PL01

Physiological limits to exercise performance: Influence of gender

M.J. Joyner

Mayo Clinic, Rochester, MN, USA

In this talk I will review ideas about the physiological limits of human performance using primarily endurance exercise and especially distance running as a template for discussion. A key focus of the lecture will be on how sex and gender influence the physiological limits to performance and adaptations to training. How these limits and adaptations have interacted over the years with social change will also be reviewed.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PL02

Physiological Limits to Human Performance: Insight from the Elite Cross-Country Skier

H. Holmberg

Swedish Winter Sports Research Centre, Department of Health Sciences, Mid Sweden University, Ostersund, Sweden

Successful cross-country skiing, one of the most demanding of endurance sports, involves considerable physiological challenges posed by the combined upper- and lower-body effort of varying intensity and duration, on hilly terrain, often at moderate altitude and in a cold environment. During the competitions lasting from 12 min (4 3-min sprint skiing races) to more than 2 h (for a 50-km race), cross-country skiers must employ a variety of techniques and change often between these, which is highly demanding, both physiologically and in terms of coordination. Over the years, research on this unique sport has also helped physiologists gain novel insights into the limits of human performance and regulatory capacity and there is a long-standing tradition of researchers in this field working close together with coaches and athletes to improve training routines, monitor progress, and refine skiing techniques. Integration of physiological and biomechanical approaches has and continues to contribute to more detailed and accurate analysis of several determinants of the performance of cross-country skiers, thereby promoting significant improvement.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PL03

Sports nutrition and exercise metabolism: how we got where we are today – An historical perspective

R.J. Maughan

Loughborough University, St. Andrews, UK

Early scientists recognised the importance of foods as a source of energy for muscular contraction, observing that periods of starvation resulted in feelings of fatigue, lethargy and a difficulty even in standing (Mosso, 1891). The introduction of a reliable exercise ergometer and improved methods for expired air analysis during the early years of the 20th century meant that the study of the metabolic response to exercise gathered pace and the rates of carbohydrate and fat use at rest and during bouts of prolonged exercise were measured and shown to be affected by the composition of the preceding diet (Krogh & Lindhard, 1920). Subjects were reported to feel less fatigue after eating a diet high in carbohydrate. Debate about the interconversion of fat and carbohydrate continued for some years, however. Measurement of blood glucose concentrations in finishers of the Boston marathon showed the hypoglycaemia was present in some and carbohydrate ingestion was suggested as a remedy (Levine et al, 1924). The first study to definitively show beneficial effects on performance – albeit on only three subjects – was that of Christensen and Hansen (1939).

The introduction of tissue biopsy techniques to human physiology in the 1960s enabled muscle glycogen utilization and storage to be directly measured, yielding new mechanistic insights into the relationship between diet, carbohydrate availability and fatigue (Bergstrom and Hultman, 1966; Bergstrom et al, 1967). This work spawned a vast body of literature examining both acute and chronic manipulation of dietary intake and its effects on pre-exercise preparation, performance and recovery. The overwhelming conclusion was that ingestion of carbohydrate prior to and during exercise was beneficial to endurance exercise. In contrast, recent suggestions that a low-carbohydrate diet can benefit performance lack a strong evidence base and also have limited theoretical support. It has been known for more than a century that the energy available per litre of oxygen uses if greater for carbohydrate than for fat (Zuntz, 1901) and that the oxygen cost of exercise is less when carbohydrate is the substrate than when fat is being oxidised (Frentzel and Reach, 1901). Where oxygen availability is critical, therefore, carbohydrate should be the preferred fuel: it is difficult to see an advantage of increasing fat oxidation. The recent growth in the application of tracer and molecular techniques has enabled researchers to track the fate of ingested nutrients and better understand the signalling events that take place in skeletal muscle in response to exercise. Current work is characterising how alterations in substrate availability modulate many adaptive processes (Hawley et al, 2011), and these approaches will likely open the door to the design of personalised diet and exercise interventions to optimise training outcomes.

A relatively recent development in exercise science has been the recognition that the fatigue process may depend more on events occurring within the brain than in peripheral tissues. Newsholme's central fatigue hypothesis was an elegant attempt to describe a possible underlying mechanism that embraced the known metabolic changes occurring in the periphery (Blomstrand et al, 1988). This, however, was not a new idea, and Bainbridge wrote in 1919 that "There appear to be two types of fatigue, one arising entirely within the central nervous system, the other in which fatigue of the muscles themselves is superadded to that of the nervous system." This seems a remarkable insight that remains largely unrecognised even today. Even earlier, Lagrange (1889) wrote that "Fatigue is . . . a kind of regulator, warning us that we are exceeding the limits of useful exercise, and that work will soon become dangerous. Numerous physiological phenomena show us that the sensation of fatigue has its seat rather in the nerve-centres than in the muscles." As with so much else in science, the concept of a "central governor" that regulates or limits exercise performance is not new.

Bainbridge FW (1919) The Physiology of Muscular Exercise. London Longmans, Green and Co.

Bergstrom J and E Hultman (1966) Muscle Glycogen Synthesis after Exercise: an Enhancing Factor localized to the Muscle Cells in Man Nature 210, 309–310

Bergstrom, J. Hermansen, L. Hultman, E. Saltin, B. (1967). Diet, muscle glycogen and physical performance. Acta Physiologica Scandinavica, 71, 140–150.

Blomstrand E, F Celsing, EA Newsholme (1988) Changes in plasma concentrations of aromatic and branched-chain amino acids during sustained exercise in man and their possible role in fatigue, Acta Physiol Scand 133, 115–121

Christensen EH and O Hansen (1939) Arbeitsfähigkeit und Ernährung. Skandinavisches Archiv Für Physiologie 81, 160–171

Frentzel J and F Reach (1901) Untersuchungen zur Frage nach der Quelle der Muskelkraft. Pflugers Arch. 83, 477–508

Hawley JA, LM Burke,SM Phillips, LL Spriet (2011) Nutritional modulation of training-induced skeletal muscle adaptations. J Appl Physiol 110, 834–845

8. Krogh A, Lindhard JL (1920). The relative values of fat and carbohydrate as sources of muscular energy. Biochemical Journal, 14, 290–363.

9. LaGrange F (1889) Physiology of bodily exercise. London, Kegan Paul

10. Levine SA, G Burgess, CL Derick (1924) Some changes in the chemical constituents of the blood following a marathon race With special reference to the development of hypoglycaemia. JAMA 82(22), 1778–1779

11. Mosso A (1891) La Fatica. Milano, Treves

12. Zuntz, N. (1901). Über die Bedeutung der versehiedenen Nährstoffe als Erzeuger der Muskelkraft. Pfluger's Arch. 83, 557–571

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA01

Respiratory complications in the elite athletes – is the treatment also affecting muscle performance

V. Backer

University of Copenhagen, Denmark, Denmark

Marginal variation in performance produces highly significant implications at the elite athletic level participating in endurance sport. Athletic performance is dependent upon the optimisation of a number of physiological determinants including several respiratory limiting factors suggested to affect exercise performance negatively. However, in elite athletes, the capacity of the cardiovascular and muscular-skeletal systems can exceed the structural and functional capacities of the lung and airways. The normal responses of the airways during exercise are relaxation of the bronchial smooth muscle as a result of withdrawn parasympathetic tone, increases in tidal end inspiratory lung volumes, activation of upper airway dilator skeletal muscles and a continuous recruitment of expiratory muscles with increasing work rate or intensity. However, in a significant proportion of the elite athletes (40%) exercise triggers a constriction in the smooth muscle surrounding the airways. The diagnosis of exercise-induced bronchoconstriction (EIB), also called exercise-induced asthma (EIA), is a complex process. The impact of untreated or mistreated EIB on performance is unknown. During the summer Olympic Games of 2006 and 2010, the use of beta2-agonists was between 7.1 and 7.7%, with a higher use among endurance athletes of 19.1 and 17.3% in Olympic swimmers and cyclists, respectively. A substantial amount of research has been conducted investigating the effects of treatment on EIB and asthma over the years. EIB can be prevented with inhaled corticosteroids (ICS), either alone with a short-acting $beta_2$-agonist (SABA) as rescuer, or in combination with a long-acting $beta_2$-agonist (LABA). Given the high prevalence of asthma and exercise-induced bronchoconstriction among elite athletes, there is a high use of $beta_2$-agonists. It is still debated whether inhaled $beta_2$-agonists are performance enhancing. Notably, Olympic asthmatic athletes that use inhaled $beta_2$-agonists win more medals than their non-asthmatic counterparts. While it has been speculated that this may reflect that asthmatic athletes are better prepared at training and in competition, recent studies suggest that high dose inhalation of $beta_2$-agonist, even within the current anti-doping regulations, is performance enhancing. Performance enhancing effects of $beta_2$-agonist are a well-known phenomenon when administered in prohibited oral doses, which may be attributed to the systemic effects that $beta_2$-agonists elicit in various tissue. Pharmacokinetic data indicate that the systemic bioavailability of inhaled $beta_2$-agonists is higher than oral, why it is likely that inhaled $beta_2$-agonists possesses same systemic effect as oral. The systemic effects of $beta_2$-agonists that may be relevant for performance include enhanced ion handling and increased rate of glycogenolysis and glycolysis in skeletal muscle. The

aim of this lecture is to present the most recent data on beta$_2$-agonists in relation to performance, muscle physiology and doping. Furthermore, to discuss whether the 2015 World Anti-doping Agency's regulation of beta$_2$-agonists is adequate to ensure fair competition and to detect supratherapeutic misuse of beta$_2$-agonists.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA02

Central limits to maximal oxygen consumption

J.A. Calbet

Dep. of Physical Education and Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain

Maximal oxygen uptake (VO_2max) is the main determinant of the exercise capacity and is a good predictor of life expectancy. VO_2max is determined by the maximal O2 delivery (ODmax) x fractional O_2 extraction (OEmax). Two main factors determine ODmax: arterial O_2 content (CaO_2) and maximal cardiac output (Qmax). Qmax is the product stroke volume (SV) x heart rate (HR). Since training does not increase maximal HR and barely affects CaO_2, the only mechanism that could explain an increase of ODmax is a greater maximal SV. Stroke volume depends on intrinsic and extrinsic factors. Intrinsic factors are determined by the structural and functional properties of the heart, principally heart size, contractility and compliance. Extrinsic factors include: venous return, and central blood volume, mean arterial pressure (via its influence on preload). Other extrinsic factors such as sympathetic neural activity, may influence SV by regulating contractility and altering HR (and filling times). Several experiments have shown that Qmax can hardly be increased without cardiac remodelling; at most 1 l.min^{-1} extra Qmax can be achieved by plasma volume expansion of by reducing the afterload in some experimental settings. Since top-level endurance athletes may achieve VO_2max values ~ 85 ml.kg^{-1}.min^{-1} (or 6.0 l.min^{-1}, for a body mass of 70 kg), it turns that they must be able to reach a Qmax ~ 35 l.min^{-1}. This is achieved with an SV of 185-190 ml and a HRmax=185-190 beats.min^{-1}. For a maximal ejection fraction of 80%, these athletes must be able to reach ~230 ml end diastolic volumes at maximal exercise, what requires a big heart. Since current studies indicate that SV can be hardly increased more tan 20-30 ml after one year of training despite remarkable heart remodelling elite athletes must have a big heart before starting training. Given that the standard deviation for VO_2max is 0.6-0.7 l.min^{-1} in sedentary subjects, at least 2.5% of the human sedentary population could have a VO_2max $\geq$ 4.8 l.min^{-1} (69 ml.kg^{-1}.min^{-1}) and a Qmax=28-34 l.min^{-1} for OEmax=0.70-0.85). These are the only humans than may have a possibility to become top-level endurance athletes. The transfer of O_2 in the lung may also contribute to limit VO_2max under certain circumstances; however the functional reserve of O_2 lung diffusing capacity is remarkable.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA03

