen storage seen before BR. ABR had no impact on insulin-stimulated carbohydrate (CHO) and lipid oxidation, but CBR reduced CHO oxidation ($p<0.05$) and blunted the magnitude of insulin-mediated inhibition of lipid oxidation ($p<0.05$). Neither ABR nor CBR increased muscle intramyocellular lipid content. Plentiful mRNA abundance changes from before BR were detected following ABR, which had waned following CBR and generally reflected the changes in fuel oxidation and muscle glycogen storage seen at this time point.

Conclusion

ABR suppressed insulin-stimulated GD and glycogen storage, and the extent of suppression was increased no further after CBR. However, insulin-mediated inhibition of fat oxidation after CBR was less than after ABR, and was accompanied by blunted CHO oxidation. Moreover, this shift in substrate oxidation after CBR was not explained by IMCL accumulation, but was reflected by muscle mRNA abundance changes, pointing to the lack of muscle contraction *per se* as the primary signal for the adaptations observed.

Reference 1 :- Mikines, K. J., Richter, E. A., Dela, F. & Galbo, H. Seven days of bed rest decrease insulin action on glucose uptake in leg and whole body. J Appl Physiol **70**, 1245-1254 (1991).

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SA18

An update on D2O applications to investigate human muscle adaptations

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Skeletal muscle is vital for the completion of activities of daily living and is becoming increasingly recognised for its essential roles in whole body metabolism, acting as the largest reservoir for amino acids in the body and a storage point for glucose and lipids. Maintenance of muscle mass is key to the promotion of health, with muscle atrophy as a result of inactivity, disease or ageing, resulting in increased frailty, metabolic comorbidities and mortality. As such, muscle hypertrophy is desirable in the general population due to the rising interest in muscle health and wellbeing, in addition to athletes looking to optimise performance. There have been considerable research efforts aimed at unravelling the regulation of muscle mass, however, many aspects of hypertrophy remain unclear and debated. Understanding the mechanisms and most effective strategies to induce skeletal muscle hypertrophy would offer great resource in combating muscle wasting and add clarity to optimal exercise practices. The use of stable isotope tracers enables the accurate quantification of dynamic metabolism and are key to understanding the mechanisms regulating muscle adaptation. Advances in the application of deuterium oxide (D2O) techniques now permit the simultaneous dynamic measurement of a range of substrates (i.e., protein, lipid, and nucleic acids, along with the potential for OMICs methodologies) with minimal invasiveness further creating opportunities for long-term 'free living' measures. As successful muscle adaptation requires a coordinated response of protein turnover, ribosomal biogenesis and satellite cell activity, D2O can provide a more dynamic holistic picture of muscle adaptation.

SA19

Imaging skeletal adaptation to exercise

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Exercise can stimulate adaptation in bone mass, geometry and architecture, that may affect risk of stress fracture and future osteoporosis. A variety of imaging approaches can be used to study such changes. Dual X-ray absorptiometry (DXA) has long been used to assess bone mineral density of sites

relevant to osteoporosis risk. It also allows estimation of some structural parameters, and detection of some localised adaptation, e.g. in the lumbar spine contralateral to the bowling arm in fast bowlers. Quantitative computed tomography (QCT) provides 3D imaging that allows discrimination of cortical and trabecular compartments. QCT data can be used with statistical mapping approaches to localise adaptations and finite element models that combine density and location of bone to estimate strength related parameters. High resolution peripheral QCT allows assessment of changes in bone microarchitecture. The presentation will review these methods and their applications in studies of unilateral loading to demonstrate localised adaptations that may have important effects on bone dimensions and strength.

SA20

Mitochondrial measurements in human muscle

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Introduction: Pre-clinical evidence suggests that nicotinamide adenine dinucleotide (NAD^+) precursors can improve disease-associated mitochondrial dysfunction, however, clinical studies using the precursor nicotinamide riboside have so far failed to recapitulate these mitochondrial-enhancing benefits. Nicotinic acid (NA) is an alternative NAD^+ precursor, which enters the NAD^+ pool via a different metabolic pathway to nicotinamide riboside, and has proven effective at enhancing mitochondrial function in diabetics. However, the efficacy of NA on skeletal muscle mitochondrial function in ageing muscle remains to be determined. **Aim:** Using a battery of mitochondrial-focused measurements, we investigated the efficacy of NA supplementation on skeletal muscle and mitochondrial function in healthy, physically inactive, older (65-75 years) males. **Methods:** In a double-blind, randomised, placebo-controlled fashion, eighteen older males were randomly assigned to 2-weeks of NA analogue supplementation (250 mg acipimox thrice daily, *n*=8) or placebo supplementation (*n*=10). Pre, during (week 1) and post (week 2) supplementation, (an)aerobic muscle function was measured via moderate incremental and ramp incremental cycling, 10-second Wingate cycling and the Short Physical Performance Battery Test (SPBBT). Muscle biopsies obtained from the *m. vastus lateralis* were used to quantify mitochondrial function via high resolution respirometry, mitochondrial content via citrate synthase, electron transport chain content via immunoblotting and mitochondrial and myofibrillar muscle protein synthesis via gas-chomotography-pyrolysis-isotope ratio mass spectrometry. Volunteers provided written informed consent prior to participation and all study procedures were approved by the South West Frenchay Research Ethics Committee (16/SW/0099). **Results:** NA supplementation reduced respiratory exchange ratio during moderate and ramp exercise ($p<0.05$) but did not influence other aerobic performance indices (i.e., V_{O2}, V_{CO2} or V_E) ($p>0.05$). Anaerobic minimum power, determined by Wingate, increased from week 1 to week 2 in NA supplemented volunteers ($p<0.05$) but SPPBT performance did not change ($p>0.05$). NA supplementation tended to increase mitochondrial

respiration (p=0.10), with coupled respiration through complex I increased at week 2 and maximal uncoupled respiration increased at week 1 and 2, compared to baseline. NA supplementation also tended to increase citrate synthase (p=0.10) but had no significant impact on electron transport chain content (p>0.05), mitochondrial or myofibrillar fractional synthesis rate (p>0.05). **Conclusion:** NA supplementation alters substrate utilisation towards fat oxidation and may improve mitochondrial function/content in older healthy adults, however due to the limited *n*, larger clinical trials are needed to substantiate these findings.

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SA21

All these analytical tools! A case study of multi-application

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Molecular and technical evolution in bioscience has led to an explosion of methodologies to interrogate key questions surrounding human physiological regulation, including in exercise and health. For instance, advances in OMICS, stable isotope tracers, medical imaging, and myriad means to quantify intra/inter-cellular metabolic dynamics (e.g., mitochondrial function, vascular remodelling) offer an increasing number of analytical options. Pressing this agenda, UK Research and Innovation (UKRI) has schemes such as *ALERT* (BBSRC) to fund capital equipment of strategic basic science importance, while the MRC's "*better methods, better research*" call is of a similar goal in a translational research space. As such, when planning research, considerations arise as to the most appropriate techniques and technologies to harness for best scientific effect. The areas of coverage within this presentation will focus upon aspects relating to: i) stable isotope tracers, ii) OMIC's, and iii) study design/methodology relevant both to an exercise physiologist, but equally to the broader study of human health.

The first method/technique to be discussed is the recently developed *Combined Oral Stable Isotope Assessment of Muscle* (COSIAM). The aim of COSIAM was to develop a versatile and minimally invasive methodology to simultaneously quantify muscle mass (d3-creatine dilution), muscle protein synthesis (D_2O direct incorporation) and muscle protein breakdown (d3-methyl-histidine dilution) via an orally consumed stable isotope tracer cocktail (1). In doing so, we demonstrate for instance, robust links between d3-creatine dilution and biomarkers of healthy muscle physiology. In future, COSIAM will prove a valuable tool in monitoring cross-sectional and longitudinal alterations at the core of muscle health in experimental medicine. Next, transcriptomics (2) and metabolomics (3) in human ageing/exercise responses will be briefly discussed as they pertain to discovery, while also highlighting limitations of single or limited time-point approaches. Reflecting these very limitations,

the penultimate aim of this presentation will cover the concept of “dynOMICS”; currently recognised as fluxomics (of metabolites) and dynamic proteomics. Data will be presented to illustrate how stable isotope methods linked to OMICS may provide a conduit to the best of both worlds: big-data linked to dynamic cellular metabolism. To illustrate this methodology, we report on muscle cells in vitro being subject to dynamic proteomics over a time-course of 48h (murine C2C12) when treated with compounds to induce mitochondrial biogenesis (AICAR/GW501516) and also to inhibit protein synthesis (cycloheximide). Notably, of ~2000 proteins identified, 250 were accurately quantified across treatments, with treatments showing significant effects on rates of individual protein turnover e.g., cycloheximide showing an expected pattern for decline in many proteins. Finally, other challenges require briefly highlighting: 1) Which OMIC(S) should be applied and why? Multi-OMICS is progressive but not without complexities and associated high-costs, and what about external validation; 2) What sampling is possible, and will this generate sufficient material; 3) What issues surround study control e.g. feeding/physical activity; and 4) A dearth in skills of physiologically trained bioinformaticians; not least a shift toward a service industry.

Reference 1 :- Cegielski J, Wilkinson DJ, Brook MS, Boereboom C, Phillips BE, Gladman JFR, Smith K, **Atherton PJ.** Combined in vivo muscle mass, muscle protein synthesis and muscle protein breakdown measurement: a 'Combined Oral Stable Isotope Assessment of Muscle (COSIAM)' approach. Geroscience. 2021 May 27.

Reference 2 :- Wilkinson DJ, Rodriguez-Blanco G, Dunn WB, Phillips BE, Williams JP, Greenhaff PL, Smith K, Gallagher IJ, **Atherton PJ**. Untargeted metabolomics for uncovering biological markers of human skeletal muscle ageing. Aging (Albany NY). 2020 Jun 24;12.

Reference 3 :- Bethan E Phillips, John P Williams, Thomas Gustafsson, Claude Bouchard, Tuomo Rankinen, Steen Knudsen, Michael Rennie, Kenneth Smith, James A Timmons, and **Philip J Atherton**. Molecular and Genomic Networks of human muscle adaptation to exercise and age. PLoS Genetics. 2013 Apr; 9(4).

OC1

The effects of very low-calorie diet (VLCD) or adjuvant resistance exercise training (RET) or high intensity interval training (HIIT) on metabolism and body composition

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Background: Very low-calorie diets (VLCD) are a front-line strategy, to propagate rapid weight loss in obesity and diabetes; however, a quarter of the total weight loss is in the form of lean mass, including muscle - representing a major deleterious concurrent consequence. Exercise in the form of resistance exercise training (RET) promotes muscle hypertrophy, while high-intensity interval training

(HIIT) improves muscle mass more marginally but also cardiopulmonary fitness (CPF). However, no study has been conducted contrasting how RET and HIIT adjuvant to VLCD interact in relation to metabolism and body composition in humans. We therefore explored the effects of VLCD with or without RET or HIIT on body composition, muscle protein synthesis (MPS), metabolic markers, muscle strength and CPF.

Methods: Overweight and obese middle-aged (male) participants were randomly allocated into interventions of VLCD only (N=10; 46±8y; 104±12kg; BMI 32±4, (means±SD)), VLCD+RET (N=8; 40±8y; 99k±10kg; BMI 32±2) or VLCD+HIIT (N=8; 46±11y; 101±12kg; BMI 33±3). All groups received 6-weeks of VLCD meal replacement (conforming to <800kcal/d). VLCD adjuvant exercise groups received supervised training (HIIT/RET) 3X/per week. DXA scans were conducted pre-and-post interventions to assess changes in body composition. Biopsies of vastus lateralis thigh muscle were performed via conchotome methods to quantify muscle protein synthesis (MPS) using deuterium oxide (D_2O) incorporation technique over the 6-week period. Lipid profiles, insulin sensitivity biomarkers, and blood pressure were assessed as markers of metabolic health. One repetition maximum (1-RM) was used to assess upper limb/lower limb strength (i.e., supine chest press, latissimus pull-down, seated row, leg curl, knee-extension, leg press), while cardiopulmonary exercise testing was used to assess CPF in the form of VO_2 max and anaerobic threshold.

Results: All groups (VLCD only, VLCD+RET and VLCD+HIIT) exhibited significant weight loss (11±3 kg, 11±4kg, 12±4kg respectively), fat mass loss (7±2kg, 7±2kg, 8±3kg, and LM loss (4±2kg, 4±2kg, 4±1kg) ($P<0.0001$) with no between-groups difference being observed. MPS rates after the 6-weeks of intervention were 0.8%±0.2d^{-1}, 0.9%±0.2d^{-1} and 1.2%±0.2d^{-1} for VLCD only, VLCD+RET and VLCD+HIIT, respectively - with significant differences being seen between VLCD+HIIT compared to VLCD only ($P=0.004$). Significant improvements were seen in insulin sensitivity biomarker indices (i.e., HOMA-IR, QUICKI, Cederholm), lipid profiles (cholesterol, triglycerides) and blood pressure (systolic and diastolic) in all groups ($P<0.05$), with no group differences. Only did the VLCD+RET group illustrate a significant 1-RM improvement ($P<0.01$), although notably the other two groups exhibited no reductions in 1-RM. No intra- or inter-group differences were observed for VO_2 max/anaerobic threshold.

Conclusion: VLCD with or without adjuvant distinct exercise modes, induced marked weight loss. Despite marked LM loss all groups similarly improved metabolic biomarkers in the form of insulin indices, lipid profiles and blood pressure, with no differences between the groups. CPF was preserved in all groups. HIIT was associated with significantly higher MPS compared to VLCD only, and thus may represent a means to offset LM loss during longer-term calorie deficits. Addition of RET, while on VLCD, improved 1-RM despite reductions in LM, suggesting maintenance of muscle strength on VLCD is beyond muscle mass per se.

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OC2

Factors affecting muscle fiber size in athletes

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Introduction: Muscle fiber cross-sectional area (CSA) significantly contributes to gains in strength after resistance training, and can be affected by numerous factors, including genetics, age, nutrition, training parameters and habits. However, the majority of research seems to include only males in the sampled cohort. **The aim** of the study was to identify the associations between various factors and muscle fiber CSA of the vastus lateralis in 157 physically active subjects. **Methods:** The retrospective, observational study involved 55 power-trained (19 females) and 102 endurance-trained (27 females) subjects. Athletes' nutrition, training parameters and habits were recorded using a survey. Muscle fiber composition and CSA of m. vastus lateralis were determined by immunohistochemistry. Genotyping was performed using micro-array analysis. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Federal Research and Clinical Center of Physical-chemical Medicine. Written informed consent was obtained from each participant. **Results:** As expected, power-trained subjects had significantly greater fast- and slow-twitch muscle fiber CSA than endurance-trained subjects ($P<0.05$). In female power-trained subjects, the CSA of the fast-twitch muscle fibers negatively correlated with age ($r=-0.48$, $P=0.037$), but positively associated with training frequency ($r=0.68$, $P=0.0014$), protein/BCAA intake ($r=0.46$, $P=0.046$), meat consumption ($r=0.65$, $P=0.0028$), water consumption ($r=0.48$, $P=0.037$) and sleep duration ($r=0.48$, $P=0.039$). Multiple regression analysis showed that these factors explained 76.6% of fast-twitch muscle fiber CSA variation in female power-trained subjects. In female endurance-trained subjects, only sleep duration ($r=0.52$, $P=0.0059$) reported a significant interrelation with CSA of the fast-twitch muscle fibers. In the whole group (n=157), the CSA of the fast-twitch muscle fibers negatively correlated with age ($r=–0.26$, $P=0.0011$) and alcohol consumption ($r=–0.17$, $P=0.032$). Furthermore, in the combined group of male and female endurance-trained subjects, the CSA of the fast-twitch muscle fibers positively correlated with creatine consumption ($r=0.24$, $P=0.015$). Next, using two panels of DNA-markers associated with fat-free mass (1981 SNPs) and testosterone levels (855 SNPs) in the UK Biobank cohort, we identified that 40 SNPs (rs11632750, rs12040325, rs12564492, rs12602084, rs1321080, rs13237404, rs1480474, rs150352963, rs1582931, rs190930099, rs2227138, rs2241388, rs2306862, rs2595104, rs26866, rs2926247, rs343935, rs34706136, rs3803573, rs4309038, rs4665244, rs6121042, rs62260729, rs693906, rs6988769, rs7184768, rs72630041, rs73198802, rs77031559, rs7727774, rs7740107, rs8086627, rs8192589, rs850294, rs853985, rs9268249, rs9271657, rs9291834, rs9329259, rs9599996) were significantly ($P<0.05$) associated with both CSA of the fast-twitch muscle fibers in our group (n=157) and handgrip strength in the UK Biobank cohort. **Conclusions:** These results demonstrate that genetics, training parameters, nutrition and habits are associated with muscle fiber CSA in physically active subjects.

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OC3

Leucine-induced mTOR translocation and peripheral activity is associated with myofibrillar fractional synthetic rates in human skeletal muscle

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Introduction Leucine is commonly considered to be the most anabolic amino acid and its ingestion or infusion alone stimulates myofibrillar muscle protein synthesis (MyoPS)[1,2], purportedly through its ability to increase mTORC1 recruitment to the lysosomal surface[3]. Our lab has also reported a further mechanism of mTORC1 activation in human skeletal muscle where anabolic stimuli elicit mTOR-lysosome translocation to the cell periphery concomitant with elevated mTORC1 activity. However, whether leucine ingestion alone is able to initiate either of these cellular events in human skeletal muscle and whether they are related to MyoPS is yet to be established.

Aims Therefore, the aim of this study was to investigate the impact of leucine ingestion on mTOR translocation, protein-protein interactions and localized activity in human skeletal muscle. A secondary aim was then to determine if these cellular events were associated with MyoPS rates.

Methods Eight young, healthy, recreationally active males (age – 23±3yrs, BMI – 24.2±1.9kg/m^2, BF% – 22.8±3.6%) volunteered to undertake a primed continuous infusion of L-[ring-$^{13}C_6$]phenylalanine with repeated blood and skeletal muscle biopsy samples. All procedures were approved by the research ethics board at the University of Toronto, Canada (Protocol No. 00036752) and conformed to the Declaration of Helsinki (revised 2013). Biopsies were obtained at baseline and 30, 60 and 180 minutes after ingestion of 2g leucine and subjected to immunofluorescent microscopy analysis for mTOR colocalization/activity analysis[4]. MyoPS was determined by LC-MS/MS and has been previously published elsewhere[5]. One-factor repeated measures ANOVAs were conducted to determine differences in mTOR localization, protein-protein interactions and region-specific RPS6$^{Ser240/244}$ phosphorylation, a mTORC1-mediated event, with appropriate *post hoc* comparisons corrected for

multiple comparisons (Holm-Bonferroni). Pearson's correlation coefficients were used to assess potential associations between these measures and MyoPS.

Results Leucine ingestion elicited increases in mTOR colocalization with WGA (sarcolemmal marker) and LAMP2 (lysosomal marker) at 30 and 60 minutes post-ingestion (18-29% & 16% respectively, $p<0.025$) suggesting leucine initiated mTOR translocation to the periphery and lysosomal surface respectively. p-RPS6$^{Ser240/244}$ displayed increased staining intensity at all post-ingestion timepoints indicating elevated mTORC1 activity across this entire post-prandial period assessed (14-30%, $p<0.03$). Moreover, when assessed in a region-specific manner, peripheral (outer 5.5µm of fibre) RPS6$^{Ser240/244}$ phosphorylation was elevated at all postprandial timepoints (16-33%, $p<0.02$) whereas central phosphorylation was only elevated at 180 minutes post-ingestion. This suggests that the primary site of mTORC1 activation following leucine ingestion is the area of the fibre to which mTOR-lysosome complexes translocate. Total fibre p-RPS6$^{Ser240/244}$ staining intensity at 60 min was positively associated with MyoPS rates ($r=0.74$, $p=0.036$), however, this association became stronger when considering only peripheral RPS6$^{Ser240/244}$ phosphorylation ($r=0.8$, $p=0.016$).

Conclusions Combined, these results demonstrate, for the first time, that leucine ingestion is able to stimulate mTOR translocation to the fibre periphery and lysosomal surface, two predominant mechanisms of mTORC1 activation, in human skeletal muscle. In addition, leucine-stimulated peripheral mTORC1 activity was associated with MyoPS rates, further confirming the importance of this cellular region for skeletal muscle anabolism.

Reference 1 :- 1. Wilkinson et al. (2013) *J Physiol*. doi: 10.1113/jphysiol.2013.253203

Reference 2 :- 2. Smith et al. (1992) *Am J Physiol.* doi: 10.1152/ajpendo.1992.262.3.E372

Reference 3 :- 3. Wolfson et al. (2016) *Science.* doi: 10.1126/science.aab2674

Reference 4 :- 4. Hodson et al. (2022) *Am J Physiol Cell Physiol* doi: 10.1152/ajpcell.00357.2021

Reference 5 :- 5. Paulussen et al. (2021) *J Appl Physiol.* doi: 10.1152/japplphysiol.00295.2021

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OC4

Six Weeks of Intensified Training maintains Mitochondrial Oxidative Capacity despite a Reduced Training Volume in Highly-Trained Cyclists

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Introduction

Given that performance in endurance events relies heavily on aerobic metabolism, endurance athletes need to possess a high oxidative capacity of skeletal muscle. While it is well-described that exercise training induces mitochondrial biogenesis and enhances muscle oxidative capacity (1,2), there is still debate as to the importance of training intensity versus volume in inducing mitochondrial biogenesis and enhancing mitochondrial respiratory capacity (2,3,4). This is particularly important to examine in endurance athletes, as this population often has a high training volume for which most of the training is conducted at low-to-moderate intensity. Herein, we examined adaptations in mitochondrial respiratory capacity following 6 weeks intensified training consisting of speed endurance training (SET) and a reduction of the overall training time spend at low-to-moderate intensity in highly-trained cyclists. We hypothesized that an increased training intensity would compensate for a reduction in training volume and thus maintain mitochondrial respiratory capacity.

Methods

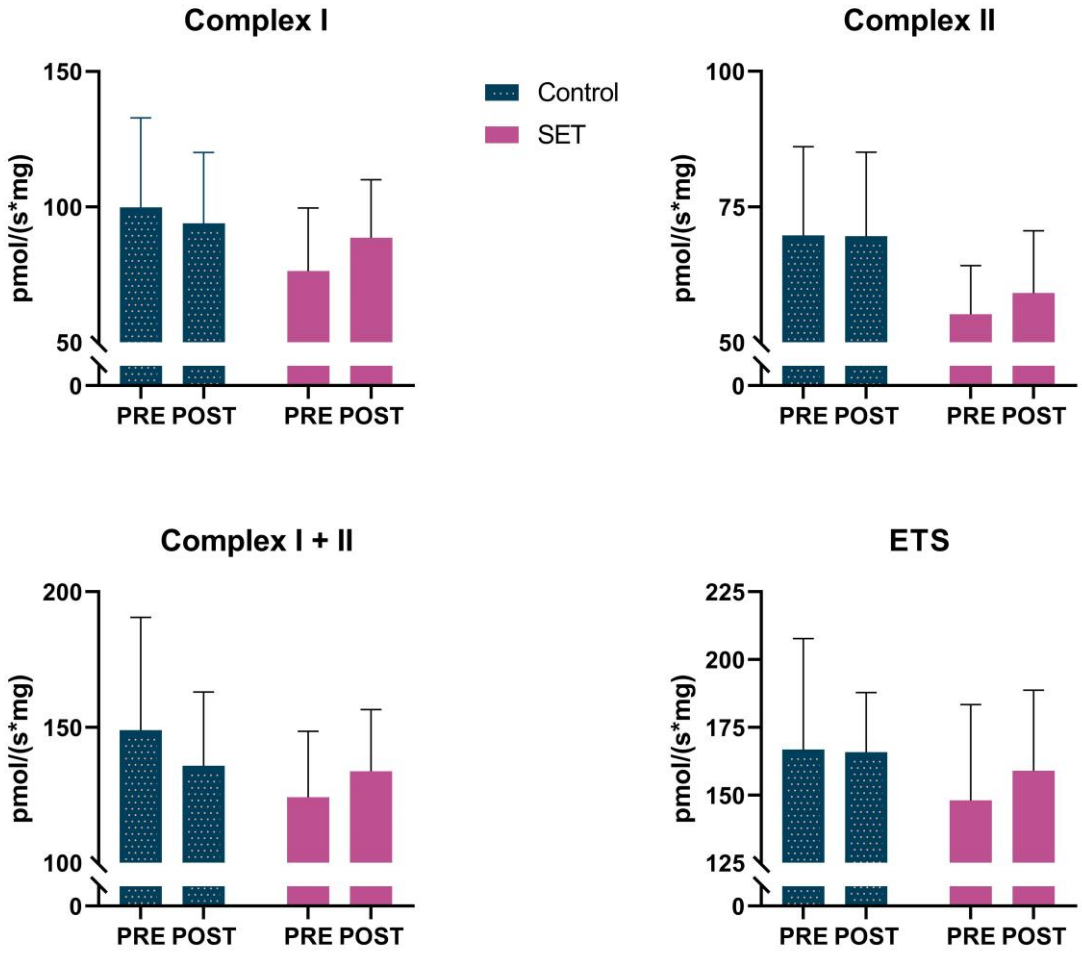
We enrolled 14 endurance-trained cyclists (mean ± SD; Age: 25±6 years, height: 183±6 cm, weight: 74±9 kg, and VO_{2max}: 69±4 mL×min^{-1}×kg^{-1}, training volume: 457±157 min/week) and randomly assigned them to either 6 weeks of maintained training intensity and volume (CON) (n=7) or a SET group (n=7), consisting 2 weekly sessions of 6 repetitions 30 s sprints, separated by 3 min active recovery. In the SET group, weekly training volume was reduced by 25%. Resting skeletal muscle biopsies were obtained from the *vastus lateralis* before and after the 6-week intervention for measurement of mitochondrial respiratory capacity using high-resolution respirometry in permeabilized muscle fibers. $\dot{V}O2_{max}$ was measured before and after the intervention and determined as the highest value achieved during any 30 s period. Weekly training time were compared by a paired t-test. A linear mixed model for repeated measurements was used to estimate within- and between-period effects of OXPHOS capacity and VO_{2max}. The level of significance was set to P≤0.05. Data are presented as means±SD. The study was approved by the local scientific ethics committee of Copenhagen (H-18007889) and was performed in accordance with the Helsinki II Declaration.

Results

Weekly training volume did not change (P=0.3) during the intervention in CON (-38±31 min), while it declined (P<0.001) by 26 % (-122±26 min) in SET. Respiratory capacity was similar between the groups at baseline. No changes in respiratory capacity of complex I, II, I+II or ETS were observed in any of the groups with the intervention (CI, CII, CI+II, ETS: CON: -6% (-6±35 pmol/(s×mg)) -9% (-13±47 pmol/(s×mg)), -1% (-1±39 pmol/(s×mg)), 0% (0±22 pmol/(s×mg)); SET: 16% (12±15 pmol/(s×mg)), 8% (10±18 pmol/(s×mg)), 7% (11±24 pmol/(s×mg)), 7% (4±16 pmol/(s×mg)), respectively). $\dot{V}O_{2max}$ did not change in any of the groups during the intervention (-0.3±4.9 and -0.3±3.9 mL×min^{-1}×kg^{-1} for CON and SET, respectively).

Conclusion

Collectively, these findings suggest that an increased training intensity using SET maintains skeletal muscle mitochondrial respiratory capacity and $\dot{V}O_{2max}$ despite a reduced training volume in endurance-trained cyclists



Reference 1 :- Lundby, C., and Jacobs, R. A. (2016) Adaptations of skeletal musclemitochondria to exercise training. Exp. Physiol. 101, 17–22

Reference 2 :- MacInnis MJ & Gibala MJ (2017). Physiological adaptations to interval training and the role ofexercise intensity. J Physiol 595, 2915–2930.

Reference 3 :- MacInnis MJ, Skelly LE & Gibala MJ (2019).CrossTalk proposal: Exercise training intensityis more important than volume to promoteincreases in human skeletal musclemitochondrial content. J Physiol 597,4111–4113.

Reference 4 :- Bishop DJ, Botella J & Granata C (2019).CrossTalk opposing view: Exercise trainingvolume is more important than trainingi ntensity to promote increases inmitochondrial content. J Physiol 597,

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OC5

Ageing and Cardiorespiratory Function: A Nine-Year Longitudinal Study of Master Cyclists

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Ageing is associated with a decline in cardiorespiratory function. However, in many older people lifestyle factors, particularly physical inactivity, may account for a significant proportion of this decline. To eliminate this confounder and investigate the inherent ageing process, healthy physically active people should be studied (Lazarus and Harridge 2010). Most studies of human ageing are cross-sectional and confounded by the natural heterogeneity in levels of function that exist between different individuals. To overcome both limitations a longitudinal study of a subset of highly active master cyclists (Pollock et al., 2015) was performed. The cyclists were retested to determine how cardiorespiratory function has changed over 9 years. The overarching hypothesis is that with a maintenance of health and exercise levels, any changes observed could be attributed to the biological ageing process unaffected by negative lifestyle factors.

Fifteen healthy master cyclists (men n = 14; female n = 1), now aged 64–85 years (74±6 years) returned for testing. Body composition was measured using dual-energy X-ray absorptiometry (DEXA). Resting blood pressure and heart rate were measured after 10 minutes of supine rest. R function (Forced Vital Capacity, FVC, Forced Expiratory volume, FEV1 and Peak Expiratory Flow, PEF) was assessed in accordance with ATS guidelines. An incremental exercise test on a cycle ergometer was used to determine maximal aerobic power (VO_{2max}) and ventilatory threshold (VT). Oxygen uptake kinetics were also determined. All data are presented as mean±SD and compared pre and post 9 years using a paired t-test.

After 9 years, cycling volume was unchanged (585±388 vs. 570±407 km/month; p = 0.88). Body mass (70±7.0 vs. 67±6.8 kg; p = 0.009) and fat free mass ((FFM); 55±4.6 vs. 50±4.1 kg; p< 0.001) were reduced by 3% and 8%, respectively, whilst fat mass increased 15% (14.4±3.5 vs. 16.5±3.7 kg; p < 0.005). Resting systolic blood pressure (128.7±13.8 vs. 129.9±22.1 mmHg; p = 0.80), and diastolic blood pressure (69.4±8.9 vs. 64.1±13.5 mmHg; p = 0.08) were unchanged after 9 years. FVC (5.6±1.1 vs. 4.0±1.0 L; p < 0.001), and FEV_1 (3.6±0.6 vs. 2.9±0.6 L p < 0.001) declined by 28% and 19%, respectively whilst PEF remained unchanged (8.2±2.3 vs. 8.1±1.5 L/s; p = 0.76). The FEV_1/FVC ratio increased by 13% (64.9±8.8 vs. 73.1±7.4; p < 0.001). VO_{2max} declined by 18% (47.3±3.5 vs. 38.9±4.7 ml kg^{-1} min^{-1}; p < 0.001), but less so (12%) when normalised to FFM (59.4±3.9 vs. 52.1±5.5 ml (kg

FFM)$^{-1}$ min^{-1}; p < 0.001). There was a decline in maximum heart rate (165.1±10.9 vs. 157.9±14.3 bpm; p = 0.02). VT decreased by 11% (35.7±3.7 vs. 31.9±4.5 ml/kg/min; p = 0.001), and by 6% when normalised to FFM (44.8±3.9 vs. 42.2±5.2 ml mls/kg/min; p = 0.03). O_2 kinetics did not change (tau; 22.2±8.3 vs. 24.3±3.3 s; p = 0.30).

These data show a decline in many indices of cardiorespiratory function in master cyclists over a nine-year period, which in the context of a maintenance of both their health status and exercise training can be interpreted as being a result of the inherent ageing process.

Reference 1 :- Lazarus NR, Harridge SDR. Exercise, Physiological Function, and the Selection of Participants for Aging Research. Journals Gerontol Ser A. 2010;65A(8):854-857. doi:10.1093/gerona/glq016

Reference 2 :- Pollock RD, Carter S, Velloso CP, et al. An investigation into the relationship between age and physiological function in highly active older adults. J Physiol. 2015;593(3):657-680. doi:https://doi.org/10.1113/jphysiol.2014.282863

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OC6

Functional impairments of motor units following unilateral lower limb immobilisation underpin reductions in strength

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The loss of muscle mass and strength caused by unloading or immobilisation is common in various clinical settings, including nerve injury, joint trauma, and intensive care. Loss of strength cannot be wholly attributed to loss of mass, with, for example, power reduction being strongly associated with bed rest duration while muscle size was not (1). Mechanistic insight into muscle mass loss is well supported by data on muscle protein turnover, mitochondrial dysfunction and insulin resistance (2), yet the mechanistic underpinning of divergent losses of muscle function is less well studied. Furthermore, it is unclear if findings based on normalised strength are influenced by the consistently reported large reductions in total strength occurring alongside disuse atrophy. This research aimed to investigate functional adaptations of vastus lateralis (VL) motor units (MU) following unilateral immobilisation, sampled at forces normalised to both baseline and post-disuse maximum force.

10 young, healthy males underwent 15-days unilateral leg immobilisation. VL cross-sectional area (CSA) and knee extensor maximal voluntary contraction (MVC) were assessed pre- and post-intervention. Individual MU of the VL were sampled with intramuscular electromyography (iEMG) during isometric contractions at 25% MVC. This was performed relative to baseline MVC and the markedly lower post-immobilisation MVC. MU potential (MUP) characteristics were derived from decomposed iEMG signals using decomposition-based quantitative electromyography software. Neuromuscular junction (NMJ) transmission instability was quantified using near-fibre jiggle (3) and MU FR was measured as the frequency of observations. CSA and MVC were analysed using repeated-measures 2-way ANOVA. MUP characteristics were analysed using multi-level mixed-effects linear regression. Significance was accepted at $p<0.05$.

VL CSA and MVC reduced by 4.3±0.6 cm^2 and 158±25.03 N respectively in the immobilised leg (both $p<0.001$), remaining unchanged in the control limb ($p=0.99$ and $p=0.50$). NMJ transmission instability increased in the immobilised leg using baseline MVC ($\beta = 1.923$, 95% CI: 0.508–3.337, $p<0.01$) and using post-disuse MVC ($\beta = 2.04$, 95% CI: 0.27–3.80, $p<0.05$), remaining unchanged in the control limb ($p=0.67$). Firing rate was reduced in the immobilised leg using baseline MVC ($\beta = -0.925$, 95% CI: -1.199 to -0.651, $p<0.001$) and using post-disuse MVC ($\beta = -0.95$, 95% CI: -1.34 to -0.56, $p<0.001$), remaining unchanged in the control limb ($p=0.56$).

Following immobilisation, VL size and strength were reduced as expected, with a disparity in degree of change. The greater reduction in muscle strength is partly explained by an increase in NMJ transmission instability and suppression of MU FR. Dysfunction at the NMJ may be attributable to a partial denervation and selective reinnervation process also observed in healthy ageing (4). Notably, both NMJ transmission instability and MU FR were similarly changed after immobilisation at contraction levels normalised to both baseline and post-disuse maximum force. As such, any observed reductions in individual MU function cannot be explained by the sampling of MUs recruited during lower absolute contraction levels. Rather, the current findings highlight the importance of impaired neural input to muscle in actively inhibiting force-generating capacity. Future interventions aiming to mitigate neuromuscular decline should specifically target neural input to muscle.

Reference 1 :- Di Girolamo FG et al. (2021). Front Nutr. 8(August), 1–13.

Reference 2 :- Rudrappa SS et al. (2016). Front Physiol. 7(August), 1-10.

Reference 3 :- Piasecki M et al. (2021). Clin Neurophysiol. 132(5), 1089-104.

Reference 4 :- Piasecki J et al. (2021). J Physiol. 599(1), 193-205.

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OC7

Aerobic exercise training alters the acute satellite cell response to damaging exercise

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Introduction

Skeletal muscle stem cells, commonly referred to as satellite cells (SC), play a key role in the skeletal muscle's adaptive response. Following various stimuli, such as damage-inducing exercise, SC are activated, proliferate and can fuse to existing myofibres to support repair and remodelling. We have previously demonstrated that greater skeletal muscle capillarization resulted in enhanced SC responses to damaging exercise[1], however the effect of aerobic training to alter the SC response to damaging exercise has yet to be explored.

Aims/objectives

The aim of the study was to determine the effect of aerobic exercise training on acute SC responses to a bout of damaging exercise. We hypothesized that aerobic exercise would augment the acute SC response to damaging exercise.

Methods

Fourteen (n=8 males; n=6 females; 21 ± 2years) healthy, recreationally active individuals were recruited. Appropriate ethical approval was granted (HiREB #3885) and procedures conformed to the Declaration of Helsinki. Participants completed 6 weeks of unilateral aerobic training on a cycle ergometer (3x45min sessions/wk; at 50% unilateral work peak) followed by an acute bout of 300 maximal eccentric contractions (180 degrees/second). Muscle biopsies were taken from the m.vastus lateralis of the aerobically trained (EX) and control (CTL) legs before (Pre), 24h and 48h hours post eccentric contractions. Immunofluorescence microscopy was used to determine SC content (Pax7+ cells), activation status (Pax7+/MyoD+ cells) and capillary to fibre perimeter exchange ratio (CFPE), a measure of capillarization. qPCR was used to determine mRNA expression of Pax7 and MyoD.

Results

Following unilateral aerobic training type I fibre CFPE (capillaries/1000 μm) was greater in EX (7.0 ± 1.2) compared to CTL (5.1 ± 1.1) ($p<.05$). Type I SC content ($Pax7^+$ cells/100 fibres), was greater in EX (Pre 6.21 ± 2.86, 24h 11.31 ± 6.21, 48h 9.77 ± 3.70) compared to CTL (Pre 4.97 ± 2.23, 24h 7.11 ± 2.20, 48h 8.25 ± 6.25) across all time points ($p<0.05$). Type II SC content ($Pax7^+$ cells/100 fibres) increased from Pre (3.84 ± 1.50) to 48h (10.85 ± 5.42) in the EX leg and at 24h (8.27 ± 3.71) and 48h (8.73 ± 3.64) compared to Pre (4.03 ± 1.67) in the CTL leg ($p<0.05$). Aerobic training did not affect the activation of SC (Pax7+/MyoD+ cells) in either fibre type ($p>0.05$).

Damaging exercise increased Pax7 mRNA expression ($p<.05$) to a greater extent in EX (Pre 1.20±0.52, 24h 1.01±0.41, 48h 1.62±0.36) compared to CTL (Pre 1.00±0.21, 24h 1.04±0.34, 48h 1.18±0.26; $p<0.05$) across all time points. MyoD mRNA expression also increased following damaging exercise ($p<0.05$) but no difference was observed between legs ($p>0.05$).

Discussion

Our results suggest that aerobic training augments the SC response to a bout of damaging exercise and potentially due to an increase in capillarization. Maximising the SC response can accelerate the muscle repair process which can have far reaching implications from athletic populations to compromised individuals e.g. older persons.

Reference 1 :- Nederveen et al. (2018). J Physiol 15;596(6):1063-1078.

Acknowledgements :- This work was supported by a National Science and Engineering Research Concil of Canada (NSERC) grant awarded to GP.

OC8

Lifelong recreational exercise preserves satellite cells and improves muscle fibre innervation status

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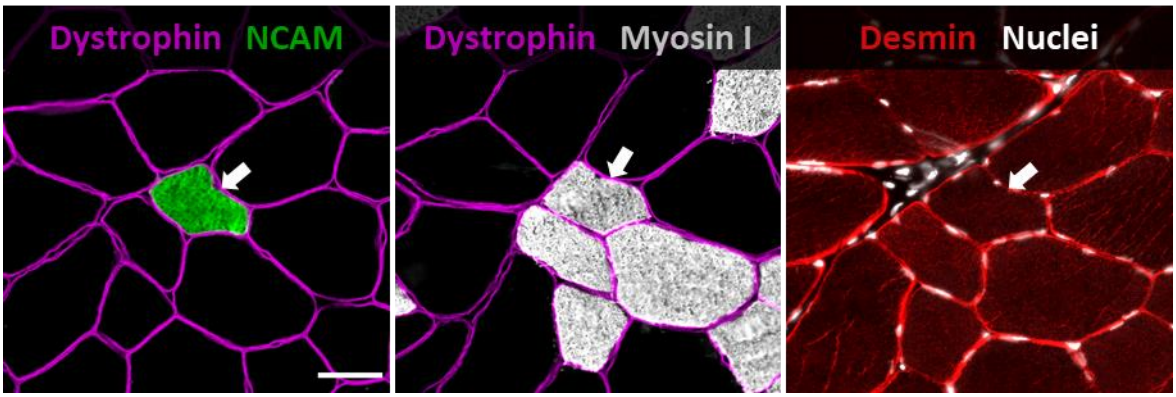
Background: Exercise can mitigate the loss of muscle mass and function with ageing (Mikkelsen *et al.*, 2017), although the protective effect of exercise specifically on muscle fibre denervation in humans has not received much attention. Similarly, muscle innervation status and satellite cells, which are interlinked (Borisov *et al.*, 2001), have not been studied together in humans. Master athletes are commonly studied to decipher the relative contribution of ageing and inactivity to the age-related decline in muscle function (Lazarus & Harridge, 2017). However, this group is highly selected and represents a minor proportion of the general population. In contrast, more than 60% of people aged ≥60 are recreationally active (Hallal *et al.*, 2012), underlining the relevance of studying this group. The purpose of this study was to investigate muscle function, denervation and satellite cell quantity and function in lifelong recreationally active elderly men, compared to sedentary young and elderly men.

Methods: Following ethical approval, 16 elderly men who had performed lifelong recreational exercise (73±4 y, LLEX), such as ball-games, resistance-exercise, racket-sports and cycling at non-competitive levels, were recruited. For comparison, 15 age matched sedentary individuals (73±4 y, SED), and 15 young sedentary individuals (26±5 y), were also recruited. Muscle function was evaluated by maximal voluntary contraction (MVC) and an acute bout of resistance exercise. Muscle biopsies were collected and analysed by immunofluorescence for markers of muscle fibre denervation (figure 1), satellite cell quantity and muscle fibre morphology. RT-qPCR was used to evaluate muscle innervation status, and primary cell cultures were established to test satellite cell

function. Parametric (mean±SD) or nonparametric (median with range) statistical analyses were used, where data displayed a normal or skewed distribution, respectively.

Results: MVC was similar between LLEX and SED (204±41 vs. 186±50 Nm, p=0.291), but higher in young compared to old (318±75 vs. 196±46 Nm, p<0.001). Muscle function under challenged conditions was superior in LLEX compared to both SED and young (p<0.05). The proportion of denervated fibres was higher in old compared to young (1.03 [0-5.45] vs. 0.42 [0-1.40] % p=0.003), but was not different between LLEX and SED (0.996 [0-5.45] vs. 1.062 [0.33-4.08] %, P=0.984). In contrast, LLEX had higher mRNA levels of gamma (p=0.026) and beta (p=0.022) acetylcholine receptors (AChR) compared to SED, and the overall profile was remarkably similar between LLEX and young. LLEX had more type II fibre associated satellite cells compared to SED (0.061±0.021 vs. 0.045±0.012 %, p=0.016), whilst no differences in satellite cell function in vitro between the groups were observed. However, several age-related differences in satellite cell function were observed (myogenin, neonatal myosin, p16, p<0.05). Muscle fibre size was similar in LLEX and SED (3138±892 vs. 3107±1045 um^2, p=0.930), but smaller in old compared to young (3123±953 vs. 5380±845 um^2, p<0.001).

Conclusion: The muscle of lifelong recreationally active elderly men is characterized by a larger type II fibre associated satellite cell content, a youthful AChR profile, and better muscle function under challenged conditions. These findings indicate that recreational physical activity offers partial protection from muscle fibre denervation with ageing.



Reference 1 :- Borisov AB, Dedkov EI & Carlson BM (2001). Interrelations of myogenic response, progressive atrophy of muscle fibers, and cell death in denervated skeletal muscle. *Anat Rec* **264,** 203–218.

Reference 2 :- Hallal PC, Andersen LB, Bull FC, Guthold R, Haskell W & Ekelund U (2012). Global physical activity levels: surveillance progress, pitfalls, and prospects. *The Lancet* **380,** 247–257.

Reference 3 :- Hepple RT & Rice CL (2016). Innervation and neuromuscular control in ageing skeletal muscle. *J Physiol (Lond)* **594,** 1965–1978.

Reference 4 :- Lazarus NR & Harridge SDR (2017). Declining performance of master athletes: silhouettes of the trajectory of healthy human ageing? *J Physiol (Lond)* **595,** 2941–2948.

Reference 5 :- Mikkelsen UR, Agergaard J, Couppé C, Grosset JF, Karlsen A, Magnusson SP, Schjerling P, Kjaer M & Mackey AL (2017). Skeletal muscle morphology and regulatory signalling in endurance-trained and sedentary individuals: The influence of ageing. *Exp Gerontol* **93,** 54–67.

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PC01

Does the distribution pattern of daily dietary protein intake influence amino acid utilization and muscle protein synthesis?

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Intro

Dietary intake of protein is the vital source of essential amino acids, and a sufficient intake is needed to ensure a balance in protein turnover in order to maintain the protein pool of the body. For older adults, the dietary intake of protein has shown to be skewed towards the evening meal, with less protein consumed at breakfast and lunch. Resultingly, at some daily meals the protein turnover is suboptimal stimulated, while at the evening meal all dietary amino acids may not be utilized.

Aims

The aim of the present study was to explore if a more even distribution of the dietary protein could improve the dietary amino acid utilization and the subsequent anabolic protein synthetic response and protein turnover net-balance.

Methods

A total of 24 healthy elderly men and women (65-80 years of age) were included in a randomized controlled trial. Seven days of habituation to either an EVEN (n=12) or SKEWED (n=12) protein intake, was followed by a 4-day trial that was initiated by an oral intake of D_2O to measure integrated muscle protein synthesis (MPS) over the initial 3-day trial period. Through the entire trial period all meals were served with intrinsically $^2H_5$-phenylalanine labeled meat as the primary protein source, thereby enabling an assessment of the dietary amino acid utilization. Plasma ^2H-alanine and muscle protein bound, ^2H-alanine and $^2H_5$-phenylalanine enrichments were measured by mass spectrometry from daily blood samples and vastus lateralis muscle biopsies taken at baseline and at the end of the 3-day trial period. Lastly, an acute trial was conducted on the fourth trial-day where an infusion of $^2H_8$-phenylalanine and $^2H_2$-tyrosine was used to measure whole body protein turnover from blood samples taken throughout the day under influence of the EVEN or SKEWED dietary distribution. All subjects gave their written consent to participate in the protocol, and the study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of Copenhagen and Frederiksberg (H-18026529).

Results

During the 3-day trial period the MPS was not different between EVEN (2.16 +/- 0.46 %/hour) and SKEWED (2.23 +/- 0.32 %/hour), and the muscle protein bound $^2H_5$-phenylalanine enrichment was the same in EVEN (0.0049 +/- 0.0013 MPE%) and SKEWED (0.0054 +/- 0.0010 MPE%). During the acute trial, protein turnover net-balance was more positive in EVEN after breakfast and lunch compared to SKEWED and was the same in both groups after dinner.

Conclusion

A dietary protein intake with either and EVEN or SKEWED distribution pattern resulted in identical muscle protein synthesis and amino acid utilization when measured over 3 days. Yet, fluctuations in whole body protein net-balance were seen throughout the acute trial day, with a greater net-balance in EVEN.

PC02

The influence of the menstrual cycle on training schedules in elite female mountain bike, road and cyclocross athletes

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Female participation in elite sport is rising, the most recent Olympic games, Tokyo 2020, was the most gender-balanced games in history but research within female athletes is lacking. This study

investigated if different phases of the menstrual cycle (MC) influenced training schedules, sleep quality, arousal, and alertness in elite female mountain bike, road and cyclocross athletes. Methodological approach was coherent with Edinburgh Napier University's ethics committee. Fifteen athletes (age: 29 ± 7.4 years, height: 1.7 ± 0.1m, body mass: 61.9 ± 7.7kg) from 9 countries, were part of the three-month study. The MC was split into two phases; follicular (FP) and luteal (LP), and participants were provided ovulation kits to identify the phases. As well as uploading training data over the three months, athletes tracked their MC symptoms, basal body temperature (BBT), body mass, perceived sleep, and psychological measures daily. BBT was significantly higher ($p<0.05$) in the LP (0.21, p=0.00, d=0.4), body mass was greater in the FP (0.3kg, p=0.54, d=0.04) but not significant. Perceived sleep quality (FP; 7.1 ± 1.7 LP; 7.0 ± 1.8, p=0.6, d= 0.04) and length (FP; 8.0 ± 1 LP; 8.0 ± 1 hrs, p=0.32, d=0.06), alertness (FP; 6.4 ± 1.9, LP; 6.5 ± 2.0, p=0.78, d=0.07) and arousal (FP; 3.4 ± 1.8, LP; 3.4 ± 1.8, p=0.28, d=0.03) did not significantly change between the phases ($p>0.05$). Further, no differences ($p>0.05$) between phases for the training variables such as average heart rate (FP; 126 ± 36, LP; 126 ± 38 bpm, p=0.85, d=3.82), training load (bTRIMP) (FP; 160 ± 155, LP; 170 ± 168 A.U, p=0.71, d=1.45), average speed (FP; 160 ± 155, LP; 170 ± 168, p=0.75, d=1.52) and rating of perceived exertion (RPE) (FP; 14 ± 3, LP; 14 ± 3, p=0.87, d=5.87) were observed. The MC phases did not appear to influence training schedules, sleep, or psychological changes. However, due to the online nature of the research, future studies are required for more controlled, lab-based research to determine the effects of MC in elite female athletes.

PC03

Effects of 6-week VLCD and Adjuvant High-Intensity Interval Training (HIIT) or Resistance Exercise Training (RET) on the Plasma Metabolome in Healthy Overweight/Obese Men

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Very low-calorie diets (VLCD) induce significant weight loss and metabolic benefits, such as improvements in whole-body glucose disposal, and are commonly employed in the treatment of obesity and its related comorbidities (i.e diabetes). However, 25% of VLCD induced weight loss is attributable to declines in lean body mass, comprised largely of muscle (1). Given the essential role of muscle in maintaining whole-body metabolic health it is desirable to maintain muscle mass during caloric restriction. Furthermore, this may be achievable with chronic resistance exercise training (RET). However, it is unclear whether adjuvant RET has an additive metabolic health benefit when provided alongside VLCD. In the present study we implemented an unbiased approach, in the form of untargeted metabolomics, to determine the impact of exercise training in the form of RET and high

intensity interval training (HIIT) adjuvant to VLCD, and to further understand the biology potentially underlying the metabolic benefits of VLCD.

Obese and overweight men (n=26) were recruited to a 6-week intervention study. Participants were randomly allocated into VCLD (800kcal/day) only (n=10; 46±8y; 104±12kg; BMI 32±4, mean±SD), VLCD+RET (n= 8; 40±8y; 99k±10kg; BMI 32±2) or VLCD+HIIT (n=8; 46±11y; 101±12kg; BMI 33±3). Body composition was determined by dual-energy X-ray absorptiometry (DXA) before and after the intervention period. Fasted plasma samples collected before and after intervention were analysed using ultra-high performance liquid chromatography operating dual phase HILIC and C18 RP separation coupled to a high resolution mass spectrometer, with data acquired in both positive and negative ionisation modes. The Random Forest (RF) algorithm was used to identify metabolite features most informative in classifying pre- and post-intervention samples which formed a reduced dataset for analysis of differences in metabolite abundance, metabolite identification and network mapping. Annotation of metabolite identity was assigned using network integration performed with the PIUMet algorithm.

Loss of lean body mass was larger in VLCD only (4.4±1.9kg) than VLCD+RET or VLCD+HIIT (3.9±1.8kg and 3.9±1.3kg, respectively), although not significantly. The RF algorithm identified the top 10 metabolites from each polarity and ionisation mode which classified pre and post intervention. Principal component analysis (PCA) using the reduced dataset resulted in clear separation of pre- and post-intervention samples (Fig. 1A). When further stratified into groups, excellent clustering was seen in VLCD+HIIT (Fig. 1D) with low separation in VLCD (Fig. 1B) and no separation in VLCD+RET (Fig. 1C). Metabolite abundance revealed similar patterns of expression for all groups from pre- to post-intervention, however differences were larger and significant ($p<0.05$) in VLCD only and VLCD+HIIT while expression changes in VCD+RET were not significant. Putative metabolite identification found benzenoids, derivatives of carboxylic acids, and several species of lipids were primarily involved in separation of samples before and after intervention.

These results demonstrate that although the addition of exercise training to a VLCD intervention did offer some protection against the loss of lean body mass, albeit non-significantly, adjuvant RET did not alter the fasting state metabolomic signature from VLCD alone. Conversely adjuvant HIIT did elicit some differences which may warrant further investigation.

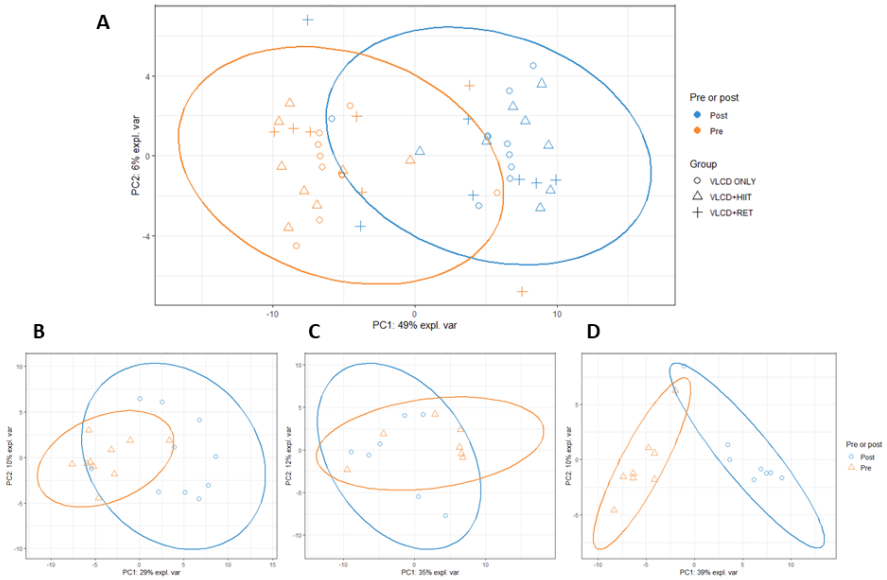

Figure 1 Representative principal component analysis (PCA) plots after RF classification showing separation of polar negative data pre- and post-intervention with **(A)** all groups combined, and further stratification into **(B)** VLCD only, **(C)** VLCD+RET, **(D)** VLCD+HIIT

Reference 1 :- Weinheimer EM, Sands LP & Campbell WW (2010). A systematic review of the separate and combined effects of energy restriction and exercise on fat-free mass in middle-aged and older adults: implications for sarcopenic obesity. Nutr Rev 68, 375–388

Acknowledgements :- This work was supported by the Medical Research Council [MR/K00414X/1] and Versus Arthritis [19891].

PC04

Effects of Dietary Nitrate Supplementation on Performance during Single and Repeated bouts of Short-duration High-intensity Exercise: A Systematic Review and Meta-analysis of Randomised Controlled Trials

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Background Inorganic nitrate (NO_3^-), which can be reduced to nitrite (NO_2^-) and subsequently nitric oxide (NO), has emerged as a potential ergogenic aid in a variety of exercise settings. While recent systematic reviews and meta-analyses suggest some small positive effects of NO_3^- supplementation on performance, the effect of NO_3^- supplementation on performance during single and repeated bouts of short-duration, high-intensity exercise is unclear.

Objective To conduct a systematic review and meta-analysis to evaluate the effects of NO_3^- supplementation on performance during single and repeated bouts of short-duration, high-intensity exercise in healthy humans. A secondary purpose was to evaluate whether performance outcomes were influenced by the NO_3^- supplementation regime (dose and duration of supplementation) and high-intensity exercise protocol (number and duration of exercise bouts) performed.

Methods This review was conducted following the Preferred Reporting Items for Systematic reviews and Meta-analyses (PRISMA) guidelines. MEDLINE and SPORTDiscus were strategically searched for relevant articles, which were screened based on pre-determined inclusion/exclusion criteria, and critically evaluated using the Revised Cochrane Collaboration Risk of Bias tool 2 for crossover trials. Paired analysis for cross-over trials was used to perform a random effects meta-analysis for each performance outcome and generate standardized mean differences (SMD) between the NO_3^- and placebo supplementation conditions. Heterogeneity between studies was assessed using the Chi^2 and I^2 statistics.

Results Of 1147 records identified, 26 studies were eligible for the systematic review with 22 eligible for meta-analysis. Five studies had a low risk of bias, 18 studies had some concerns, and three studies had a high risk of bias. Compared to placebo, time to reach peak power was lower during all-out sprint exercise after NO_3^- supplementation (SMD=0.75, P=0.02) (Figure 1). In addition, NO_3^- supplementation had a small positive effect on mean power output (MP) (SMD=0.20, P=0.02) (Figure 2). Sub-group analyses revealed that MP was greater after NO_3^- supplementation when NO_3^- dose was ≥ 8 mmol (SMD=0.27, P=0.04) but not < 8 mmol (SMD=0.19, P=0.08), during a single sprint (SMD=0.31, P=0.004) but not during repeated sprints (SMD=0.09, P=0.23), and when sprint time was >15 s - ≤ 30 (SMD=0.31, P=0.001), but not ≤ 15 s (SMD=0.14, P=0.15). Although total work done (TWD) was not different between conditions (SMD=0.06, P=0.52), sub-group analyses revealed TWD was greater after NO_3^- doses ≥ 8 mmol (SMD=0.23, P=0.08) compared to < 8 mmol (SMD= -0.11, P=0.22), and multiple day (SMD=0.34, P=0.008) compared to single day (SMD=-0.10, P=0.26) NO_3^- supplementation. Total distance covered in the Yo-Yo intermittent recovery level 1 test was greater after NO_3^- supplementation (SMD=0.14, P < 0.0001) (Figure 3).

Conclusion NO_3^- supplementation can enhance some performance outcomes during single and repeated bouts of high-intensity exercise, but such effects are generally small and influenced by the NO_3^- supplementation regime and the high-intensity exercise protocol.

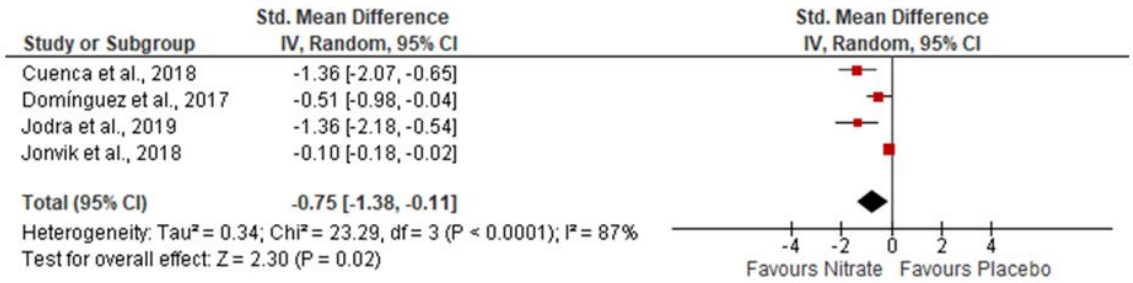

Figure 1. Forrest plot for time to reach peak power in the nitrate and placebo trials.

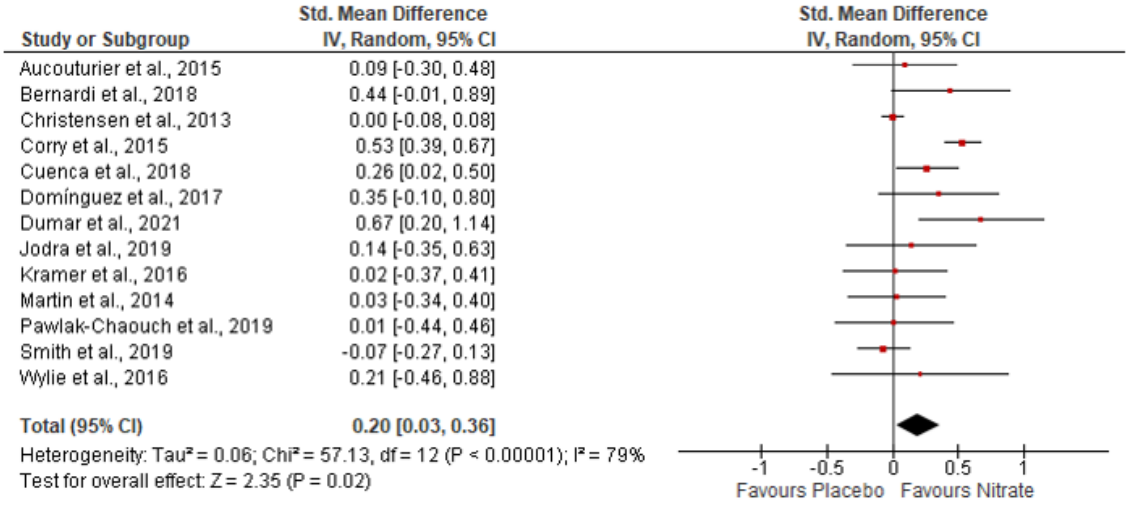

Figure 2. Forrest plot for mean power output in the nitrate and placebo trials.

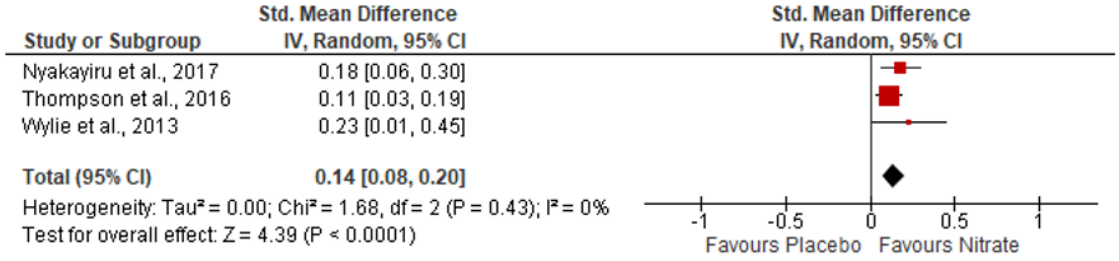

Figure 3. Forrest plot for total distance covered in the Yo-Yo intermittent recovery level 1 test the nitrate and placebo trials.

PC05

Temporality and muscle specificity of disuse atrophy in human lower leg muscles

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Introduction: Skeletal muscle disuse atrophy (DA) through inactivity and immobilization is not fully understood, and as such remains a major goal of global muscle biology science efforts. Nonetheless, remarkably not all leg muscles atrophy at the same rate or total degree despite similar anatomical roles/ proximity (1). Moreover, temporal aspects of DA remain ambiguous, with rates of muscle loss over chronic periods (i.e., >7 days) often inferred between repeated measures spanning several days (2), which lack resolution. We thus investigated this temporality amongst atrophy resistant and atrophy susceptible muscles of the lower leg, i.e. the *tibialis anterior* (TA) and *medial gastrocnemius*(MG), respectively (3).

Methods: We recruited 10 healthy young men (22±1y, BMI 23±1kg·m^2) who underwent 15 days unilateral leg immobilisation (ULI) using a knee and foot brace to immobilise both the upper and lower leg. To assess muscle mass, participants received sequential magnetic resonance imaging (MRI) scans at baseline and thereafter on alternate days to quantify the volume of individual muscles (focussing upon the TA and MG) throughout the 15 days of immobilisation. Functional assessments were also undertaken (i.e., knee extensor 1-RM, dorsiflexion/ plantarflexion MVC) before and after immobilisation. Data are mean±SEM, analysed by repeated measures 2-way ANOVA.

Results: After 7 days ULI, MG volume decreased from baseline values by 8.8±2.9% (p=0.02), and thereafter remained largely stable at this lower level throughout the remaining immobilisation period (i.e., day 9: -7.7±4.4% (p=0.02), day 16: -9.7±3.1%, (p=0.006)). TA muscle volume remained unchanged in both legs at all time-points as did the non-immobilised MG. In the immobilised leg, knee extensor 1-RM (-19±7%, p<0.001) and MVC (-31±5%, p=0.0003) each decreased from baseline, yet were unchanged in the non-immobilised leg. Following the 15 days of ULI, a similar degree of muscle strength loss was observed in measures of plantarflexion MVC (i.e., in MG) (-16±18%, p=0.04) and dorsiflexion MVC (i.e., in TA) (-25±9%, p=0.001).

Conclusion: Investigating atrophy susceptible vs. atrophy resistant muscles is a novel paradigm to study the mechanistic basis of DA. Moreover, muscle mass decreases in a non-linear manner; with early losses (i.e., 5-7 days) remaining consistent up to 15 days of ULI. Importantly, despite divergent responses in DA, the disparity between relative mass and functional losses of both muscles may be due to detrimental changes in neuromuscular aspects. We contend that targeting early atrophy is central to mitigating even long-term losses.

Reference 1 :- 1. Belavý DL, Miokovic T, Armbrecht G, Richardson CA, Rittweger J, Felsenberg D. Differential atrophy of the lower-limb musculature during prolonged bed-rest. Eur J Appl Physiol. 2009

Reference 2 :- 2. Kilroe SP, Fulford J, Jackman SR, Van Loon LJC, Wall BT. Temporal Muscle-specific Disuse Atrophy during One Week of Leg Immobilization. Vol. 52, Medicine and Science in Sports and Exercise. 2020

Reference 3 :- 3. Bass JJ, Hardy EJO, Inns TB, Wilkinson DJ, Piasecki M, Morris RH, et al. Atrophy Resistant vs. Atrophy Susceptible Skeletal Muscles: "aRaS" as a Novel Experimental Paradigm to Study the Mechanisms of Human Disuse Atrophy. Front Physiol. 2021

Acknowledgements :-

This work was funded through a grant BBSRC (BB/R010358/1). This work was also supported by the Medical Research Council, United Kingdom [grant number: MR/P021220/1] as part of the MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research awarded to the Universities of Nottingham and Birmingham, and the National Institute for Health Research, United Kingdom, Nottingham Biomedical Research Centre.

PC06

The impact of genetic variation within the Vitamin D axis upon skeletal muscle function: a systematic review and validation study

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[1]MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research and Nottingham National Institute for Health Research (NIHR) Biomedical Research Centre (BRC);, Nottingham, United Kingdom [2]Musculoskeletal Physiology Research Group, Sport, Health and Performance Enhancement Research Centre, School of Science and Technology, Nottingham Trent University, Nottingham, United Kingdom [3]Sport and Exercise Sciences, Science and Engineering, Manchester Metropolitan University, Manchester, United Kingdom [4]Metabolism and Systems Research, University of Birmingham, Birmingham, United Kingdom

Background: Vitamin D-deficiency or insufficiency (i.e., low serum 25-hydroxyvitamin D, 25D) is associated with skeletal muscle myopathy [1,2] in addition to being linked to impaired sports performance. We recently identified a molecular role for the 1,25-dihydroxyvitamin D (1,25D) system in muscle mass regulation, via the Vitamin D Receptor (VDR). Yet, an understanding of the role of the vitamin D-axis in muscle health is unresolved. Reflecting this, the impact of vitamin D-related genetic variation in relation to skeletal muscle health and performance is unclear. We therefore aimed i) to review studies relating to muscle function and single nucleotide polymorphisms (SNPs) in vitamin D-axis genes, and ii) to investigate relationships between VDR SNPs and biochemical and physiological parameters of masters athletes (MA) [3].

Methods: Firstly, A systematic review of articles published between January 2000 and July 2021 was performed by searching PubMed, EMBASE and Web of Science for articles investigating associations between functionally relevant variants of genes within the vitamin D pathway and skeletal muscle function outcomes. Human research articles were included regardless of language or article type;

article quality/risk of bias was assessed by using the Quality of Genetic Association Studies (Q-Genie) tool. Secondly, an elite master athlete (MA) cohort (N = 48) and age-matched controls (N = 48) were genotyped for 6 distinct VDR SNPs (BsmI, FokI, ApaI, Cdx2, TaqI, and A1012G) using the tetra-primer amplification refractory mutation system (tetra-ARMS) and TaqMan allelic discrimination assays. Physiological parameters in cohorts included body composition, handgrip strength (HGS) and muscle function; biochemical markers were examined in controls only [3]. Statistical analysis was performed by one-way ANOVA with Tukey's post-hoc test for multiple comparisons (significance $P<0.05$).

Results: Twenty-one articles were included in the systematic review with 81% solely including participants aged ≥50 y, and of the 9 studies that included individuals of ethnic minorities, only 2 included black participants. A total of 20 different VDR SNPs were investigated in relation to muscle function, with multiple studies identifying associations with muscle physiology and BsmI, FokI, ApaI, and A1012G. Notably, A1012G was associated with improved handgrip strength, whereas results for other SNPs were conspicuously variable between studies. In agreement, MA carrying A1012G displayed greater HGS ($P = 0.0455$), whilst controls carrying this SNP also displayed higher jump power ($P = 0.0132$). Interestingly, controls homozygous for A1012G or Cdx2 had significantly higher levels of serum sex hormone binding globulin (SHBG) ($P = 0.048$ and 0.0227, respectively).

Conclusions: To conclude, our initial systematic review exemplifies that research into the impact of genetic polymorphisms of vitamin D-related genes in relation to muscle health and function is largely restricted to the *VDR* gene. In addition, our findings from genetic association analyses in elite MA validate previous findings of associations between A1012G and improved muscle/physical function while also suggesting a putative link to athletic prowess. Finally, we provide evidence of potential novel associations between the vitamin D system and sex hormones warranting further study.

Reference 1 :- Bollen SE, Atherton PJ. Myogenic, genomic and non-genomic influences of the vitamin D axis in skeletal muscle. *Cell Biochem Funct*. 2020; 39:48-59

Reference 2 :- Pfeifer M, Begerow B, Minne HW. Vitamin D and muscle function. *Osteoporos Intl*. 2002;13:187-194.

Reference 3 :- Crossland H, Piasecki J, McCormick D, Phillips BE, Wilkinson DW, Smith K, McPhee JS, Piasecki M, Atherton, PJ. Targeted genotype analyses of GWAS-derived lean body mass and handgrip strength-associated single-nucleotide polymorphisms in elite master athletes. 2020; 319:184-194

Acknowledgements :- This work was supported by the Medical Research Council [grant number MR/J500495/1]

PC07

Peak cardiac output determined using inert gas rebreathing: A comparison of two exercise protocols using a non-inferiority, randomized crossover design

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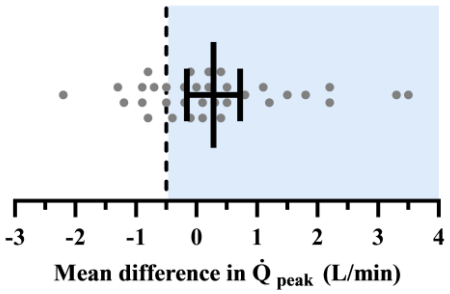
Introduction: Peak cardiac output ($\dot{Q}_{peak}$) can be estimated non-invasively using inert gas rebreathing (IGR). IGR-derived measurements of $\dot{Q}_{peak}$ are highly correlated with the gold-standard direct Fick and thermodilution methods (r≥0.94), have reasonable Bland-Altman limits of agreement with the Fick method, and may be superior to the thermodilution method during submaximal exercise in terms of reliability (1,2). There is no consensus on the optimal exercise protocol to estimate $\dot{Q}_{peak}$ using IGR, which requires a rebreathing period of ~10 s as close to "maximal" effort as possible.

Aims/objectives: To compare the $\dot{Q}_{peak}$ elicited by two different protocols using a non-inferiority, randomized crossover trial and considering a $\dot{Q}_{peak}$ non-inferiority margin of 0.5 L/min. The comparison involved a constant load protocol modelled after peak oxygen uptake ($\dot{V}O_{2peak}$) tests that include a verification phase ($\dot{Q}_{CL}$) (3) and an incremental step protocol ($\dot{Q}_{step}$) (4).

Methods: Sample size was estimated using G*Power for a two-tailed paired t-test to detect a 0.5 effect size (Cohen's d) at 80% power and alpha at 5%. Thirty-four participants (19 females; 25±5 y) performed two baseline $\dot{V}O_{2peak}$ tests to determine peak heart rate (HR_{peak}) and peak work rate (W_{peak}). Participants then performed the $\dot{Q}_{CL}$ and $\dot{Q}_{step}$ protocols on two separate occasions (four total visits) in random order. $\dot{Q}_{peak}$ was measured using IGR (Innocor, COSMED, Italy). The $\dot{Q}_{CL}$ protocol involved a $\dot{V}O_{2peak}$ test followed 10 minutes later by cycling at 90% (W_{peak}) with IGR initiated after 2 minutes. The $\dot{Q}_{step}$ protocol involved a step test with IGR initiated when HR reached five beats/min below the participant's HR_{peak}. We tested whether the first $\dot{Q}_{CL}$ and $\dot{Q}_{step}$ tests were non-inferior and estimated the reproducibility of each method (typical error; TE) with the second set of tests. Data are presented as mean±SD and between-groups confidence intervals (CI). We estimated the difference between means through a two-tailed paired t-test. To claim non-inferiority, the lower bound 95% CI of the difference between the $\dot{Q}_{peak}$ exercise protocols ($\dot{Q}_{CL} - \dot{Q}_{peak}$) had to be within the non-inferiority margin. The project was approved by the Hamilton Integrated Research Ethics Board (13339).

Results: The $\dot{Q}_{CL}$ protocol was non-inferior to $\dot{Q}_{step}$ ($\dot{Q}_{CL}$=17.1±3.2, $\dot{Q}_{step}$=16.8±3.1 L/min, p=0.20; Figure 1: 95% CI =-0.16-0.72 L/min [error bars], non-inferiority margin=0.5 L/min [dotted line]). The baseline $\dot{V}O_{2peak}$ (3.13±0.83 L/min) was achieved during both the $\dot{Q}_{CL}$ (3.12±0.72 L/min, p=0.87) and $\dot{Q}_{step}$ protocols (3.12±0.80 L/min, p=0.82). The TE in $\dot{Q}_{peak}$ was 6.6% for the $\dot{Q}_{CL}$ protocol and 8.3% for the $\dot{Q}_{step}$ protocol and did not differ between retesting (p=0.43 and p=0.12, respectively).