Local regulation of skeletal muscle blood flow

Y. Hellsten

Department of Nutrition, Exercise and Sports, University of Copenhagen, Copenhagen, Denmark

During exercise, blood flow to active skeletal muscle is increased to precisely meet the oxygen demand required for energy production. The precise regulation of blood flow is overall achieved through a balance between constriction via sympathetic nervous activity, locally formed vasoconstrictors and vasodilators and compounds that can modulate the constrictive effect of the sympathetic nervous system (functional sympatholysis). The regulation of local formation of vasoactive and sympatholytic compounds in the muscle is complex and not yet fully understood but a number of mechanisms and cells have been proposed to contribute. Central for most vasodilator mechanisms are the endothelial cells that receive signals and respond to chemical and mechanical stimuli by hyperpolarization and release of vasodilators including nitric oxide (NO) and prostacyclin. The chemically induced stimulation is achieved by compounds such as ATP and adenosine, originating from erythrocytes in response to oxygen desaturation of the hemoglobin molecule and, on the interstitial side, from skeletal muscle cells in response to contraction. Interestingly ATP is also a potent sympatholytic compound. A strong mechanical stimulus particularly for NO formation, is the frictional force that the blood exerts on the endothelial membrane (shear stress) which is sensed and transduced by mechanosensors. Substantial advancements in the area of skeletal muscle blood flow regulation have been made in the recent years but key aspects that remain to be resolved are the mechanisms underlying the close coupling between oxygen demand and regulation of blood flow and fully understanding functional sympatholysis.

Work of the author has been supported by The Danish Council for Independent Research-Medical Sciences, The Novo Nordisk Foundation, The Lundbeck Foundation, The Danish Ministry of Culture and The University of Copenhagen.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA04

Brain vascular control during exercise

J. van Lieshout

Internal Medicine - Acute Admissions Unit, Academic Medical Center, Amsterdam, Netherlands

Brain blood flow (BBF) is critical for maintaining oxygen and substrate supply to the brain and is secured by several mechanisms, of which the partial pressure of arterial carbon dioxide, mean arterial pressure, and cerebral metabolism are the most important. The historical controversy about whether BBF is altered during exercise relates to the methodology used to quantify BBF. The present understanding is that when the brain is activated, as during exercise, an increment in BBF enhances cerebral oxygenation.

An increase in near-infrared spectroscopy determined oxygenated hemoglobin (Hb) and a reduction in deoxygenated Hb in response to a motor task support that cerebral oxygenation exceeds the increase in O_2 demand. During exercise, the arterial O_2 content may increase and, together with increased BBF in response to cerebral activation, enhance brain oxygen delivery. The cerebral hyperperfusion in the early phase of exercise may be an important precaution because BBF declines in the later stages of exercise, mainly by the dominant negative effect of progressive hypocapnia on BBF. Brain function deteriorates when its oxygenation is reduced by more than 10% from the resting level. In contrast, skeletal muscles tolerate oxygen desaturation down to 10%.

During exercise, reduced cerebral oxygenation precedes development of so-called central fatigue. Debate continues on whether the subsequent reduction in cerebral oxygenation in the later stages of exercise could play a role in the development of central fatigue with reduced motor drive to working muscles. Cardiac output may also influence BBF during exercise. Inability to increase cardiac output sufficiently during exercise may jeopardize cerebral perfusion and thereby the ability of the central nervous system to drive the motoneurons adequately. This is illustrated by the BBF response to exercise in subjects with type 2 diabetes which could contribute to their high perceived exertion.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA05

Development and application of stable isotope tracers to exercise physiology

P.J. Atherton

Clinical, Metabolic and Molecular Musculoskeletal Physiology, MRC-ARUK Centre of Excellence, University of Nottingham, Derby, UK

Stable isotope tracers are molecules where one or more of the atoms of the molecule is/are substituted for an atom, such as hydrogen, nitrogen and carbons, of the same chemical element (same number of protons) but with an additional neutron, engendering a "stable isotope"; these are easily detected and precisely quantitated using mass spectrometry. Commonly used examples of this include: 1) for protein metabolism: ^{13}C phenylalanine, where 1 of the carbons are of the ^{13}C, rather than ^{12}C motif, 2) for fat metabolism, ^{13}C palmitate, or 3) ^{2}H glucose for carbohydrate metabolism. There are a large number of such molecules available with distinct atomic labelling profiles, which may be applied to a vast number of applications; of particular relevance to this audience, the synthesis, release and oxidation of fat, carbohydrates and protein constituents. The application of stable isotope labelled essential amino acid tracers (particularly ^{2}H or ^{13}C motifs on phenylalanine or leucine) have been long used in exercise research and been successfully applied to increasing our understanding of how exercise regulates energy and protein metabolism. Yet, a major limitation to the application of these tracers has been their restriction to studies of a shorter-term nature (less than 24 h) and under controlled laboratory environments. This is in addition to the invasive requirements of these approaches e.g. the need for cannulation for I.V infusions and concurrent arterial cannulation or arterialization of blood (for arterio-venous balance techniques to quantify synthesis and breakdown across a vascular bed), as well as the need for multiple muscle tissue biopsies. Not to mention the sterility requirements for isotope I.V infusates and clinical consumables. Recent technological advances in analytical instrumentation (e.g. pyrolysis IRMS and LC-MS/MS) have opened up a number of new avenues of research application and in a less invasive manner. Firstly, the re-introduction of heavy water (deuterium oxide (D_2O or $^{2}H_2O$)) has made it now possible to simultaneously study longer term changes in protein metabolism that were previously implausible due to the restraints of I.V infusions. Although there remain few studies, we have shown that this methodology can be used to investigate links between longer-term muscle protein synthesis and muscle hypertrophy (1). Others have done the same in relation to mitochondrial biogenesis and skeletal muscle stem cell activity (2). We have also shown these methods to be equally effective for studying protein metabolism over hours or days (3) and for testing the efficacy of interventions, albeit without the need for tracer infusions. Interesting recent work using these D_2O methodologies has also shown that these approaches can be combined with proteomics (4) to establish the synthesis rates of plasma proteins, such as muscle

creatine kinase, that act as a proxy of muscle protein synthesis, and moreover, to determine which individual proteins are being synthesized in muscle in response to interventions. In addition to this, metabolomics approaches are now being used where elements of a specific labelled metabolite (e.g. ^{13}C valine) can be tracked and its fate related to metabolic capacity; these fluxomics methods have been successfully applied to track the impact of exercise capacity upon branched chain amino acid oxidation (5). Finally, other recently developed tracer methods include the use of orally administered d3-methylhistidine (6) and d3-creatine (7), which are now being used: i) to determine the normally difficult to quantify muscle protein breakdown (typically through A-V balance techniques) and, ii) to quantify whole body muscle mass without the need for imaging (DXA, CT, MRI). Both of these entail minimally invasive means of sampling labelled metabolites (methylhistidine and creatinine, respectively) in subject's urine. Combining contemporary stable isotope methodologies with established approaches heralds a new era for both biological insight and engendering less invasive approaches to studying exercise physiology and biochemistry.

1. Brook MS, Wilkinson DJ, Mitchell WK, Lund JN, Szewczyk NJ, Greenhaff PL, et al. Skeletal muscle hypertrophy adaptations predominate in the early stages of resistance exercise training, matching deuterium oxide-derived measures of muscle protein synthesis and mechanistic target of rapamycin complex 1 signaling. FASEB J [Internet]. 2015 Jul 13 [cited 2016 Jan 10];29(11):4485–96. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26169934

2. Robinson MM, Turner SM, Hellerstein MK, Hamilton KL, Miller BF. Long-term synthesis rates of skeletal muscle DNA and protein are higher during aerobic training in older humans than in sedentary young subjects but are not altered by protein supplementation. FASEB J [Internet]. 2011 Sep [cited 2012 Jul 30];25(9):3240–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21613572

3. Wilkinson DJ, Franchi M V, Brook MS, Narici M V, Williams JP, Mitchell WK, et al. A validation of the application of D2O stable isotope tracer techniques for monitoring day-to-day changes in muscle protein subfraction synthesis in humans. Am J Physiol Endocrinol Metab [Internet]. 2014 Mar [cited 2014 Mar 20];306(5):E571–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24381002

4. Shankaran M, King CL, Angel TE, Holmes WE, Li KW, Colangelo M, et al. Circulating protein synthesis rates reveal skeletal muscle proteome dynamics. J Clin Invest [Internet]. 2015 Dec 14 [cited 2015 Dec 15];126(1):288–302. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26657858

5. Overmyer KA, Evans CR, Qi NR, Minogue CE, Carson JJ, Chermside-Scabbo CJ, et al. Maximal oxidative capacity during exercise is associated with skeletal muscle fuel selection and dynamic changes in mitochondrial protein acetylation. Cell Metab [Internet]. 2015 Mar 3 [cited 2016 Jan 10];21(3):468–78. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25738461

6. Sheffield-Moore M, Dillon EL, Randolph KM, Casperson SL, White GR, Jennings K, et al. Isotopic decay of urinary or plasma 3-methylhistidine as a potential biomarker of pathologic skeletal muscle loss. J Cachexia Sarcopenia Muscle. 2014 Mar;5(1):19–25.

7. Stimpson SA, Turner SM, Clifton LG, Poole JC, Mohammed HA, Shearer TW, et al. Total-body creatine pool size and skeletal muscle mass determination by creatine-(methyl-d3) dilution in rats. J Appl Physiol. 2012 Jun;112(11):1940–8.