Conclusion: The $\dot{Q}_{CL}$ protocol was non-inferior to the $\dot{Q}_{step}$ protocol to estimate $\dot{Q}_{peak}$. Both protocols elicited $\dot{V}O_2$ values that were comparable to those obtained at the end of a ramp $\dot{V}O_{2peak}$ test, indicating both protocols elicit a near-maximal exercise response. We conclude that both protocols are feasible and appropriate to determine $\dot{Q}_{peak}$. The $\dot{Q}_{CL}$ protocol may be more convenient because it allows for the measurement of $\dot{Q}_{peak}$ during the same session used to determine $\dot{V}O_{2peak}$. This may reduce participant and experimenter burden, e.g., the number of laboratory visits required.



Reference 1 :- Agostoni P et al. (2005). J Am Coll Cardiol 46(9), 1779–1781

Reference 2 :- Hardin EA et al. (2020). J Am Heart Assoc 9(17), e015794

Reference 3 :- Bostad W et al. (2021). Eur J Appl Physiol 121, 2449–58

Reference 4 :- Montero D et al. (2015). J Physiol 593(20), 4677–88

Acknowledgements :- This project was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC).

PC08

Axon sub-population sensitivity to high frequency stimulus in adult mouse optic nerve

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Axon diameter determines conduction velocity, where increasing size confers an evolutionary advantage in prey-predator confrontations, the squid giant axon, which initiates the animal's escape reflex, an obvious example. In mammals myelin increases axon conduction velocity (CV) without significantly increasing diameter; in the mammalian cortex only 20% of axons exceed 3 µm in diameter. However, the importance of increased CV with diameter diminishes in short central tracts, e.g., the optic nerve. Mitochondrial density in optic nerve axons is constant such that energy capacity increases as a squared function of diameter (Perge *et al.*, 2009), whereas membrane bound ion channel and Na pump density increase linearly with axon size, bestowing larger axons with an increased capacity to re-equilibrate the ion perturbations that result from action potential firing. We describe differential sensitivity of mouse optic nerve (MON) axon sub-populations to sustain firing at high stimulus frequencies.

All procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986 under appropriate authority of project and personal licenses. Adult male CD-1 mice were killed by

cervical dislocation and decapitated. Optic nerves were dissected, placed in a superfusion chamber, and bathed with aerated aCSF containing 10 mM glucose flowing at 2 ml min^{-1}. The stimulus evoked compound action potential (CAP), whose profile displayed three distinct peaks, was evoked with supra-maximal stimuli. The MONs were stimulated at 1 Hz under baseline conditions.

The areas of the three separate peaks recruited with increasing stimulus voltage were fit as cumulative histograms. The resulting estimates of mean ± SD values (n = 12) allowed creation of corresponding probability distributions, which yielded three distinct peaks whose profile matched that of the CAP, evidence that axon size underlies the separation of the CAP into three distinct peaks. In MONs in which high frequency stimulus was imposed for 5 minutes, the 3rd peak loss occurred at 10 Hz, the 2nd peak at 20 Hz and the 1st peak at 67 Hz. During stimulus axons release K^+ thus the effect of increasing aCSF [K^+] from the baseline value of 3 mM on the CAP was studied. The CAP started to fall when [K^+] was increased to 9 mM and a sigmoidal fit showed an IC_{50} value of 12.5 mM with a slope of -9.38 (n = 8). Post-stimulus recovery revealed peak amplitude transiently exceeded baseline values in a frequency dependent manner in agreement with a decrease in [K^+] below baseline (Ransom *et al.*, 2000). Reducing the aCSF [Na^+] from 153 mM in steps to 30 mM decreased rate of rise and amplitude of the individual peaks in a logarithmic manner (n = 10). From this data, based on the Nernstian relationship, stimulus induced elevations in $[Na^+]_i$ were calculated for each peak.

Our data provides evidence that the ability of axons sub-populations to 'follow' at high frequency stimulus is determined by axon size, and that elevations in $[Na^+]_i$, not $[K^+]_o$, is the primary determinant that causes decrease of the CAP.

Reference 1 :- Perge JA, Koch K, Miller R, Sterling P & Balasubramanian V. (2009). How the optic nerve allocates space, energy capacity, and information. *J Neurosci* **29,** 7917-7928.

Reference 2 :- Ransom CB, Ransom BR & Sontheimer H. (2000). Activity-dependent extracellular K^+ accumulation in rat optic nerve: the role of glial and axonal Na^+ pumps. *J Physiol* **522,** 427-442.

PC09

Heat stress associated with aerosol personal protective equipment (PPE) and its impact on mood, cognitive and motor function: a randomised controlled cross-over study

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Introduction

The COVID-19 pandemic has resulted in the widespread use of aerosol personal protective equipment (PPE) to prevent disease transmission in healthcare environments. Other forms of PPE are a heat stress[1] and heat stress has a negative impact upon cognitive and motor function[2,3]. The use of aerosol PPE is reported subjectively to have a negative impact upon healthcare workers' performance and wellbeing[4], but the consequences have not been assessed objectively.

Aims

This study aimed to quantify the heat stress associated with aerosol PPE and to investigate its impact upon mood, cognitive and motor function, and task performance.

Methods

Ethical approval was obtained from Imperial College Research Ethics Committee (20IC6445). Sixteen healthy subjects (eight male) undertook an exercise protocol, which simulated the metabolic expenditure of hospital work[5]: once wearing aerosol PPE (PPE visit) and once wearing standard surgical attire (control visit). The order of the visits was randomised and they were scheduled approximately one week apart. Subjects walked on a treadmill for 2 hours followed by a 30-minute recovery period. Core temperature, heart rate, urine specific gravity, grip strength, mood state (Bond-Lader scales), executive function (One-Touch Stockings task) and task performance (Intubation of a Manikin) were recorded. Data are presented as mean (SD) and analysis performed with two-way repeated measures ANOVA.

Results

Core temperature was greater on the PPE visit than on the control visit from 90 minutes of exercise until the end of the rest period ($p < 0.05$, Figure 1). Maximum core temperature was 38.1 (0.2)°C on the PPE visit and 37.8 (0.3)°C on the control visit. Heart rate was greater on the PPE visit from 30 minutes of exercise until 15 minutes of the rest period ($p < 0.01$). Maximum heart rate was 121 (9) bpm on the PPE visit and 109 (9) bpm on the control visit. Urine specific gravity was greater following exercise on the PPE visit (1.023 (0.006)) than the control visit (1.019 (0.008), $p = 0.04$). Alertness ($p < 0.001$) and contentment ($p < 0.001$) were less following exercise on the PPE visit, but calmness did not differ between the visits ($p = 0.24$). One-Touch Stockings latency ($p = 0.93$) and accuracy ($p = 0.19$) did not differ between the visits. Grip strength was less on the PPE visit following exercise in the right (-4 (6) N) and left hands (-4 (4) N) compared to the control visit ($p < 0.01$). Time to complete the intubation task was greater on the PPE visit after donning PPE (+43 (66) s; $p < 0.05$) and after the exercise period (+65 (103) s; $p < 0.01$, Figure 2).

Conclusion

This study demonstrates that wearing aerosol PPE for 2 hours in a simulated hospital environment results in heat exhaustion and has a negative impact upon mood, motor function and task performance. Cognitive function was not affected, but this may be due to insufficient heat stress. Whilst wearing PPE is important to prevent disease transmission, strategies should be developed to limit its impact upon healthcare workers' performance and wellbeing.

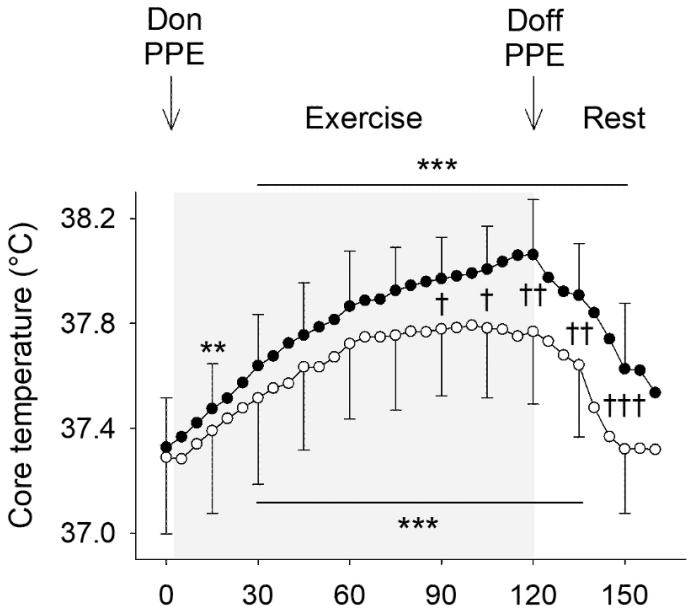

Figure 1 Mean (SD) core temperature on the PPE visit (black symbols) and the control visit (white symbols). Baseline values are displayed at 0 minutes and the exercise period is denoted by the shaded areas. Difference to the baseline value is indicated by ** $p < 0.01$ and *** $p < 0.001$. Difference between the PPE visit and the control visit is indicated by † $p < 0.05$, †† $p < 0.01$ and ††† $p < 0.001$.

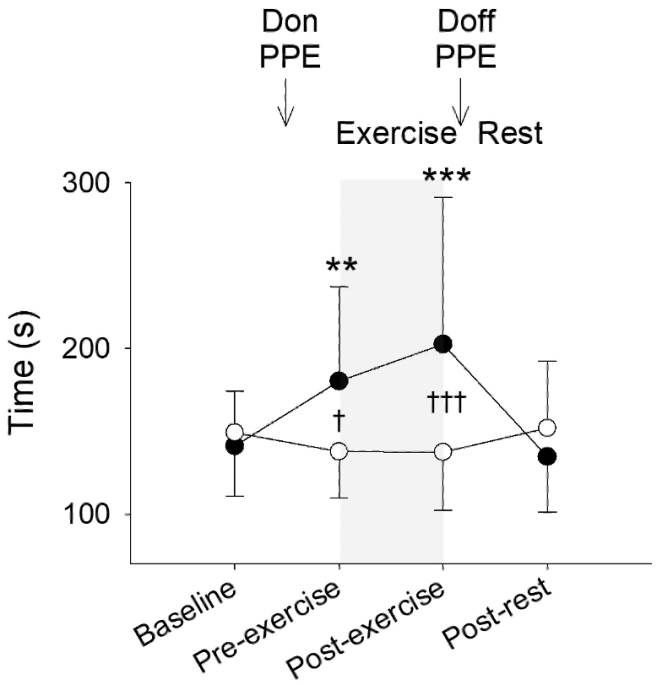

Figure 2 Mean (SD) intubation task time on the PPE visit (black symbols) and the control visit (white symbols). The exercise period is denoted by the shaded areas. Difference to the baseline value is indicated by ** $p < 0.01$ and *** $p < 0.001$. Difference between the PPE visit and the control visit is indicated by † $p < 0.05$, †† $p < 0.01$ and ††† $p < 0.001$.

Reference 1 :- Schlader ZJ, Temple JL, Hostler D. Exercise in personal protective equipment in a hot, humid environment does not affect risk propensity. Temperature. 2016;3(2):262-270. doi:10.1080/23328940.2016.1148526

Reference 2 :- Caldwell JN, Patterson MJ, Taylor NAS. Simulated Helicopter Flight Performance is Affected by Heat Strain. Journal of the Human-Environment System. 2006;9(1):13-18. doi:10.1618/jhes.9.13

Reference 3 :- Radakovic SS, Maric J, Surbatovic M, et al. Effects of Acclimation on Cognitive Performance in Soldiers during Exertional Heat Stress. Military Medicine. 2007;172(2):133-136. doi:10.7205/MILMED.172.2.133

Reference 4 :- Davey SL, Lee BJ, Robbins T, Randeva H, Thake CD. Heat stress and PPE during COVID-19: impact on healthcare workers' performance, safety and well-being in NHS settings. Journal of Hospital Infection. 2021;108:185-188. doi:10.1016/j.jhin.2020.11.027

Reference 5 :- Ainsworth BE, Haskell WL, Leon AS, et al. Compendium of physical activities: Classification of energy costs of human physical activities. Medicine and Science in Sports and Exercise. 1993;25(1):71-74. doi:10.1249/00005768-199301000-00011

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PC10

Intramyocellular lipid content is unaffected by acute and chronic bed rest in volunteers maintained in energy balance, and is not responsible for impaired glucose disposal.

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Introduction:

Musculoskeletal unloading during bed rest (BR) reduces insulin-mediated glucose disposal (GD) at whole-body and limb levels [1], which develops within 24h [2]. An increase in intramyocellular lipid (IMCL) content has been proposed as a mechanistic driver of reduced GD in sedentary lean and obese individuals [3]. However, an increase in IMCL during BR could also reflect that a positive energy balance state occurred, and IMCL accumulation was not causative in any impairment of GD. We therefore measured GD and IMCL content during acute and chronic bed rest (BR) during which individuals were maintained in energy balance.

Methods:

A 3-day (acute) BR study (n=10, age 24±1.25 years, BMI 22.7±0.60 kg/m^2), and a 56-day (chronic) BR study (n=20, age 34±1.8 years, BMI 23.8±0.41 kg/m^2) were performed. In both, participants were exposed to -6° head-down tilt and maintained in energy balance (resting metabolic rate x 1.2) and hyperinsulinaemic euglycaemic clamps (60 mU/m^2/min) were performed before and after BR to quantify whole-body GD. IMCL content was determined in pre-clamp vastus lateralis muscle biopsies before and after BR via staining of transverse, 14 µm thick sections with the fluorescent dye Bodipy-493/503, which stains neutral lipids [4]. Additionally, fibre-type specific IMCL content was quantified following immunohistochemical staining of myosin heavy chain isoforms. Student's T-tests and two-way repeated measures ANOVA were used for statistical analyses. Values represent mean ± SD.

Results

Steady-state insulin mediated GD was reduced from pre BR (baseline) following acute BR (13.01 ± 6.75 vs 7.75 ± 7.08 mg/kg/min, p=0.0037) and chronic BR (10.16 ± 1.89 vs 7.90 ± 1.26 mg/kg/min, p<0.0001). The magnitude of decline in GD was similar in both acute BR (17%) and chronic BR (22%).

IMCL content was unchanged from baseline following acute BR (4.0±1.3% vs. 5.7±1.8%, p=0.15) and chronic BR (11.3±6.9% vs. 9.7±6.6%, p=0.69). There was a fibre type difference in IMCL content in both studies, such that type I fibre IMCL content was greater than IIA and IIX fibres, but the IMCL content of all fibre types was unchanged from baseline following acute and chronic BR (Table 1).

Conclusion

Reductions in GD following acute and chronic BR could not be explained by an increase in IMCL content or fibre-type specific IMCL responses. The IMCL accumulation reported during BR [5] appears to be a confounding factor arising from participants being in a state of positive energy balance.

	IMCL Content (%) by Fibre Type					
	Acute Bed Rest			Chronic Bed Rest		
	Pre Bed Rest	Post Bed Best	P Value	Pre Bed Rest	Post Bed Best	P Value
Type I	6.1 ± 1.7	9.0 ± 4.3	0.32	15.3 ± 9.5	13.0 ± 10.0	0.86
Type IIA	3.4 ± 0.7	4.3 ± 2.1	0.64	7.6 ± 5.7	7.4 ± 5.6	0.99
Type IIX	2.6 ± 0.8	4.0 ± 1.8	0.39	5.1 ± 4.0	6.1 ± 4.4	0.89

Table 1: *Muscle fibre type IMCL content, before and after 3 days and 56 days bed rest in healthy participants. Values are mean ± SD.*

Reference 1 :- Mikines, K.J., Richter, E.A., Dela, F. and Galbo, H. (1991) Seven Days of Bed Rest Decrease Insulin Action on Glucose Uptake in Leg and Whole Body. *Journal of Applied Physiology*. 70 (3), pp. 1245-1254.

Reference 2 :- Burns, A.M., Nixon, A., Mallinson, J., Cordon, S.M., Stephens, F.B., and Greenhaff, P.L. (2021) Immobilisation induces sizeable and sustained reductions in forearm glucose uptake in just 24 h but does not change lipid uptake in healthy men. *The Journal of Physiology*. 599 (8), pp. 2197-2210.

Reference 3 :- Bergouignan, A., Rudwill, F., Simon, C. and Blanc, S. (2011) Physical inactivity as the culprit of metabolic inflexibility: evidence from bed-rest studies. *Journal of Applied Physiology*. 111 (4), pp. 1201-1210.

Reference 4 :- Prats, C., Gomez-Cabello, A., Nordby, P., Andersen, J.L., Helge, J.W., Dela, F., Baba, O. and Ploug, T. (2013) An optimized histochemical method to assess skeletal muscle glycogen and lipid stores reveals two metabolically distinct populations of type I muscle fibers. *PloS One*. 8 (10), p. e77774.

Reference 5 :- Cree, M.G., Paddon-Jones, D., Newcomer, B.R., Ronsen, O., Aarsland, A., Wolfe, R.R. and Ferrando, A. (2010) Twenty-eight-day bed rest with hypercortisolemia induces peripheral insulin

resistance and increases intramuscular triglycerides. *Metabolism: Clinical and Experimental*. 59 (5), pp. 703-710.

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PC11

Central and peripheral fatigue induced by 100 purely explosive isometric contractions

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Introduction. The fatigue following explosive contractions (≈ 200 ms in duration) is involved in most actions, especially sporting gestures (such as shot put, vertical jumps, and sprinting during kayaking, cycling and running) are based on explosive contractions of short duration (< 200 ms). Previous studies investigated the fatiguing effect of rapid isometric contractions maintained for longer duration (1 – 5 s). Thus, so far the fatiguing effect of purely explosive isometric (which is the most ecological contraction modality) are unknown. The early phase of explosive contractions is mainly dependent on central mechanisms such as motor unit recruitment and firing rate. Nevertheless, it is unknown if repeated explosive isometric contractions induce mainly central or peripheral fatigue. Thus, we aimed to verify the extent of central and peripheral fatigue induced by repetitive purely explosive contractions of knee extensors.

Methods. 30 volunteers performed 100 fast and brief (< 200 ms in duration) explosive isometric knee extensions interspersed by 3 s of rest. High-density surface electromyography (HDsEMG) was recorded from the vastus medialis and lateralis muscles. During the first (PRE) and last (POST) 10 contractions, we calculated the rate of force development (RFD) over the first 50, 100, and 150 ms of contractions. We also evaluated the contractile and EMG responses of electrically evoked single and octet stimuli (eight stimuli, 300 Hz). Neural efficacy was calculated as the ratio between octet-evoked and voluntary force during the first 50 ms of contraction. Student's t-test was used to evaluate the difference between PRE vs. POST. This study was approved by the Ethical Advisory Committee and performed in accordance with the Helsinki Declaration.

Results. Voluntary RFD decreased by 12±8% at 50ms and 6±5% at 100ms (all p values < 0.01). Root mean square EMG (normalized to M-wave amplitude) decreased by around 20±10% in all time intervals from 0 to 150 ms (all p values <0.001); conduction velocity (normalized to M-wave) decreased by 2.5±1.5% at 100 and 150 ms (p<0.05). Neural efficacy also declined by 15±8% (p<0.01).

Some metrics related to peripheral function did not show fatigue but even increased: the RFD assessed at 0-50 ms through the electrically evoked octet increased by 3±2% ($p<0.01$).Similarly, the M-wave amplitude and conduction velocity increased by 7±5% ($p<0.05$).

Conclusions. The neural efficacy was impaired following 100 purely explosive contractions. However, the peripheral mechanisms did not show evidence of fatigue. Therefore, the impairment in explosive capacity was mainly driven by central fatigue.

Reference 1 :- Buckthorpe M et al., (2014). Exp Physiol. 99(7): 964-973

Reference 2 :- D'Emanuele S et al., (2021). Front Hum Neurosci. 387

Reference 3 :- de Ruiter CJ et al., (2004). J Appl Physiol. 97(5): 1693–170

Reference 4 :- Del Vecchio A et al., (2018). J Appl Physiol. 125(4): 1218-1226

Reference 5 :- Maffiuletti NA et al., (2016). Eur J Appl Physiol. 116(6): 1091-1116

PC12

Longer neurophysiological vs. clinical recovery following sport concussion

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Introduction: Sport related concussion (SRC) is a public health issue. The true incidence of sport concussion is believed to be higher than reported in most studies due to misdiagnosis and under-reporting (1). To safeguard players against the consequences of concussion, the 5th consensus statement on concussion in sport developed comprehensive return to play (RTP) guidelines (2). Progression between RTP stages is based on results from the sport concussion assessment tool (SCAT-5), a standardised questionnaire used to evaluate players suspected of having sustained a SRC (3). However, it remains unknown if the SCAT-5 is accurate in assessing concussed states at days 3-5 post injury. Consequently, combining the SCAT-5 with objective neurophysiological measures may help shed light on the validity of this tool for assessing concussion recovery.

Objectives: The objective of this study was to assess if injury-related alterations in the SCAT-5 are matched by changes in transcranial magnetic stimulation (TMS) derived intracortical inhibition. We hypothesised that neurophysiological measures would take longer to return to normal than recovery assessed by the SCAT-5 following SRC.

Methods: Thirteen male contact sport athletes (20.5 ± 4.5 years), who reported a concussion were recruited from local Rugby and American football clubs. Participants were tested at 4 timepoints throughout the concussion recovery period: within 24h of concussion (day 0), and at 7, 9 and 11 days after concussion. All participants completed the SCAT-5 and underwent TMS to assess cortical silent period duration (CSp), a measure of intracortical inhibition.

Results: After concussion CSp significantly declined from day 0 (122 ± 28 ms) to day 11 (106 ± 15 ms) ($F(3,33) = 7.80$, $p<.001$). SCAT-5 measures of symptom number and severity were significantly decreased (symptom number: $\chi2(3) = 30.44$, $p<0.01$; symptom severity: $\chi2(3) = 25.75$, $p <.001$) between the day 0 timepoint and each of the other timepoints. SCAT-5 balance errors (mBESS) decreased significantly ($F(3,33) = 19.55$, $p <.001$) between the day 0 timepoint and each of the other timepoints. CSp and SCAT-5 recovery patterns were different. SCAT-5 domains recovered faster showing no further significant changes after day 7, whilst CSp was still decreasing between days 7 and 9. Due to the small sample size we also used a Bayesian linear model to investigate the recovery of CSp and mBESS. The posterior distribution of our Bayesian model provided evidence that CSp decreased at day 7 and it continued to decrease at day 9, unlike mBESS which decreased at day 7 and then reached a plateau.

Conclusion: There are clinically important discrepancies between clinical and neurophysiological measures of concussion recovery. This finding has important implications for return to play protocols and the prevention of complications after sport concussion.

Reference 1 :- McCrory P et al. 2013. BJSM, 47(5), 250– 497 258.

Reference 2 :- McCrory P et al. 2017 BJSM, 51(11), 838–847

Reference 3 :- Echemendia et al. 2017 BJSM, 51(11), 848–850

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PC13

Training induced improvements in knee extensor force accuracy are associated with reduced motor unit firing variability

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Background: Muscle force output during sustained submaximal isometric contractions fluctuates around an average value and is influenced by variability of motor unit (MU) firing (1). Traditional exercise training interventions have been shown to reduce such fluctuations in muscle force (2, 3),

therefore, improving muscle force control/accuracy, which is often attributable to reduced variability in MU firing rate (4). However, much less is known with respect to MU properties following low intensity training. We therefore investigated whether targeted force accuracy training could lead to improved muscle functional capacity and control, in addition to determining any alterations of individual MU features.

Methods: Ten healthy participants (7 females, 27±6 years, 170±8 cm, 68.7±15.7 kg) underwent 2 bilateral assessment visits (pre- and post-training) separated by 4-weeks of fully supervised unilateral knee extensor force accuracy training. Force accuracy training occurred 3x/week and consisted of 6 sinusoidal force tracking contractions at 10, 25 and 40% MVC in each training session. Knee extensor strength was assessed via maximal voluntary contractions. Unilateral balance was assessed during static one-legged standing. The coefficient of variation for force ($FORCE^{CoV}$) and sinusoidal wave force tracking accuracy ($FORCE^{Sinu}$) was quantified pre- and post-training at 25% MVC. Intramuscular electromyography (iEMG) was utilised to record individual MU potentials from the vastus lateralis (VL) muscles at 25% MVC during sustained contractions. MU firing rate variability was calculated as the coefficient of variation of the interdischarge interval. Data were analysed via two-way repeated measures ANOVA for muscle strength, $FORCE^{CoV}$, $FORCE^{Sinu}$ and unilateral balance, with bonferroni post hoc tests used to identify statistical differences as a result of training. Multi-level mixed effects linear regression models used to analyse MU data. Statistical significance was accepted at $p<0.05$.

Results: Knee extensor muscle strength remained unchanged following training in both legs (trained: 464.4±173.4 N *vs.* 447.0±173.1 N, $p=0.97$; untrained: 450.6±171.6 N *vs.* 403.0±160.7 N, $p=0.13$). Displacement of centre of pressure during unilateral static standing did not change, thus no improvements in unilateral balance were observed, following training ($n=9$; trained: 352±105 mm *vs.* 407±138 mm, $p=0.42$; untrained: 397±139 mm *vs.* 406±189 mm, $p>0.99$). $FORCE^{CoV}$ significantly improved in the trained leg by ~13% (2.80±0.58% *vs.* 2.39±0.40%, $p=0.01$) but not the untrained leg (3.02±0.71% *vs.* 3.00±0.65%, $p>0.99$). Similarly, $FORCE^{Sinu}$ significantly improved in the trained leg by ~30% (34.59±7.26 N·s *vs.* 23.40±3.85 N·s, $p<0.0001$) but not the untrained leg (32.22±6.76 N·s *vs.* 28.57±5.93 N·s, $p=0.19$). MU firing rate variability, significantly reduced by ~16% in the trained VL ($n=8$; ß=-2.018, 95% CI=[-3.202]-[-0.835], $p=0.001$), with no changes in the untrained leg (ß=0.862, 95% CI=[-0.271]-[1.995], $p=0.14$).

Conclusion: A 4-week period of targeted force accuracy training leads to improved muscle force control and accuracy in young healthy participants, which is associated with reduced MU firing rate variability. Importantly, these adaptations and possible mechanisms were evident in the trained limb only. These findings may influence interventional strategies to improve force accuracy in older and clinical populations to potentially aid improved balance and subsequently reduce the risk of future falls, a future direction of this work.

Reference 1 :- 1 - ENOKA, R. M. & FARINA, D. 2021. Force Steadiness: From Motor Units to Voluntary Actions. *Physiology (Bethesda),* 36**,** 114-130.

Reference 2 :- 2 - TRACY, B. L. & ENOKA, R. M. 2006. Steadiness training with light loads in the knee extensors of elderly adults. *Med Sci Sports Exerc,* 38**,** 735-745.

Reference 3 :- 3 - KOBAYASHI, H., KOYAMA, Y., ENOKA, R. M. & SUZUKI, S. 2014. A unique form of light-load training improves steadiness and performance on some functional tasks in older adults. *Scand J Med Sci Sports,* 24**,** 98-110.

Reference 4 :- 4 - VILA-CHA, C. & FALLA, D. 2016. Strength training, but not endurance training, reduces motor unit discharge rate variability. *J Electromyogr Kinesiol,* 26**,** 88-93.

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PC14

Intra-individual characteristics of physiological adaptation to resistance versus endurance exercise training

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Introduction: Endurance (EET) and resistance exercise training (RET) each initiate specific physiological adaptions; EET enhances aerobic capacity(1) and RET muscle mass and strength(2). Yet both promote similar health benefits e.g. in insulin sensitivity and blood pressure. Despite it being known that adaptive responses to both EET and RET vary markedly among individuals(3,4), how, a) the patterns of intra-individual adaptations to distinct exercise modes vary, and; b) the core physiological adaptations to EET/RET relate to health benefits, is unknown.

Method: We conducted a 14-week randomised cross-over trial (4-weeks RET/EET; 6-week wash-out; then 4-weeks EET/RET) in (n=16) young male subjects (23.4±4.6 years). Aerobic capacity and muscle mass/strength were measured before and after each training cycle. Paired t-tests determined primary adaptive responses, as above. Two-way ANOVA was used to assess clinical health-related changes. Linear correlation and linear discriminant analysis (LDA) were used to associate changes in VO_2max and appendicular lean body mass (ALBM) and to identify biomarkers predicting training responses, respectively. Individuals were categorized as responders or non-responders according to calculated cut-off values (typical error). $P<0.05$ was considered significant. Data are mean±SD.

Result: As expected, the 4-weeks EET enhanced aerobic capacity (VO_2max: 43.2±9.7 vs. 46.2±9.2 ml/kg/min, P<0.001), while 4-weeks RET increased lean body mass (ALBM: 26.1±4.4 vs. 26.8±4.2 kg, P<0.01). The health benefits of both types of exercise were seen on body composition variables; e.g. both EET and RET reduced total body (EET: 22.5±6.2 vs. 21.8±6.2%, *P<0.05*; RET: 22.7±6.0 vs. 21.8±5.9%, P<0.01) and abdominal fat % (EET: 23.0±10.2 vs. 21.6±10%; RET: 23.7±9.5 vs. 22.3±10%, both P<0.05). Notably, there was a high probability (~85%) for a RET "non-responder" to be a responder to EET. In contrast, responders to RET had low probability (~22%) of being a responder to EET. Mixed model logistic regression confirmed these relationships with <1 odds ratio for individuals to respond to both training modes (0.046, *P<0.05*). In terms of health-related outcomes, ~63% of the non-responders to EET (of 8) demonstrated improvement in at least one of the three health parameters (diastolic blood pressure (DBP), HOMA-IR, fat %). Conversely, ~86% of the non-responders to RET (of 7) demonstrated no improvement in any. Finally, LDA analysis suggested reduction in abdominal fat was the most defining feature in individuals' response to EET, while changes in insulin sensitivity and body fat were the best predictors of response to RET.

Conclusion: Four weeks of EET/RET elicited predicted mean exercise-mode adaptions i.e., aerobic capacity gains with EET and muscle mass gains with RET, while promoting improvements in health-related parameters such as in DBP and body composition even in healthy individuals. Strikingly, individuals showed favour for adaptation to one exercise mode over another i.e., it is unlikely a given individual be a responder to both modes. Intriguingly, health benefits were observed with EET regardless of improvement in aerobic capacity, while gaining muscle mass seems essential to promote health benefits by RET. Finally, changes in biomarkers such as abdominal fat and insulin sensitivity can help predict an individual's responses to EET/RET, respectively.

Reference 1 :- Hoier B, Passos M, Bangsbo J, Hellsten Y. Intense intermittent exercise provides weak stimulus for vascular endothelial growth factor secretion and capillary growth in skeletal muscle. Exp Physiol. 2013;98(2):585–97.

Reference 2 :- Phillips B, Williams J, Atherton P, Smith K, Hildebrandt W, Rankin D, et al. Resistance exercise training improves age-related declines in leg vascular conductance and rejuvenates acute leg blood flow responses to feeding and exercise. J Appl Physiol. 2012;112(3):347–53

Reference 3 :- Green DJ, Eijsvogels T, Bouts YM, Maiorana AJ, Naylor LH, Scholten RR, et al. Exercise training and artery function in humans: Nonresponse and its relationship to cardiovascular risk factors. J Appl Physiol. 2014;117(4):345–52.

Reference 4 :- Davidsen PK, Gallagher IJ, Hartman JW, Tarnopolsky MA, Dela F, Helge JW, et al. High responders to resistance exercise training demonstrate differential regulation of skeletal muscle microRNA expression. J Appl Physiol. 2011;110(2):309–17.

PC16

Investigating the effect of New Zealand blackcurrant extract on blood glucose responses to post-exercise carbohydrate feeding: Implications for glycogen resynthesis

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Nutritional strategies undeniably contribute to recovery from one training bout to another; crucial to achieving training adaptations. Athletes often train multiple times per day, requiring well considered eating strategies to optimise recovery; specifically replenishing muscle glycogen stores. Post-exercise carbohydrate availability is a key factor determining the rate of glycogen resynthesis. Nutritional guidelines suggest to consume carbohydrate at 1.2 g/kg body mass in the hours following exercise, however many athletes struggle to consume this. Therefore, it is important to maximise the availability of glucose to support muscle glycogen resynthesis in the face of sub-optimal carbohydrate consumption.

Blackcurrants, one of the richest sources of polyphenols, includes high concentrations of anthocyanins, a major flavonoid subclass. New Zealand blackcurrant (NZBC) extract supplementation has shown potential ergogenic properties that induce physiological and metabolic responses. Cell culture investigations revealed that anthocyanins enhance glucose transporter protein 4 (GLUT4) translocation to the plasma membrane, increasing cellular glucose uptake. We have shown that one week of supplementation with anthocyanin-rich NZBC extract improves glucose clearance both following a mixed meal and in response to an oral glucose load. Supplementation with NZBC extract could therefore augment the uptake of exogenous carbohydrate during exercise recovery. Thus, we aimed to investigate the effects of 7 days of NZBC supplementation on blood glucose responses following post-exercise carbohydrate ingestion.

Eight amateur cyclists (mean ± SD: age, 22 ± 4 years; body mass, 77.4 ± 4.7 kg; height, 1.81 ± 0.09 m; VO_{2max} 55.5 ± 5.6 ml/kg/min) completed a randomised, cross-over, double-blind, placebo-controlled study. On day 6 of supplementation, participants performed a cycling protocol lasting ~90–120 min to deplete muscle glycogen, followed by a low-carbohydrate evening meal. The following morning (before breakfast), participants cycled for 45 min at 50% W_{max} before performing repeated intervals to exhaustion to measure exercise capacity. Following exercise, participants consumed a sub-optimal dose of carbohydrate (0.8 g/kg BW) for 4 hours, every 15 min.

Exercise capacity was not different (P=0.095) between trials (placebo, 415 ± 44s; NZBC 454 ± 38s). There was no significant difference in blood glucose concentrations following exercise (P=0.702) between trials (placebo, 3.17 ± 0.69 mmol/L; NZBC, 3.24 ± 0.77 mmol/L). Glucose area under the curve was also not different between trials (Placebo, 1363 ± 72; NZBC, 1334 ± 56). However, NZBC lead to noticeably lower glucose concentrations 30 min post-exercise (P = 0.091 ± 1.4 mmol/L). although this did not reach significance.

Currently, the results show little difference between conditions in the blood glucose response during 4 hours of carbohydrate feeding in a glycogen-depleted state. However, blood glucose concentrations appear to be greater in the placebo trial at 30 min post-carbohydrate ingestion. This

could indicate greater rates of blood glucose, augmenting the availability of glucose for muscle glycogen resynthesis. To investigate this hypothesis, we will now analyse muscle biopsies obtained at 0, 60- and 240-minutes following exercise to determine the rate of glycogen resynthesis. If this data supports our hypothesis, NZBC supplementation could be used to aid faster glycogen resynthesis, benefiting athletes who require short term recovery.

PC17

Human primary fibroblastic cells derived from different tissues have similar marker expression but exhibit different responses to an adipogenic stimulus

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Skeletal muscle resident fibroblastic cells are considered to be the origin of the intermuscular adiposity that develops in pathophysiological conditions such as myopathies, type-2 diabetes, and obesity (Contreras et al 2021). Isolated human skeletal muscle TE7$^+$/collagen VI$^+$/PDGFRα$^+$ fibroblastic cells have been shown to have the potential to differentiate into adipocytes when exposed to media containing fatty acids and/or an adipocyte inducing medium (AIM; Agley et al 2013). However, it is not clear if similar behaviour is apparent in fibroblastic cells from other tissues. The aim of the present study was to compare marker expression and response to an adipogenic stimulus of human primary fibroblastic cells from different tissues (muscle, skin and fat) compared to pre-adipocytes.

Human skeletal muscle fibroblastic cells were isolated from muscle biopsies from the *vastus lateralis* of young healthy adult volunteers (n=6, aged 22.3±3.2 yrs). Human primary skin and lung fibroblasts, as well as human primary visceral pre-adipocytes were purchased and cultured as per manufacturer's instructions (n=3 for each, PromoCell). For adipogenic differentiation, all cell types were grown for seven days in skeletal muscle growth medium before applying AIM+oleic treatment. This consisted of a 3-day incubation in a pre-adipocyte differentiation medium followed by a 15-day incubation in an adipocyte nutrition medium (PromoCell) supplemented with 600 µM oleic acid and 15 mg/ml BSA (AIM+oleic). Protein expression and cell morphological changes were analysed using immunocytochemistry. Expression of secreted adipokines, adipsin and adiponectin were analysed from conditioned media collected over 24 hours before and after treatment using a Luminex platform.

Expression of fibroblast marker proteins TE-7, PDGFRα, collagen VI, fibronectin, vimentin and TCF4 were indistinguishable between the four cell types under proliferation conditions. After AIM+oleic treatment three of six skeletal muscle fibroblastic and all pre-adipocyte cell populations showed protein expression of adipocyte marker perilipin (34.3±4.8% and 22.7±3.1% perilipin$^+$ cells

respectively, $p<0.01$), exhibited protein expression of other adipocyte protein markers acetyl-CoA carboxylase and fatty acid synthase, as well as having a classical adipocyte signate ring morphology. None of the skin or lung-derived fibroblasts showed perilipin expression or other adipocyte marker expression. Indeed, although all cell types increased secretion of adipsin (skin:17276±7225 pg/ml, lung:1211±6837 pg/ml, muscle:18504±359 pg/ml, pre-adipocyte: 12183±2592 pg/ml, n=3, $p<0.05$) and showed visible signs of increased lipid accumulation. Only the groups of perilipin$^+$ skeletal muscle fibroblastic cells and pre-adipocyte populations reached statistical significance for total cellular lipid accumulation as measured by integrated density of oil red O staining ($3.3x10^7$±$2.0x10^7$ AU and $4.8x10^7$±$1.3x10^7$ AU respectively, n=3, $p<0.05$). These cells also showed secretion of adiponectin (1722±318 pg/ml and 748±715 pg/ml for muscle fibroblasts and pre-adipocytes respectively, $p<0.05$). Non perilipin$^+$ cell populations were characterised by accumulating multiple small lipid droplets whereas lipid-filled the cytoplasm of perilipin$^+$ cell populations.

These data show that despite being indistinguishable on the basis of their fibroblast marker expression, skin and lung cells differ in their responses to an adipogenic stimulus compared with skeletal muscle origin fibroblastic cells. However, only muscle origin fibroblastic cells derived from some individuals exhibited the full potential for differentiation into adipocytes.

Reference 1 :- Contreras O, Rossi FMV & Theret M (2021). Origins, potency, and heterogeneity of skeletal muscle fibro-adipogenic progenitors—time for new definitions. *Skelet Muscle* **11,** 1–25.

Reference 2 :- Agley CC, Rowlerson AM, Velloso CP, Lazarus NR & Harridge SDR (2013). Human skeletal muscle fibroblasts, but not myogenic cells, readily undergo adipogenic differentiation. *J Cell Sci* **126,** 5610–5625.

Acknowledgements :-

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PC18

Pistachio ingestion reduces muscle soreness in recovery from downhill running in trained team-sport athletes

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Introduction: Pistachio nuts are considered a rich source of leucine and other essential amino acids, as well as being a good source of antioxidants. These properties suggest that pistachio ingestion could potentially influence recovery from exercise induced muscle damage. **Aim**: We aimed to determine if pistachio nut consumption would influence lower limb muscle soreness and/or function in a dose (0oz/d, 1.5oz/d, 3oz/d) dependent manner. **Method**: Following local ethics committee

approval, we used a randomized cross-over approach in trained team-sport players (n=18 males, mean(SEM) age 23.1 ± 1.2y, stature 180.7 ± 1.5cm, mass 77.0 ± 3.0kg, and VO_{2max} 47.5 ± 2.3ml·kg^{-1}·min^{-1}). These players completed three experimental trials, each separated by a minimum period of 3 weeks. Trials were conducted following 2 weeks of pistachio nut ingestion and involved a 40-minute downhill treadmill run to induce muscle damage. Lower limb muscle soreness (visual analogue scale), muscle function (maximal voluntary isokinetic torque and vertical jump), and blood markers of muscle damage / inflammation (creatine kinse, C-reactive protein, myoglobin, superoxide dismutase) were assessed before the damaging exercise (baseline) and at 24h, 48h, and 72h of recovery following exercise. Data were analysed using a repeated measures ANOVA with post-hoc paired T-tests, or one-way ANOVA when appropriate. Initial analysis examined for trial order effects in the model. Where no order effect was observed this variable was removed from the model and main effects of trial and time and their interaction were examined. **Results**: No trial order effects were observed for any of the key outcome measures (soreness, isokinetic torque, vertical jump height, or blood parameters) across trials. Subjective measure of muscle soreness was reduced for mean soreness response ($p<0.05$) across the whole recovery period in the non-dominant quadriceps between 0oz/d and 3oz/d trials (mean difference (95%CI): 13(1 to 25) AU). Although not significant, mean soreness in the dominant quadriceps also was lower in the 3 oz per day group compared to the 0 oz per day control group ($p=0.06$; 13(-1 to 26) AU). No main effects were observed in mean soreness of hamstrings across the recovery period. No main effects were observed for isokinetic torque of knee extensors or knee flexors. A time effect was observed in vertical jump height ($p<0.01$) but no group, or group´time interactions were evident. A significant time effect was noted for serum creatine kinase, but no group effect or group´time interaction. Serum creatine kinase concentration peaked at 24h post-damage (mean(SEM): 763(158)µg/L) from baseline (300(87)µg/L), but had returned to baseline by 72h post (398(80)µg/L). No differences were observed between trials in any of the other blood parameters assessed. **Conclusions**: These data suggest that pistachio nut ingestion at 3oz/d dose provides some alleviation of muscle soreness, but not upon muscle function. This specific effect on soreness rather than on muscle function suggests a mechanism of action related to blunting of the inflammation response. However, further work is required to explore these effects when greater damage is induced, or during longer term follow-up.

Acknowledgements :- This work was supported by a grant from the American Pistachio Growers

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PC19

The effectiveness of eccentric exercises compared to other exercise interventions or rest on patient-oriented outcomes among adults with Achilles tendinopathy: A systematic review and meta-analysis

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Background: Achilles tendinopathy (AT) is a common musculoskeletal condition characterised by the presence of pain, stiffness, swelling, impaired function and performance. Eccentric exercises (EEs) are well-established and commonly prescribed for the treatment of AT. Previous reviews on this topic have compared EEs with or without co-interventions for AT, primarily focusing on pain and function.

Aim/s: To determine the efficacy of EEs compared to other exercise interventions or rest on pain, disability, activity participation, quality of life (QoL) and patient-rating of condition outcomes in managing AT.

Methods: The current review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2020 guidelines. A systematic search was undertaken using eight databases: Web of Science, SCOPUS, PubMed, AMED, EMBASE, Medline, PsycINFO and PEDro. Studies with at least one group receiving EEs for AT were included. The quality assessment of included studies was performed using the PEDro scale. The certainty of the evidence was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE).

Results: Out of 1880 records obtained, 11 studies (n=412) met the eligibility criteria. Meta-analysis indicated that EEs had no significant effect on pain [SMD=0.06 (95% CI: -1.14, 1.25), p=0.92, I^2=92%] or disability [MD=0.77 (95% CI: -5.67, 7.22), p=0.81, I^2=31%] relative to other exercises. However, EEs were superior to rest for pain [SMD=-0.90 (95% CI: -1.32, -0.49), p<0.0001] and disability [MD=20.60 (95% CI: 11.69, 29.51), p<0.0001]. There were no significant differences between EEs and other exercises on activity participation, QoL and patient-rating of condition. Nevertheless, the eccentric exercise (EE) group reported a significantly better patient-rating of condition when compared to rest (p<0.001).

Discussion and conclusion: Eccentric exercises reported similar effects to other exercises on pain, disability, activity participation, QoL and patient-rating of condition. However, EEs was superior compared to rest for pain, disability, and patient-rating of condition. Nevertheless, the results should be interpreted with caution as the overall certainty of the body of evidence was low to very low as per the GRADE system. The limitations pertaining to the current review warrant future research.

Trial registration: PROSPERO (CRD42021247014), date of registration: 16.04.2021

Keywords: Achilles Tendinopathy, Physiotherapy, Eccentric, Exercises

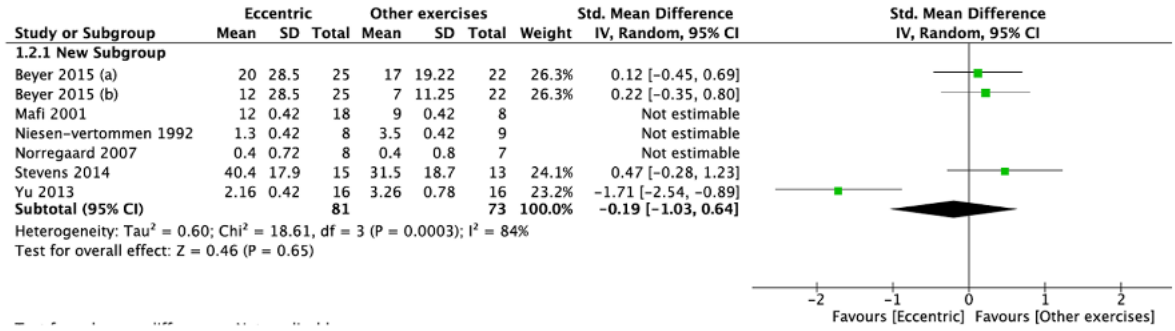

Figure 1. Sensitivity analysis for effect on pain between eccentric exercises vs. other exercises
a=VAS in running, b=VAS in heel-raises

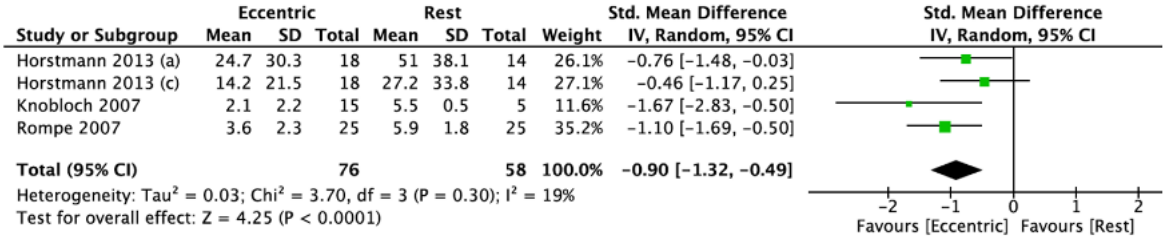

Figure 2. Meta-analysis for effect on pain between eccentric exercises vs. rest
a=VAS in running, b=VAS in heel-raises

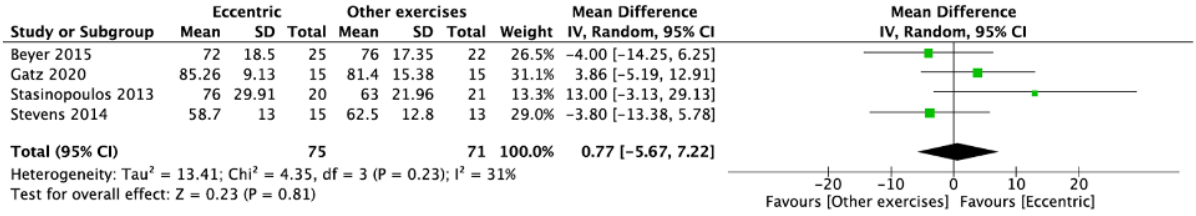

Figure 3. Post-intervention effects on disability between eccentric exercises vs. other exercises

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PC20

Acute respiratory responses to moderate-intensity exercise at -15°C in atopic and non-atopic subjects.

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Background: Strenuous exercise in sub-zero environments may cause airway injury and exercise-induced bronchoconstriction (EIB) (1). Atopic disposition is a risk factor for EIB development (2). However, it is currently unknown whether atopic disposition influences the acute respiratory responses to exercise in a sub-zero climate. Aim: To examine whether the respiratory responses to short- and long-duration exercise at -15^{o}C differ between atopic and non-atopic subjects. Methods: Eighteen non-asthmatic, endurance-trained volunteers (males/females: 14/4, age: 29.4 ± 5.9 years old, maximal oxygen consumption ($\dot{V}O_{2max}$): 61.3 ± 8.7 ml/kg/min) were screened for atopy via the Allergy Questionnaire for Athletes (3) and completed two moderate-intensity (60% $\dot{V}O_{2max}$) environmental chamber running trials at -15^{o}C lasting for 30 and 90 min in a randomized cross-over design. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Swedish Ethical Review Authority. Dynamic spirometry (4) was performed at baseline and 15 and 55 min post-exercise to measure forced expiratory volume in 1 sec (FEV1). Venous blood samples collected pre-exercise and 10 and 65 min post-exercise were analysed for serum Clara cell secretory protein (CC16) using an enzyme-linked immunosorbent assay. A respiratory questionnaire (5) was administered before, immediately after and 20 min after exercise to examine the proportion of affirmative responses ('YES') to the occurrence of four symptoms (cough, wheezing, chest tightness and hypersecretion of mucus) associated with lower airways. To examine 2- and 3-way interaction effects on the relative change in FEV1 from baseline as well as the CC16 concentration, a 3-factor repeated measures ANOVA and a linear mixed-effects model were employed, respectively. A two-proportion z-test was performed to compare the symptom frequency between the two groups. Analyses entailing multiple comparisons were adjusted with the Benjamini-Hochberg method. Results: Atopy was identified in 10 subjects (56%, 7/3: men/women). There were no significant interaction effects for FEV1 or CC16 concentration (group x trial, FEV1: $p = 0.35$, CC16: $p = 0.50$; group x time, FEV1: $p = 0.10$, CC16: 0.10; group x trial x time, FEV1: $p = 0.39$, CC16: $p = 0.51$). Nevertheless, immediately after the 90-min trial, the onset of airway symptoms was significantly more frequent in atopic volunteers than their non-atopic peers (22.5% vs 0%, $p < 0.01$) with no intergroup differences observed 20 min post-trial. Atopic status did not affect the occurrence of the lower airway symptoms immediately after (10% in atopic vs 0% in non-atopic, $p = 0.08$) or 20 min after (5% in atopic vs 0% in non-atopic, $p = 0.19$) the 30-min trial. Conclusion: Atopy is not a major risk for bronchoconstriction when moderate-intensity exercise of either short or long duration is performed in a sub-zero climate by non-asthmatic subjects. Although the extent of bronchial epithelial damage did not differ between the two groups, atopic disposition may transiently elicit more lower airway symptoms after prolonged exercise.

Reference 1 :- Hanstock, H.G.; Ainegren, M.; Stenfors, N. "Exercise in Sub-zero Temperatures and Airway Health: Implications for Athletes With Special Focus on Heat-and-Moisture-Exchanging Breathing Devices." Frontiers in Sports and Active Living. 2020, 2: 34.

Reference 2 :- Bauza, D.; Silveyra, P. ''Asthma, atopy, and exercise: Sex differences in exercise-induced bronchoconstriction.'' Experimental Biology and Medicine. 2021, 246, 1400-1409

Reference 3 :- Bonini, M.; Braido, F.; Rasi, G.; Bonini, S.; Baiardini, I.; Del Giacco, S.; Gramiccioni, C.; Manara, M.; Tagliapietra, G.; Scardigno, A.; Sargentini, V.; Brozzi, M. "AQUA : Allergy Questionnaire

for Athletes. Development and Validation." Medicine and Science in Sports and Exercise. 2009, 41, 1034-1041

Reference 4 :- Graham, B. L., Steenbruggen, I., Miller, M. R., Barjaktarevic, I. Z., Cooper, B. G., Hall, G. L., Hallstrand, T. S., Kaminsky, D. A., McCarthy, K., McCormack, M. C., Oropez, C. E., Rosenfeld, M., Stanojevic, S., & Swanney, M. P. Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. Am J Respir Crit Care Med. 2019; 200: E70–88

Reference 5 :- Kennedy, M.D.; Faulhaber, M. "Respiratory Function and Symptoms Post Cold Air Exercise in Female High and Low Ventilation Sport Athletes." Allergy, Asthma & Immunology Research. 2018, 10, 43-51.

PC21

Influence of aerobic training on exercise capacity and mitochondrial function in type 1 diabetes and matched healthy controls

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Introduction: Type 1 diabetes mellitus (T1DM) is a disease characterized by the destruction of the insulin-producing, pancreatic β-cells, where the inability to maintain glucose homeostasis causes multi-organ problems. The influence of T1DM on exercise performance is complex, with some studies displaying no difference (Baldi et al., 2010) and others finding an impaired exercise capacity in T1DM compared to healthy individuals (Goulding et al., 2020). When rigorous matching of adults with T1DM for age-, sex- and physical activity to healthy controls is performed, however, the balance of evidence indicates a reduced exercise capacity in T1DM (Eckstein et al., 2020). The cause of this lower exercise capacity seems to be related to an impaired mitochondrial function in individuals with T1DM irrespective of physical activity (Monaco et al., 2018). Whether skeletal muscle mitochondria of people with T1DM are less responsive to exercise training has not been systemically studied.

Objectives: To compare aerobic exercise capacity and mitochondrial function between adults with T1DM and matched adults without T1DM both before and after an aerobic exercise training intervention.

Methods: Data collection is still ongoing, but so far 17 adults with T1DM and 5 matched healthy control subjects (18-65 years) have performed a maximal ramp incremental test on a cycle ergometer, before and after a 4-week moderate-intensity aerobic exercise training intervention. Muscle biopsies were obtained from the vastus lateralis before and after the intervention. Mitochondrial respiration was assessed in permeabilized fibres using high-resolution respirometry and transmission electron microscopy was to study mitochondrial ultrastructure.

Results: The current low sample size and unequal groups preclude statistical analysis at present. Maximal oxygen uptake (VO_{2peak}) was 37±15 vs. 47±11 $mL.kg^{-1}.min^{-1}$ for people with T1DM (n=17, 40±17 yrs) and controls (n=5, 30±15 yrs). Maximal OXPHOS capacity in permeabilized fibers was 80±44 vs. 98±28 $pmol.s^{-1}.mg^{-1}$ in T1DM and individually-matched controls, respectively (i.e., n=5 for both groups). Peak power (pre: 279±81 vs. post: 292±96 W), VO_{2peak} (pre: 40±13 vs. post: 41±13 $mL.kg^{-1}.min^{-1}$) and OXPHOS capacity (pre: 83±26 vs. post: 107±44 $pmol.s^{-1}.mg^{-1}$) have been assessed before vs. after training in a subgroup of 5 people with T1DM, with matched controls currently undergoing the same intervention. Moreover, we observed mitochondrial cristae damage in electron microscopy images from one healthy, and moderately-trained (VO_{2peak} 55 $mL.kg^{-1}.min^{-1}$), person with T1DM (Figure 1).

Conclusion: These data will allow us to comprehensively study mitochondrial alterations and mechanisms underpinning the impairment in exercise tolerance in people with T1DM. Moreover, this study will reveal to whether skeletal muscle mitochondria of people with T1DM show plasticity after aerobic exercise training. It is expected that data collection and analysis is complete mid-April 2022.

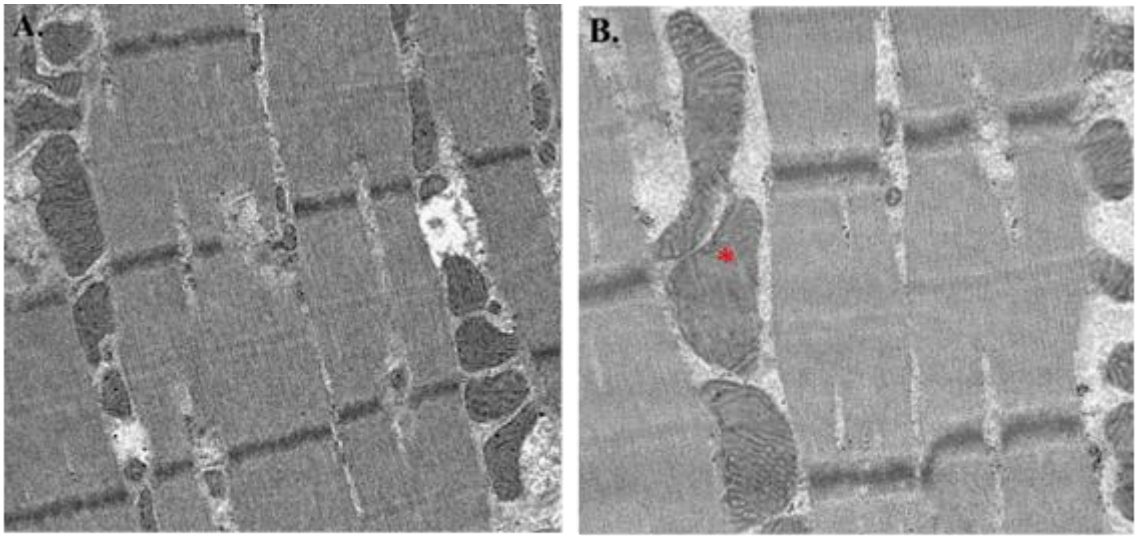

Figure 1. *Pilot data.* A) Electron microscopy showing structured and highly dense cristae in a 27-year old healthy subject B) Electron microscopy image showing cristae damage (indicated by red dot) in a 20-year old individual with T1DM despite a $\dot{V}O_{2max}$ of 55 $mL.kg^{-1}.min^{-1}$.

Reference 1 :- Baldi, J.C., Cassuto, N.A., Foxx-Lupo, W.T., Wheatley, C.M., Snyder, E.M., 2010. Glycemic status affects cardiopulmonary exercise response in athletes with type I diabetes. Med Sci Sports Exerc 42, 1454–1459.

Reference 2 :- Eckstein, M.L., Farinha, J.B., McCarthy, O., West, D.J., Yardley, J.E., Bally, L., Zueger, T., Stettler, C., Boff, W., Reischak-Oliveira, A., Riddell, M.C., Zaharieva, D.P., Pieber, T.R., Müller, A., Birnbaumer, P., Aziz, F., Brugnara, L., Haahr, H., Zijlstra, E., Heise, T., Sourij, H., Roden, M., Hofmann, P., Bracken, R.M., Pesta, D., Moser, O., 2020. Differences in Physiological Responses to Cardiopulmonary Exercise Testing in Adults With and Without Type 1 Diabetes: A Pooled Analysis. Diabetes Care 44, 240–247. https://doi.org/10.2337/dc20-1496

Reference 3 :- Goulding, R.P., Roche, D.M., Scott, S.N., Koga, S., Weston, P.J., Marwood, S., 2020. Limitations to exercise tolerance in type 1 diabetes: the role of pulmonary oxygen uptake kinetics and priming exercise. Journal of Applied Physiology. https://doi.org/10.1152/japplphysiol.00892.2019

Reference 4 :- Monaco, C.M.F., Hughes, M.C., Ramos, S.V., Varah, N.E., Lamberz, C., Rahman, F.A., McGlory, C., Tarnopolsky, M.A., Krause, M.P., Laham, R., Hawke, T.J., Perry, C.G.R., 2018. Altered mitochondrial bioenergetics and ultrastructure in the skeletal muscle of young adults with type 1 diabetes. Diabetologia 61, 1411–1423. https://doi.org/10.1007/s00125-018-4602-6

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PC22

Divergent motor unit rate modulation in strength-matched male and female vastus lateralis

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Background: Increases in skeletal muscle force generation are mediated by the recruitment of additional, progressively larger motor units (MU) and modulation of MU firing rate (FR) (Enoka, 2017). Males typically have larger muscles and greater muscle strength compared to females, and we have previously shown that females exhibit higher MU FR in contractions normalised to absolute strength. However, it remains unclear if these differences in MU FR are attributable to inherent sex characteristics or the substantial sex-based differences in absolute force output. The purpose of this study was to determine sex differences in individual MU features of the vastus lateralis in strength-matched males and females.

Methods: Twenty-four healthy participants (18-35 years), 12 males and 12 females, were recruited to this study, based on similar knee-extensor isometric strength (<4% mean difference). Vastus

lateralis electromyography data were collected by intramuscular electrodes during 10% and 25% of maximum isometric voluntary contractions (MVC). Decomposition-based quantitative electromyography (DQEMG) software was used to identify individual motor unit potentials (MUPs) from each contraction, and their corresponding MUP trains. MU FR was assessed as the rate of MUP occurrences within a MUP train, and MUP area was taken as the total area within the MUP duration (onset to end). Multi-level mixed-effect linear regression models were used to investigate the effect of sex at each contraction level. Significance was assumed when $p<0.05$.

Results: There was no sex difference in maximum muscle torque ($p=0.576$). When compared to males, females had a 16% higher MU FR at 10% MVC (M:7.9 Hz; F: 9.2 Hz, $p=0.002$) and 8% higher at 25% MVC (M:8.7 Hz; F: 9.4 Hz, $p=0.052$). There was a significant interaction between sex and contraction level in MU FR ($p=0.024$) with males exhibiting a 10% increase ($p<0.001$) when moving from low to mid-level contraction, with only a 2% increase in females ($p=0.488$). Males exhibited a 27% (M: 880 mV·ms; F: 565 mV·ms, $p=0.036$) and 17% (M: 1114 mV·ms; F: 944 mV·ms, $p=0.434$) larger MUP area at 10% and 25% MVC, respectively, when compared to females. Both males and females showed significant increases in MUP area (24% and 50%, respectively) from low- to mid-level contractions (both $p<0.001$) with no interactions between sex and contraction level detected ($p=0.5$).

Conclusions: Our findings demonstrate that females exhibit higher MU FR than males to generate absolute force, with a suppressed modulation of MU FR and greater increase in MUP size in females when moving from a low to mid-level contraction, highlighting divergent sex-based MU recruitment strategies to generate similar forces. Although assessed at the lower end of the contraction range of similar absolute forces, females rely more on additional MU recruitment and males rely more on MU FR modulation to increase force.

Ethics approval: This research was approved by the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee ((C16122016, 160-0121, 186-1812, 103-1809, 302-1903) and was conducted between 2019 and 2021 in accordance with Declaration of Helsinki.

Reference 1 :- Enoka RM, Duchateau J. Rate Coding and the Control of Muscle Force. Cold Spring Harb Perspect Med. 2017;7(10).

Acknowledgements :-

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PC23

Comparable rates of exogenous CHO oxidation and trivial gastrointestinal discomfort when ingesting 120 g/h from a drink, gel, jelly chew or co-ingestion approach

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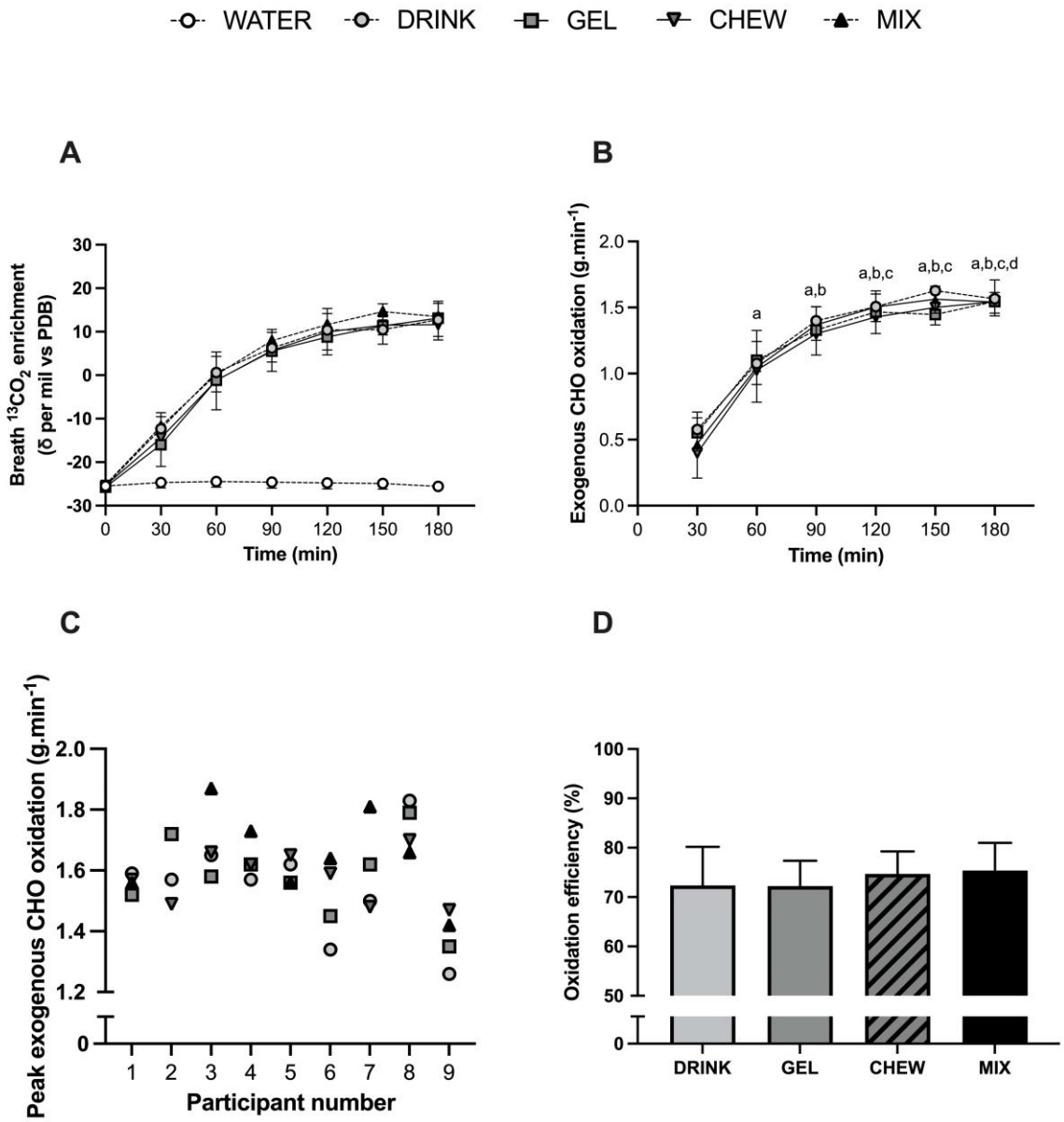
We examined the effects of carbohydrate (CHO) delivery format on rates of exogenous CHO oxidation, gastrointestinal discomfort, and exercise capacity. All participants gave written informed consent prior to participation and the study was approved by the Ethics Committee of Liverpool John Moores University. In a randomised repeated measures design (after 24 h of high CHO intake (8 g·kg^{-1}) and pre-exercise meal (2 g·kg^{-1})), nine trained male cyclists ingested 120 g CHO·h^{-1}from fluid (DRINK), semi-solid gel (GEL), solid jelly chew (CHEW), or a co-ingestion approach (MIX). Participants cycled for 180 min at 95% lactate threshold followed by an exercise capacity test (150% lactate threshold). Peak rates of exogenous CHO oxidation (DRINK, 1.56 ± 0.16; GEL, 1.58 ± 0.13; CHEW, 1.59 ± 0.08; MIX, 1.66 ± 0.02 g·min^{-1}) and oxidation efficiency (DRINK, 72 ± 8; GEL, 72 ± 5; CHEW, 75 ± 5; MIX, 75 ± 6%) were not different between trials (all $P > 0.05$). Despite ingesting 120 g·h^{-1}, participants reported trivial symptoms of gastrointestinal distress across all individual exercise trials. Exercise capacity was not different (all $P < 0.05$) between conditions (DRINK, 446 ± 350; GEL, 529 ± 396; CHEW, 596 ± 416; MIX, 469 ± 395 sec), all of which were greater than a water only trial completed during familiarisation (231 ± 244 sec; all $P < 0.05$). Importantly, these data represent the first time that exogenous rates of CHO oxidation (via stable isotope methodology) have been assessed using feeding strategies (i.e., formats and a co-ingestion approach) commonly adopted by elite endurance athletes. Data demonstrate that consumption of 120 g·h^{-1} CHO (in a 1:0.8 ratio of maltodextrin (or glucose) to fructose) is a practically tolerable strategy to promote high CHO availability and oxidation during exercise, independent of the delivery format.



(A) Breath $^{13}CO_2$ enrichment and (B) exogenous CHO oxidation during 180 minutes of exercise during the WATER, DRINK, GEL, CHEW and MIX trials. [a]Significant difference from 30 min, [b]significant difference from 60 min, [c]significant difference from 90 min, [d]significant difference from 120 min, $P < 0.05$. Individual participant's peak exogenous CHO oxidation during exercise (C) and mean oxidation efficiency (D). $N = 8$ for MIX trial (missing individual data point for participant 2).

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PC24

Regenerating human skeletal muscle: fibre branching and fibre typing

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Skeletal muscle fibre type and structural alterations, as seen during regeneration and in certain muscle diseases, can be challenging to interpret. Earlier we have described the spatial and temporal cellular processes occurring in the first 7 days after electrically induced myofibre necrosis in human skeletal muscle (*1*). Furthermore, we previously reported remnants of regeneration in the myofibre and its basement membrane 30 days post stimulation (*2*), where changes in the occurrence of small myofibres and encroachment of sarcolemma and basement membrane (suggestive of myofibre branching/splitting) were observed through a serial sectioning approach of the biopsy samples. The purpose of this study was 1) to investigate these phemonena further in a systematic manner, and 2) to determine the reliability of fibre-typing human muscle tissue undergoing regeneration.

Methods: Muscle biopsies from 3 individuals demonstrating muscle regeneration were sectioned serially and stained for ATPase, and antibodies against type I and II myosin, embryonic and neonatal myosin, as well as dystrophin and laminin to label the sarcolemma and basement membrane, respectively. Single fibres and tissue blocks were examined by confocal and electron microscopy, respectively.

Results: A classification guide of approximately 210 regenerating muscle fibers and 180 control fibres was created, consisting of 8 “fibre type” profiles. Comparing the staining patterns of the control and the regenerating muscle revealed solely type II muscle fibres were affected by electrical stimulation damage. In addition, confocal and electron microscopy images revealed regular branching of small myofibre segments, most of which were observed to fuse further along the fibre. Central nuclei were frequently observed at the point of branching/fusion.

Conclusions: Human muscle fibres expressing embryonic and neonatal myosins complicate the determination of fibre type using traditional methods such as ATPase or type I and II myosin antibodies. So-called myofibre branching or splitting is likely to be explained by incomplete regeneration after a necrosis-inducing event.

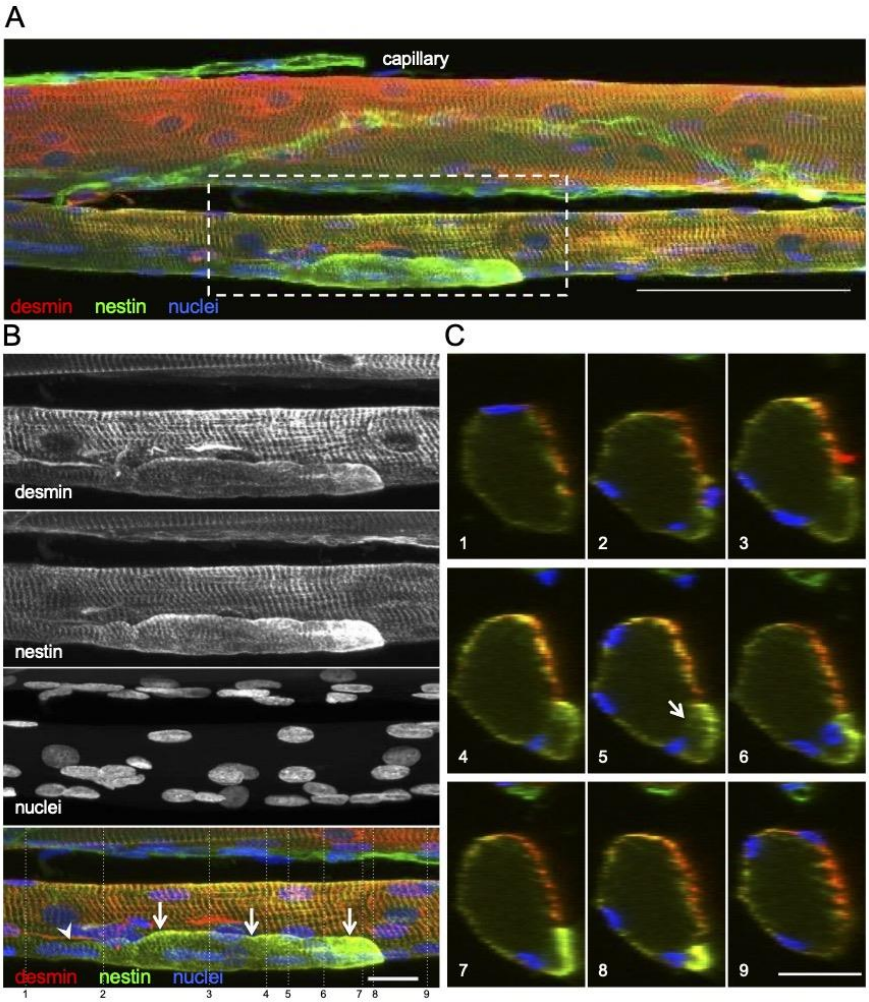

Figure 1:Confocal microscope images of a regenerating human muscle fibre, 30 days post injury, stained for desmin (red), nestin (green) and nuclei (blue). A) Maximum intensity projection of a 14-slice z-stack (100 um scale bar). The area in the dashed line box was imaged at higher magnification (B). B) Maximum intensity projection of a 25-slice z-stack, with single channel grey scale and merged images displayed (20 um scale bar). Note the striated and strongly nestin+ segment (arrows) tightly associated with the main fibre. This segment is approximately 100 um, extending (from left to right) from the point where it first begins to be distinguishable from the main fibre (point of branching or fusion) to the point where it is no longer visible, gradually increasing in nestin immunoreactivity towards its "end". C) YZ orthogonal views (20um scale bar) of B at various points from left to right, as indicated.

Reference 1 :- A. L. Mackey, M. Kjaer, The breaking and making of healthy adult human skeletal muscle in vivo. *Skelet. Muscle*. **7**, 1–18 (2017).