I would like to thank Dr Kenny Smith and Dr Daniel Wilkinson for their analytical expertise.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA06

Studying physiological adaptation through metabolic systems biology

L. Edwards

GlaxoSmithKline, London, UK

[1]*Centre of Human & Aerospace Physiological Sciences, King's College London;* [2]*Respiratory Therapy Area Unit, GlaxoSmithKline Medicines Research.*

Systems biology is 'an iterative process of computational model building and experimental model revision with the aim of understanding or simulating complex biological systems' (PMID23849719). In practice, systems biology rests on three pillars: computational modelling, high-throughput 'omics methods, and systematic perturbation of the biological system of interest. By experimentally disrupting a biological system, by measuring a broad range of targetted (preferably unbiased) variables and by building and refining computational models describing these (and related) variables, the experimenter aims to uncover the fundamental properties of a system without recourse to a hypothesis. Computational models that can accommodate such a paradigm naturally fall into two categories: those that model the data and those that model the mechanism. In both cases, a recursive approach is recommended, leading to the concept of the 'model as hypothesis'. In this talk I present a mechanistic modelling approach that can encompass many different types of data and that lends itself to recursive model refinement: constraint-based metabolic modelling, using metabolic network reconstructions. I will outline the principles and assumptions that underlie constraint-based modelling. I will also present case studies showing that constraint-based modelling can aid interpretation of high-throughput data (including metabolomics and transcriptomics) and can predict genes whose mutations are likely to be favoured in a human population under strong selective pressure (hypoxia).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA07

New insights from microscopy imaging – Obesity induced skeletal muscle insulin resistance and Gastric-by-pass induced remission

C. Prats

Department of Biomedical Sciences, University of Copenhagen, Copenhagen, UK

The etiology of obesity-induced skeletal muscle insulin resistance remains elusive. Several molecular mechanisms have been proposed as link between metabolic alterations and decreased cell insulin response. Obesity has been linked to increases in skeletal muscle ectopic accumulation of lipid and decreases in skeletal muscle mitochondrial content. However, the athlete paradox clearly shows that skeletal muscle with high lipid accumulation can be very responsive to insulin. During the last years our group has investigated whether the combination of qualitative and quantitative measurements of skeletal muscle lipid storage and mitochondrial networks can explain changes in skeletal muscle insulin sensitivity in a morbid obese population undergoing gastric-by-pass. Several high resolution microscopy techniques have been used for the qualitative measurements of both lipid storage and mitochondrial dynamics.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA08

Can we predict the response to aerobic exercise training?

C. Bouchard

Human Genomics Laboratory, Pennington Biomedical Research Center, Baton Rouge, LA, USA

A common assumption is that cardiorespiratory fitness (CRF), as evaluated by the measurement of VO_2max, and cardiometabolomic risk factors improve as a result of exposure to aerobic exercise programs. Up until recently, this was considered to apply to all adult males and females. However, in a series of studies in which the exercise prescription was rigorously standardized, it was consistently observed that there are young, middle-aged and older adults who experienced little to no increase in CRF or in the risk factor profile. It is therefore of great importance for exercise medicine to understand the biological basis for the large individual differences in the trainability of CRF and the variability in responsiveness of cardiometabolomic risk factors. In this regard, exercise training studies performed in pairs of monozygotic twins and a cohort of about 200 nuclear families have revealed that CRF trainability was characterized by a heritable component of the order of 45%.

In contrast, the heritability of the risk factor trait responses is heterogeneous and ranges from 20% to 50%. These low to moderate genetic components sugests that it may not be feasible to generate powerful predictive algorithms based on DNA sequence variants alone. Attempts based on a combination of single nucleotide polymorphisms and skeletal muscle gene expression profiling have been reported and have led to disgnostic improvements. However, these studies have typically been hindered by low statistical power and lack of adequate replication material. Diagnostics aimed at predicting the level of responsiveness to regular exercise must met stringent sensitivity and specificity standards and achieve a high level of predictive power in order to be of sufficient quality for applications in a personalized exercise medicine context. This is unlikely to be attained without a comprehensive approach to diagnostic development that incorporates information on personal characteristics, morphological and physiological traits, genomics, epigenomics, transcriptomics and metabolomics. Bioinformatics explorations of the signals generated in early genomic studies have yielded a number of pathways and networks that contribute to variation in CRF trainability but also reveal that it will be a challenge to develop powerful diagnostics. For instance, genes with allelic variations contributing to CRF trainability are enriched in developmental, regulation of gene expression, angiogenesis, skeletal and cardiac muscle growth, and apoptosis pathways. Based on our study of the variation in insulin sensitivity in response to regular endurance execise, one can predict that many other key pathways will emerge when the biology of the changes in cardiometabolomic risk factor traits has been more thoroughly investigated. The global topic of predicting the response the responsiveness to endurance exercise (and resistance exercise as well) is one that will benefit greatly from the science being pursued by the large NIH-funded MoTrPAC project.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA09

Gene doping – where are we now?

D.J. Wells

Department of Comparative Biomedical Sciences, Royal Veterinary College, London, UK

Since the first demonstration that rodent muscles could be genetically modified to increase strength (Barton-Davis et al., 1998) and subsequent publications showing that muscles may also be genetically modified to improve endurance, there has been substantial interest in the potential for a genetic approach to doping. In 2004 gene doping was included in the WADA prohibited list with the following definition: ''Gene or cell doping is defined as the non-therapeutic use of genes, genetic elements and/or cells that have the capacity to enhance athletic performance''. Based on animal studies, a wide range of potentially athletically advantageous genes can

be transferred into target cells using a gene vector. Genes that have been shown to enhance athletic performance in rodents with increased expression include insulin-like growth factor 1, erythropoietin, peroxisome-proliferator-activated receptor (PPAR) delta, PPAR gamma co-activator alpha or beta and phosphoenolpyruvate carboxykinase. Down regulation of myostatin activity has also been demonstrated to increase muscle mass. It should be noted that many of the genetic effects were observed in transgenic mice that inherited the genetic modification in the germline and thus developed in the presence of the difference in gene expression whereas the potential application for gene doping would be through the modification of somatic tissues in juvenile or adult humans.

The likelihood of gene doping is enhanced by the research into gene therapy for muscle diseases such as Duchenne muscular dystrophy. The current method of choice for gene therapy in the clinical setting is through the use of adeno-associated viral (AAV) vectors carrying the therapeutic gene and these have been shown to be very effective in animal models of various muscular dystrophies but to date use in man has been limited to local delivery to single muscles. We are likely to see systemic delivery of AAV in human clinical trials for a number of neuromuscular diseases over the next few years which, if successful, could increase the possibility of athletes using gene therapy techniques. However, one significant limitation to potential gene dopers is that it is very technically demanding to produce the quantity of AAV required for high efficiency gene delivery to teenage or adult humans due to the large mass of muscle.

A new and emerging area of concern relates to gene editing. A number of systems have been developed to allow researchers to make precise modifications to the genome and these are being refined rapidly. All of these methods involve some system for targeting specific genetic sequences couple to a nuclease that cleaves the genomic DNA. Methods developed to date include the zinc-finger nucleases, TAL effector nucleases (TALENs) and CRISPR/Cas9. The latter system developed rapidly in 2015 and has been used to modify the genome when delivered systemically using an AAV viral vector (Nelson et al., 2015).

To the best of our knowledge gene doping has yet to be used by athletes. This is likely due to the current technical hurdles that prevent high efficiency gene delivery to muscle. There have also been a number of pro-active studies to identify methods for detecting gene doping that have shown potential to catch cheating athletes. Finally like many other forms of doping there are significant health risks and unlike conventional pharmaceuticals an athlete cannot stop taking the treatment if they have undergone genetic modification which increases the level of risk.

Barton-Davis ER *et al*. (1998). *Proc Natl Acad Sci U S A* **95**, 15603–15607.

Nelson CE *et al*. (2015) *Science* aad5143. [Epub ahead of print]

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA10

Pharmacological agents in human skeletal muscle adaptation

A.L. Mackey[1,2], M. Heisterberg[1,2] and M. Kjaer[1,2]

[1]Department of Biomedical Sciences, Center for Healthy Aging, University of Copenhagen, Copenhagen, Denmark and [2]Institute of Sports Medicine, Bispebjerg Hospital, Copenhagen, Denmark

One of the remarkable aspects of skeletal muscle is its capacity to adapt in response to mechanical loading and disuse throughout life, albeit to a gradually lesser extent with advancing age. Recovery of lost muscle mass and strength following periods of disuse is a challenge for ageing societies where loss of independent living is at stake. The ability of muscle to regenerate fully following injury is equally impressive and animal studies have clearly shown that satellite cells, the resident stem cells of skeletal muscle, are essential for this process. Whether satellite cells are required for hypertrophy has been keenly debated, but it would seem that maladaptation of the muscle as a whole occurs in response to overload where contribution from satellite cells is inhibited. In this context, the action of various pharmacological agents on muscle adaptation is receiving increasing attention. This talk will focus on a range of drugs, from the widely used over-the-counter available non-steroidal anti-inflammatory drugs to blood pressure-lowering prescription medication (Angiotensin II type I receptor blockers). Banned substances such as growth hormone and testosterone will also be touched upon. These will be addressed from the perspective of specific action on satellite cell function as well as skeletal muscle as a whole, with a focus on human data.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA11

Novel roles for satellite cells in muscle growth

C.A. Peterson[1], C. Fry[2], T. Kirby[3] and J. McCarthy[3]

[1]College of Health Sciences, University of Kentucky, Lexington, KY, USA, [2]School of Health Professions, University of Texas Medical Branch, Galveston, TX, USA and [3]Department of Physiology, Unviersity of Kentucky, Lexington, KY, USA

Satellite cells are the primary stem cell in skeletal muscle, required for postnatal muscle growth and adult muscle regeneration. Satellite cells are activated to proliferate and normally contribute nuclei to growing myofibers in response to hypertrophic stimuli, although considerable growth can occur in the absence of myonuclear accretion through the expansion of the myonuclear domain. Satellite cells reside

within an interstitial niche between myofibers and the basal lamina of the extracellular matrix (ECM). The muscle ECM is largely composed of collagens secreted by fibroblasts. We utilized the discrete expression of Pax7 in satellite cells to develop the Pax7-DTA mouse, whereby the use of *Cre*-lox technology allows for the specific and inducible depletion of satellite cells following tamoxifen-induced expression of diphtheria toxin. Synergist ablation surgery, where removal of synergist muscles places functional overload on the plantaris, was used to stimulate robust hypertrophy. Depletion of satellite cells in the adult mouse during mechanical overload of muscle resulted in ECM dysregulation and muscle fibrosis. We characterized interactions of activated satellite cells and their daughter cells, myogenic progenitor cells (MPCs), with muscle fibroblasts. We found that MPC-derived exosomes are capable of down-regulating fibroblast collagen expression. Interfering with microRNA processing, resulting in loss of microRNAs in MPC exosomes, reduced their ability to downregulate fibroblast ECM gene expression. These findings provide the first evidence for a new role for satellite cells in the regulation of fibroblast ECM production and suggest MPCs are actively involved in the remodeling of the skeletal muscle extracellular environment during muscle hypertrophy.