Reference 2 :- A. L. Mackey, S. Brandstetter, P. Schjerling, J. Bojsen-Moller, K. Qvortrup, M. M. Pedersen, S. Doessing, M. Kjaer, S. P. Magnusson, H. Langberg, Sequenced response of extracellular matrix deadhesion and fibrotic regulators after muscle damage is involved in protection against future injury in human skeletal muscle. *FASEB J.* **25**, 1943–1959 (2011).

PC26

Specific force of single, permeabilised, human skeletal muscle fibres studied using two activation strategies from healthy young, healthy older cyclists and elderly hip fracture patients.

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Peak isometric force (P_o) normalised to cross-sectional area (CSA), termed specific force (SF), reflects muscle contractile quality. An age-related decrease in SF has been reported inconsistently at the myofilament level in human skeletal muscle. This has been attributed to both varying physical activity and health status between elderly cohorts and to methodological differences between research groups studying chemically skinned skeletal muscle fibres. Notably, the different activating solutions used (Kalakoutis et al 2021). To address these two issues the present study aimed to compare single fibre SF from both physically active master cyclists (MCs), elderly hip fracture patients (HFPs) and healthy young controls (YCs) using two different activating solutions to determine if this would reveal further insights into ageing and exercise effects on skeletal muscle.

Needle biopsy samples were obtained from the quadriceps of YCs (*n* = 6, age 26 ± 2) and MCs (*n* = 5, age 75 ± 1), and surgical biopsy samples from HFPs (*n* = 5, age 75 ± 4). Skinned fibre isometric force was measured (at 15°C, sarcomere length 2.75µm, pCa 4.5) in each fibre using activating solutions (A and B) which differed in their pH buffer. Solution A used Imidazole and Solution B used TES pH buffer. Fibres were then characterised based on myosin heavy chain (MHC) isoform composition. P_o was normalised to CSA calculated assuming an elliptical shape and also to myosin protein content.

The data are summarised in Table 1. In MHC-I fibres P_o and SF were significantly ($p < 0.05$) greater in solution B than A in YCs (*n* = 45 fibres), MCs (*n* = 72) and HFPs (*n* = 72), whilst in MHC-IIA fibres, P_o and SF were only greater in solution B in YCs (*n* = 45). Between groups, SF was similar in both solutions, as was fibre myosin content. However, a difference was observed when the ratio between

P_o in solutions B:A was compared. The ratio was significantly ($p < 0.05$) greater in MHC I fibres from YCs (1.86) compared to HFPs (1.32), but not compared to MCs (1.57).

The present study has demonstrated that human skinned fibre force is highly sensitive to the chemical constituents of activating solutions used and did not identify any differences in SF between the three participant groups in either activating solution. However, the differing responses to the two solutions within the groups suggests that using two different activating solutions might be used to probe for functional differences occurring at the myofilament level between participants which may not be observed when only a single activation approach is used.

	P_o (mN)				P_o Ratio B:A		SF (kPa)			
	MHC I		MHC IIA		MHC I	MHC IIA	MHC I		MHC IIA	
	A	B	A	B			A	B	A	B
YC	0.36 ± 0.09	0.62[a] ± 0.13	0.41 ± 0.15	0.7[a] ± 0.23	1.86 ± 0.15	2.01 ± 0.27	78 ± 14	139[a] ± 18	73 ± 20	126[a] ± 20
MC	0.40 ± 0.08	0.60[a] ± 0.11	-	-	1.57 ± 0.24	-	88 ± 10	130[a] ± 9	-	-
HFP	0.34 ± 0.06	0.46[a] ± 0.09	0.18 ± 0.014	0.22[b] ± 0.02	1.32[b] ± 0.06	1.23 ± 0.2	88 ± 15	119[a] ± 22	89 ± 6	109 ± 6

Table 1. MHC-I and -IIA P_o and SF measured in either solutions A or B, from young controls (YCs), master cyclists (MCs) and hip fracture patients (HFPs). The ratio of P_o measured in solution B:A is also reported. MHC-I P_o measured in either solution A or B was compared between groups using a Kruskal-Wallis test. MHC-IIA P_o between groups and P_o in solution A vs B within groups were compared using t-tests or Mann-Whitney tests. The MHC-I B:A ratio was compared between groups using a Kruskal-Wallis test and posthoc multiple t-tests. The MHC-IIA B:A ratio was compared using a t-test. MHC-I and -IIA SF were compared between groups using a one-way ANOVA and t-test respectively. SF in solution A vs B within each group was compared using a t-test. [a] significantly ($p < 0.05$) different from solution A; [b] significantly different from YCs. Data are mean ± SEM.

Reference 1 :- Kalakoutis, M., Di Giulio, I., Douiri, A., Ochala, J., Harridge, S. D. R., & Woledge, R. C. (2021). Methodological considerations in measuring specific force in human single skinned muscle fibres. *Acta Physiologica*, *n/a*(n/a), e13719. https://doi.org/https://doi.org/10.1111/apha.13719

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PC27

Influence of cardiorespiratory fitness on performance in simulated mixed martial arts bouts.

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Mixed martial arts (MMA) performance is characterised by high lactate production (9-20mmol·L^{-1}), heart rate (HR) >90%HRmax in between rounds and effort-pause ratio (E:P) ~1:1-4:1 over 9-25 mins(1,2). Performance is therefore likely influenced by the athlete's ability to maintain energy resynthesis at a high percentage of their aerobic capacity ($\dot{V}O_2$max). There is currently no accepted method for measuring the physiological performance of MMA bouts. This study aimed to determine the relationship between the external load of MMA simulated competition and markers of cardiorespiratory fitness. Six human, male participants (25±3 years; 75±8kg; 178±9cm) were recruited for this study following institutional ethical approval. Each participant completed the following procedures at least once. Two participants completed each procedure twice, separated by a 7-week training program thus providing 8 distinct data points. Participants completed a treadmill based graded exercise test (GXT) to volitional failure to determine $\dot{V}O_2$max (ml·kg·min^{-1}), ventilatory thresholds (VT_1 and VT_2, ml·kg·min^{-1}) and running velocity at $\dot{V}O_2$max ($v\dot{V}O_2$max, km·$hour^{-1}$). Gas exchange variables were recorded via direct gas analysis using a Metalyzer 3B (Cortex Medical, Germany). Each participant also completed a 3x5mins simulated MMA bout within 4 days of the GXT. External load of simulated bouts was measured via Catapult Optimeye S5 accelerometers (Catapult Innovations, Australia) worn on the T3-4 vertebrae for the full duration of each simulated bout to record Playerload (PLd_{ACC}) and Playerload per minute (PLd_{ACC}·min^{-1}) in AU(3). Internal load in AU was calculated using sessional rating of perceived exertion (sRPE) for the overall simulated bout(4). Relationships between cardiorespiratory and performance variables were calculated using Pearson's r correlation (Bayes factor [BF_{10}] ≥3)(5) using JASP 0.16.0 (JASP Team, Netherlands). PLd_{ACC} (158±26 AU) was found to have a strongly supported, very large correlation (r = .824, BF_{10} = 12) with $v\dot{V}O_2$max (16±2km·$hour^{-1}$), a strongly supported, very large correlation (r = .828, BF_{10} = 13) with VT_1 (28±5 ml·kg.min^{-1}); a moderately supported, very large correlation (r = .734, BF_{10} = 5) with VT_2 (39±6ml·kg.min^{-1}); a very strong, very large correlation (r = .886, BF_{10} = 30) with VT_1% of $\dot{V}O_2$max (49±11%), but not VT_2% of $\dot{V}O_2$max (78±8%). PLd_{ACC}·min^{-1} (11±2AU) shared a strongly supported, very large correlation (r = .820, BF_{10} = 12) with $v\dot{V}O_2$max; a strong, very large correlation (r = .819, BF_{10} = 12) with VT_1; a moderately supported, very large correlation (r = .743, BF_{10} = 5) with VT_2; a strong, very large correlation (r = .879, BF_{10} = 27) with VT_1% of $\dot{V}O_2$max, but not VT_2% of $\dot{V}O_2$max. There were no statistically relevant correlations between $\dot{V}O_2$max (50±5 ml·kg.min^{-1}), sRPE (119±24AU) and any other variable. Results indicate that MMA athletes capable of higher intensity work at their $\dot{V}O_2$max are also capable of performing more total work in simulated bouts. This is potentially influenced by enhanced metabolic thresholds. Cardiorespiratory training for MMA may be improved by aiming to achieve VT_1 and VT_2 at a higher % of $\dot{V}O_2$max. The strong relationships presented support the in-field use of accelerometry in monitoring improvements in sport specific fitness of MMA athletes in response to training.

Reference 1 :- Petersen C, Lindsay A. Movement and physiological demands of amateur mixed martial art fighting. The Journal of Sport and Exercise Science. 2020;4(1).

Reference 2 :- Kirk C, Clark D, Langan-Evans C, Morton J. The physical demands of mixed martial arts: A narrative review using the ARMSS model to provide a hierarchy of evidence. Journal of Sport Sciences. 2020;

Reference 3 :- Hurst HT, Atkins S, Kirk C. Reliability of a portable accelerometer for measuring workload during mixed martial arts. Journal of Athletic Enhancement . 2014;5(2).

Reference 4 :- Foster C. Monitoring training in athletes with reference to overtraining syndrome. Medicine and Science in Sports and Exercise. 1998;30:1164–8.

Reference 5 :- Wetzels R, Wagenmakers E-J. A default Bayesian hypothesis test for correlations and partial correlations. Psychonomic Bulletin & Review. 2012;19(6):1057–64.

PC28

A cell culture model to investigate contraction-mediated skeletal muscle glucose uptake

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Background: Skeletal muscle plays essential roles in whole body metabolism and is the largest sink for postprandial glucose disposal (DeFronzo *et al,* 1981). As such, impaired muscle glucose uptake contributes significantly to whole-body insulin resistance. Physical activity is key to the maintenance of glycaemic control, with insulin resistance developing rapidly with inactivity (Burns et al. 2021). Yet the mechanisms regulating contraction/inactivity mediated glucose uptake remain unclear. The 2-deoxyglucose (2DG) tracer is commonly used to determine glucose uptake, yet over long time periods can interfere with cellular metabolism (Suginohara *et* al, 2021). We aimed to optimise a valid *in vitro* model of contraction-mediated glucose uptake.

Methods: C2C12 cells were grown in DMEM containing 10% v/v FBS, 1% L-glutamine and 1% penicillin/Streptomycin to a confluency of 90%. Media was switched for differentiation by substituting FBS with 2% horse serum, until myotubes formed. Media was changed 24hr before experiments. One hr of [pre-] electrical pulse stimulation (EPS; C-Pace) was applied at 11.5V and 1Hz with a pulse duration of 2ms to induce formation of contractile sarcomeres. 2DG was dissolved in water and applied at final concentrations of 25µM or 200µM. EPS was applied for 24hr at the same settings as pre-stimulation with samples collected at 30min, 4, 8 and 24hr (n=6). Cells were scraped into homogenisation buffer for immunoblotting or 75% methanol solution for mass spectrometry. Non-stimulated time controls were run alongside treated cells. Glucose uptake was determined by

measurement of 2DG 6-phosphate (n=3). Methanol scraped cells were homogenised and derivatised to methoxime-TMS. Samples were measure on a GC-MSMS (Thermo) TRACE 1310 Gas Chromatograph connected to TSQ 8000 triple quadrupole GC-MS/MS (Thermo Scientific) alongside a standard curve of 25 to 0.78µM 2-DG6P. Simple linear regression was performed to assess glucose uptake linearity, whilst two way-ANOVA was used to analyse changes in media lactate.

Results: At 200µM, cell 2DG6P levels plateaued after 4 hr, presumably due to impaired glucose uptake. At 25µM a linear accumulation of 2DG6P over 24hr was observed (r2= 0.9797 ,P= 0.01). Further, 24 hr EPS showed a linear increase in 2DG6P over 24hr (r2=0.9479, P=0.005). In response to 24hrs EPS, media lactate increased from ~10mM at baseline to ~22mM at 24hrs (P<0.0001), with no effect of 25µM 2DG. There was no change in media lactate concentration in unstimulated cells. Finally, we observed increases in anabolic signalling over 24hr, with EPS e.g. increases in P70 phosphorylation (P=0.03).

Conclusion: At a concentration of 25µM 2-DG is suitable for use in C2C12 cells as a glucose tracer over a 24hr study period. An in vitro model of skeletal muscle glucose uptake over 24 hr has been established that is valid under conditions for EPS mediated contraction.

Reference 1 :- Burns AM et al (2021), *The Journal of Physiology,* 599(8), 2197-2210

Reference 2 :- DeFronzo RA et al (1981), *Diabetes,* 30(12), 1000-1007.

Reference 3 :- Suginohara T et al (2021), *Metabolism,* 114, 154419.

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PC29

Periodised Manipulation of Energy Availability without Symptoms of Athlete Triad or RED-S in an Elite Female Combat Sport Athlete: A 2 Year Long Case Study.

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INTRODUCTION

Combat sport athletes experience transient periods of low energy availability (LEA) when making weight for competition, leading to potential consequences of Athlete Triad (TRIAD) and Relative

Energy Deficiency in Sports (RED-S) (1). Previous research has highlighted that females may be particularly susceptible to these consequences when exposed to both acute and chronic periods of LEA, although recent evidence in this area is equivocal (2, 3). The aim of this case study was to outline the impact of repeatedly making weight, utilising a structured nutrition and training intervention across a two year long period, on symptoms of TRIAD and RED-S in a female combat sport athlete who competes in the Ultimate Fighting Championship (UFC).

METHODS

Over 26 months and within 6 individual 8-week periods, the athlete adhered to periodised daily energy intakes (1,600 ± 305 kcal.day^{-1}; 2.2 g.kg^{-1} protein, 4.2 g.kg^{-1} carbohydrate and 1.0 g.kg^{-1} fat) equivalent to resting metabolic rate (RMR). Training consisted of sport specific, cardiorespiratory conditioning and strength sessions equating to 20-22 hours.wk^{-1} and exercise energy expenditure (EEE) was assessed utilising combined heart rate and portable actigraphy. Body composition (Dual X-ray Absorptiometry [DXA]; Bioelectrical Impedance Analysis [BIA]), RMR & VO_{2peak} (indirect calorimetry), venous blood sampling, cardiac screening (12-lead electrocardiogram and echocardiography) strength (1 repetition maximums), power (force velocity profile) and total mood disturbance (TMD) via a profile of mood states (POMS) were assessed at regular intervals. BM and menstrual cycle data were collected daily.

RESULTS

EEE varied between 776 ± 39 to 1298 ± 71 kcal.day^{-1}, equating to estimated EA ranging from 13 ± 1 to 22 ± 1 kcal.kg.day^{-1}. BM loss was 0.7 ± 0.3 kg.wk^{-1}, resulting in total BM losses of 5.1 ± 1.2 kg, predominantly attributed to decreases in fat mass of 4.1 ± 0.9 kg. Lean mass remained relatively unchanged, ranging from 44.4 to 47.0 kg and RMR was stable throughout (1534 ± 25 – 1468 ± 95 kcal.day^{-1}), eliciting no adaptive thermogenic responses. Despite LEA status, endocrine markers of reproductive/metabolic function and bone metabolism, alongside left and right ventricular structure and function, did not diverge beyond smallest worthwhile or minimal detectable change and were all within normal clinical reference ranges across all assessed timepoints. Measures of cardiorespiratory (VO_{2peak} 42.5 ml.kg.min^{-1}/2.7 L.min^{-1} – 63.8 ml.kg.min^{-1}/3.9 L.min^{-1}), strength (1RM Squat 1.7 – 2.1 x BM) and power (>N.kg^{-1} & W.kg^{-1}) based performance all consistently improved, with no detrimental effect on psychological (TMD ≤2 – 7) health status.

DISCUSSION

This nutritional strategy represented a major alteration in the athlete's habitual BM loss practices, as they previously employed several acute dehydration methods resulting in extreme states of hypohydration. The intervention demonstrates that a gradual approach to making weight in combat sport athletes can be successfully achieved, via a combination of restricted and periodised energy intake according to the daily demands of training, therefore reducing the need to employ aggressive acute BM loss strategies. Fundamentally, the repeated phases of LEA across a chronic period did not result in any negative consequences of either female TRIAD or RED-S.

Reference 1 :- Langan-Evans, C., Germaine, M., Artukovic, M., Oxborough, D. L., Areta, J. L., Close, G. L., & Morton, J. P. (2021). The Psychological and Physiological Consequences of Low Energy Availability in a Male Combat Sport Athlete. *Medicine & science in Sports & Exercise*, *53*(4), 673–683.

Reference 2 :- Lieberman, J. L., DE Souza, M. J., Wagstaff, D. A., & Williams, N. I. (2018). Menstrual Disruption with Exercise Is Not Linked to an Energy Availability Threshold. *Medicine & Science in Sports & Exercise*, *50* (3), 551–561.

Reference 3 :- Heikura, I. A., Stellingwerff, T., & Areta, J. L. (2021). Low energy availability in female athletes: From the lab to the field. *European Journal of Sport Science*, 1–11. Advance online publication.

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PC30

Mechanistic target of rapamycin (mTOR) signalling in aged human skeletal muscle

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Background: Sarcopenia, (loss of muscle mass and quality with age), is a major health problem without safe and effective pharmaceuticals. The mechanistic target of the rapamycin complex 1 (mTORc1) pathway is a cell-type agnostic master regulator of metabolism, and in skeletal muscle, positively regulates proteostasis. However, studies show mTOR signaling is counterintuitively hyperactive in aged muscle, representing a potential cause of sarcopenia (e.g. via inhibition of autophagy). This study aims to comprehensively analyze mTOR-related anabolic/autophagy cell signaling via western blot comparing protein phosphorylation site activity across age (young/old) and sex (men/women).

Methods: This project was approved by the University of Nottingham Ethics Committee and complied with the Declaration of Helsinki (2015), and was registered at https://clinicaltrials.gov/(NCT02505438). This experiment includes 29 human muscle biopsy samples (fasted-state, *vastus lateralis*) collected from young men (22±4y, BMI:24±3, N=6), older men (69±4y, BMI: 27±2, N=8), young women (22±3y, BMI:22±2, N=7) and older women (67±4y, BMI: 26±2, N=8). Following protein extraction and standardization, mTOR-related targets were quantified, including mTOR(Ser^{2448}), ribosomal protein S6 kinase 1 (p70S6K1 Thr^{389}), 4E-binding protein 1 (4E-BP1 $Thr^{37/46}$), ribosomal protein S6 (rps6 $Ser^{235/236}$), tuberous sclerosis complex 2 (TSC2 Thr^{1462}), adenosine monophosphate kinase (AMPK Thr^{172}), serine-threonine protein kinase (AKT Ser^{473}), and Forkhead family of transcription factors (FoxO families, 1a(Thr^{24}),3a(Thr^{32}), and 4a(Thr^{28})). Data were quantified via densitometry and normalized to Coomassie blue staining to account for loading error. Data were tested for normal distribution using a Shapiro-Wilk test. Comparisons between age/sex were performed using independent t-tests or non-parametric equivalent, with the alpha level of significance set at $p < 0.05$. Graph Pad Prism (version 9.0, La Jolla, US) was used for all analyses.

Results: mTOR phosphorylation (Ser^{2448}) was unaffected by age, however the mTOR target S6K1 phosphorylation (Thr^{389}) was higher in both sexes combined in old age (1.5-times) (P<0.05) than their young comparative group. In line with p70S6K1 data, phosphorylation of rps6 ($Ser^{235/236}$) in both older men and both sexes combined were higher than their young counterparts (2-times; P<0.05 and 1.9-times; P<0.01, respectively). In contrast, there were no differences in 4E-BP1 ($Thr^{37/46}$). AMPK phosphorylation (Thr^{172}) in older men and women was 5.2-times (P<0.001), and 3.2-times (P<0.05) greater than their younger counterparts; and both sexes combined were 5.6-times (P<0.01) higher. Upstream Akt phosphorylation (Ser^{473}) exhibited no difference, while TSC2 phosphorylation (Thr^{1462}) was lower two-thirds (P<0.01) in young than older subjects (sex combined). For autophagy targets, FoxO1a and FoxO3a phosphorylation were not different (Thr^{24}/Thr^{32}), while FoXO4a (Thr^{28}) phosphorylation was 5.6 (P<0.01) and 3.4-times (P<0.05) greater in men and both sexes combined in older groups, respectively.

Discussion: We find key elements of hyperactive mTOR signaling in human muscle supporting the notion of mTOR inhibition strategies e.g. rapamycin interventions, to ameliorate sarcopenia in older age. That FoxO4a phosphorylation is increased in older age, implies this target might also be important in aging autophagy dysregulation.

PC31

Protein dose requirements to maximise skeletal muscle protein synthesis after repeated bouts of resistance exercise in young women

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Little is known about temporal and cumulative effects of repeated bouts of resistance exercise and protein feeding on muscle protein synthesis (MPS), or the protein dose required to maximise post-exercise MPS in women. We aimed to understand: 1) acute (4h, 8h) and extended (24h) effects of two bouts of resistance exercise with protein feeding, in women; and 2) the dietary protein requirement to maximise MPS within this time window.

Method: Following written informed consent and screening, 24 resistance exercise-trained women (26.6 ± 0.7 years) were recruited into the study. A DXA scan was performed and participants consumed 500ml of 70% D_2O in 100ml boluses over a 5h period. Twenty-four h later, after an overnight fast, resting state saliva, venous blood and vastus lateralis muscle biopsy samples were

obtained, and volunteers performed a whole-body resistance exercise protocol consisting of 3x8 repetitions at 75% 1 rep max. (2 min rest between sets) involving latissimus dorsi pull down, single-leg press and chest press. Immediately after exercise, subjects ingested a whey protein drink containing either 15g, 30g or 60g total protein (n=8/group). After resting for 4h, saliva, venous blood and muscle biopsy samples were collected again, and volunteers then repeated the exercise and protein ingestion regimen. Saliva, venous blood and muscle samples were taken again 4h later (8h) and the following day (24h). Subjects were fed a controlled diet throughout the study and only performed the exercise prescribed. Plasma leucine was quantified using GC-MS. Body water and muscle protein D_2O enrichment were measured to quantify the rate of MPS as previously described (1). Statistical analysis was performed using two-way repeated measures ANOVA with Dunnett's post-hoc test. Values in the text represent mean ± SEM.

Results: Post-exercise ingestion of 15g protein did not alter plasma leucine concentration or MPS over time. Following post-exercise ingestion of both 30g and 60g protein, plasma leucine concentration was increased above baseline (105.5 ± 5.3 μM; 120.2 ± 7.4 μM, respectively) at 4h (151.5 ± 8.2 μM, $p<0.01$; 224.8 ± 16.0 μM, $p<0.001$ respectively) and 8h (176.0 ± 7.3 μM, $p<0.001$; 281.7 ± 21.6 μM, $p<0.001$, respectively). Post-exercise ingestion of 30g protein increased MPS above baseline (0.068 ± 0.005 %/h) from 0 to 4h (0.140 ± 0.021 %/h, $p<0.05$), 0 to 8h (0.121 ± 0.012 %/h, $p<0.001$) and 0 to 24h (0.099 ± 0.011 %/h, $p<0.01$). Post-exercise ingestion of 60g protein increased MPS above baseline (0.063 ± 0.003 %/h) from 0 to 4h (0.109 ± 0.011 %/h, $p<0.01$), 0 to 8h (0.093 ± 0.008 %/h, $p<0.01$) and 0 to 24h (0.086 ± 0.006 %/h, $p<0.01$).

Conclusion: Post-exercise ingestion of 30g or 60g protein increased MPS above baseline, acutely, after one bout of exercise (0 to 4h), after two bouts of exercise (0 to 8h) and extended the anabolic window over 24h. The magnitude of the post-exercise increase in MPS was no greater following the ingestion of 60g, suggesting the maximal MPS stimulatory effect was achieved with 30g protein.

Reference 1 :- Wilkinson DJ, Franchi MV, Brook MS, Narici MV, Williams JP, Mitchell WK, et al. (2014) A validation of the application of D(2)O stable isotope tracer techniques for monitoring day-to-day changes in muscle protein subfraction synthesis in humans. American Journal of Physiology Endocrinology and metabolism, 306(5):E571-9

PC32

Acute exogenous ketone body supplementation reduces exercise cardiac output independent of blood acidosis: A case study

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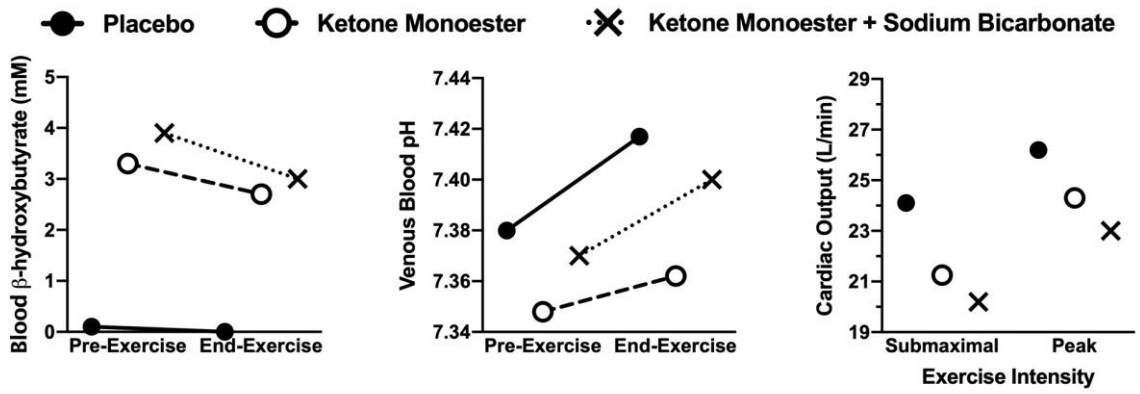
Introduction: We previously reported that acute ingestion of 600 mg/kg body mass of a ketone monoester (KE) supplement increased heart rate (HR) and ventilation during submaximal constant-load cycling compared to placebo (1). The mechanistic basis for the increased cardiorespiratory stress is unknown but could be related to blood acidosis (2,3) and in turn affect cardiac output (Q̇).

Aims/objectives: The present case study examined the effect of increasing blood ketone bodies and manipulating pH on Q̇ during exercise. It informed the design of a larger comprehensive project that is ongoing (n=15). We hypothesized that KE versus placebo ingestion would increase Q̇ during exercise and that supplementing the KE with a blood pH buffer would attenuate this response.

Methods: A male competitive triathlete (21 y, 74 kg) ingested either 0.6 g/kg of KE or a flavour-matched placebo, and 0.2 g/kg body mass of sodium bicarbonate (BICARB) or a salt control. The various manipulations created three experimental conditions: low ketone bodies and neutral pH (placebo), high ketone bodies and acidic pH (KE), and high ketone bodies and neutral pH (KE+BICARB). This was verified through venous blood gas analysis (EPOC, Siemens, Canada) (Figure 1). After supplementation, the participant cycled on an ergometer (Excalibur Sport v2.0, Lode, the Netherlands) for 30 minutes at ventilatory threshold intensity. HR was recorded throughout (Polar Electro model A300, Finland), expired gases were collected from 10-20 minutes using a metabolic cart (Quark CPET, COSMED, USA), and Q̇ was measured in duplicate at ~25 and ~29 minutes using inert gas rebreathing (Innocor, Innovision, Denmark). The constant-load exercise period was followed by an incremental protocol to fatigue for determination of peak oxygen uptake (VO_{2peak}) and peak Q̇ peak as we have described (4). All procedures were approved by the Hamilton Integrated Research Ethics Board (#12811).

Results: During constant-load exercise in both KE-supplemented trials HR was ~8% higher (KE=158, KE+BICARB=156) compared to placebo (145 beats/min) and ventilation was 10% higher (placebo=102, KE=114, KE+BICARB=111 L/min). In contrast, Q̇ was ~15% lower in KE (20.9) and KE+BICARB (20.2) compared to in placebo (24.1 L/min) (Figure 1). This change in Q̇ exceeded day-to-day variability determined using Q̇ measured in duplicate on two separate days without nutritional manipulation (day 1=22.7, day 2=22.0 L/min, coefficient of variation=2.4%). VO_2 varied by <1% between conditions (placebo=4.16, KE=4.15, KE+BICARB=4.19 L/min). Estimated arterial-venous oxygen difference during constant-load exercise, calculated using the Fick principle, was higher in both KE supplemented trials compared to placebo (placebo=172, KE=195, KE+BICARB=208 ml/L). Peak Q̇ was also 8-12% lower in the KE supplemented trials (KE=24.3, KE+BICARB=23.0) compared to placebo (26.2 L/min) (Figure 1). VO_{2peak} (placebo=5.73, KE=5.57, KE+BICARB=5.45 L/min) and peak power output (placebo=470, KE=458, KE+BICARB=451 Watts) were also lower in the KE supplemented trials compared to placebo. Peak HRs were placebo=177, KE=181, and KE+BICARB=177 beats/min.

Conclusions: This case study suggests that KE supplementation, independent of blood acidosis, reduced Q̇ during both submaximal and peak exercise. VO_2 was maintained during submaximal exercise potentially related to increased HR and oxygen extraction whereas VO_{2peak} and peak power output were seemingly reduced.



Reference 1 :- McCarthy DG et al. (2021). Appl Physiol Nutr Metab 46(8), 986-993.

Reference 2 :- Poffe C et al. (2021). Med Sci Sports Exerc 53(2), 431-441.

Reference 3 :- Poffe C et al. (2021). Med Sci Sports Exerc 53(5), 1068-1078.

Reference 4 :- Bostad W et al. (2021). Eur J Appl Physiol 121(9), 2449-2458.

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PC33

Ageing and Neuromuscular Function: A Nine-Year Longitudinal Study of Master Cyclists

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It is generally reported that both ageing, and inactivity, which generally increases with age (Troiano et al, 2008), are associated with a decline in neuromuscular function. In order to eliminate the effects of inactivity and on ageing processes highly active, healthy older adults should be studied (Lazarus & Harridge, 2010). In addition, with ageing research there is a reliance on cross-sectional studies which can have large inter-subject variation in many physiological measures. Thus, the relationship between neuromuscular function and healthy ageing is still not fully clear. Nine years ago, a cross sectional study of master cyclists aged 55–79 was undertaken (Pollock et al., 2015) with numerous neuromuscular indices measured. In the present study a number of these cyclists were re-tested in a longitudinal investigation of the effects of healthy active ageing on neuromuscular function.

Fifteen highly active master cyclists (male=14, female=1) now aged 74±6 years returned to the laboratory. Body composition was measured using dual-energy X-ray absorptiometry (DEXA). Neuromuscular testing comprised: nerve conduction velocity (NCV) of the peroneal nerve, assessment of motoneuron excitability via the H-reflex in the soleus, maximal voluntary isometric strength (MVT) and voluntary activation (VA) of the knee extensors (using the twitch interpolation technique), grip strength, peak explosive cycling power (PP) and the timed up and go (TUG). All data are presented as mean±SD and compared pre and post 9 years using paired t-tests.

On re-testing, cycling volume was unchanged (585±388 versus 570±407 km/month; p=0.88). However, total body mass (69.2±7.1 versus 67.0±6.8 kg; p=0.009) and fat free mass (FFM; 54.8±4.6 versus 50.5±4.1 kg; p<0.001) showed significant reductions of 3% and 8%, respectively, whilst fat mass increased by 15% (14.4±3.5 versus 16.5±3.7 kg; p=0.005). NCV (42.8±4.9 versus 39.2±8.4 $m.s^{-1}$, p=0.06), H reflex (H-wave/M-wave: 0.35±0.29 versus 0.29±0.26; p=0.25) and VA (90.5±7.5 versus 90.2±5.7 %; p=0.86) were unchanged. MVT (167.8±49.4 versus 152.6±34.7 Nm; p=0.30), grip strength (459.2±71.6 versus 442.5± 68.5 N; p=0.32) and performance in the TUG (5.28±0.69 versus 4.99±0.84 seconds; p=0.25) were also unchanged after nine years of ageing. However, PP decreased significantly in absolute terms by 11% (1028±150 versus 913±171 W; p<0.001), with this age-related decline being reduced (8%) when PP was expressed relative to body mass (14.9±2.2 versus 13.7±2.7 W/kg body mass; p=0.021) and removed when normalised to FFM (18.8±2.3 versus 18.1±3.3 W/kg FFM; p=0.30).

Overall, these data show significant changes in body composition, but a general maintenance of neuromuscular function over nine years of healthy active ageing. The exception being the decline in PP which was removed when normalised to FFM.

Reference 1 :- Lazarus, N.R. and Harridge, S.D. (2010). Exercise, physiological function, and the selection of participants for aging research. Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences, 65(8), pp.854-857.

Reference 2 :- Pollock, R.D., Carter, S., Velloso, C.P., Duggal, N.A., Lord, J.M., Lazarus, N.R. and Harridge, S.D. (2015). An investigation into the relationship between age and physiological function in highly active older adults. The Journal of physiology, 593(3), pp.657-680.

Reference 3 :- Troiano, R.P et al (2008). “Physical Activity in the United States Measured by Accelerometer.”. Med Sci Sports Exerc 40(1):181-88.

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PC34

Determination of critical speed from training data: a comparison between field-based tests

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Introduction

The relationship between the intensity of exercise (e.g. running speed) and time until task-failure is characterised by a hyperbolic function. The asymptote of the hyperbola, critical speed (CS), represents the highest intensity at which a metabolic steady-state may be achieved, whereas the curvature constant, labelled as *D'*, represents a finite exercise capacity above CS [1]. CS is therefore an important physiological threshold and, together with *D'*, offers insight into the limits of human performance [2]. CS and *D'* can be calculated from a series of maximal efforts, such as time-trials [TT], constant-intensity tests to task failure, or from a 3-minute all-out test (3MT) [3]. Alternatively, the fastest times recorded over a range of distances during training have been used to estimate CS and *D'* [4]. In this study, we aimed to 1) compare estimates of CS and *D'* derived from unsupervised field tests (TT and 3MT), and those derived from six weeks of habitual training (HAB); and 2) calculate the test-retest repeatability of the 3MT.

Methods:

Twenty-three recreational runners (females *n*=4; mean ± SD age 45±7 years) volunteered to participate in this study, and agreed to have their training monitored through a foot-pod (Stryd Inc., Boulder CO, USA) for 8 weeks. Participants followed their habitual training in weeks 1-6. In weeks 7-8, a series of TT (3, 7, and 12 min) and 3MTs were performed in a randomised order. CS and *D'* were calculated using the fastest 3, 7, and 12 min recorded at any point in the HAB, from the TT, and in duplicate, from the 3MT. Estimations of CS and *D'* derived from TT, 3MT, and HAB were compared using repeated-measures ANOVA.

Results

There was a significant difference between CS estimations across methods (*P*<0.001, Table 1). Bonferroni post-hoc tests revealed CS_{3MT} was higher than CS_{HAB} and CS_{TT} (P<0.05), but there was no difference between CS_{HAB} and CS_{TT} (*P*=1.00). Furthermore, CS_{HAB} and CS_{TT} were strongly associated (r=0.86), and the coefficient of variation (CoV) between both estimates was 4.3% [95% CI: 3.1–5.6%]. Similarly, estimations of *D'* were different between methods (*P*<0.004, Table 1), but post-hoc tests did not detect differences (*P*>0.05) between D'_{TT}, D'_{HAB}, and D'_{3MT}. The repeated 3MTs resulted in estimates of CS which were not different (3.75±0.58 and 3.78±0.58 $m \cdot s^{-1}$; *P*=0.276), and had a high agreement (r=0.93, CoV: 3.2% [2.1–4.3%]) with each other. There was no difference between *D'* derived from repeated 3MT (87±46 m and 75±46 m; *P*=0.276), but these values exhibited low agreement (r=0.71; CoV: 25% [13-37%]).

Conclusion

The close agreement between CS_{HAB} and CS_{TT} is promising and of great practical relevance, and warrants future research to investigate the sensitivity of such estimations to assess human performance. However, CS_{HAB} and CS_{TT} were 7-9% lower than CS_{3MT}. Since data collection was performed remotely, and unsupervised, participants may have paced themselves during the 3MT, which may overestimate CS. Therefore, although repeated 3MT resulted in similar estimations of CS and *D'*, caution is warranted when performing the 3MT in unsupervised conditions.

Reference 1 :- Jones AM et al. (2019). Physiol Rep 7(10), e14098

Reference 2 :- Burnley M & Jones AM. (2018). Eur J Sport Sci 18(1), 1-12

Reference 3 :- Muniz-Pumares D et al. (2019). J Strength Cond Res 33(2), 584-596

Reference 4 :- Smyth & Muniz-Pumares D. Med Sci Sports Exerc 52(12), 2637-2645

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PC35

Oxidation of combined ingestion of galactose and glucose during exercise

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Introduction: Glucose co-ingestion has been shown to enhance splanchnic extraction and metabolism of ingested galactose at rest, but effects during exercise are unknown. The aim of the present study was to examine whether combined ingestion of galactose and glucose during exercise enhances exogenous galactose oxidation. Methods: 14 endurance-trained participants (age, 27 [5] years; $\dot{V}O_2$peak, 58.1 [7.0] ml·kg^{-1}·min^{-1}) performed stationary cycle ergometry for 150 min at 50% peak power output while ingesting beverages providing carbohydrates at rates of 0.4 g.min^{-1} galactose (GAL), 0.8 g.min^{-1} glucose (GLU) and on two occasions 0.8 g.min^{-1} total galactose-glucose (GAL+GLU; 1:1 ratio). Single-monosaccharide ^{13}C-labelling (*) was used to enable calculation of individual (GAL, GLU, GAL*+GLU, GAL+GLU*) and combined (GAL*+GLU*, COMBINE) exogenous-monosaccharide oxidation between 60-150 min of exercise. The research was approved by the Science, Technology, Engineering and Mathematics Ethics Committee, University of Birmingham, Birmingham, UK in accordance with the Declaration of Helsinki. Results: Plasma galactose concentrations with GAL+GLU (0.4 mmol.L; 95%CL 0.1, 0.6) were lower (contrast: 0.5 mmol.L; 95%CL 0.2, 0.8; P<0.0001) than when GAL alone (0.9 mmol.L; 95%CL 0.7, 1.2) was ingested during exercise. Exogenous carbohydrate oxidation with GAL alone (0.31 g·min^{-1}; 95%CL 0.28, 0.35) was marginally reduced (contrast: 0.05 g·min^{-1}; 95%CL -0.09, 0.0; P=0.01) when combined with glucose (GAL*+GLU 0.27 g·min^{-1}; 0.24, 0.30). Total combined exogenous-carbohydrate oxidation (COMBINE: 0.57 g·min^{-1}; 0.49, 0.64) was similar (contrast: 0.02 g·min^{-1}; 95%CL -0.05, 0.09; P=0.63) when compared with isoenergetic GLU (0.55 g·min^{-1}; 0.52, 0.58). Conclusion: glucose co-ingestion did not enhance exogenous galactose oxidation during exercise. When combined, isoenergetic galactose-glucose ingestion elicited similar total exogenous-carbohydrate oxidation to glucose suggesting that galactose-glucose blends are a valid and equivalent alternative for glucose as an exogenous-carbohydrate source during exercise.

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PC36

The effects of treadmill exercise in normobaric hypoxia on gastrointestinal symptoms and injury in trained runners

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Introduction: During intense exercise, splanchnic blood flow is reduced to enhance oxygen supply to peripheral tissue and maintain thermoregulatory control (Rowell 1974). Splanchnic hypoperfusion results in gut epithelia cell hypoxia and increases barrier injury, endotoxemia and gastrointestinal (GI) symptoms. Exercising in hypoxia can increase splanchnic hypoperfusion by 12-30% (Joyner & Casey 2014). Whether this exacerbates GI symptoms and injury is unclear. Intestinal fatty acid-binding protein (I-FABP) is a sensitive marker of epithelia injury within the small intestine. I-FABP appears in plasma rapidly after injury has occurred and has been shown to correlate with exercise-induced splanchnic hypoperfusion (van Wjick et al. 2011). The purpose of the present study was to investigate the impact of a single bout of submaximal treadmill running in normobaric-hypoxia on GI symptoms and I-FABP. **Methods:** Twelve well trained male runners performed two 1-hour bouts of treadmill exercise (85% gas exchange threshold) in a randomised order. One bout was performed in normoxia (NORM: fraction of inspired oxygen (F_IO_2) = 20.9%) and the other in normobaric-hypoxia (HYP: (F_IO_2) = 14%). Plasma I-FABP was assessed pre-exercise, immediately post-exercise and 1 hour-post-exercise for the assessment of GI injury. Peripheral oxygen saturation (SpO_2) was assessed from the forehead throughout each trial. Capillary samples were collected for the assessment of lactate every 15-mins, while heart rate (HR) and rating of perceived exertion (RPE) were recorded every 5-mins. Global gastrointestinal symptoms were collected at 15-min time points during treadmill exercise with all scores summed to give a total score (Pugh et al. 2017). A symptom specific GI questionnaire was completed following exercise (Gaskell et al. 2019). Physiological data were analysed using two-way (time x trial) repeated-measures ANOVA with Bonferroni adjusted post hoc tests where appropriate. Paired sample t-tests and Wilcoxon tests were used to analyse gastrointestinal symptom data. Statistical significance was accepted at $P < 0.05$. **Results:** Global GI symptom scores collected during exercise and from the post-exercise questionnaire were higher during HYP compared to Norm

(P < 0.05). The number of severe symptoms reported from the post-exercise questionnaire were also higher in HYP (1.5 ± 2.0) compared to NORM (0 ± 0) (P = 0.027). A two-way ANOVA revealed a time x trial interaction for I-FABP (P = 0.045), with higher concentrations immediately post-exercise in HYP when compared to NORM (1121.1 ± 438.7 vs 830.2 ± 422.3 pg/mL) (P = 0.008). Global gastrointestinal symptoms across both trials correlated with pre-post ΔI-FABP (P = 0.047). SpO_2 was significantly lower at all time points in HYP, whereas lactate, HR and RPE were all higher in HYP when compared to NORM (P < 0.05). **Conclusion:** Taken together, our results indicate that submaximal treadmill exercise in normobaric-hypoxia causes more GI symptoms and intestinal injury than in normoxia. It is likely that the combination of reduced SpO_2 and enhanced splanchnic hypoperfusion increased intestinal ischemia, causing greater injury during the HYP trial. The correlation of I-FABP and global GI symptoms was a novel finding and implies GI injury may partly be responsible for the onset of symptoms.

Reference 1 :- Rowell LB (1974). Physiol Rev 54, 75-159

Reference 2 :- Joyner M & Casey D (2014). Journal of Applied Physiology, 116(7), 852-857

Reference 3 :- Van Wjick K et al. (2011). PloS one 6.7, e22366

Reference 4 :- Pugh JN et al. (2017). Applied Physiology, Nutrition, and Metabolism 42.9, 941-947

Reference 5 :- Gaskell SK et al. (2019). International Journal of Sport Nutrition and Exercise Metabolism 29.4, 411-419

PC37

Validity and reliability of the Manikin Assessment Tool for endoTracheal Intubation (MATTI) task

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Introduction

Endotracheal intubation is a core skill of anaesthetists, intensive care physicians and pre-hospital practitioners [1]. Trainees usually learn how to perform the procedure on a manikin before they are permitted to practise on patients. However, there is no validated method to assess proficiency at endotracheal intubation of a manikin. The MATTI (Manikin Assessment Tool for endoTracheal Intubation) task was developed, using a task analysis approach, by two consultant anaesthetists to assess endotracheal intubation proficiency [2]. The MATTI task has fourteen components (Table). Each component may be awarded either 2 (if completed correctly on the first attempt), 1 (if partially completed, completed out of sequence or completed after a prompt) or 0 marks (if not completed).

Total score (out of 28) and time taken to complete the MATTI task are the recorded parameters. This study aimed to assess the construct validity, the test-retest reliability and the inter-rater reliability of the MATTI task [3].

Methods

Ethical approval was obtained from the Imperial College Research Ethics Committee (20IC6445). Thirty-six participants (12 Novices with no anaesthetic experience; 12 Intermediates with <2 years anaesthetic experience; 12 Experts with >2 years anaesthetic experience) were recruited to test the construct validity and the test-retest reliability (intubation group). A separate 20 participants (5 with anaesthetic experience; 5 with teaching experience; 5 with both; 5 with neither) were recruited to assess the inter-rater reliability (rater group). Both groups watched an instructional video before participating and the intubation group were allowed to practise the task. The intubation group were asked to perform the MATTI task three times, with an interval of 1 hour between attempts. The rater group were asked to score five pre-recorded videos of the MATTI task which contained between 0 and 10 deliberate mistakes. The "gold standard" score for each video was decided by two investigators (NC and CJM).

Results

Intermediates and Experts performed the MATTI task faster than Novices ($p < 0.05$; Figure). The median (IQR [range]) total score was 27 (26-28 [23-28]) out of 28, but the total score did not differ with experience level ($p = 0.54$). Neither time ($p = 0.64$) nor score ($p = 0.69$) differed between attempts. The median (IQR [range]) difference between raters' scores and the "gold standard" was 1 (0-2 [0-11]) per video and Cronbach's alpha for the rater group's scores was 0.99.

Conclusion

The results demonstrate that the MATTI task is a valid and reliable tool to examine proficiency at endotracheal intubation of a manikin. Although the total score in the intubation group did not differ with anaesthetic experience level, its inclusion in the assessment is necessary in order to demonstrate correct completion of the MATTI components. The rater group results demonstrate that neither anaesthetic nor teaching experience is required in order to mark candidates' proficiency. Further work should assess the validity and reliability of MATTI to assess endotracheal intubation in patients.

Table The fourteen components of the MATTI task.

1	Place the face mask on the manikin to pre-oxygenate
2	Select the size 7.0 ETT and check the pilot balloon is functional with 20 ml of air (clear syringe)
3	Select the MAC 4 laryngoscope and check that the laryngoscope light is working
4	Give medication with a 20 ml syringe (white syringe)
5	Remove the oxygen
6	Pick up the laryngoscope in the left hand
7	Tilt the head back and insert the laryngoscope into the mouth
8	Obtain a view of the vocal cords without breaking the teeth (only 1 point if teeth are broken)
9	Pick up the ETT with the right hand
10	Successfully insert the ETT into the trachea
11	Inflate the ETT cuff with 20 ml of air (clear syringe)
12	Attach the capnometer, catheter mount and self-inflating bag to the ETT and ventilate the lungs
13	Auscultate both lungs
14	Secure the ETT with a tube tie (half hitch and bow)

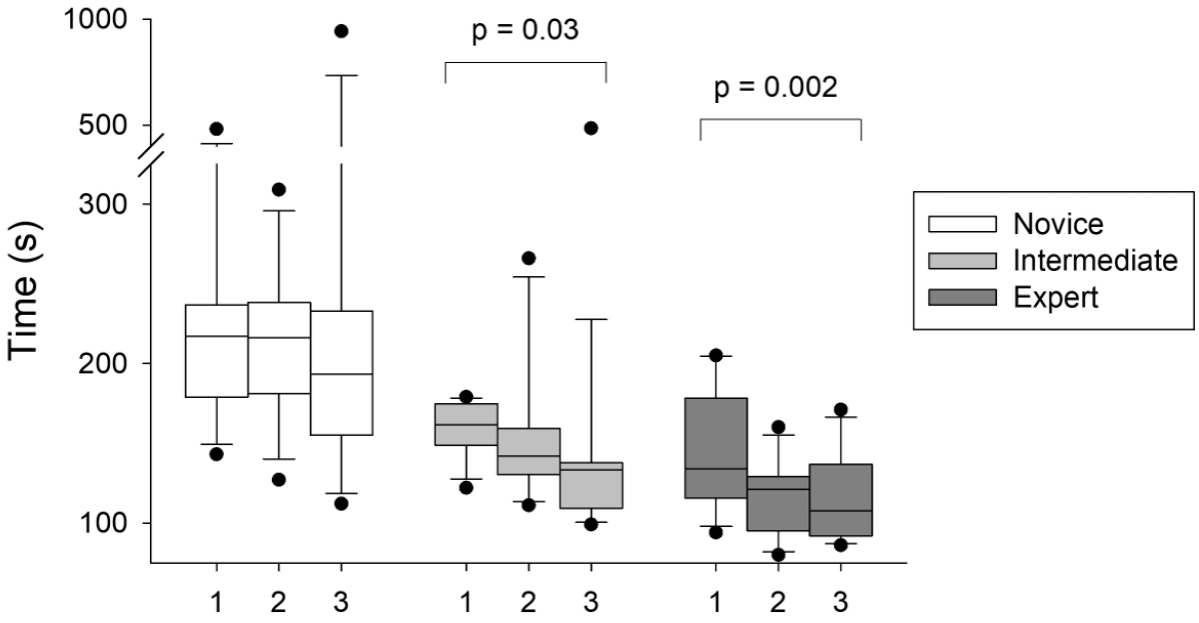

Figure Time taken to perform the MATTI task on three occasions. Boxes are median and interquartile range; whiskers are 10th and 90th centiles; dots are 5th and 95th centiles. p-values indicate difference from the "Novice" group.

Reference 1 :- Royal College of Anaesthetists CCT in Anaesthetics Annex B Core Level Training (2010), 30-3.

Reference 2 :- Weinger MB et al. (1994) Anesthesiology 80, 77-92.

Reference 3 :- Heale R, Twycross A. (2015) Evid Based Nurs 18, 66-7.

Acknowledgements :- We would like to thank the students, teachers, lecturers and anaesthetists who particpated in this study.

PC38

Ischemic preconditioning blunts exercise induced mitochondrial dysfunction, speeds $\dot{V}O_2$ kinetics but does not alter severe-intensity exercise capacity.

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This study examined the effect of ischemic preconditioning (IPC) on severe-intensity exercise performance, pulmonary $\dot{V}O_2$ kinetics, skeletal muscle oxygenation (TSI) and mitochondrial function. Eight males underwent contralateral IPC (4×5 min at 220 mmHg) or sham-control (SHAM; 20 mmHg) before performing a cycling time-to-exhaustion test (TTE; 92% maximum aerobic power). $\dot{V}O_2$ kinetics and vastus lateralis (VL) TSI were measured throughout trials. Muscle biopsies (VL) were obtained before IPC and SHAM and ~1.5 min post-exercise to determine mitochondrial respiration and citrate synthase activity (CS). TTE did not differ between SHAM and IPC (249 ± 37 vs 240 ± 32 s; P = 0.62). Pre- and post-exercise mitochondrial respiration through protein complexes (C) I-IV did not differ between trials (P > 0.05). Mass and CS corrected CI leak respiration (CIL) increased post-exercise compared with baseline in SHAM (1.9 ± 1.8 vs 4.5 ± 1.7 $pmol.s^{-1}.mg^{-1}$; P ≤ 0.05 and 0.10 ± 0.07 vs 0.29 ± 0.10 $pmol.s^{-1}.mg^{-1}.CS^{-1}$; P = 0.003, respectively), but not IPC (3.2 ± 1.6 vs 3.9 ± 2.2 $pmol.s^{-1}.mg^{-1}$; P = 0.19 and 0.21 ± 0.10 vs 0.26 ± 0.15 $pmol.s^{-1}.mg^{-1}.CS^{-1}$; P = 0.16, respectively). $\dot{V}O_2$ mean-response time was faster (51.3 ± 15.5 vs 63.7 ± 14.5 s; P = 0.003) with a smaller slow component (270 ± 10 vs 377 ± 188 $ml{\cdot}min^{-1}$; P = 0.03) in IPC compared with SHAM. TSI did not differ between trials (P > 0.05). IPC attenuated increased CIL, evoked by severe-intensity exercise, and expedited $\dot{V}O_2$ kinetics but did not improve exercise performance. All experimental procedures were approved by the Loughborough University Ethics Approvals Human Participants Sub-Committee (R19-P138),

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PC39

The effect of a 3-day dietary intervention on maximal fat oxidation and the regulation of metabolic genes in moderately trained men

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Short-term high-fat diets may be a possible strategy to optimize the maximal fat oxidation (MFO) with the aim of increasing the contribution of fat to whole-body metabolism during prolonged exercise in order to preserve the glycogen stores and potentially postpone the onset of fatigue and improve performance[1]. Short-term dietary intervention may change the utilization of exo- and endogenous fat and carbohydrate sources and may influence muscle genes encoding proteins involved in lipid and carbohydrate metabolism underlying the changes in substrate utilization[2]. Yet, the effect of a short-term dietary intervention on MFO and the relation between changes in MFO and changes in lipid and carbohydrate metabolism-associated mRNAs following a short-term dietary intervention has not previously been examined. Therefore, this study aimed to investigate how MFO and the relative intensity that elicits MFO (Fatmax) were affected by a dietary intervention with marked changes in the distribution of dietary fat and carbohydrate intake. Moreover, it was investigated if concurrent changes in mRNAs encoding proteins involved in lipid and carbohydrate metabolism in skeletal muscle could be a contributing mechanism to the expected changes in MFO. Forty moderately trained men with a maximal oxygen uptake (VO_2max) of 56.2±1.2 ml/kg/min (mean ±SEM) were allocated by stratified randomization to a 3-day isocaloric high-fat diet (HiFat) (65 E% fat, 20 E% carbohydrate, and 15 E% protein) (n=20) or high-carbohydrate diet (HiCho) (15 E% fat, 70 E% carbohydrate, and 15 E% protein) (n=20). Before and after the dietary intervention the participants underwent two identical test days in a fasted state, where a muscle biopsy was obtained and a Fatmax-test and VO_2max-test were performed in order to determine MFO and Fatmax[3]. Real time PCR was applied to quantify changes in the mRNAs of interest, and PCR-data were normalized to the amount of total ssDNA in each sample. Mixed linear models were applied to determine possible interactions (group*time) of the dependent variables and pairwise comparisons were used to locate the differences within- and between groups. P-values < 0.05 were considered statistically significant. MFO and Fatmax were increased from 0.41±0.05 g/min to 0.59±0.05 g/min (p>0.001) and 37±2% to 44±2% (p>0.001), respectively, in response to HiFat and decreased from 0.36±0.04 g/min to 0.28±0.06 g/min (p>0.001) and 37±2% to 33±2% (p>0.001), respectively, after HiCho. Lipid transporter cluster of differentiation 36 (CD36) and uncoupling protein-3 (UCP3) mRNA content were increased by 3.1 and 1.7-fold (p=0.018, p=0.046), respectively, after HiFat. Perilipin-5 (PLIN5) mRNA content was greater after HiFat compared to after HiCho (p=0.014), and hexokinase II (HKII) mRNA content was increased by 3.2-fold after HiCho (p=0.032). No changes were found in the mRNA content of hormone sensitive lipase(HSL), glucose transporter type 4 (GLUT4), pyruvate dehydrogenase kinase 4 (PDK4), nucleotide translocator-1 (ANT1), beta-hydroxyacyl-CoA dehydrogenase (ß-HAD), or carnitine palmitoyltransferase I (CPT-1). These findings demonstrate that a 3-day dietary intervention affects the MFO and Fatmax and that concurrent changes in mRNAs encoding proteins related to lipid and carbohydrate metabolism in the skeletal muscle could be a potential contributing mechanism to the changes observed in MFO after HiFat and HiCho.

Reference 1 :- Maunder E, Plews DJ, Kilding AE. Contextualising Maximal Fat Oxidation During Exercise: Determinants and Normative Values. *Front Physiol*. 2018;9:599. doi:10.3389/fphys.2018.00599

Reference 2 :- Yeo WK, Carey AL, Burke L, Spriet LL, Hawley JA. Fat adaptation in well-trained athletes: effects on cell metabolism. *Appl Physiol Nutr Metab*. Feb 2011;36(1):12-22. doi:10.1139/h10-089

Reference 3 :- Achten J, Gleeson M, Jeukendrup AE. Determination of the exercise intensity that elicits maximal fat oxidation. *Med Sci Sports Exerc*. Jan 2002;34(1):92-7. doi:10.1097/00005768-200201000-00015

PC40

Knee extensor force control as a predictor of dynamic balance

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Previous research has demonstrated that force control in various muscles of the lower limb (measured according to the magnitude of force fluctuations) explains a significant amount of variance in static balance (Davis *et al*., 2020). Given the dynamic nature of many sports and activities of daily living (Ringhof and Stein, 2018), and the fact that most fall-related events occur under dynamic conditions (Blake *et al*., 1998), assessment of balance and its determinants under dynamic conditions is of critical importance. The aim of the present study was, therefore, to determine whether muscle force control also explains significant variance in dynamic balance. 20 healthy participants (9 males, 11 females; mean ± SD: age 31.6 ± 12.9 years; height 1.72 ± 0.09 m; body mass 76.7 ± 21.3 kg) provided written informed consent to participate in the study, which was approved by the ethics committee of the University of Essex (Ref. ETH2021-0394), and which adhered to the Declaration of Helsinki. Participants visit the laboratory on a single occasion during which balance was measured using the Y balance test performance and knee extensor muscle force control were measured was isokinetic dynamometry. The Y balance test involved stance on an elevated central footplate with the right leg and attempting maximal reach along reach indictor pipes with the left leg in the anterior, posteromedial and posterolateral directions. Force control was assessed during isometric knee extension contractions of the right leg at 10, 20 and 40% maximal voluntary contraction (MVC) and was quantified according to the magnitude of force fluctuations, using the coefficient of variation (CV), and according to the temporal structure of force fluctuations, using approximate entropy (ApEn) and detrended fluctuation analysis (DFA) α. A significant negative correlation was observed for Y balance test anterior reach and muscle force CV during contractions at 40% MVC (r = –0.44, P = 0.05) and a significant positive correlation was observed for anterior reach and muscle force ApEn during contractions at 40% MVC (r = 0.53, P = 0.015). A subsequent stepwise, linear, multiple regression model demonstrated that muscle force CV and ApEn during contractions

at 40% MVC significantly explained 32.3% of variance in Y balance test anterior reach. These results are the first to indicate that a moderate amount of variance in dynamic balance can be explained by measures of isometric force control. Based on the purported significance of muscle force CV and ApEn (Pethick *et al*., 2021) and the Y balance test (Lockie *et al*., 2013), these results indicate that greater force steadiness and adaptability are associated with greater dynamic balance and stability. Consequently, improving muscle force control should, in theory, result in a predictable improvement in dynamic balance performance; an observation that could be of importance for athletes and those at risk of falls alike.

Reference 1 :- Davis *et al*., 2020, Exp Brain Res 238, 487-497

Reference 2 :- Ringhof and Stein, 2018, Hum Mov Sci 58, 140-147

Reference 3 :- Blake *et al*., 1998, Age Ageing 17, 376-372

Reference 4 :- Pethick *et al*., 2021, Exp Physiol 106, 2046-2059

Reference 5 :- Lockie *et al*., 2013, Isokint Exerc Sci 21, 301-309

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PC42

Modelling the relationship between skin blood flow, cutaneous heat flux and skin temperature during passive heat stress

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Introduction

During cold stress, increased cutaneous vasomotor tone (vasoconstriction) may be inferred from reductions in skin blood flow (SkBF), skin temperature (T_{Sk}) or cutaneous heat flux (Q_{Sk})[1, 2]. However, in other scenarios, such as direct local heating or intrapartum epidural anaesthesia, this proportionate relationship is absent[3, 4]. We propose that the relationship between skin blood flow, skin temperature and cutaneous heat flux may be modelled using a conservation of energy principle:

$$Q_D = Q_{Sk} + Q_{St} \quad (1)$$

where Q_D is the rate of heat delivery to the skin and Q_{St} is the rate of heat storage in the skin. The rate of heat delivery may be estimated as follows:

$$Q_D \alpha \ SkBF \bullet (T_C - T_{Sk}) \quad (2)$$

and the rate of heat storage as:

$$Q_{St} = p_{Sk} \bullet d_{Sk} \bullet c_{Sk} \bullet \delta T_{Sk} \quad (3)$$

where T_C is core temperature, p_{Sk} is the density of skin (1.02 g/cm^2), d_{Sk} is the thickness of skin (1.5mm), c_{Sk} is the specific heat capacity of skin (3.47 KJ/kg) and δT_{Sk} is the rate of change in skin temperature. Inserting equations (2) and (3) into (1), we hypothesise that the relationship between skin blood flow, skin temperature and cutaneous heat flux may be modelled as:

$$SkBF \bullet (T_C - T_{Sk}) \alpha Q_{Sk} + (8.85 \bullet \delta T_{Sk})$$

The aim of this mechanistic study was to test the performance of the proposed model in two scenarios: indirect heat stress and direct local heating.

Methods

Ethical approval was obtained from Imperial College Research Ethics Committee (20IC6475). Sixteen healthy subjects (14 male) undertook a heat stress protocol using a water-perfused mattress and blanket. The mattress and blanket covered the chest, abdomen and legs, but the arms and head were uncovered. Forearm (indirect heat stress) and thigh (direct local heating) skin blood flow (laser doppler flowmetry), cutaneous heat flux, and skin temperature were recorded. Core temperature was recorded with an ingestible telemetry pill. Linear regression was used to evaluate the relationships between parameters.

Results

The relationships between skin blood flow, skin temperature and cutaneous heat flux were proportionate in the forearm ($p < 0.001$), but in the thigh there was no correlation between skin blood flow and cutaneous heat flux ($p = 0.827$; Figure 1). Cutaneous heat delivery correlated with cutaneous heat loss + storage in both the forearm and the thigh ($p < 0.001$; Figure 2).

Conclusions

Skin temperature and cutaneous heat flux can be considered indices of cutaneous vasomotor tone during indirect heat stress but not during direct local heating. The relationship between skin blood flow, skin temperature and cutaneous heat flux during indirect heat stress and direct local heating can be quantified using a conservation of energy model. The difference in the model regression line gradients between the forearm and the thigh may be due to an unmeasured parameter, such as cutaneous heat permeability. Further work should investigate the validity of the cutaneous conservation of energy model in other scenarios, such as exercise and anaesthesia.

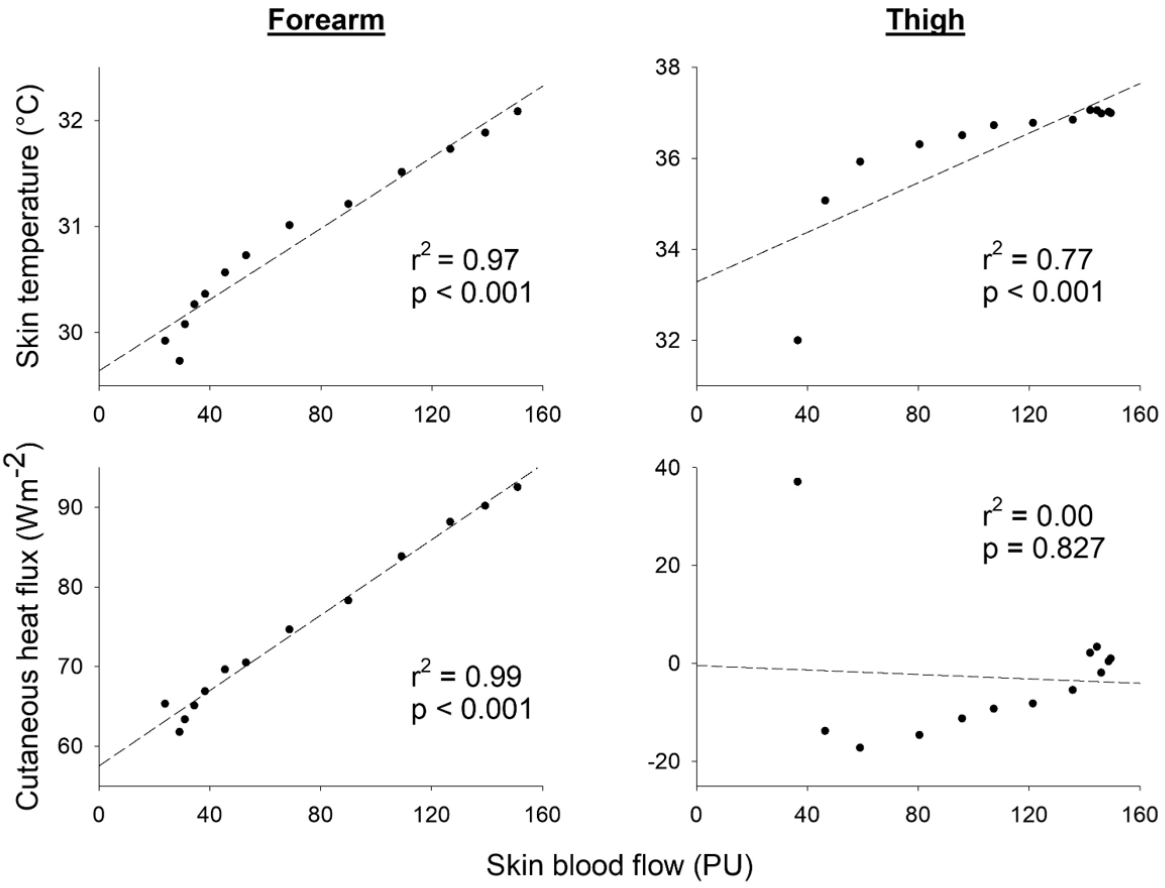

Figure 1 The relationships between skin blood flow, skin temperature and cutaneous heat flux.

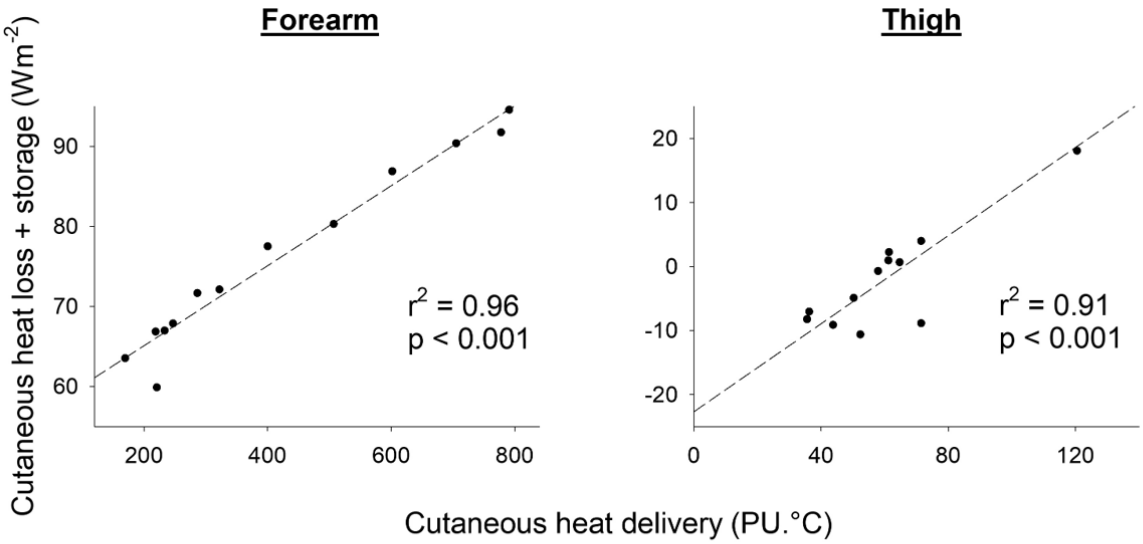

Figure 2 The performance of the proposed conservation of energy model of cutaneous heat transfer.

Reference 1 :- Sessler DI et al. (1990) Anesthesiology 73, 656-60.

Reference 2 :- House JR & Tipton MJ. (2002) Eur J Appl Physiol 88, 141-5

Reference 3 :- Mullington CJ et al. (2018) Anaesthesia 73, 1500-6

Reference 4 :- Sessler DI & Moayeri A. (1990) Anesthesiology 73, 218-24

Acknowledgements :- We would like to thank the subjects who particpated in this study.

PC43

Evaluating the leucine trigger hypothesis to explain the postprandial regulation of muscle protein synthesis in young and older adults: A systematic review

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Introduction

The anabolic potential of a protein source is dependent on factors related to protein digestibility, amino acid kinetics, and amino acid composition. The leucine trigger hypothesis predicts that the magnitude (amplitude and rate) of postprandial increase in blood leucine concentrations, termed leucinemia, serves to regulate the magnitude of postprandial muscle protein synthesis (MPS) response to an ingested protein source. The aim of this qualitative systematic review was to systematically examine the leucine trigger hypothesis by comparing a range of supplemental protein and/or whole-food protein sources in young and older adults at rest and post-exercise.

Methods

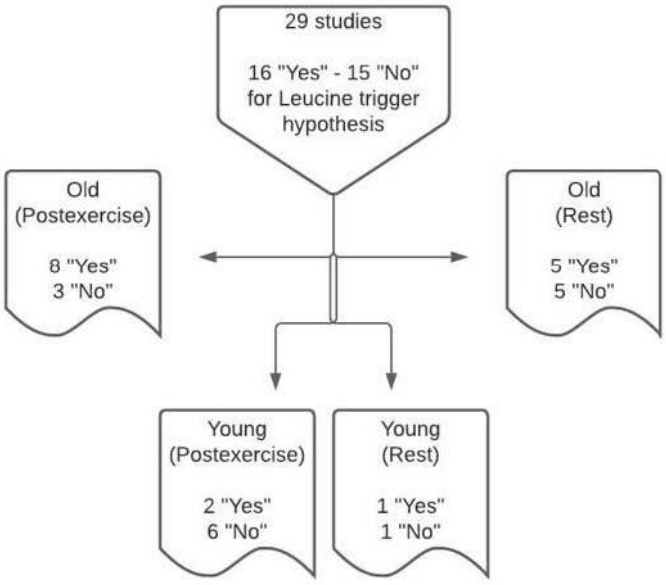
This qualitative systematic review extracted data from studies that combined measurements of postprandial blood leucine concentrations and rates of MPS following ingested protein at rest and following exercise in young and older adults. Data relating to blood leucine concentration profiles and postprandial MPS rates were extracted from all studies and reported as providing sufficient or insufficient evidence for the leucine trigger hypothesis.

Results

Overall, 16 of the 29 eligible studies provided sufficient evidence to support the leucine trigger hypothesis for explaining divergent postprandial rates of MPS in response to different ingested protein sources. Of these 16 studies, 13 were conducted in older adults (8 of which conducted measurements post-exercise) and 14 studies included the administration of isolated proteins.

Conclusions

This systematic review underscores the merits of the leucine trigger hypothesis for the explanation of the regulation of MPS. However, our data indicate that the leucine trigger hypothesis confers most application in regulating the postprandial response of MPS to ingested proteins in older adults. Consistent with our hypothesis, we provide data to support the idea that the leucine trigger hypothesis is more relevant within the context of ingesting isolated protein sources rather than protein-rich whole foods. Future mechanistic studies are warranted to understand the complex series of modulatory factors beyond blood leucine concentration profiles within a food matrix that regulate postprandial rates of MPS.


29 studies
16 "Yes" - 15 "No"
for Leucine trigger
hypothesis
Old
(Postexercise)
8 "Yes"
3 "No"
Old
(Rest)
5 "Yes"
5 "No"
Young
(Postexercise)
2 "Yes"
6 "No"
Young
(Rest)
1 "Yes"
1 "No"

Reference 1 :- West, D.W.D., Burd, N.A., Coffey, V.G., Baker, S.K., Burke, L.M., Hawley, J.A. (2011) Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise. Am. J. Clin. Nutr. 95, 795-803.

Reference 2 :- Bukhari, S.S.I., Phillips, B.E., Wilkinson, D.J., Limb, M.C., Rankin, D., Mitchell, W.K. (2015) Intake of low-dose leucine-rich essential amino acids stimulates muscle anabolism equivalently to bolus whey protein in older women at rest and after exercise. Am. J. Physiol. Metab. 308, 1056-1065.

Reference 3 :- Devries, M.C., Mcglory, C., Bolster, D.R., Kamil, A., Rahn, M., Harkness, L. (2018) Protein leucine content is a determinant of shorter- and longer-term muscle protein synthetic responses at rest and following resistance exercise in healthy older women: a randomized , controlled trial. Am. J. Clin. Nutr. 107(2), 217-226.

Reference 4 :- Devries, M.C., McGlory, C., Bolster, D.R., Kamil, A., Rahn, M., Harkness, L. (2018) Leucine, Not Total Protein, Content of a Supplement Is the Primary Determinant of Muscle Protein Anabolic Responses in Healthy Older Women. J. Nutr. 148(7), 1088-1095.

Reference 5 :- Wilkinson, D.J., Bukhari, S.S.I., Phillips, B.E., Limb, M.C., Cegielski, J., Brook, M.S. (2017) Effects of leucine-enriched essential amino acid and whey protein bolus dosing upon skeletal muscle protein synthesis at rest and after exercise in older women. Clin. Nutr. 37(6), 2011-2021.

PC44

Alternative Substrates in the Critically Ill Subject (ASICS): A feasibility study and Metabolomic Analysis

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INTRODUCTION

Ketogenic feeding (KF) may have health-related benefits for healthy individuals and does not appear to cause negative effects on moderate-to-vigorous intensity exercise. It may also be relevant to muscle wasting and recovery from injury in sports science, and in increasing muscle performance under physiological stress. Critically ill patients represent an extreme phenotype of metabolic and physiological stress, and lose muscle mass rapidly. We performed a randomised controlled feasibility study to see if Intensive Care Unit (ICU) patients can be recruited into a study of enteral KF; if feed can be prepared, administered, and tolerated; and if it raises plasma and urine ketone body levels. Metabolomic analysis of plasma and urine samples sought to identify changes in the nutritional metabolic pathways during ketogenic versus standard feeding.

METHODS

Patients recruited ≥ 48hrs after ICU admission in two UK ICUs; randomised to 10d KF or standard feed (SF) enterally. Inclusion: (i) > 18yrs old (ii) prescribed enteral feed (iii) likely to have mechanical ventilation > 48hrs, to be on ICU ≥ 5d, surviving ≥ 10d (iv) multi-organ failure (SOFA score > 2 in > 2 areas). ClinicalTrials.gov NCT04101071[1]. Data were collected for feasibility of recruitment, retention (receipt of 10d randomised feed and those on ICU ≥ 5d if < 10d feed); Adverse Events (AEs); blood glucose levels; urine and plasma ketone body levels. Samples were analysed via untargeted metabolomics using ultra-high performance liquid chromatography (UHPLC) operating dual phase HILIC and C18 RP separation coupled to a high resolution mass spectrometer, with data acquired in both positive and negative ionisation modes. Metabolomics data were assessed for differential metabolite signatures to provide additional biological insight into the effects of the intervention.