Acknowledgements: This work was supported by grants from the National Institutues of Health (National Institute on Aging and National Institute of Arthritis and Musculoskeletal and Skin Disorders).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA12

The muscle "memory"

K. Gundersen

University of Oslo, Norway, Norway

Individuals with a history of previous training of are more easily retrained even after prolonged de-training, and this phenomenon has been dubbed "muscle memory". This has been an unfortunate term since it was attributed solely to motor learning in the CNS. Motor learning has, however not been a satisfactory explanation for why muscle force is more easily required if you have once been strong. Until recently muscle hypertrophy and atrophy was considered reversible processes as illustrated in Fig. 1. When muscle mass was built myonuclei was added to the growing fibres from activated satellite cells to support the larger cytoplasm. During atrophy the "excess" nuclei was believed to be lost through a selective nuclear apoptosis within the intact fibres. With observations based on in vivo imaging in rodents we have challenged this view. Thus, during a variety of inactivity conditions we observed no loss of myonuclei during atrophy, apoptosis was confined to other cell types. Muscle is a postmitotic tissue and myonuclei are very stable; a low estimate for humans would be a half-life of 15 years. Thus, the increased number

of nuclei could represent a long lasting "memory" of previous size. We also investigated the ascending limb of the model in Fig. 1, and confirmed that during de novo overload hypertrophy the number of myonuclei are increased, but seemed to precede the growth in size. In recent experiments with satellite cell ablation we found that such cells are obligatory for de novo hypertrophy. In contrast, in muscles that were first atrophied by hindlimb suspension and then grew back to the previous size, new myonuclei were not recruited during regrowth. These observations led us to propose a new model (Fig. 2) in which there is a first training route where new myonuclei are added, and then a re-training route where the fibres can grow without adding new nuclei. We then asked if the retraining route was faster. Female mice were treated with testosterone that created hypertrophy. When the testosterone was withdrawn the fibre size, but not the elevated number of nuclei reverted to control levels within 3 weeks. After a 3 months washout period the muscles were subjected to overload, the testosterone group then grew 36% within the first 6 days, while the control group grew insignificantly (6%). We hypothesize that the elevated number of nuclei represents a novel form of epigenetic memory in the form of an elevated number of myonuclei aiding muscle strength building in muscles that has previously been strong. If applicable to humans this mechanisms has important implications for public health and for evaluation of doping exclusion times.

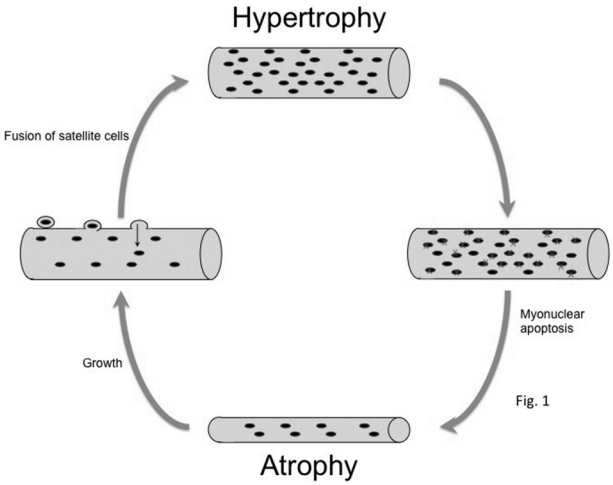


Fig. 1

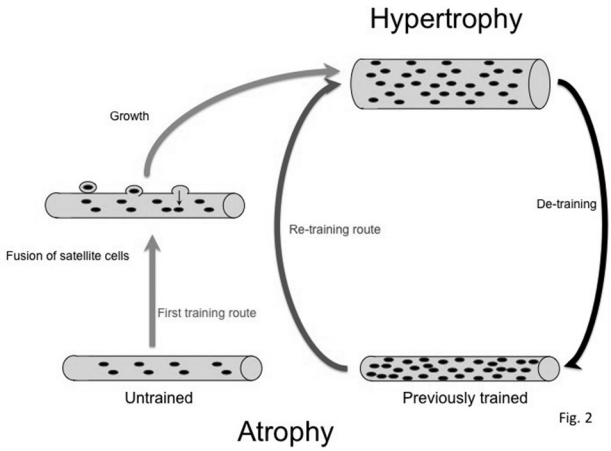


Fig. 2

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA13

Physiological adaptations to interval exercise training: New insights

M.J. Gibala

Department of Kinesiology, McMaster University, Hamilton, ON, Canada

Interval exercise refers to the basic pattern of alternating periods of more intense effort with period of less intense effort, or complete rest, within a single training session. Coaches and athletes have employed the practice since the early 20th century, and English language publications on physiological adaptations to interval training date back to the 1960s[1]. The wide variety of terms used to describe this basic type of exercise has led to a dizzying array of acronyms and a lack of consistency in the literature. In an effort to standardize terminology, a classification scheme was recently proposed to delineate "high intensity interval training" (HIIT) from "sprint interval training" (SIT)[2]. HIIT generally refers to submaximal exercise protocols in which the workload elicits a relative intensity of ≥80% of peak heart rate. SIT describes protocols in which the intensity corresponds to ≥100% of the workload that elicits maximal oxygen uptake (VO_{2max}). It has long been appreciated that both HIIT and SIT elicit physiological adaptations that resemble, and indeed can be superior to, changes normally associated with traditional moderate-intensity continuous training (MICT)[3,4]. Studies that have directly compared MICT to HIIT protocols matched for total work or energy expenditure, as summarized in several recent systematic reviews and meta-analyses[2,5,6], have generally concluded that interval training elicits superior physiological adaptations in both average healthy

individuals and people with lifestyle-induced cardiometabolic disease. Research over the last decade in particular has shed new light on the potency of *low-volume* interval training, which involves a relatively small total amount of exercise, to elicit physiological adaptations that are comparable to MICT in a time-efficient manner[7]. Studies that have directly compared MICT to low-volume HIIT or SIT protocols have reported similar improvements in markers of aerobic energy metabolism, as well as clinical indices of health status, despite large differences in total exercise and training time commitment. These findings are noteworthy given that lack of time is the most cited barrier to regular physical activity; however, while the efficacy of interval training is increasingly recognized, it's effectiveness and potential impact on public health remains controversial[8]. Recent evidence supports the general contention that exercise intensity is more important than duration for training-induced increases in cardiorespiratory fitness[9]. In contrast, the specific roles of intensity, duration and volume on aspects of exercise-induced skeletal muscle remodelling, in particular mitochondrial biogenesis, are equivocal[10]. Recent work suggests that SIT promotes greater and faster mitochondrial adaptations in skeletal muscle of moderately trained men than does HIIT and MICT despite a much lower training volume[11]. With respect to highly trained endurance athletes, interval training has long been considered an essential component of programs designed to maximize performance, although the underlying mechanisms are likely different compared to less trained individuals[12]. Inserting a short period of HIIT or SIT for up to several weeks, either by replacing a portion of usual training or in combination with an overall reduction in total training volume, can further enhance performance in highly trained individuals. It has been proposed that a polarized approach, in which ~75% of total training volume be performed at low intensities, with ~10-15% performed at very high intensities, may be the optimal training intensity distribution for elite athletes who compete in endurance events[13].

Christensen EH, et al. Acta Physiol Scand 50:269-286, 1960.

Weston KS, et al. Br J Sports Med 48:1227-1234, 2014

Knuttgen HG, et al. Med Sci Sports. 5:220-226, 1973.

Fox EL, et al. Med Sci Sports. 5:18-22, 1973.

Bacon AP, et al. PLoS One. 8:e73182, 2013.

Milanović Z, et al. Sports Med. 45:1469-1481, 2015.

Gibala MJ, et al. J Physiol. 590:1077-1084, 2012.

8. Biddle SJ, Batterham AM. Int J Behav Nutr Phys Act. 12:95, 2015.

9. Ross R, et al. Mayo Clin Proc. 90:1506-1514, 2015.

10. Bishop DJ, et al. Biochim Biophys Acta. 1840:1266-1275, 2014.

11. Granata C et al. FASEB J. 2015 Nov 16.

12. Bangsbo J. Scand J Med Sci Sports. 25:S88-S99, 2015.

13. Laursen PB. Scand J Med Sci Sports 20:S1-10, 2010.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA14

Eccentric exercise: physiology and application in sport and rehabilitation

H. Hoppeler

University of Bern, Bern, Switzerland

Muscle activation that results in torque that is greater than the resistance encountered, leads to muscle shortening, a so called *concentric contraction*. During a concentric contraction a muscle performs positive work; it accelerates a load or it imparts potential energy to an object. If however, the load that is applied to a muscle is larger than the torque that it produces, the activated muscle undergoes lengthening and this is called an *eccentric contraction*. In eccentric contractions, a muscle performs negative work i.e. it decelerates an object or it absorbs potential energy such as during walking downhill. There are several physiological properties that differ notably between concentric and eccentric contractions. The torque that can be produced by muscle tissue can be several times higher during eccentric than during concentric contractions at similar angular velocity. This implies that muscle tissue can be subjected to much higher stress during eccentric than during concentric contractions, potentially leading to muscle damage and delayed onset muscle soreness (DOMS). Furthermore, the metabolic energy required to provide negative work is typically four fold lower than the metabolic energy required to produce the same amount of positive work. We have used eccentric exercise in rehabilitation of patients with limited metabolic performance capacities to subject muscle tissue to high mechanical loads that could not have been achieved by concentric exercise. In a similar vein, eccentric exercise was used in octogenarians in the prevention of sarcopenia. Eccentric exercise further proved effective in preparing competitive alpine skiers to the enormous eccentric muscle loads seen in alpine ski racing.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA15

Ultra-endurance exercise: muscle adaptations and metabolism

J.W. Helge, T.H. Morville, M. Rosenkilde, A. Vigelsoe and F. Dela

Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

Regular endurance training increases fat oxidation during exercise at a given exercise load and decreases carbohydrate utilisation. In addition to training status the daily dietary macronutrient intake as well as the consumption of carbohydrate containing beverages and/or food during exercise will influence fuel utilisation during exercise. When ultra-endurance exercise including several different exercise

modalities is performed more or less continuously for several days fat oxidation during exercise is increased both at rest and during submaximal exercise (1). However, the increased fat oxidation may well be due to decreased glycogen stores and inability to maintain energy balance, both of which will increase fat utilization during exercise. To study the effect of excessive prolonged exercise on maximal fat oxidation during exercise 14 days of very prolonged exercise was performed by 6 elderly male subjects. The study adhered to the Declaration of Helsinki and was approved by the Science Ethical Committee of the Copenhagen Region (H3-2011-008) and also registered at clinicaltrials.gov (NCT02353624). Over the 14 days the subjects performed approximately 10½ hours of cycle exercise per day. During the 14 days the subjects consumed an ad lib high carbohydrate diet and before and approx. 30-34 hours after completion of the last of the 14 exercise days maximal fat oxidation as well as maximal oxygen uptake was measured during a graded cycle exercise protocol in the overnight fasted condition. Before and after the 14 days muscle biopsies from vastus lateralis and a blood sample under resting conditions were obtained. Interestingly a marked decrease in maximal fat oxidation (32 ± 8 %) and a decreased maximal oxygen uptake (6 ± 2 %) were observed after regular excessive prolonged exercise (Authors unpublished findings). The blood glucose was unchanged, but the plasma FA concentration at rest was massively decreased by approx. 60 % after the 14 days. Muscle glycogen and triacylglycerol concentrations were unchanged after the 14 days. Muscle GLUT4 and HKII expression was increased after the 14 days and ATGL and LPL expression but not HSL expression, were also increased (Authors unpublished findings). Based on these data very prolonged exercise lead to a decreased maximal fat oxidation possibly due to a decreased exogenous fat availability. In a recent paper also based on the above described study, we demonstrated that energy expenditure during the 14 days were above 30 ± 2 MJ per day and although the body weight was unchanged there was a shift in body composition with a decreased body fat content (-2.2 +/- 0.7 kg and an increased lean mass (2.5 +/- 0.6 kg) (2). This implies that maximal fat oxidation after 14 days of prolonged exercise was decreased despite similar muscle glycogen and a sizeable energy deficit in these older men. Interestingly Slivka and colleagues observed a higher fat oxidation across a range of exercise intensities in 10 trained younger men after 21 days of very prolonged road cycling (4½ - 5 hours per day) (3). Clearly this difference may be explained by the differences in age, exercise load and the energy balance during the prolonged exercise.