RESULTS

Of 286 patients screened, 29 were recruited and 24 were retained (12/14: SF; 12/15 KF). It was feasible but labour-intensive to calculate and prepare feed constituents to meet patients' daily nutritional needs, and for bedside nurses to administer KF. No related or unexpected serious AEs were found. AE rates for episodes of daily vomiting and high gastric residual volume (GRV; > 350mls) were similar in SF and KF arms; episodes of diarrhoea (3d in a row) were more prevalent with KF than SF (76.92% vs. 52.33%, respectively). Daily blood glucose concentration remained ≥ 3.9mmol/L in all KF patients but was ≥ 10mmol/L in a greater proportion of SF than KF patients (57.48% vs. 26.85%, respectively). KF was associated with mild plasma ketosis (up to 2.7mmol/L) and a greater urinary ketosis (up to 8mmol/L). Metabolomics data highlighted key groups of metabolites changing due to the intervention.

CONCLUSIONS

Enteral KF in ICU patients is safe, tolerated and does induce ketone body production, although the availability of ready-made feed would improve feasibility. Additional insights provided via the application of unbiased approaches, such as metabolomics, could help yield significant biological insight for novel interventional nutritional research in the future.

Reference 1 :- [1] Langan, A et al. (2020). Intensive Care Med. Exp. 8(S2):43.

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PC45

Changes in aerobic fitness during 6 days of Mountain Walking

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Mountain walking (trekking) is a popular activity and is associated with large increases in energy expenditure (Ainslie et al., 2002 *J. Appl. Physiol.* 93:714-723) similarly trekking has been identified as having cardiovascular health benefits (Kang, 2014 *J. Ex. Rehab*. 10:225-229). The aim of the present study was to investigate any physiological changes associated with a 6-day mountain walk. Over a 12-year period 134 male (age, 21.0±1.4 years; height 1.80±0.08 m; body mass 78.7±11.3 kg) and 124 female undergraduates (age, 20.8±1.6 years; height 1.67±0.06 m; body mass 63.5±10.3 kg) participated in a 6-day mountain trek. Height was measured using a wall-mounted stadiometer (Seca LS225) in minimal clothing and bare feet. Body mass and composition were measured using a free-standing body composition segmental analyser (Tanita, BC-418). The initial gradient for the submaximum treadmill test was 0%, for 4 minutes. At the end of four minutes gradient increased by 4% (to 4, 8, 12, and 16% respectively) at constant speed 1.5 m s^{-1} (3.3 mph). After a 30-min rest period, the maximal treadmill test took place (1.5 m s^{-1} with gradient increasing 2% very minute from 0 to 20%; thereafter the speed of the treadmill increased 0.13 m s^{-1} (0.3 mph) every minute until volitional exhaustion). During submaximal and maximal exercise oxygen uptake (VO_2) was measured using a breath-by-breath system (Cortex Metalyzer) and heart rate was measured by Polar telemetry. The same measures were made at the same time of day after trek completion. The mountain walk was part of an optional module which had received approval from Oxford Brookes University Ethics Committee (UK). Participants were treated in accordance with the principles laid down in the Declaration of Helsinki (1986). Two way mixed ANOVAs were conducted using SPSS (Version 27) followed by a *post hoc* Bonferroni correction to investigate the influence of sex on submaximal and maximal measures during mountain walking. Differences between men and women were analysed

using an independent samples t-test with an alpha set at $p<0.05$. Post-trek body mass was lower than pre-trek in both men 78.7±11.3 vs. 77.6±10.9 and women 63.5±10.3 vs. 62.6±9.5 kg. There was also a significant reduction in body fat 14.3±5.1 vs. 12.9±4.8%, men and 26.5±7.0 vs. 25.5±6.7%, women ($p<0.001$). Heart rate was significantly lowered in men (mean: 128±15 vs. 119±12) and women (mean: 142±6 vs. 137±10 b min^{-1}) at the same treadmill gradients after the trek. Oxygen pulse was significantly higher after the 6-day mountain walk 0.179±0.023 (pre-) vs. 0.185±0.026 ml kg^{-1} b^{-1} (post-) (mean for all treadmill gradients). The ventilatory equivalent for oxygen (V_E/VO_2) was significantly higher after the 6-day mountain walk 22.9±4.2 (pre-) vs. 23.8±4.2 (post-) (mean for all treadmill gradients) ($p<0.001$). There was a significant, although modest, increase in the maximal aerobic fitness (VO_2max values) for men (2.7%) and women (2.9%). The present study suggests that breathing efficiency and oxygen delivery during submaximum exercise was improved together with a lowered heart rate and favourable changes in body composition and an enhanced maximal aerobic fitness as a result of a 6-day mountain walk.

Reference 1 :- Ainslie, P. N., Campbell, T., Frayn, K. N., Humphreys, S. M., Maclaren, D. P. M., Reilly, T. and Westerterp, K. R. (2002) Energy balance, metabolism, hydration, and performance

during strenuous hill walking: the effect of age *J Appl Physiol* 93: 714–723, doi: 10.1152/japplphysiol.01249.2001.

Reference 2 :- Kang, S-J. (2014) Trekking exercise promotes cardiovascular health and fitness benefits in older obese women *Journal of Exercise Rehabilitation*,10 (4): 225-229. doi.org/10.12965/jer.140136.

Acknowledgements :- N/A

PC46

Are handball wingers resistant to birthdate discrimination? The relative age effect in Polish handball.

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The aims of this study were to a) identify the birthdate distribution in Polish handball teams from 2007 to 2020, b) evaluate differences between sex, level and position on the court and c) analyse the relations between points per game and the player's birth quarter. We hypothesized that there is discrimination between players in contact sports based on birth quarter, as well as throwing efficiency during the game. The study involved 94,617 male and 60,202 female handball players registered in the official database of the Polish Handball Association between 2007 and 2020 and their corresponding birthdates in the Polish population between 1994 and 2003. The following levels were categorised: PRO, SEMI-PRO (S-P), AMATEUR (AM) , CENTRAL YOUTH (CY) and REGIONAL YOUTH (RY). The differences between the observed distributions were analyzed in relation to specific variables: the position of the player on the court and the category of competition. The quarterly

differences between points per game according to the on-court position were analysed. Surprisingly, no relative age effect (RAE) was identified in the wingers position in handball, regardless of sex or competition level. Additionally, an RAE was identified regardless of sex in most of the seasons analysed (apart from the 2009-2010 and 2010-2011 seasons for female). Subjects representing certain positions born in the last quarter of the calendar year also scored fewer points per game than other respective players. The strongest disproportion in the relationship between the birth quarter and the points per game was observed in the group of playmakers (for female: Q1>Q2>Q3>Q4; for male: Q1>Q2>Q3>Q4 for $p<0.01$ to $p<0.001$ between quarters). An almost complete absence of the RAE among wingers is the unique finding of this study, one that had not been observed before

Table 2: Distibution of birth quater in Polish Handballl in 2007-2020.

SEASON	Q1 (%)	Q2 (%)	Q3 (%)	Q4 (%)	TOTAL	x2	p	df	V	Effect
				MALE						
2007-08	251 (36,06)	179 (25,72)	154 (22,13)	112 (16,09)	696,00	23,2800	<,0001	3	0,13	small
2008-09	245 (34,85)	184 (26,17)	149 (21,19)	125 (17,78)	703,00	18,5300	0,0003	3	0,11	small
2009-10	223 (32,27)	179 (25,9)	165 (23,88)	124 (17,95)	691,00	10,3000	0,0162	3	0,09	small
2010-11	228 (32,9)	186 (26,84)	164 (23,67)	115 (16,59)	693,00	14,8200	0,0020	3	0,10	small
2011-12	1553 (32,33)	1344 (27,98)	1147 (23,88)	760 (15,82)	4804,00	111,1300	<,0001	3	0,11	small
2012-13	1757 (31,92)	1568 (28,48)	1261 (22,91)	919 (16,69)	5505,00	113,7900	<,0001	3	0,10	small
2013-14	3913 (31,64)	3394 (27,44)	2974 (24,05)	2087 (16,87)	12368,00	214,4600	<,0001	3	0,09	small
2014-15	3247 (31,19)	2773 (26,64)	2609 (25,06)	1781 (17,11)	10410,00	152,73	<,0001	3	0,09	small
2015-16	3264 (31,53)	2761 (26,67)	2520 (24,34)	1808 (17,46)	10353,00	151,82	<,0001	3	0,09	small
2016-17	3152 (31,72)	2599 (26,15)	2460 (24,76)	1726 (17,37)	9937,00	148,54	<,0001	3	0,09	small
2017-18	3979 (30,81)	3373 (26,12)	3232 (25,03)	2329 (18,04)	12913,00	144,71	<,0001	3	0,07	small
2018-19	4024 (30,5)	3484 (26,41)	3281 (24,87)	2404 (18,22)	13193,00	136,42	<,0001	3	0,07	small
2019-20	3618 (29,29)	3258 (26,38)	3100 (25,1)	2375 (19,23)	12351,00	77,00	<,0001	3	0,06	small
				FEMALE						
2007-08	147 (32,45)	109 (24,06)	105 (23,18)	92 (20,31)	453,00	5,68	0,1283	3	0,08	small
2008-09	185 (32,86)	142 (25,22)	133 (23,62)	103 (18,29)	563,00	9,1	0,028	3	0,09	small
2009-10	158 (29,26)	148 (27,41)	124 (22,96)	110 (20,37)	540,00	3,98	0,2636	3	n,s,	-
2010-11	157 (29,29)	138 (25,75)	138 (25,75)	103 (19,22)	536,00	3,32	0,3449	3	n,s,	-
2011-12	853 (31,18)	753 (27,52)	668 (24,42)	462 (16,89)	2736,00	44,47	<,0001	3	0,09	small
2012-13	891 (30,88)	806 (27,94)	680 (23,57)	508 (17,61)	2885,00	42,75	<,0001	3	0,09	small
2013-14	2345 (31,92)	1954 (26,6)	1755 (23,89)	1293 (17,6)	7347,00	114,87	<,0001	3	0,09	small
2014-15	1860 (30,5)	1608 (26,36)	1493 (24,48)	1138 (18,66)	6099,00	59,13	<,0001	3	0,07	small
2015-16	1782 (31,09)	1478 (25,79)	1456 (25,41)	1015 (17,71)	5731,00	71,51	<,0001	3	0,08	small
2016-17	1748 (29,6)	1543 (26,13)	1511 (25,59)	1103 (18,68)	5905,00	45,13	<,0001	3	0,06	small
2017-18	2630 (29,82)	2295 (26,02)	2187 (24,8)	1707 (19,36)	8819,00	61,24	<,0001	3	0,06	small
2018-19	2897 (30,11)	2498 (25,97)	2399 (24,94)	1826 (18,98)	9620,00	76,86	<,0001	3	0,06	small
2019-20	2666 (29,73)	2320 (25,87)	2264 (25,25)	1718 (19,16)	8968,00	61,89	<,0001	3	0,06	small

Legend: Q – qauter of the yerar, x2 – chi-square , p – test value, df- deegre of freedom, V- Cramer`s V

Table 4: Distibution of birth quater by players postion and level in Polish Handballl in 2007-2020.

			Q1	Q2	Q3	Q4	TOTAL	χ 2	p	df	V	Effect
PIVOTS	PRO (%)	M	556 (28,94)	571 (29,72)	439 (22,85)	335 (18,48)	1921	28,39	<.0001	3	0.086	small
		F	400 (34,39)	271 (23,30)	315 (27,09)	177 (15,22)	1163	34,68	<.0001	3	0.122	small
	SEMI-PRO (%)	M	524 (36,64)	329 (23,01)	330 (23,08)	247 (17,27)	1430	44,97	<.0001	3	0,125	small
		F	156 (29,43)	158 (29,81)	137 (25,85)	79 (14,91)	530	12,6	0.0069	3	0,107	small
	AMAUTER (%)	M	187 (33,94)	142 (25,78)	135 (24,50)	87 (15,78)	551	14.19	0.0027	3	0.113	small
		F	*LESS THEN <30 cases*									
	CENTRAL YOUTH (%)	M	466 (33,84)	391 (28,40)	318 (23,09)	202 (14,67)	1377	45.84	<.0001		0.13	small
		F	337 (31,03)	281 (25,87)	292 (26,89)	176 (16,21)	1086	18.74	0.0003	3	0.093	small
	REGIONAL YOUTH (%)	M	2240 (32,24)	1860 (26,77)	1666 (23,98)	1182 (17,01)	6948	124.28	<.0001	3	0.094	small
		F	1155 (28,53)	1077 (26,60)	1092 (26,97)	725 (17,91)	4049	33.43	<.0001	3	0.064	small
WINGERS										3		
	PRO (%)	M	925 (28,29)	843 (25,78)	775 (23,70)	727 (22,23)	3270	9,18	0.027	3	0.037	negligible
		F	646 (27,71)	671 (28,79)	538 (23,08)	476 (20,42)	2331	15,06	0.0018	3	0.057	negligible
	SEMI-PRO (%)	M	673 (28,05)	622 (25,93)	605 (25,22)	499 (20,08)	2399	5,92	0,1156	3	0,035	n.s.
		F	279 (26,62)	255 (24,33)	286 (27,29)	228 (21,76)	1048	1,07	0,7843	3	0,026	
	AMAUTER (%)	M	317 (30,19)	270 (25,71)	283 (26,95)	180 (17,14)	1050	13	0,0046	3	0,079	small
		F	LESS THEN <50 cases							3		
	CENTRAL YOUTH (%)	M	632 (25,86)	648 (26,51)	679 (27,78)	485 (19,84)	2444	7	0,0719	3	0,038	n.s.
		F	646 (28,11)	559 (24,33)	598 (26,02)	495 (21,54)	2298	4,64	0,2001	3	0,032	n.s.
	REGIONAL YOUTH (%)	M	3800 (26,21)	3639 (25,1)	3969 (27,38)	3088 (21,30)	14496	14,92	0,0019	3	0,027	n.s.
		F	2656 (27,31)	2428 (24,96)	2503 (25,73)	2140 (22,00)	9727			3		
PLAYMAKERS										3		
	PRO (%)	M	2022 (34,58)	1565 (26,77)	1358 (23,23)	902 (15,43)	5847	178,17	<.0001	3	0.123	small
		F	1258 (31,70)	989 (24,92)	985 (24,82)	736 (18,55)	3968	47,15	<.0001	3	0,077	small
		M	1356 (32,65)	1147 (27,62)	966 (23,26)	684 (16,47)	4153	90,98	<.0001	3	0,105	small

Table 5. Points per game according to quater of birth and positon on the court in Polish Handball in 2007-2020.

			N	Q1 (SD)	Q2 (SD)	Q3 (SD)	Q4 (SD)	Q1-Q4	Post hoc
PIVOTS	PRO	M	1921	24,01 (1,26)	25,68 (1,26)	25,18 (1,47)	24,82 (1,59)	-0,82	Q1=Q2=Q3=Q4
		F	1163	20,07 (1,32)	22,14 (1,62)	26,78 (1,92)	28,75 (2,86)	-8,75	Q1=Q2* <Q3*<Q4
	SEMI-PRO	M	1430	18,91 (1,02)	19,64 (1,27)	20,77 (1,43)	19,91 (1,72)	-1	Q1=Q2=Q3=Q4
		F	530	12,89 (1,3)	12,32 (1,23)	13,09 (1,29)	12,42 (1,52)	0,45	Q1=Q2=Q3=Q4
	AMAUTER	M	551	12,03 (1,17)	11,42 (1,3)	10,3 (1,12)	10,53 (1,21)	1,5	Q1=Q2=Q3=Q4
		F	not included						
	CENTRAL YOUTH	M	1377	8,74 (0,56)	7,75 (0,53)	7,16 (0,52)	7,79 (0,71)	0,95	Q1=Q2=Q3=Q4
		F	1086	7,10 (0,53)	8,14 (0,72)	6,78 (0,57)	7,72 (0,85)	-0,62	Q1=Q2=Q3=Q4
	REGIONAL YOUTH	M	6948	15,67 (0,44)	14,36 (0,45)	14,55 (0,48)	12,97 (0,56)	2,7	Q1=Q2=Q3>Q4*
		F	4049	11,51 (0,56)	10,74 (0,52)	11,11 (0,49)	10,18 (0,57)	1,33	Q1=Q2=Q3=Q4
WINGERS	PRO	M	3270	36,49 (1,27)	37,28 (1,43)	37,15 (1,51)	37,86 (1,43)	-1,37	Q1=Q2=Q3=Q4
		F	2331	30,91 (1,54)	25,70 (1,17)	27,72 (1,54)	27,60 (1,48)	3,31	Q1=Q2=Q3>Q4*
	SEMI-PRO	M	2399	30,41 (1,36)	31,61 (1,53)	31,46 (1,37)	29,9 (1,44)	0,51	Q1=Q2=Q3=Q4
		F	1048	19,04 (23,89)	19,96 (1,53)	19,08 (1,32)	15,98 (1,34)	3,06	Q1=Q2=Q3>Q4*
	AMAUTER	M	1050	17,28 (1,19)	15,81 (1,25)	17,52 (1,27)	16,67 (1,56)	0,61	Q1=Q2=Q3=Q4
		F	not included						
	CENTRAL YOUTH	M	2444	10,99 (0,61)	9,23 (0,49)	11,05 (0,53)	9,54 (0,58)	1,54	Q1=Q2=Q3=Q4
		F	2298	11,15 (0,65)	9,4 (0,55)	9,39 (0,55)	8,36 (0,56)	2,79	Q1=Q2=Q3>Q4*
	REGIONAL YOUTH	M	14496	15,91 (0,39)	15,26 (0,36)	15,67 (0,36)	12,86 (0,36)	3,05	Q1=Q2=Q3>Q4*
		F	9727	14,41 (0,450	12,04 (0,42)	11,6 (0,38)	12,24 (0,46)	2,17	Q1>Q2***=Q3=Q4
PLAYMAKERS	PRO	M	5847	37,65 (0,92)	35,65 (0,92)	34,68 (1,03)	36,86 (1,29)	0,79	Q1=Q2=Q3=Q4
		F	3968	34,96 (1,21)	29,76 (1,22)	35,46 (1,33)	33,63 (1,47)	1,06	Q1>*Q2*<Q3,Q4
	SEMI-PRO	M	4153	31,24 (0,96)	31,79 (1,06)	33,04 (1,26)	35,7 (1,46)	-4,46	Q1=Q2=Q3=Q4
		F	1836	22,68 (0,99)	20,64 (1)	18,71 (1,07)	22,11 (1,43)	0,57	Q1=Q2=Q3=Q4
	AMAUTER	M	1674	17,6 (0,88)	17,32 (1,04)	18,56 (1,04)	21,04 (1,5)	-3,44	Q1=Q2=Q3=Q4
		F	not included						
		M	4211	14,99 (0,44)	14,18 (0,51)	13,22 (0,48)	14,38 (0,71)	0,61	Q1=Q2=Q3=Q4

	SEMI-PRO (%)	F	605 (32,95)	490 (26,69)	414 (22,55)	327 (17,81)	1836	35,06	<.0001	3	0,097	small
	AMAUTER (%)	M	534 (31,90)	475 (28,37)	389 (23,24)	276 (16,49)	1674	35	<.0001	3	0,102	small
		F	LESS THEN <130 cases				128			3	-	
	CENTRAL YOUTH (%)	M	1522 (36,14)	1134 (23,93)	973 (23,11)	582 (13,82)	4211	181,97	<.0001	3	0,147	small
		F	1191 (34,51)	893 (25,88)	809 (23,44)	558 (16,17)	3451	93,55	<.0001	3	0,116	small
	REGIONAL YOUTH (%)	M	7889 (34,33)	6217 (27,06)	5448 (23,71)	3425 (14,9)	22979	728,68	<.0001	3	0,126	small
		F	5039 (32,48)	4213 (27,15)	3669 (23,65)	2594 (16,72)	15515	312,06	<.0001	3	0,1	small
GOALKEEPERS	PRO (%)	M	612 (28,28)	546 (25,23)	547 (25,28)	459 (21,21)	2164	5,02	0,1703		0,034	n.s.
		F	452 (30,81)	362 (24,68)	253 (24,06)	300 (20,45)	1367	33,29	<.0001		0,109	small
	SEMI-PRO (%)	M	417 (26,83)	472 (30,37)	373 (24)	292 (18,79)	1554	14,4	0,0024		0,068	small
		F	182 (29,98)	143 (23,56)	143 (23,56)	139 (22,90)	607	7,98	0,0464		0,081	small
	AMAUTER (%)	M	179 (29,68)	176 (29,19)	135 (22,39)	113 (18,73)	603	7,84	0,0494		0,081	small
		F	*LESS THEN <50 cases*									
	CENTRAL YOUTH (%)	M	439 (30,07)	402 (27,07)	351 (24,04)	268 (18,36)	1460	15,18	0,0017		0,072	small
		F	362 (30,55)	325 (27,43)	290 (24,47)	208 (17,55)	1185	15,84	0,0012		0,082	small
	REGIONAL YOUTH (%)	M	2088 (30,27)	1937 (28,08)	1642 (23,81)	1230 (17,83)	6897	87,68	<.0001		0,08	small
		F	1248 (29,40)	1102 (29,40)	1164 (27,42)	731 (17,22)	4245	47,72	<.0001		0,075	small
MORE THAN ONE	REGIONAL YOUTH (%)	M	380 (33,13)	287 (25,02)	311 (27,11)	169 (14,73)	1147	32,15	<.0001		0,118	small
		F	187 (29,97)	134 (21,47)	173 (27,72)	130 (20,83)	624	5,16	0,1604		0,06	n.s.`
UNDEFIDED	REGIONAL YOUTH (%)	M	1392 (27,55)	1353 (26,78)	1267 (25,08)	1040 (20,59)	5052	12,89	0,0049		0,036	negligible
		F	1302 (30,26)	1114 (25,89)	1092 (25,38)	795 (18,48)	4303	39,17	<.0001		0,067	small

Legend: Q – qauter of the yerar, x2 – chi-square , p – test value, df- deegre of freedom, V- Cramer`s V

	CENTRAL YOUTH	F	3451	14,79 (0,54)	14,44 (0,6)	13,78 (0,56)	11,5 (0,570	3,09	Q1=Q2=Q3>Q4*
	REGIONAL YOUTH (%	M	22979	24,91 (0,34)	23,26 (0,37)	22,04 (0,37)	19,62 (0,44)	5,29	Q1>Q2*>Q3>Q4***#&
		F	15515	20,2 (0,38)	18,71 (0,4)	19,39 (0,44)	16,39 (0,48)	3,81	Q1>Q2*>Q3***#>Q4***#&
MORE THEN ONE	REGIONAL YOUTH	M	1147	22,35 (1,35)	17,77 (1,35)	18,26 (1,33)	19,7 (2,41)	2,65	Q1>Q2*=Q3=Q4
		F	624	13,08 (1,34)	16,25 (1,91)	15,24 (1,75)	17,08 (1,99)	-4	Q1=Q2=Q3=Q4
UNDEFIDED	REGIONAL YOUTH	M	5052	10,96 (0,51)	9,44 (0,42)	8,96 (0,47)	7,94 (0,45)	3,02	Q1=Q2>Q3*>Q4*
		F	4303	5,80 (0,35)	6,11 (0,36)	6,33 (0,36)	4,72 (0,39)	1,08	Q1=Q2=Q3=Q4

Legend: Q- quater of year, Q1-Q4 -diffrence beetwen first and four quater of the year, *, **, *** - p<0.01, p<0.001, p<0.0001 for Q1-Q4, #- p<0.01 for Q2-Q4, & -p<0.01 for Q3-Q4.

Reference 1 :- ieseler G, Hermassi S, Hoffmeyer B, Schulze S, Irlenbusch L, Bartels T, et al. Differences in 557 anthropometric characteristics in relation to throwing velocity and competitive level in professional 558 male team handball: a tool for talent profiling. J Sports Med Phys Fitness. 2017;57(7-8):985-92.

Reference 2 :- Rubia A, Bjorndal CT, Sanchez-Molina J, Yague JM, Calvo JL, Maroto-Izquierdo S. The 536 relationship between the relative age effect and performance among athletes in World Handball 537 Championships. PLoS One. 2020;15(3):e0230133

Reference 3 :- Sanchez-Rodiguez C, Sancho AY, Quintana MS, Rivilla-Garcia J. The relative age effect in 531 the Spanish elite male handball. E-Balonmano Com. 2012;8(3):181-90

Reference 4 :- Bjorndal CT, Luteberget LS, Till K, Holm S. The relative age effect in selection to 498 international team matches in Norwegian handball. PLoS One. 2018;13(12):e0209288.

Reference 5 :- Karcher C, Buchheit M. On-Court Demands of Elite Handball, with Special Reference to 496 Playing Positions. Sports Medicine. 2014;44(6):797-814.

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TMS-Derived Corticomotor Inhibition is Reliable to Evaluate Brain Health in Soccer Players

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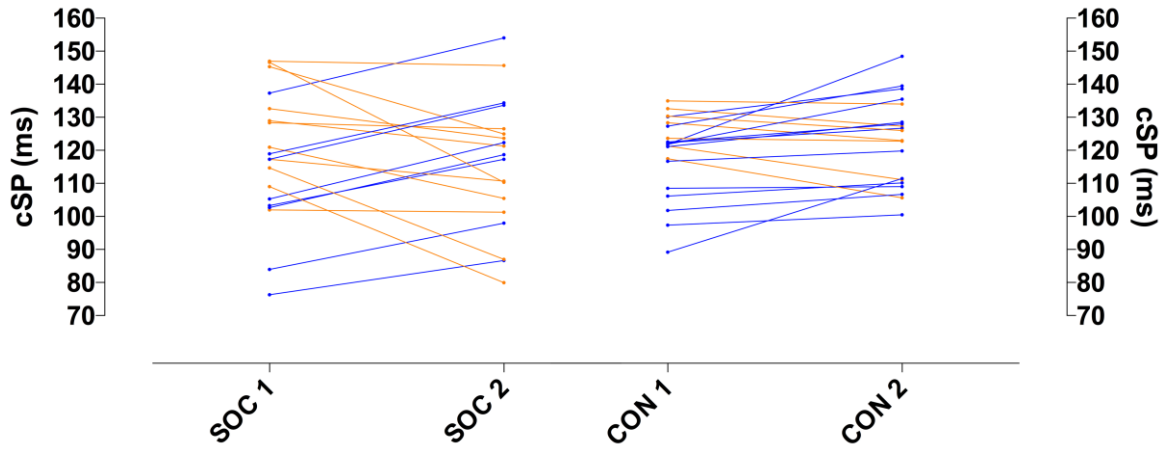
Introduction: Concussive and subconcussive head impacts in soccer can negatively affect player availability. Assessment of concussive and subconcussive impacts in field settings is limited to indirect methods such as balance as a proxy for motor control, or subjective methods involving cognitive function. Changes to corticospinal excitability and corticomotor inhibition in relation to subconcussive/concussive impacts can be measured using transcranial magnetic stimulation (TMS) (Di Virgilio et al, 2016; 2019; Ntikas et al, 2021). This study will inform practitioners and researchers regarding the reliability of TMS to monitor concussion and subconcussion when assessing soccer players.

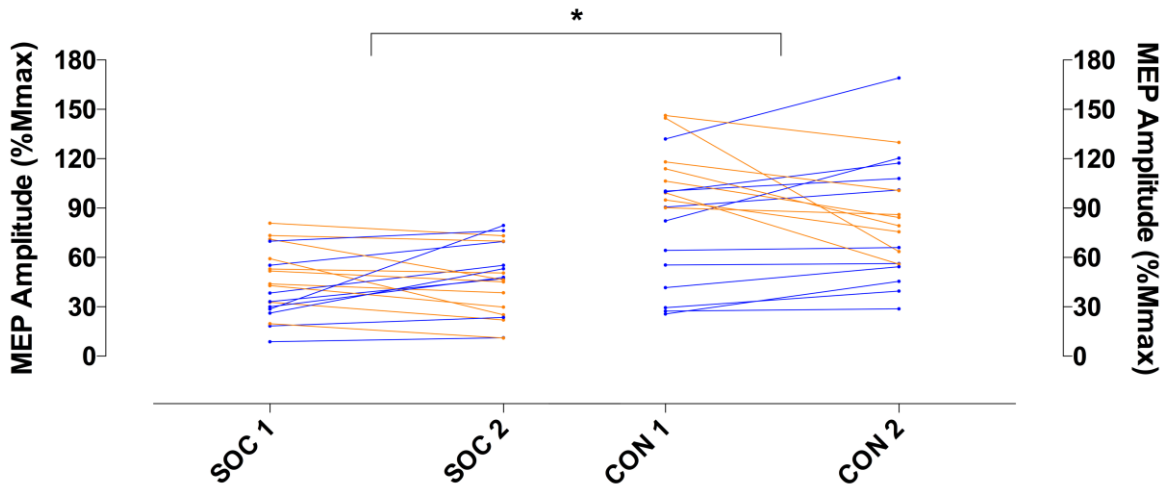
Methods: Nineteen soccer players (14 Males, 5 Females, Age = 22 ± 3 years, body mass = 72.9 ± 8.3 kg, height = 175.4 ± 10.2 cm) and twenty healthy active controls (16 Males, 4 Females, Age = 24.3 ± 4 years, body mass = 76.4 ± 13.6 kg, Height = 174.9 ± 9.8 cm) completed two identical experimental sessions spaced 7-14 days apart, where their corticospinal excitability and corticomotor inhibition were assessed. Surface electromyography (EMG) electrodes were affixed to the rectus femoris of the

dominant leg. Participants performed three 5 second knee extension maximum isometric voluntary contractions (MVC) at a 60° joint angle, with a 60 second rest period between contractions. A single pulse transcranial magnetic stimulation was delivered over the primary motor cortex (M1) during each MVC to measure corticomotor inhibition, which was quantified as the EMG silent period from TMS stimulus artefact to resumption of normal EMG activity. To assess corticospinal excitability, participants performed a 20% MVC contraction, while 20 single pulse stimulations were delivered over M1. Corticomotor excitation was measured as the average of the motor evoked potentials' peak-to-peak amplitudes and expressed relative to the maximal excitability of the muscle (Mmax). Reliability of these measures within population and inter-rater (n = 2) was measured using a two-way random model of intraclass correlation coefficient, and coefficient of variation. Group differences were assessed using a 2-way ANOVA.

Results: Good inter-day reliability was evident for corticomotor inhibition in soccer players (ICC = 0.61) and controls (ICC = 0.70) and corticospinal excitability in soccer players (ICC = 0.59) and controls (ICC = 0.70). Corticomotor inhibition also showed excellent inter-rater reliability (ICC = 0.87). Corticomotor inhibition showed lower coefficient of variation than excitability in soccer players (Inhibition=15.2%; Excitability=41.6%) and in controls (inhibition = 9.7%; Excitability = 39.5%). No group differences between soccer players and healthy active individuals were found on the corticomotor inhibition value ($p > 0.05$), but levels of corticospinal excitability were significantly lower in soccer players (45.1±20.8 vs 85.4±6.2%Mmax, $p < 0.001$).

Discussion: We show that corticomotor inhibition and corticospinal excitability are stable and maintain good degrees of reliability when assessed over different days and between investigators in soccer players, despite their routine exposure to head impacts. However, based on intra-group reliability and group differences of the levels of excitability, we conclude that corticomotor inhibition is best suited for the evaluation of outcomes and monitoring of recovery following head impacts in contact sport.





Reference 1 :- Di Virgilio, T.G., Hunter, A., Wilson, L., Stewart, W., Goodall, S., Howatson, G., Donaldson, D.I. and Ietswaart, M. (2016). Evidence for acute electrophysiological and cognitive changes following routine soccer heading. EBioMedicine, 13, pp.66-71.

Reference 2 :- Di Virgilio, T.G., Ietswaart M., Wilson, L., Donaldson, D.I. and Hunter, A.M. (2019) Understanding the Consequences of Repetitive Subconcussive Head Impacts in Sport: Brain Changes and Dampened Motor Control Are Seen After Boxing Practice. Frontiers in Human Neuroscience, 13:294.

Reference 3 :- Ntikas, M., Hunter, A.M., Gallagher, I.J. and Di Virgilio, T.G. (2021). Longer neurophysiological vs. clinical recovery following sport concussion. Frontiers in sports and active living, 3.

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Bisphosphonates as a sarcopenia countermeasure in *Caenorhabditis elegans*.

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Background: Age-related muscle decline (sarcopenia) is a major, unresolved societal health challenge. Repurposing existing prescription drugs represents a highly cost- and time-efficient approach to sarcopenia treatment discovery. For decades, bisphosphonates have been prescribed for osteoporosis treatment, however their potential as a sarcopenic therapy remain unclear. We

examined the efficacy of bisphosphonates for preserving muscle health across the entire lifespan, using a *C. elegans* model of ageing muscle health.

Methods: Using zoledronic acid (ZA) as a potent, third-generation bisphosphonate, we assessed life/healthspan using a microfluidic device ('NemaLife'). Wild-type *C. elegans* were treated with 100 nM – 500 µM ZA from larval stages (~300 animals per condition) and analysed for longevity and healthspan (total animal movement across the lifecourse). Muscle architecture was examined in response to ZA using transgenic animals expressing green fluorescent protein reporters for myofibres and mitochondria, until wild-type sarcopenic onset (~day 4-6 adulthood; ~60 animals / 300 muscle cells per condition/ time point). Pharmaco-genetic life/healthspan experiments of ZA + RNAi knockdown of muscle-bone crosstalk, and ZA-responsive genes were performed to establish potential mechanisms of ZA-mediated healthspan extension (~140 animals per condition/ time point).

Results: Higher ZA concentrations (100 and 500 µM) were lethal, and 10 µM had no effect on lifespan but reduced healthspan (P<0.01). Conversely, lower ZA doses of 100 nM, 500 nM and 1µM extended lifespan (median lifespan = 13-16 *vs.* 9 days post-adulthood in untreated controls, all P<0.0001) and preserved healthspan (AUC for movement rate across lifespan = 784-878 vs. 678 in untreated controls, all P<0.05). ZA (1 µM) also preserved muscle sarcomeric structure at day 6 post-adulthood (18% loss of sarcomeric arrays *vs.* 58% loss in untreated controls, P<0.05). Age-related mitochondrial fragmentation was not preserved by ZA (P=0.177). Of the five mechanistic candidates examined, *agxt-2*/ β-aminoisobutyric acid (BAIBA) was required for ZA-mediated healthspan preservation. Whilst healthspan extension was retained with ZA + *sir-2.2*/ *sir-2.3* (mitochondrial sirtuins) or *igdb-1* (FDNC5) knockdown, healthspan was not synergistically improved, putatively implicating ZA-induced healthspan acts *via* downregulation of these targets.

Conclusion: Low doses of bisphosphonates significantly improve lifespan, healthspan and preserves muscle integrity across age. Bisphosphonates might, therefore, hold significant potential as an immediately exploitable sarcopenia therapeutic.

PC51

Exercise timing before and during COVID-19 social distancing: opportunities, barriers, preferences, and proximity to eating

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Background The time of day that exercise is performed may affect adherence and influence physiological outcomes, due to circadian rhythms and nutrition-exercise interactions. For example, exercise performed in the evening may be associated with reduced perception of effort (Maraki et

al., 2005) and may improve glycaemic control more than morning exercise (Savikj et al., 2019). Also, pre-exercise feeding is known to affect metabolism, with morning exercise performed before, compared to after a meal, shown to improve insulin sensitivity to a greater extent (Van Proeyen et al., 2010; Edinburgh et al., 2020). Before designing exercise interventions to improve health outcomes, we first need to understand when people choose to exercise and in what proximity from the previous meal. Therefore, this study surveyed exercise timing behaviours, preferences, and opportunities, to inform future exercise interventions to improve health. **Methods** An online survey was completed by 512 adults to assess typical times of day that exercise is performed and proximity of exercise to the previous meal. Due to the timing of release of this survey, responses to these questions were assessed for both before, and during COVID-19 social distancing measures. In addition, preference and opportunity-based determinants of exercise timing were assessed. Descriptive statistics and between-group comparisons were calculated, and associations between categorical variables were examined using Pearson's chi-squared analyses. **Results** The most common time to exercise was the early evening (40.4%) during the week and the morning (57.6%) during the weekend. During social distancing, morning was the most common exercise time during the week (32.0%) and weekend (49.2%). Exercise in the morning was more likely to occur following a period of extended (>5 h) fasting, whereas exercise later in the day occurred sooner following a prior meal (P<0.001). Most participants (52.3%) preferred to exercise during the morning, however, only 30.7% reported having opportunity to exercise at their preferred time. The greatest opportunity to exercise was the early evening (59.0%), and 'job/work commitments' (63.9%) was the most frequently reported barrier preventing exercise at preferred times. **Conclusions** The largest proportion of people perform exercise sessions in the early evening during the week. However, this trend is inverted towards morning exercise during the weekend, which more closely aligns with peoples' preferences. This indicates that weekday temporal restrictions, possibly resulting from full-time employment, is a primary factor governing exercise timing, with the post-work period clearly identified as the preferential time in which to exercise. As circadian rhythms and feeding status may impact the metabolic response to exercise at different times of the day, future studies should aim to explore the specific effects of evening exercise to maximise ecological validity.

Reference 1 :- Maraki M et al. (2005). Appetite 45, 272-278.

Reference 2 :- Savijk M et al. (2019). Diabetologia 62, 233-237.

Reference 3 :- Van Proeyen K et al. (2010). J Physiol 588, 4289-4302.

Reference 4 :- Edinburgh RM et al. (2019). J Clin Endocrinol Metab 105, 660-676.

PC52

The role of resistance exercise training for improving cardiorespiratory fitness in healthy older adults: a systematic review.

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Background:

The UK, as with most of the Western World, has an ageing population due to decades of improving life expectancy. However, this increase in lifespan has not been matched by an increase in healthy life expectancy ("health span"), and as such an increased number of people are living in poor health in later life. Declines in cardiorespiratory fitness (CRF) and muscle mass are both associated with advancing age and each of these declines are associated with worse health outcomes. Resistance exercise training (RET) has previously been shown to improve muscle mass and function in both young and older adults. However, if RET is also able to improve the CRF of older adults, as it has been shown to do in younger populations, it has the potential to improve multiple health outcomes in the expanding older population.

Methods:

This systematic review aimed to identify the role of RET for improving CRF in healthy (i.e., not disease-specific cohorts) older (>60 y) adults. A search across CINAHL, MEDLINE, EMBASE, and EMCARE databases was conducted using a PICO (population, intervention, comparison, outcome) protocol, with meta-analysis performed on eligible papers (randomised control trials only) to identify improvements in established CRF parameters (VO_2 peak, anaerobic threshold (AT), blood pressure and peak heart rate) following RET-only interventions. Search terms used included "healthy ageing", "resistance training", "weightlifting", "muscle strengthening", "aged", and "cardiorespiratory fitness", and only papers published in the English language were included.

Results:

Thirteen eligible studies were identified, the majority of which used weight machines or free-weights to provide resistance. Three studies used resistance bands and 1 study used water-based resistance. Meta-analysis revealed a significant improvement in VO_2 peak (MD 2.59 ml/kg/min; 95% CI 1.73 ml/kg/min to 3.45 ml/kg/min) and AT (MD 1.40 ml/kg/min; 95% CI 0.06 ml/kg/min to 2.74 ml/kg/min) when RET was compared to control (no intervention). Blood pressure (systolic: MD 2.32 mmHg; 95% CI -5.76 mmHg to 10.41 mmHg; diastolic: MD -0.38 mmHg; 95% CI -4.19 mmHg to 3.43 mmHg) and peak heart rate (MD 2.05 bpm; 95% CI 0.73 bpm to 3.36 bpm) were not altered by RET compared to control.

Discussion:

The predominant finding of this systematic review is a clear benefit of RET on both VO_2 peak and AT when compared to habitual activity controls. This review also highlights that despite exercise being well recognised as beneficial to human health, including for older adults, there is limited high-quality evidence for one of the most common forms of exercise; RET, to improve a known significant contributor to whole body health i.e., CRF. The main finding of this review adds to a growing body of

evidence supporting the implementation of RET in the older population for improving whole-body health across the life course.

Acknowledgements :-

Acknowledgements: The authors would like to thank Suzanne Toft, Clinical Librarian, Royal Derby Hospital for her assistance

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PC53

High intensity interval training attenuates the loss of proteostasis in obese insulin-resistant muscle.

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High-intensity interval training (HIIT) is an attractive training strategy for improving performance and protecting against chronic metabolic disease. Changes to muscle function are underpinned by dynamic proteome remodeling but little is known regarding how exercise alters the turnover of individual proteins in humans. We compared individual protein abundance and turnover rates in obese insulin resistant (OIR; n = 3) and healthy, trained (TR; n = 4) male participants (38 ± 8 years), and conducted longitudinal analysis of proteome changes in OIR after 10-weeks HIIT. Newly synthesised proteins were labelled by oral consumption of deuterium oxide (D_2O; 50 ml of 99.8 atom %) taken 4 times per day, for 14-days. Biopsies of vastus lateralis were performed before D_2O administration (day 0) and after 4, 9, and 14 days of labeling. The OIR group trained 3 times/ week for 10-weeks (4 x 60 s cycling at 100% maximum power output interspersed by 60 s recovery periods). Training volume was increased by 1 interval bi-week and the experimental protocol was repeated over the final 2 weeks of HIIT. Physiological measurements were taken > 72 h before and after HITT and D_2O incorporation and protein abundance data were quantified by liquid chromatography-tandem mass spectrometry. Baseline data were compared by between subject ANOVA and within-subject ANOVA was used to compare pre- vs post-HIIT responses. By design, the average BMI ($kg.m^{-2}$) of OIR (34.0 ± 5.8) was greater (P = 0.02) than TR (24.2 ± 2.4 $kg.m^{-2}$) participant, whereas peak oxygen consumption (VO_{2peak}; $ml.kg^{-1}.min^{-1}$) of OIR (26.1 ± 4.4) was significantly (P < 0.01) less than TR (45.5 ± 7.9) and OIR had significantly (P < 0.01) lower insulin sensitivity (Matsuda Index; OIR = 1.7 ± 0.6 vs TR = 5.7 ± 1.4). HIIT increased VO_{2peak} by 9 % (P = 0.25) and peak power

output by 14 % (P = 0.06) in OIR. Abundance and turnover rate data were quantified for 880 and 301 proteins, respectively, in all OIR and TR participants. In total 352 proteins exhibited significant (p < 0.05, false discovery rate < 5%) differences in abundance at baseline (Figure 1). OIR muscle was enriched with markers of cellular stress and protein unfolding, including small heat shock proteins (HSPB; 1, 2, 3, and 6) and members of the 90 kDa HSP family. Additionally, HSP72, and cytoprotective enzymes such as alcohol dehydrogenases (AK1A1, and ALDH2) exhibited significantly slower turnover rates in OIR. HIIT significantly (p < 0.05) altered the abundance of 53 proteins and increased the turnover rate of 20 proteins in OIR muscle. Four proteasome subunits that were more abundant in OIR muscle compared to TR at baseline, significantly decreased (p < 0.05) in abundance post-exercise. Significant (p < 0.05) increases were also observed in the turnover rate of HSP7C, HS90B, as well as the antioxidant and detoxifying enzymes PRDX2 and ALDH1. These data indicate greater proteotoxic stress and impaired proteome dynamics in OIR muscle and provide insight to exercise-induced changes in protein-specific abundance and turnover rates that act to restore muscle proteostasis.

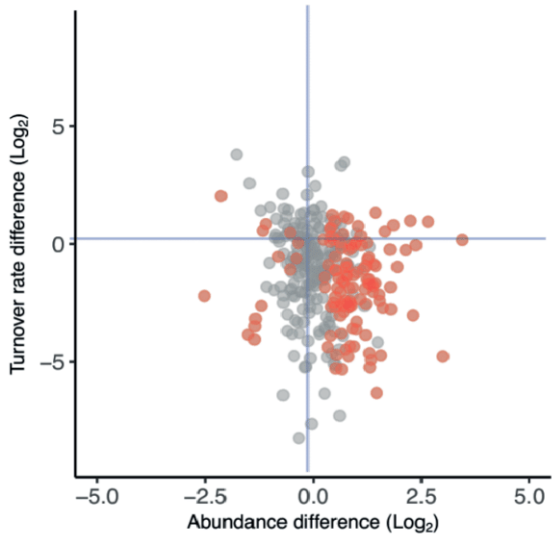

Figure 1 – Muscle proteins exhibit slower rates of turnover in obese insulin resistant humans
Scatter plot reporting Log2-fold differences between the abundance (x-axis) and turnover rate (y-axis) of 301 proteins analysed in vastus lateralis of obese insulin resistant (OIR; n = 3) and endurance trained (TR; n = 4) participants. Data points to the right of centre represent proteins that are more abundant in OIR muscle and data points below centre represent proteins that have slower rates of turnover in OIR compared to TR muscle. Red points indicate statistically significant differences in abundance (between subjects ANOVA; p < 0.05, false discovery rate <5 %). Most proteins (248 of 301; 82 %) exhibit slower turnover in OIR muscle and the majority (185 of 248; 75 %) of these proteins are also of greater abundance (bottom right quadrant) in the muscle of OIR humans.

PC54

Mitochondrial peptides MOTS-c and Humanin are upregulated in skeletal muscle in response to acute exercise and training.

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Mitochondria-derived peptides are newly discovered small bioactive peptides thought to communicate metabolic status to other cells. Two such peptides, MOTS-c and Humanin, have been shown to have particularly strong regulatory effects on metabolism and oxidative stress in tissues, but their response to exercise training remains unknown. Thigh muscle biopsies were obtained from nine healthy young subjects, before, and at 0 and 2h after one acute bout of cycling exercise (1 hour at 70% of peak VO_2) and after 3, 5, 7, and 14 days of exercise training at same cycling intensity and duration. A total of 8 training sessions were conducted in the 14 days. The skeletal muscle samples were analysed in duplicates for mRNA content of MOTS-c and Humanin. A one-way repeated measure analysis of variance (ANOVA) was performed to evaluate the effect of acute exercise or 14 days of exercise training. Tukey's honestly significant difference post-hoc test was used to locate differences and FDR-adjusted *p*-values are reported. Data are reported as mean ± SEM.

The results showed that MOTS-c and Humanin mRNA content was higher by 19% and 24%, respectively (fig. 1a, $p<0.05$), at 2h after acute exercise compared to at rest. Furthermore, MOTS-c and Humanin content were 43% and 35% higher, respectively, than pre-training (fig. 1b, $p<0.05$) after the 14-days of exercise training.

In conclusion, we show that MOTS-c and humanin are elevated both by acute exercise and exercise training at moderate intensity. Further studies are required to elucidate the role of these mitochondrial peptides for metabolic and performance adaptations to exercise training.

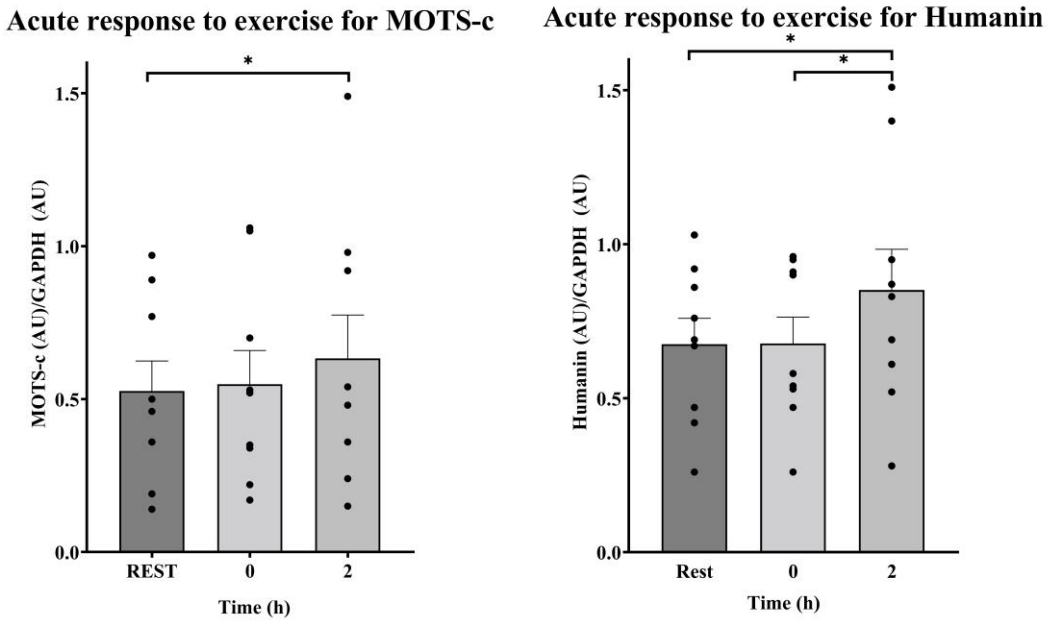

Fig. 1a. mRNA levels of MOTS-c and Humanin after an acute bout of exercise.

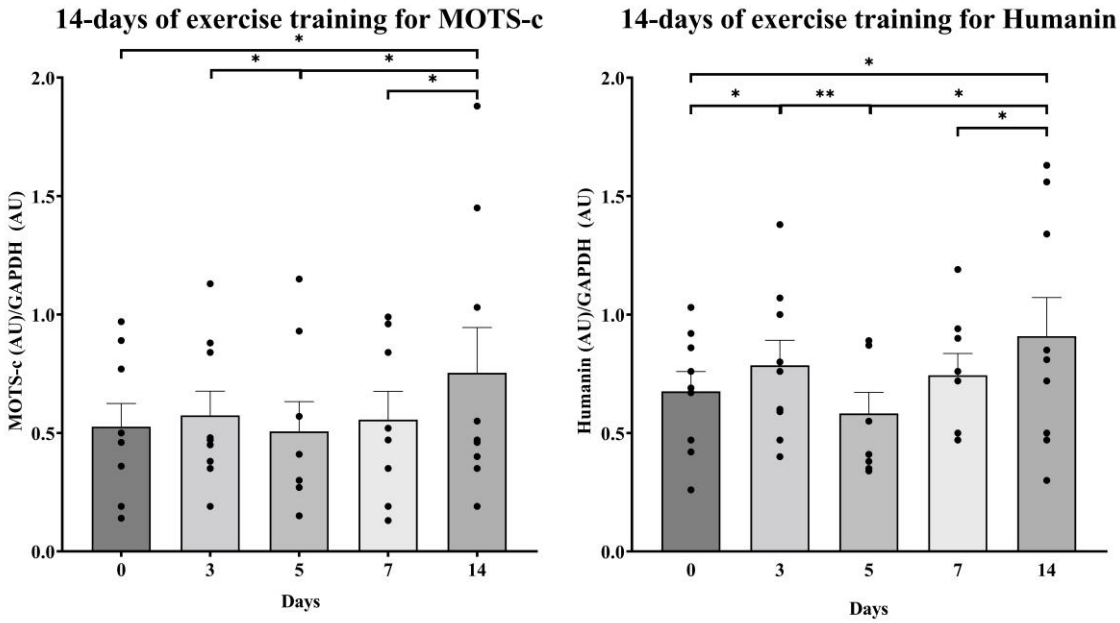

Fig. 1b. mRNA levels of MOTS-c and Humanin after 14-days of exercise training.

Acknowledgements :- The authors thank Gemma Kroos for expert technical assistance.

PC55

The Impact of Acute Montmorency Tart Cherry Supplementation Timing on Sub-Maximal Exercise Economy and 15km Cycling Time-Trial Performance

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Introduction

Montmorency cherry (MC) can improve endurance performance in trained participants following chronic supplementation [1, 2]. However, optimal pre-exercise timing of acute supplementation and the effect of training status on efficacy is not known. We investigated the impact of training status and supplement timing on the effects of acute MC concentrate (MCC) supplementation on exercise economy and performance.

Methods

Twenty participants (10 recreationally active (RA), 10 trained) completed a 10-minute steady state bout of exercise at 40%Δ (SSE), and a 15 km time trial (TT) on 4 separate occasions following consumption of a standardised breakfast (30.5 g carbohydrate) 90-minutes pre-exercise, and either no supplement (unsupplemented, US), or MCC supplementation 30-, 90- or 150-minutes pre-exercise ($MCC^{30/90/150min}$). Venous blood samples were taken pre-supplement, pre-exercise and post-exercise; heart rate (HR) and capillary [lactate] pre- and post-SSE, and pre-, 5km, 10km, and post-TT. Muscle oxygenation was measured continuously by near-infra-red spectroscopy (NIRS) during SSE and TT. Venous blood was analysed for [TNFα] and [NO_3^-]. Data are presented as mean±SD and analysed by one-way repeated measures ANOVA.

Results

MCC significantly improved TT performance, but not SSE economy, with the greatest improvement in performance following MCC^{90min} compared to US (US 1603.1±248 s vs MCC^{90min} 1554.8±226.7 s, 2.8±3.8% improvement in performance; MCC^{30min}: 1563.4±209.7 s, 2.1±4.3% improvement; MCC^{150min}: 1587.3±267.2 s, 1.1±3.9% improvement; p=0.037, N=20) (figure 1a). Performance was significantly enhanced in trained (US 1496.6±173.1 s vs MCC^{90min} 1466.8±157.6 s, N=10, p=0.005) but not RA participants (figure 1b). Capillary [lactate] (US 8.9±3.8 mmol.L^{-1} vs MCC^{90min} 11.2±4.2 mmol.L^{-1} at 10 km, p=0.001, N=20) (figure 2) and HR (US 165±14 BPM vs MCC^{90min} 170±15 BPM at 5 km p=0.009, N=2-; US 167±15 BPM vs MCC^{90min} 172±14 BPM at 10 km, p=0.011, N=20) (figure 3) were significantly greater during TT for MCC^{90min}. There was no significant difference between conditions in muscle oxygenation status (SSE tissue saturation index (TSI): US 61.6±8.7%, MCC^{30min} 61.3±7.6%, MCC^{90min} 61.3±7.5%, MCC^{150min} 61.5±7.0%; TT TSI: US 63.0±8.2, MCC^{30min} 61.3±8.2%, MCC^{90min} 62.2±7.7%, MCC^{150min} 62.6±7.9%, N=20) or plasma nitrate concentration (pre-supplement: MCC^{30min} 27.4±15.8 µ.mol^{-1}, MCC^{90min} 30.3±12.6 µ.mol^{-1}, MCC^{150min} 36.8±15.4 µ.mol^{-1}; pre-exercise: US 31.8±14.5 µ.mol^{-1}, MCC^{30min} 28.5±16.6 µ.mol^{-1}, MCC^{90min} 28.6±11.6 µmol^{-1}, MCC^{150min}26.5±12.7 µ.mol^{-1}; post-exercise: US 25.3±17.9 µ.mol^{-1}, MCC^{30min} 30.7±17.3 µ.mol^{-1}, MCC^{90min} 33.2±11.8 µ.mol^{-1}, MCC^{150min} 30.6±14.0 µ.mol^{-1}, N=20).

Conclusion

This is the first study to show acute MC supplementation is ergogenic for endurance performance and determine the influence of pre-exercise supplement timing and training status. Optimal supplementation timing was 90 min pre-exercise, which is probably related to the peak in plasma phenolic metabolite concentrations that occurs 1-2 hours following consumption [3].

Only trained individuals, experienced significant performance benefits, likely due to greater inter- and intra-participant variability in TT^{Time} for RA participants (RA: 3.7±5.5% vs Trained: 1.9±1.9%), and thus lower statistical power. Mechanisms underpinning ergogenic effects may be related to improved nitric oxide bioavailability increasing vasodilation [4], or perhaps more likely in light of the NIRS data, an anti-hyperalgesic effect induced by anti-inflammatory effects of phenolic metabolites[5].

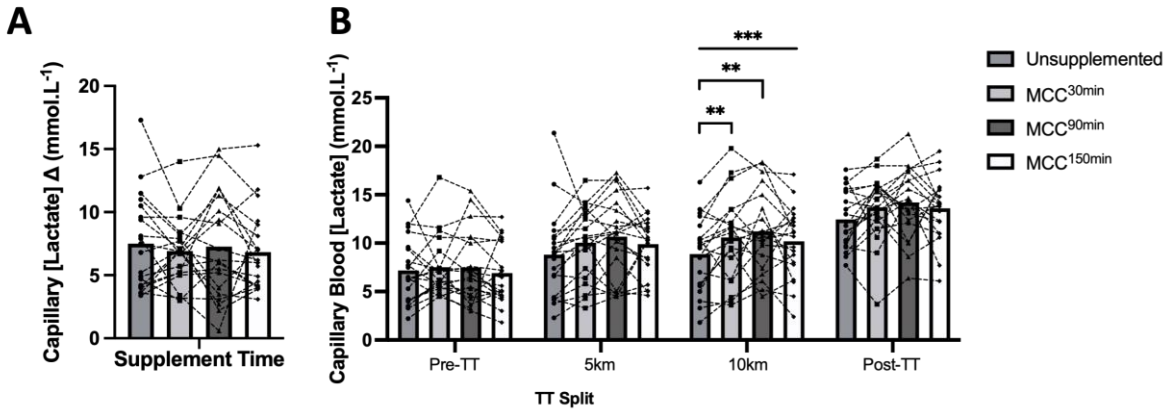

Figure 2: Comparison of capillary [lactate] for unsupplemented control and Montmorency Cherry Concentrate (MCC) supplementation 30 minutes, 90 minutes and 150 minutes prior to exercise for **A:** steady-state exercise **B:** time-trial by 5 km split. Values are displayed as means and individual data points. *denotes a significant effect of supplementation timing (*p<0.05/**p<0.01). Bars represent supplementation timing main effects, and brackets indicate where a post-hoc difference has been identified between two or more supplementation timings. N = 20.

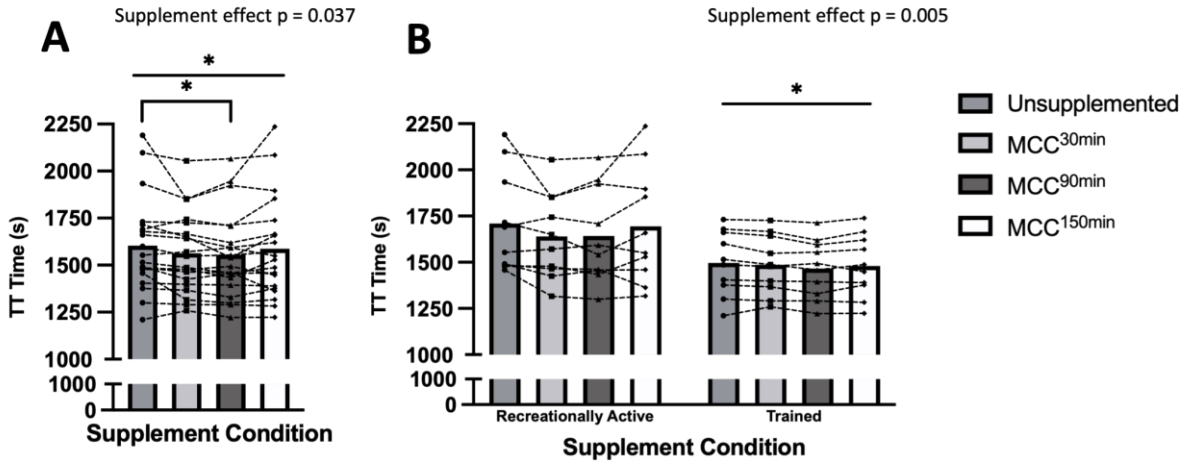

Figure 1: Comparison of time trial performance time for unsupplemented control and Montmorency Cherry Concentrate (MCC) supplementation 30 minutes, 90 minutes and 150 minutes prior to exercise for **A:** total time trial time **B:** total time trial time by training status**.** Values are displayed as means and individual data points. *denotes a significant effect of supplementation timing (*p<0.05/**p<0.01). Bars represent supplementation timing main effects, and brackets indicate where a post-hoc difference has been identified between two or more supplementation timings. N = 20.

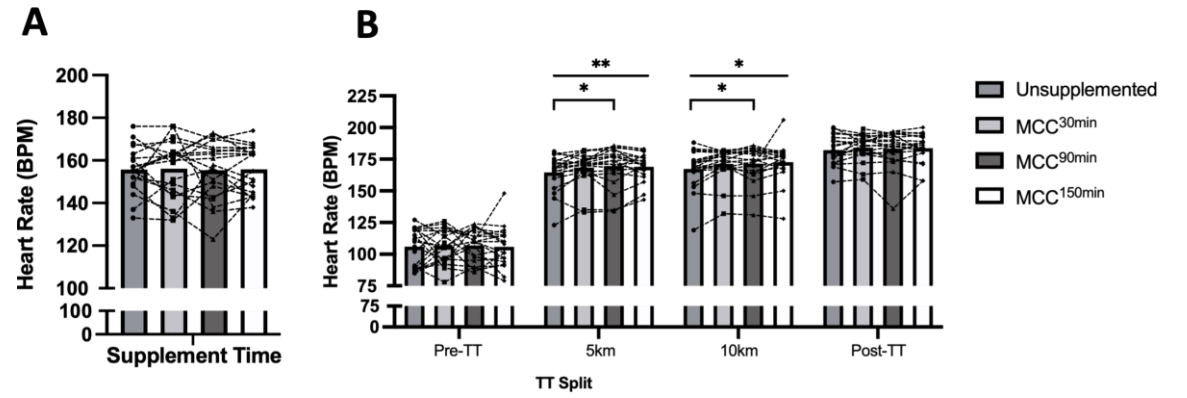

Figure 3: Comparison of heart rate for unsupplemented control and Montmorency Cherry Concentrate (MCC) supplementation 30 minutes, 90 minutes and 150 minutes prior to exercise for **A:** steady-state exercise **B:** time-trial by 5 km split**.** Values are displayed as means and individual data points. *denotes a significant effect of supplementation timing (*p<0.05/**p<0.01). Bars represent supplementation timing main effects, and brackets indicate where a post-hoc difference has been identified between two or more supplementation timings. N = 20.

Reference 1 :- Levers, K., et al., *Effects of powdered Montmorency tart cherry supplementation on acute endurance exercise performance in aerobically trained individuals.* Journal of the International Society of Sports Nutrition, 2016. **13**: p. 22.

Reference 2 :- Morgan, P.T., M.J. Barton, and J.L. Bowtell, *Montmorency cherry supplementation improves 15-km cycling time-trial performance.* European Journal of Applied Physiology, 2019.

Reference 3 :- Keane, K.M., et al., *Phytochemical uptake following human consumption of Montmorency tart cherry (L. Prunus cerasus) and influence of phenolic acids on vascular smooth muscle cells in vitro.* Eur J Nutr, 2016. **55**(4): p. 1695-705.

Reference 4 :- Senefeld, J.W., et al., *Ergogenic Effect of Nitrate Supplementation: A Systematic Review and Meta-analysis.*Med Sci Sports Exerc, 2020. **52**(10): p. 2250-2261.

Reference 5 :- Holgado, D., et al., *Analgesics and Sport Performance: Beyond the Pain-Modulating Effects.* Pm r, 2018. **10**(1): p. 72-82.

Acknowledgements :- We would like to acknowledge the University of Queensland and the QUEX Institute (University of Queensland and University of Exeter) who provided funding for the study, and ActiveEdge who provided the cherry supplement.

PC56

Incipient clot microstructure is altered in endurance trained female athletes

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Introduction

Endurance training is typically associated with high training volumes, often with insufficient rest, leading to chronic systemic stress [**1**]. This may alter factors that promote blood coagulation, such as enhanced sympathetic activity and inflammation [**2**]. Moreover, although regular exercise typically protects against cardiovascular disease, a single bout of maximal exercise can paradoxically trigger a cardiac event or stroke [**3**]. This may be attributed to exercise-induced hypercoagulability and the formation of denser blood clots [**4**]. Interestingly, little is known regarding the impact of training status on resting and post-exercise hemostasis.

Recently, a novel and sensitive rheology method for assessing hypercoagulability has been developed. The method provides a measure of incipient clot microstructure through the determination of fractal dimension (d_f), where a higher d_f is indicative of a more dense and stronger clot, and a lower d_f reflects a less dense and weaker clot [**5**].

Aims/Objectives

The purpose of this study was to assess clot microstructure in recreationally active (REC) and endurance trained (ET) females at rest and following a battery of intensive exercise. We hypothesized that ET females would have a higher d_f at rest and after maximal exercise compared to REC females.

Methods

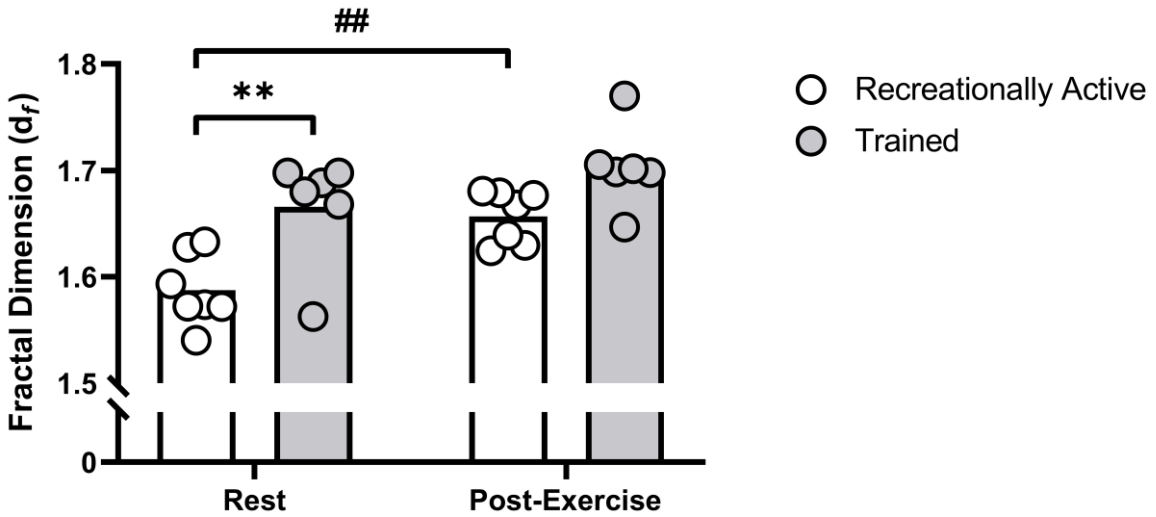
The study protocol was approved by the Ethical Committee of Copenhagen Region H (H-21032399). Seven REC ($\dot{V}O_{2max}$: 45.3 ± 0.9 mL·kg^{-1}·min^{-1}; training 2.1 ± 0.4 hours per week) and 6 ET females ($\dot{V}O_{2max}$: 52.3 ± 1.7 mL·kg^{-1}·min^{-1}; training 8.4 ± 0.6 hours per week) were recruited. Participants rested for 15 minutes before a baseline blood sample was drawn and immediately analyzed for d_f. Participants then completed a cycling battery including: a 12-minute graded warm-up, a $\dot{V}O_{2max}$ test to exhaustion, time to exhaustion at 100% watt max (W_{max}), and a 10 minute isokinetic time trial. Immediately after the exercise battery, a blood sample was drawn for determination of d_f. Statistics were performed using Graphpad Prism 9.3.1 (California, USA). An unpaired t-test was used for comparing W_{max}, a two-way ANOVA (group x time) was utilized for d_f analysis, and a Pearson correlation was used for correlational analysis. Data are reported as mean ± SEM.

Results

W_{max} during the $\dot{V}O_{2max}$ test was higher in the ET (313 ± 14 W) compared to the REC females (271 ± 10 W) (p = 0.03). However, W_{max} relative to body mass was not different (6.0 ± 0.4 W·kg^{-1} vs. 6.0 ± 0.3 W·kg^{-1}) (p = 0.97). At rest, d_f was significantly higher in the ET (1.67 ± 0.02) compared to the REC females (1.59 ± 0.01) (p = 0.002). Resting d_f correlated significantly with relative $\dot{V}O_{2max}$ (R^2 = 0.61) (p = 0.002). After the maximal exercise battery, d_f increased significantly in REC females (p = 0.004), whereas no change was observed in ET females.

Conclusions

This study provides novel insight into the impact of intensive high-volume training on clot microstructure. These findings indicate that higher training volumes and/or fitness may be associated with hypercoagulability at rest and abolition of exercise-induced changes in clot microstructure. Further studies investigating the underlying mechanisms are ongoing.



Reference 1 :- **[1]** George, K., *et al*. 2015. *British Journal of Sports Medicine*. **46**(Suppl 1): i29–36. DOI: 10.1136/bjsports-2012-091141.

Reference 2 :- **[2]** Hull, C.M. and Harris, J.A. 2013. *Circulation.* **128**: e469–71. DOI: 10.1161/CIRCULATIONAHA.113.004586

Reference 3 :- **[3]** Lee, C.D., *et al*. 2003. *Stroke*. **34**: 2475 – 81. DOI: 10.1161/01.STR.0000091843.02517.9D.

Reference 4 :- **[4]** Davies, N.A., *et al*. 2016. *Thrombosis Research*. **143**: 130–6. DOI: 10.1016/j.thromres.2016.05.018.

Reference 5 :- **[5]** Lawrence, M.J., *et al*. 2015. *Atherosclerosis*. **240**(2): 402–7. DOI: 10.1016/j.atherosclerosis.2015.04.012.

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Prevalence of disordered eating, exercise dependency, depression, and low energy availability in non-elite competitive endurance athletes compared to non-athletic individuals

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Introduction: Many athletes fail to match energy intake (EI) to exercise energy expenditure (EEE) (Bratland-Sanda and Sundgot-Borgen, 2013). Consequently, athletes are at risk of low energy availability (LEA) and relative energy deficiency in sport (RED-S) (Areta et al., 2021). Endurance athletes are a high-risk population for LEA and RED-S due to high levels of EEE, increased prevalence of disordered eating (DE), and their desire to maintain a low body mass (Mountjoy et al., 2018). Previous literature has focused on elite-level or female athletes, and limited work has explored the relationship between the psycho-physiological manifestations of LEA and RED-S (Logue et al., 2020).

The purpose of the study was to assess a) the prevalence of DE, exercise dependency (EXD), depression, and LEA in non-elite competitive endurance athletes compared to non-athletes and b) to compare DE, EXD, mood disorders, and LEA prevalence in male athletes compared to female athletes.

Methods: One hundred and seventy-five participants were recruited, 44 male and 87 female endurance athletes and 44 non-athletes. All participants completed: Eating Disorders Examination Questionnaire 6 (EDE-Q 6.0), Profile of Mood State Questionnaire (POMS), ORTO-15, Exercise Dependence Scale-21 Manual (EXDS-21), Socio-cultural Attitudes Towards Appearance Questionnaire 4, Athletic Identity Measurement Scale (AIMS).

Seventy participants (50 athletes, 20 non-athletes) completed 4-day self-reported nutritional and exercise diaries. Twenty participants (ten athletes and ten non-athletes) undertook resting metabolic rate (RMR) measurements via indirect calorimetry and body composition and bone mineral density measurements via dual-energy X-ray absorptiometry.

Results: Athletes produced significantly greater scores for *depression* and all EXDS-21 measures and significantly lower ORTO-15 scores than non-athletes ($P<0.05$). Athletes had significantly greater scores for the *eating concern* subscale of the EDE-Q ($P<0.05$), produced larger average scores for all other EDE-Q measures ($P>0.05$) (figure one), and presented with a greater prevalence of pathologic DE and EXD behaviours than non-athletes (51% vs 8%).

Athletes presented with a 35% larger prevalence of LEA, significantly lower energy balance and energy availability, and significantly greater EEE, than non-athletes ($P<0.05$). 70% of athletes presented with RMR suppression, compared to 0% of non-athletes.

Female athletes demonstrated significantly higher scores than male athletes in the '*eating concern', 'shape concern,'* and *'weight concern'* EDE-Q subscales ($P<0.05$) (figure one). Additionally, female athletes had a larger prevalence of LEA than male athletes (46% vs 32%) (table one).

Linear multiple regression analyses were conducted to calculate predictive factors of LEA, DE, and depression. Body mass, SATAQ-4 internalisation factors, and ORTO-15 and EXDS-21 scores significantly predicted EDE-Q score ($F(5,44)=20.031$, $P<0.001$, $R^2=0.70$). Additionally, body mass and ORTO-15, EXDS-21, and EDE-Q scores were significant predictors of athletic EA ($F4,45)=15.718$, $P<0.001$, $R^2=0.58$). Finally, body mass and EXDS-21 and EDE-Q scores significantly predicted depression ($F(3,127)=41.496$, $P<0.01$, $R^2=0.39$).

Conclusions: Non-elite endurance athletes are at greater risk of DE, EXD, depression, and LEA than non-athletes, with female athletes at greater risk than male athletes. The reported prevalence of DE, EXD, and LEA matches studies in elite athletes. Future research should explore the relationships between the psycho-physiological manifestations of LEA to identify potential preventive treatment plans.

Reference 1 :- Areta, J.L., Taylor, H.L. and Koehler, K. (2021) 'Low energy availability: History, definition and evidence of its endocrine, metabolic and physiological effects in prospective studies in females and males', *European Journal of Applied Physiology*, 121(1), pp.1-21. doi: https://doi.org/10.1007/s00421-020-04516-0

Reference 2 :- Bratland-Sanda, S. and Sundgot-Borgen, J. (2013) 'Eating disorders in athletes: overview of prevalence, risk factors and recommendations for prevention and treatment', *European Journal of Sport Science*, *13*(5), pp.499-508. doi: https://doi.org/10.1080/17461391.2012.740504

Reference 3 :- Logue, D.M., Madigan, S.M., Melin, A., Delahunt, E., Heinen, M., Donnell, S.J.M. and Corish, C.A. (2020) 'Low energy availability in athletes 2020: An updated narrative review of

prevalence, risk, within-day energy balance, knowledge, and impact on sports performance', *Nutrients*, *12*(3), p.835. doi: https://doi.org/10.3390/nu12030835

Reference 4 :- Mountjoy, M., Sundgot-Borgen, J.K., Burke, L.M., Ackerman, K.E., Blauwet, C., Constantini, N., Lebrun, C., Lundy, B., Melin, A.K., Meyer, N.L. and Sherman, R.T. (2018) 'International Olympic Committee (IOC) consensus statement on relative energy deficiency in sport (RED-S): 2018 update', *International Journal of Sport Nutrition and Exercise Metabolism,* 28(4), pp.316-331. doi: https://doi.org/10.1123/ijsnem.2018-0136