This talk will outline current knowledge on substrate utilization and muscle adaptation in ultra-endurance exercise and will with reference to the results of the study outlined above discuss possible mechanisms and future directions in this field.

Helge JW, Rehrer NJ, Pilegaard H, Manning P, Lucas SJ, Gerrard DF and Cotter JD. Increased fat oxidation and regulation of metabolic genes with ultraendurance exercise. *Acta Physiol (Oxf)* 191: 77–86, 2007.

Rosenkilde M, Morville T, Riis AP, Kjaer K, Rasmusen H, Holst JJ, Dela F, Westerterp K, Sjodin A and Helge JW. Inability to match energy intake with energy expenditure at sustained near-maximal rates of energy expenditure in older men during a 14-d cycling expedition. *Am J Clin Nutr* 2015.

Slivka DR, Dumke CL, Hailes WS, Cuddy JS and Ruby BC. Substrate use and biochemical response to a 3,211-km bicycle tour in trained cyclists. *Eur J Appl Physiol* 112: 1621-1630, 2012.

The skilled technical assistance of Thomas Nyegaard Beck, Regitze Kraunsøe, and Jeppe Bach is gratefully acknowledged.

Financial support from the Danish National Research Foundation, The Nordea Foundation, and the Lundbeck Foundation and by the Danish Council for Independent Research, Natural Sciences is gratefully acknowledged.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA16

Mixing your exercise modes: When opposites distract

J.A. Hawley[1,2]

[1]Faculty of Health Sciences, Mary MacKillop Institute for Health Research, Melbourne, VIC, Australia and [2]Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UK

Specificity of exercise training is essential to promote the phenotypic adaptations crucial for optimal athletic performance. The main factor contributing to the specificity of adaptation in skeletal muscle is the mode of contractile activity, which is underpinned by the volume, intensity and frequency of the training stimulus. In this regard, the diverse functional and phenotypic profiles associated with endurance or resistance/power-based training are easy to discern, and the 'molecular signatures' associated with these distinct adaptations well described. However, characterising the molecular 'footprint' responsible for the 'interference effect' of endurance-based exercise on hypertrophy and strength when divergent exercise stimuli are undertaken concurrently (in the same training session or as part of a long-term training regimen) has proven problematic.

Adding to the complexity of the molecular responses to concurrent exercise modes is the training background on which they are implemented. Divergent modes of exercise can induce similar gene expression and signalling profiles in skeletal muscle of untrained or recreationally active individuals, while chronic endurance or strength training attenuates some of the exercise specific signaling responses involved in single-mode adaptations to training. An important practical outcome of a 'time-dependent training adaptation continuum' is that it may be necessary to undertake divergent modes of exercise at different times during a periodised training program to induce the desired phenotype. Despite the potential for several key regulators of muscle metabolism to explain the incompatibility in adaptation between endurance and resistance exercise, the mechanistic underpinning of the 'interference effect' after concurrent training is not well understood. It seems likely that multiple integrated networks and

pathways with a high degree of crosstalk and feedback regulation, rather than single isolated, effectors or processes underpin this phenomenon.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA17

Manipulating muscle protein turnover to maximize exercise adaptations: evidence-based strategies

S. Phillips

Kinesiology, McMaster University, Hamilton, ON, Canada

Muscle hypertrophy is an often sought goal of athletes and mere mortals alike. The basis of hypertrophy resides predominantly in the confluence of two processes: changes in muscle protein turnover (protein synthesis and breakdown), and satellite cell function. In this presentation I will review the evidence summarizing what we know about changes in protein turnover that drive changes in muscle mass. Evidence for the impact of protein – dose, timing, and source – will be reviewed. In addition, the impact of the amino acid leucine, as a key amino acid in stimulating muscle protein synthesis (MPS), will also be examined. I will also address the role of external load and how motor unit recruitment is an independent variable determining muscle activation and muscle fibre hypertrophy. The role of what some call intensity, as a driver of MPS and muscle hypertrophy and strength gain will also be discussed. Finally, I will show evidence that demonstrates the belief-based, not evidence-based, role of endogenous hormones in stimulating MPS and hypertrophy. Attendees will gain up-to-date evidence-based knowledge on what is currently understood about the science of muscle protein turnover and exercise adaptations.

Burd NA, Mitchell CJ, Churchward-Venne TA and Phillips SM. Bigger weights may not beget bigger muscles: evidence from acute muscle protein synthetic responses after resistance exercise. *Appl Physiol Nutr Metab* 37: 551–554, 2012.

Burd NA, West DW, Staples AW, Atherton PJ, Baker JM, Moore DR, Holwerda AM, Parise G, Rennie MJ, Baker SK and Phillips SM. Low-Load High Volume Resistance Exercise Stimulates Muscle Protein Synthesis More Than High-Load Low Volume Resistance Exercise in Young Men. *PLoS ONE* 5: e12033, 2010.

Mitchell CJ, Churchward-Venne TA, West DD, Burd NA, Breen L, Baker SK and Phillips SM. Resistance exercise load does not determine training-mediated hypertrophic gains in young men. *J Appl Physiol* 113: 71–77, 2012.

Morton RW, McGlory C and Phillips SM. Nutritional interventions to augment resistance training-induced skeletal muscle hypertrophy. *Front Physiol* 6: 245, 2015.

West DW, Burd NA, Staples AW and Phillips SM. Human exercise-mediated skeletal muscle hypertrophy is an intrinsic process. *Int J Biochem Cell Biol* 42: 1371–1375, 2010.

West DW and Philips SM. Are acute post-resistance exercise increases in testosterone, growth hormone, and IGF-1 necessary to stimulate skeletal muscle anabolism and hypertrophy? *Med Sci Sports Exerc* 45: 2044–2051, 2013.

West DW and Phillips SM. Associations of exercise-induced hormone profiles and gains in strength and hypertrophy in a large cohort after weight training. *Eur J Appl Physiol* 112: 2693–2702, 2012.

The Natural Science and Engineering Research Council of Canada, the Canadian Institutes for Health Research, The Canadian Diabetes Association, The Canadian Foundation for Innovation

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA18

Regulation and limitations to fat oxidation during exercise

F. Stephens

University of Nottingham, Nottingham, UK

Fat and carbohydrate are the primary sources utilised to fuel oxidative, mitochondrial adenosine triphosphate (ATP) resynthesis for human skeletal muscle contraction. The relative contribution of these two substrates to ATP resynthesis and total energy expenditure during exercise can vary substantially, and is predominantly determined by substrate availability and exercise intensity and duration. For example, the increased ATP demand that occurs with an increase in exercise intensity is met by increases in both fat and carbohydrate oxidation up to an intensity of around 70% of maximal oxygen consumption (VO_2max) in elite athletes. However, when exercise intensity increases beyond this workload skeletal muscle carbohydrate utilisation is accelerated, which results in a reduction in the relative contribution of fat oxidation to total energy expenditure likely by inhibiting the absolute rate of fat oxidation. As muscle glycogen depletion ultimately results in the inability to maintain exercise at intensities above 70% VO_2max, elucidating the limitations to the rate of fat oxidation during exercise is desirable in order to understand, and develop strategies to improve, elite exercise performance. However, despite a considerable accumulation of knowledge that has been gained over the past half century, the precise mechanisms regulating muscle fuel selection and underpinning the aforementioned decline in fat oxidation remain unclear. This presentation will primarily address the theory that a carbohydrate-mediated reduction in the availability of muscle carnitine to carnitine palmitoyltransferase 1 (CPT1), a rate limiting step in mitochondrial fat utilisation, is a key mechanism for the decline in fat oxidation during high intensity exercise. This is discussed in relation to recent work in this area taking advantage of the discovery that skeletal muscle carnitine content can be increased in vivo in humans. Methods to measure skeletal muscle fat utilisation during exercise in humans will also be explored.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA19

Dietary carbohydrate is an obligatory requirement for the endurance athlete – or is it?

L.M. Burke

Australian Catholic University, Canberra, ACT, Australia

The importance of body carbohydrate (CHO) stores in supporting exercise capacity was established a century ago. Since then, sports nutrition guidelines have recommended that endurance athletes optimise their competition performance by implementing dietary strategies that achieve high CHO availability. Periodically, however, this principle has been challenged by the enticing view that the endurance performance could be enhanced by better utilisation of the body's relatively larger fat stores. For example, during 1985-2005, studies examined the proposal that adaptation to a low-CHO (< 25% energy) high-fat (>60% energy) diet (LCHF) to increase muscle fat utilisation during exercise could enhance performance in trained individuals by reducing reliance on muscle glycogen. As little as 5 days of training with LCHF retools the muscle to enhance fat-burning capacity with robust changes that persist despite acute strategies to restore carbohydrate availability (e.g. glycogen supercompensation, carbohydrate intake during exercise). Furthermore, 2-3 week exposure to minimal carbohydrate (< 20 g/d) intake has been shown to achieve adaptation to high blood ketone concentrations. However, the failure to detect clear performance benefits during endurance/ultra-endurance protocols, combined with evidence of impaired performance of high-intensity exercise via a down-regulation of carbohydrate metabolism led this author to dismiss the use of such fat adaptation strategies by competitive athletes in conventional sports. Recent re-emergence of interest in LCHF diets, coupled with anecdotes of improved performance by sportspeople who follow them, has created a need to re-examine the potential benefits of this eating style. Unfortunately, the absence of compelling data to support this proposal prevents a different conclusion from being made. Notwithstanding the outcomes of future research, there is a need for better awareness of current sports nutrition guidelines which promote an individualised and periodised approach to fuel availability during training, allowing the athlete to prepare for competition performance with metabolic flexibility and optimal utilisation of all muscle substrates. While there may be a few scenarios where LCHF diets are of benefit, or at least are not detrimental, for sports performance, the advantages of CHO as a fuel for the brain and muscle should not be forgotten.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA20

Mechanisms in glycogen re-synthesis after exercise

J.F. Wojtaszewski

Nutrition, Exercise and Sports, The August Krogh Centre, Copenhagen, Denmark

In 1966 the two Swedish scientists Bergström and Hultman elegantly demonstrated the glycogen super-compensation phenomenon in human skeletal muscle. By use of one-legged exercise it was revealed that the ability to super-compensate muscle glycogen is restricted to the prior exercised muscle. The authors concluded that a single bout of exercise induces local changes within the muscle that are maintained for several days after exercise increasing the set-point for glucose storage. It is noteworthy that the nature of this exercise-induced signature still remains to be established. We have now performed invasive human study alongside mechanistic studies of transgenic animals enabling us to provide a model for the molecular signature induced by exercise ensuring that skeletal muscle glycogen content is re-established and even super-compensated. Our studies point to an important regulatory role of the energy/fuel sensing kinase; AMP-activated protein kinase.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA21

Does 'altitude training' increase exercise performance in elite athletes?

C. Lundby

University of Zurich, Switzerland, Switzerland

'Altitude training' covers a wide range of different exercise training strategies combining training with hypoxic/altitude exposure in one way or the other. While such strategies in some instances may prove useful in improving exercise capacity at altitude, the scientific evidence for this also being the case for exercise performance conducted at sea level is less clear. During the presentation the pros and cons of the various approaches will be discussed and emphasis will be given to studies that have applied sound experimental designs and measurements. Finally, unpublished data will be presented from recent LHTL studies incl. elite athletes and where current recommendations for LHTL were fulfilled.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA22

Physiological Responses and adaptations to exercise in the heat

G. Havenith

Loughborough University, Loughborough, UK

Environmental heat load is a major factor influencing performance. Numerous studies have looked at the impact of different environmental components on athletic functioning. Temperature, Humidity, Solar Radiation and Wind interact as stressors, impacting the athletes' strain and performance. Repeated exposure to heat leads to one of the strongest forms of adaptation known, and reduces strain in the vast majority of exercisers. Various adaptation regimes have been evaluated. More recently, the question has arisen whether heat adaptation also has benefits that extend to exercise in cool conditions. This talk will try to provide an overview of some of these issues.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA23

Physiological and psychological responses and adaptation to cold environments

M.J. Tipton

University of Portsmouth, Portsmouth, UK

Cold water immersion is the biggest killer of sports people undertaking their sport. For example, 80 % of those that die in triathlons do so during the swim. The impact of cold on performance can include: subtle decrements in muscle function and co-ordination; increased substrate utilisation; incapacitation, drowning and sudden cardiac death. Cold affects performance by the stimulation of cold receptors in the skin and by the direct impact of cooling on nerves, muscle and connective tissue as well as the heart and brain.

On entering cold water, sudden cooling of cutaneous cold receptors evokes hyperventilation and tachycardia, components of the "cold shock" response, which is often a precursor to drowning. If the face is also submerged the coincidental activation of cold shock tachycardia and diving response bradycardia can result in "Autonomic Conflict" and, in susceptible individuals, can predispose to sudden cardiac death.

In cold air, freezing and non-freezing cold injury represent potential threats, but seldom to life. Provided an individual is reasonably well clothed and can remain active, hypothermia should not occur. However, if poorly clothed and inactive due to injury or exhaustion, then hypothermia represents a significant threat. Hypothermia affects cellular metabolism, blood flow, fluid shifts and neural activation.

In comparison with heat and altitude, adaptation to cold remains something of a mystery, in part because of the historic ability of individuals to avoid cold exposure and therefore the stimulus to adapt. One sporting group who are repeatedly exposed to cold are the rapidly growing number of open water swimmers, including many triathletes. In theory humans may show *metabolic* (raised basal metabolic rate), *hypothermic* (undefended fall in deep body temperature) or *insulative* (increased body insulation via increased subcutaneous fat or reduced peripheral blood flow) adaptation to cold. Different aboriginal groups have been reported to have all of these types of adaptation. The type of adaptation developed has been suggested to vary with i. the fitness and body morphology of those being adapted (high fitness/low body fat: metabolic adaptation; low fitness: insulative adaptation; Bittel, 1992) and ii. the duration of exposure to cold, with adaptation progressing with time from the metabolic to hypothermic to insulative form (Skreslet & Aarefjord, 1968). The adaptation observed may also vary depending on whether or not exercise is performed during the repeated cold exposures or during the assessment of adaptation (Golden & Tipton, 1998). Typically, outdoor cold water swimmers, both children and adults, tend to demonstrate a hypothermic adaptation to cold when at rest in cold water and an insulative adaptation when exercising in cold water (Bird *et al.* 2015). Repeated exposure to cold increases thermal comfort (Golden & Tipton, 1988); whilst beneficial to performance, this can also be hazardous by disassociating the thermal state of the body from the drive to behaviourally thermoregulate (the most powerful of the thermoregulatory responses); it means that cold adapted individuals are poor at assessing how cold they are.

Responses vary in their lability: the initial response to cold water immersion can be significantly attenuated by a small number of short exposures. The site of this habituation appears to be central to the peripheral cold receptors and once established the habituation lasts, in part, for at least 14 months. Repeated showers are a less effective stimulus for adaptation than repeated whole body (head out) immersions, with the rate of change of skin temperature on immersion apparently being an important determinant of the magnitude and specificity of the adaptation developed. Interestingly, the ability to breath hold on immersion in cold water can be improved by psychological skills training without repeated exposure to cold water (Barwood *et al.* 2007). In contrast, the decrement seen in neuromuscular function with cooling does not tend to improve with repeated exposure.

The metabolic (shivering) response to cooling is often attenuated with repeated cold exposure; this habituation requires falls in deep body temperature and is specific to the deep body temperatures experienced when becoming adapted, with a normal, unadapted metabolic response being evoked if individuals cool themselves more than they are used to (Tipton *et al.* 2013). In contrast, the habituation of the initial response to cold water immersion is not as specific, with habituation to warmer water temperatures (15 °C) providing some habituation to colder temperatures (10 °C). Although different in nature, habituation of both the initial and metabolic responses to cold water improve swimming performance in cold water.

Finally, "cross adaptation" can occur between cold and altitude, with repeated short term cold water exposures improving the responses of individuals at altitude. Evidence suggests that the mechanism for cross adaptation is located in the autonomic nervous system; this area is worthy of further investigation (Lunt *et al.* 2010).

Barwood M, Datta A, Thelwell R & Tipton M (2007). Breath-hold time during cold water immersion: effects of habituation with psychological skills training. *Aviat Space Environ Med* 78(11), 1029–1034.

Bittel J (1992). The different type of general cold adaptation in man. *Int J Sports Med* Suppl 1, 172–176.

Golden FStC & Tipton MJ (1988). Human adaptation to repeated cold immersions. *J Physiol* 396, 349–363.

Lunt HC, Barwood MJ, Corbett J & Tipton MJ (2010). "Cross-adaptation": habituation to short repeated cold-water immersions affects the response to acute hypoxia in humans. *J Physiol* 588 (18), 3605–3613.

Skreslet S & Aarefjord F (1968). Acclimatization to cold in man induced by frequent scuba diving in cold water. *J Appl Physiol* 24(2), 177–181.

Tipton M, Wakabayashi H, Barwood M, Eglin C, Mekjavic I & Taylor NAS (2013). *Habituation of the metabolic and ventilatory responses to cold-water immersion in humans. J Therm Biol* 38(1), 24–31.

Bird F, House JR & Tipton MJ (2015). The physiological response to immersion in cold water and cooling rates during swimming in a group of children aged 10-11 years. *Int J Aquatic Res & Educ* 9: 162-174.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA24

The athletes heart- when is adaptation good, and when is it dangerous?

M. Börjesson

Swedish School of Sport and Health Science, Sweden, Sweden

Secondary to long-term exercise, cardiac adaptations occur. These are mostly physiological adaptations and are considered benign. However, in some cases the adaptation may have negative consequences, or may not only be benign.
The normal physiological cardiac adaptation to long-term exercise is termed "athletes heart", and includes both structural, electrical (autonomic) and functional adaptation. It is considered typical for a competitive athlete in endurance sports, to have sinus bardycardia, sinus arrythmia, AV-block I (or even AV-block II, Type 1), early repolarisation changes as well as QRS-signs of left ventricular hypertrophy (LVH) on standard 12-lead ECG. The structural adaptations, as readily shown by echocardiography, includes larger cardiac dimensions (walls and cavities). The level of adaptation will depend on the age, gender, ethnicity and body size of the athlete, as well as on the type of sport, duration of the sporting career and

on external factors such as doping and drugs. Importantly, the adaptation of the athletes heart is associated with improved systolic and/or diastolic function, and is thus considered to be ”good”.
However, it has been suggested, by Heidbuchel and others, that the adaptation to long-term intensive endurance activity, in some cases may have a negative effect on the right ventricle (RV). Indeed, the RV is submitted to a disproportionate hemodynamic load, compared to the LV, during intense activity. This may lead to transient exercise-induced RV dysfunction and to chronic RV remodelling. Remodelling includes fibrosis and could in turn, lead to increased risk of ventricular arrhythmias. Importantly, a standard resting-echocardiography, focusing on the LV, will miss these changes. The RV function is most important, and may possibly be best evaluated during exercise, by either cardiac MRI and/or echocardiography.
Furthermore, physiological adaptation to exercise may be dangerous, when it is added to an existing underlying disease. For example, in patients with hypertrophic cardiomyopathy (HCM) the degree of hypertrophy is a prognostic marker, and is associated with increased risk of malignant arrhythmias and sudden cardiac death (SCD). We know, that medications (beta-blockade) may reduce the level of hypertrophy in HCM, showing that it is possible to influence. Conversely, although the majority of hypertrophy in HCM, is genetically determined, it may be aggravated by the adaptation secondary to sporting activity. When the diagnosis of HCM is made, sporting eligibility is restricted, to reduce the risk of SCD. Another example, is aortic insufficiency (AI), where sporting eligibility applies for all sports, in athletes with mild-moderate AI’s with normal LV size and function and without arrhythmias. However, bradykardia in ”athletes heart” may result in increased regurgitation by lengthening the diastolic duration, as may cardiac dilatation secondary to exercise.
Finally, physiological adaptation to exercise is associated with an increased risk of atrial fibrillation (AF). An overweight, physically inactive patient with hypertension do have a high risk of accompanying AF, much more so than a lean, regularly active individual. However, long-lasting endurance activity, is associated with an increased risk of AF, and at a younger age, than non-athletes. This is attributed to the increase in atrial dimensions as well as the autonomic changes associated with athletes heart. Many athletes with AF, have a low cardiovascular risk profile, but anticoagulation should be considered when necessary, to reduce the risk of cardiovascular complications, such as stroke.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA25

Tendon overuse and development of injury

M. Kjaer

Inst Sports Medicine, Bispebjerg Hospital, Univ of Copenhagen, Denmark, Copenhagen, Denmark

The overall turnover of the tendon in humans seems to be taking primarily within the first 17 years of life, indicating that the basic structure remains relatively unchanged through adult life. Nevertheless, mechanical loading of adult human tendon results in an up-regulation of collagen synthesis and collagen degradation relatively independent upon tendon loading intensity, and indicates a "fine-tuning" of e.g. cross link formation in relation to level of physical activity and accompanies the relatively fast change in tendon mechanical properties with either training or immobilization. Development of tendinopathy is suggested to be coupled to a mismatch between loading and adaptation, and results in pain, palpatory soreness, tendon thickening, rounded cells, disorganized matrix and GAG accumulation, plus angiogenesis. The best documented treatment is controlled strength training exercises, and other treatments have either good but short lasting effect, or minimal to no effect upon the tendinopathy. Although we today have a better grasp on the different theories behind development of tendinopathy, there are still many challenges, such as a mismatch between symptoms and imaging findings (e.g. flow), a mismatch between tissue pathology and perceived pain, and a mismatch between general tendon tissue changes and specific regional presentations, differential locations and variation in patient characteristics.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA26

The ageing athlete

S.D. Harridge and N. Lazarus

Centre of Human & Aerospace Physiological Sciences, King's College London, London, UK

The demographics of the population are changing such that life expectancy is progressively rising. However, this is not being accompanied by equivalent years of healthy living (the health span). Whilst there are growing concerns over the effects of physical inactivity in much of the older population, there are also increasing numbers of older individuals undertaking high levels of physical exercise, with many actively involved in competitive sports. These individuals, often referred to

as “veteran” or “master” athletes, are of interest from a number of physiological perspectives. AV Hill once said noted much could be learned about human physiology from analysis of athletic records, perhaps because performance in athletic competitions is arguably the greatest test of physiological integration. This can also be applied to the study of the physiology of ageing where analyses of world records have been undertaken to target the trajectory of declines in performances as athletes get older. An overview of these analyses suggests an essentially linear decline in performance with increasing age until the eighth decade after which there appears to be an accelerated decline. This “breakpoint” is interesting. It may simply reflect a progressively smaller population base of older athletes, or is possibly reflective of a breakdown in integration of physiological systems. Another perspective from which the study of master athletes is important arises from a more fundamental biological standpoint about human ageing. The ageing process is generally considered to be characterised by decrements in size, functionality and adaptability of different tissues and cells. This is reflected in declines in whole body physical function and an increased susceptibility to disease and risk of frailty. Yet, a unifying theory of ageing remains elusive. Furthermore, when it comes to integrative human physiology and function we lack clarity as to the trajectory of decline that might be attributed to an underlying or inherent ageing process – i.e. that is not influenced by other lifestyle factors. This is partly as a result of fundamental limitations to cross-sectional studies *per se*, but also by the vagaries of inclusion criteria for studies of human ageing. In the context of our hunter- gatherer evolutionary heritage, where levels of physical activity levels were believed to be much higher than those currently undertaken by most of the population, it can be argued that master athletes provide the most appropriate model in whom to study the biology of human ageing. A decline in function in an exerciser over time should represent the effects of the biological ageing process, as opposed to a decline in a sedentary person whose functional decline would represent an interaction of the inherent ageing process and the pernicious effects of inactivity and other negative lifestyle factors. This presentation will consider both the remarkable performances of master athletes as well as how these individuals contribute our understanding of the physiology of human ageing.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

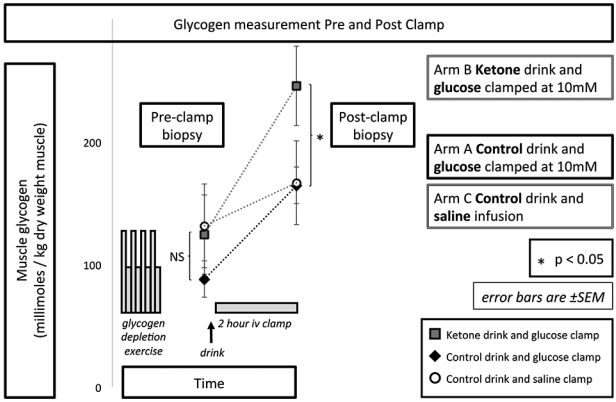
C01

Oral ketone body supplementation accelerates and enhances glycogen synthesis in human skeletal muscle following exhaustive exercise

D.A. Holdsworth, P.J. Cox and K. Clarke

Department of Physiology Anatomy and Genetics, University of Oxford, Oxford, UK

PURPOSE: Physical endurance is limited by muscle carbohydrate stores (glycogen)[1]. Glycogen depletion dramatically reduces external work[2]. Ketone bodies (D-β-hydroxybutyrate and acetoacetate) are natural 4-carbon molecules synthesised in the fasted liver. Animal studies show that ketones increase glycogen synthesis in the presence of carbohydrate[3,4]. The study hypothesis is that ketone supplementation augments replenishment of glycogen stores in man, beyond that achieved by optimum carbohydrate provision alone.
METHODS: 12 well-trained British Forces servicemen underwent a validated interval protocol to deplete muscle glycogen. They were randomised to control drink + intravenous carbohydrate (Cont-CHO), ketone drink + intravenous carbohydrate (Ket-CHO) or control drink + intravenous saline (Cont-saline) in a randomised, blinded, crossover study. Carbohydrate was delivered by standardised 2-hour 10mM glucose - hyperglcaemic clamp. Glycogen was measured in muscle biopsies (pre and post feeding).
RESULTS: Ketone supplementation achieved a D-β-hydroxybutyrate of 5.3(±0.48) vs. 0.4(±0.1) mmol/L with Cont-CHO condition. There was an associated 33% increase in whole body glucose disposal 125.8(±4.2) vs. 94.7(±3.2) g, for Ket-CHO vs Cont-CHO ($p<0.001$). Muscle glycogen increased significantly 246(±32.4) vs 164(±12.5) mmol glycosyl units/kg dry weight of muscle ($p=0.017$) and insulin levels doubled: 31.1(±5.7) vs 16.4(±2.7) mU/L ($p<0.01$) for Ket-CHO vs Cont-CHO.
CONCLUSIONS: There is increased glucose uptake and higher muscle glycogen deposition following ketone supplementation in man, beyond levels achievable by 'gold-standard' high-dose intravenous glucose infusion alone. The additional novel finding of a doubling of endogenous insulin has significant implications for the augmentation of exercise recovery and anabolic metabolism.



Bergström, J. and E. Hultman, *Muscle glycogen synthesis after exercise: an enhancing factor localized to the muscle cells in man.* Nature, 1966. **210**(5033): p. 309–10.

Bergström, J. and E. Hultman, *A study of the glycogen metabolism during exercise in man.* Scand J Clin Lab Invest, 1967. **19**(3): p. 218–28.

Maizels, E.Z., et al., *Effect of acetoacetate on glucose metabolism in the soleus and extensor digitorum longus muscles of the rat.* Biochem J, 1977. **162**(3): p. 557–68.

Laughlin, M.R., et al., *Nonglucose substrates increase glycogen synthesis in vivo in dog heart.* Am J Physiol, 1994. **267**(1 Pt 2): p. H219-23.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C02

Nitrate supplementation and peroxisome proliferator-activated receptor alpha knockout in hypoxia: Effects on skeletal muscle mitochondrial function

K.A. O'Brien[1], J.A. Horscroft[2], L.M. Edwards[1], S.D. Harridge[1] and A.J. Murray[2]

[1]Centre of Human and Aerospace Physiolocial Sciences, King's College London, London, UK and [2]Department of Physiology, Development and Nueroscience, University of Cambridge, Cambridge, UK

Oxygen insufficiency (hypoxia) is a condition prevalent amongst a range of diseased populations. Furthering our understanding of hypoxic acclimation and of potential methods to alleviate hypoxic stress is thus highly clinically relevant. A controversial aspect of acclimation is skeletal muscle metabolic remodelling, a process that may be aided by nitrate supplementation. Mechanisms of nitrate action have been shown to involve interaction with a master regulator of fat metabolism, peroxisome proliferator-activated receptor alpha (PPARα) [1]. In this study, the potential for nitrate to aid hypoxic acclimatisation in skeletal muscle (soleus) and the role of PPARα in this

process were investigated. Wild type (WT) (n=42) and PPARα knockout (KO) (n=42) mice from a SVEV129 genetic background received either nitrate or chloride supplementation via drinking water for a week prior to and during exposure to either 4 weeks of hypoxia (10% oxygen) or normoxia. Mice were then killed by dislocation of the neck and mitochondrial respiration was subsequently assessed in permeabilised soleus muscle using a Clark-Type oxygen electrode. Data was analysed using a three way ANOVA in order to assess effects of all 3 parameters (hypoxia, nitrate, PPARα), with significant interactions between parameters being investigated further using a post hoc Tukeys test. Hypoxia induced a suppression of mitochondrial function, including a decrease in mass corrected fatty acid LEAK state respiration (no addition of ATP, a non-phosphorylating resting state, normoxic chloride vs hypoxic chloride WT: 22.9±3.5 vs.15.1±4 pmolsO_2/sec/mg, ±SD, P<0.001) and carbohydrate oxidative phosphorylation (OXPHOS) capacity (with addition of ATP, normoxic chloride vs. hypoxic chloride WT: 50.6±4.2 vs. 35.9±8.3 pmolsO_2/sec/mg). These significant decreases were lost in nitrate supplemented mice, indicating a nitrate dependent recovery of mitochondrial function. A nitrate effect was observed in both WT and KO, suggesting it can act independently of any effects on PPARα. Nitrate supplementation failed to recover hypoxic induced suppression of fatty acid or mitochondrial complex 2 OXPHOS capacity. Our results confirm previous reports of hypoxia suppressing skeletal muscle mitochondrial function [2]. In addition, they support the notion that nitrate supplementation may aid hypoxic acclimation by partially recovering this suppression. In conclusion, our results indicate that nitrate can exert effects upon skeletal muscle metabolism in hypoxia independently of PPARα. Although mechanisms remain unclear, this effect may occur though improvements in skeletal muscle blood flow and/or activation of PPARβ/δ.

Ashmore, T., et al., *Nitrate enhances skeletal muscle fatty acid oxidation via a nitric oxide-cGMP-PPAR-mediated mechanism.* BMC Biology, 2015. **13**(1): p. 1–17.

Horscroft, J.A. and A.J. Murray, *Skeletal muscle energy metabolism in environmental hypoxia: climbing towards consensus.* Extreme physiology & medicine, 2014. **3**(1): p. 19.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C03

Transcranial direct current stimulation improves cycling performance in healthy individuals

L. Angius, S. Marcora, J. Hopker and L. Mauger

School of Sport and Exercise Sciences, University of Kent, Chatham, UK

Central motor command originating from motor and premotors areas have been shown to correlate with the intensity of perception of effort (RPE) (1). Recently, non-invasive brain stimulation techniques able to change excitability of targeted area have been shown to improve exercise capacity on single joint exercise (2) and

to alter perception of effort (3). In the present study we monitored whether stimulation of both motor cortexes can alter perception of effort and exercise capacity of whole body cycling exercise.

Twelve healthy volunteers were recruited and underwent a placebo (SHAM), anodal tDCS (ANODAL) and cathodal tDCS (CATHODAL) condition in a double-blind, randomised and counterbalanced experimental design. tDCS stimulation was delivered for 10 min at 2.0 mA by using two extracephalic montages with the active electrode placed over the motor cortex and the reference electrode over the shoulder. Neuromuscular assessment was performed before and after tDCS stimulation to monitor central and peripheral parameters. This consisted on a maximal voluntary contraction (MVC) of knee extensor muscles with superimposed doublet followed by a resting potentiated doublet. Then, four brief submaximal contractions at 10% MVC with superimposed transcranial magnetic stimulation and one at 10% MVC with superimposed femoral nerve stimulation were executed. Volunteers then underwent a cycling time to exhaustion (TTE) at 70% of peak power output previously assessed. Heart rate (HR), ratings of perceived exertion (RPE) and leg muscle pain (PAIN) were monitored during the TTE while blood lactate (BLa-) was measured immediately after the TTE.

TTE was significantly longer in the ANODAL ($P=0.003$) compared to the CATHODAL and SHAM conditions (13.24 ± 4.34 min; 11.1 ± 4.28 min; 10.75 ± 3.03 min). A significant reduction of RPE ($P<0.001$) and higher increase of BLa^- ($P<0.001$) were found in the ANODAL condition. No differences were found for HR ($P=0.80$) and PAIN between conditions ($P=0.27$) (Fig. 1).

MVC, voluntary activation level (VAL) and doublet were not affected by tDCS stimulation. However, an increase in cortical excitability was found following ANODAL tDCS as demonstrated by the increased motor evoked potential (MEP_{area}/M_{wave} ratio) response (Fig 2). None of the monitored parameters were significantly affected in the SHAM and CATHODAL conditions. This experiments demonstrated that ANODAL tDCS stimulation improves constant cycling performance. Moreover, the increased excitability of the motor cortex might facilitate the central command required and consequently reduced the perception of effort during exercise. These findings further demonstrate that the motor cortex plays an important role in the generation of perception of effort.

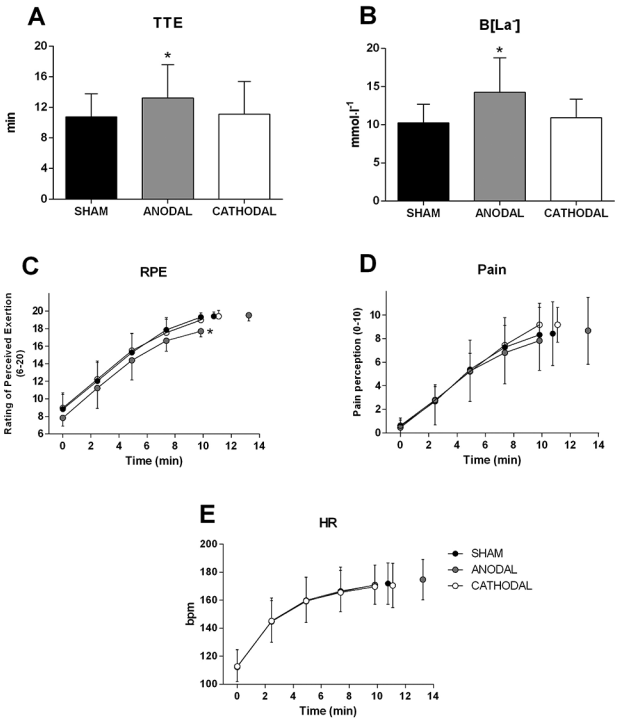


Fig 1. Panel A shows time to exhaustion (TTE) performance. Panel B shows blood lactate accumulation (BLa-) values. Time courses of rating of perceived exertion (RPE), pain perception (PAIN) and heart rate (HR) are shown in panel C, D and E. * P<0.05, denotes significant difference from CATHODAL and SHAM conditions. Data presented as mean ± SD (n=12).

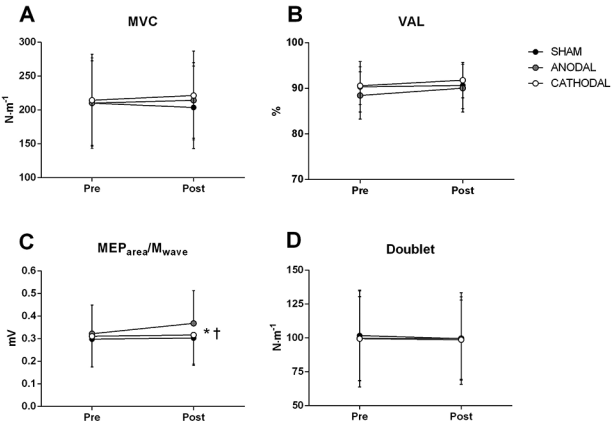


Fig 2. Panel A shows maximal voluntary contraction (MVC); Panel B shows voluntary activation level (VAL); Panel C shows motor evoked potential area (MEP_{area}) muscular wave (M_{wave}) MEP_{area}/M_{wave} ratio. Panel D shows peak torque of the doublet (Doublet); * P<0.05, denotes significant difference from CATHODAL and SHAM; † P<0.05, denotes significant condition × time interaction. Data are presented as mean ± SD (n=12).

de Morree HM, Klein C, Marcora SM. Perception of effort reflects central motor command during movement execution. Psychophysiology. 2012 Sep;49(9):1242–53.

Cogiamanian F, Marceglia S, Ardolino G, Barbieri S, Priori A. Improved isometric force endurance after transcranial direct current stimulation over the human motor cortical areas. Eur J Neurosci. 2007 Jul;26(1):242–9.

Takarada Y, Mima T, Abe M, Nakatsuka M, Taira M. Inhibition of the primary motor cortex can alter one's 'sense of effort': effects of low-frequency rTMS. Neurosci Res. 2014 Dec;89:54–60.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C04

Repetition-load and systemic hormone concentrations do not determine resistance training-mediated adaptations in trained young men

R. Morton[1], S. Oikawa[1], N. Mazara[1], C. Wavell[1], C. McGlory[1], J. Quadrilatero[2] and S. Phillips[1]

[1]Kinesiology, McMaster University, Hamilton, ON, Canada and [2]Biology, University of Waterloo, Waterloo, ON, Canada

We have previously showed that performing resistance exercise (RE) to volitional fatigue, regardless of load, results in similar increases in skeletal muscle mass, fibre size and strength. However, these studies were performed in RE-naïve trainees using unilateral leg exercises. Here we aimed to determine whether the same

was true in resistance trained (RT) young men using a whole-body RE regimen. We also aimed to address if there were any associations between post-RE systemic hormone concentrations with changes in skeletal muscle hypertrophy and strength. Forty-nine RT men (mean ± SEM, 23 ± 1 y, 86 ± 2 kg, 181 ± 1 cm) were randomly allocated into a high-repetition-low-load group (HR; 30-50% 1RM: 20-25 repetitions/set, n=24) or a low-repetition-high-load group (LR; 70-90% 1RM: 8-12 repetitions/set, n=25) and performed 12 wk of whole-body RE. Skeletal muscle biopsies, one repetition-maximums, dual-energy X-ray absorptiometry and blood draws in response to an acute bout of HR and LR were all evaluated pre- and post-intervention. Groups were matched at baseline for age, lean body mass, training experience, and strength for leg press, bench press, knee extension and shoulder press ($p > 0.05$). In response to the 12 wk intervention, muscular strength increased for all exercises in both groups ($p < 0.01$), with change in bench press significantly greater in the LR group (HR; 9 ± 1, LR; 14 ±1 kg, $p < 0.05$). Lean body mass, type I and type II muscle fibre cross sectional area increased following training ($p < 0.01$) with no significant differences between groups and no change in fibre type distribution. All hormones (cortisol, free testosterone, total testosterone, dihydrotestosterone, dehydroepiandrosterone, luteinizing hormone, free insulin-like growth factor 1, total insulin-like growth factor 1 and growth hormone) increased as a result of an acute bout of RE ($p < 0.001$). Bivariate correlations revealed that no hormone at any time point was significantly correlated with the change in hypertrophy or strength. A stepwise multiple linear regression model revealed that three hormones accounted for a significant proportion of variance related to the intervention-induced change in hypertrophy or strength. Pre-intervention, the post-RE AUC for free insulin-like growth factor 1 and cortisol explained 15% and 12% of the change in leg press and type 2 cross sectional area, respectively. Post-intervention, the post-RE AUC of cortisol explained 9% of the change in type 2 CSA. These data show that when RE is performed to volitional fatigue neither repetition-load nor post-RE systemic hormone concentrations are significant determinants of gains in strength or hypertrophy in RT individuals.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C05

Beta-adrenergic agonist, Ractopamine, increases skeletal muscle expression of enzymes involved in the biosynthesis of anabolic intermediates

D. Brown[1], H. Williams[1,2], K. Ryan[1,3], T. Wilson[4], D. Harris[4], M. Mareko[1,5], Z. Daniel[1], S. Jones[1], R. Emes[6], J. Wattis[2], I. Dryden[2], C. Hodgman[1], T. Parr[1] and J. Brameld[1]

[1]School of Biosciences, University of Nottingham, Nottingham, UK, [2]Mathematical Sciences, University of Nottingham, Nottingham, UK, [3]Guys Hospital, London, UK, [4]VMRD Global Therapeutics, Zoetis, Kalamazoo, MI, USA, [5]Botswana College of Agriculture, Gaborone, Botswana and [6]Veterinary Medicine and Science, University of Nottingham, Nottingham, UK

Manipulation of muscle mass has implications for sporting performance as well as potential health benefits. This study sought to investigate the mechanisms by which Beta-adrenergic agonists (BA) and Growth Hormone (GH) mediate their muscle hypertrophy effects, through the examination of changes in the skeletal muscle transcriptome during treatment of pigs with these anabolic agents over a 27 day timecourse. Female pigs (85 kg) were all fed a high protein/ energy diet *ad-libitum,* with the GH group receiving an intramuscular injection of porcine GH (10mg, Reporcin, Zamira, Australia) every other day, the BA group receiving Ractopamine (Eli Lilly, USA) at 20mg/kg feed, and the control group (C) simply had *ad-libitum* feed. Pigs were killed by exsanguination (following electrical stunning) after 1, 3, 7, 13 and 27 days of treatment (n=10 per treatment per time point, n=15 on day 27) and samples of the *Longissimus dorsi* (LD) muscle were collected. All animal work was done in accordance with UK Home Office regulations. The effect of treatments on the LD transcriptome was assessed using the Agilent pig microarray followed by gene cluster analysis using a modified maSigPro methodology (Conesa et al., 2006). Verification of differentially expressed genes and proteins in LD was by quantitative RT-PCR and western blotting, respectively and assessed by two-way or one-way ANOVA, respectively. BA, but not GH, significantly ($P<0.05$) increased muscle weights (*semitendinosis*: BA 529±63g vs GH 498±95g vs C 474±67g) and induced a switch to faster muscle fibre types, with expression of MyHC IIB (*myh4*) and MyHC IIA (*myh2*) mRNA being up and down-regulated respectively (Fig. 1A and 1B). MaSigPro clustering of microarray data revealed extensive coordinated up-regulation of genes involved in the serine synthesis pathway, phosphoglycerate dehydrogenase (*Phgdh*), phosphoserine-aminotransferase (*Psat1*) and phosphoserine phosphatase (*Psph*), by BA and to a lesser extent GH ($P<0.001$; Fig. 1C-E). This was accompanied by elevated PHGDH protein at days 3 and 7 ($P<0.05$; Fig. 1G and 1H). In addition, clusters included mitochondrial phosphoenol pyruvate carboxykinase (PEPCK-M) transcripts (*Pck2*), which was similarly increased by BA treatment ($P<0.001$; Fig. 1F), and there was a 2-fold increase in PEPCK-M protein at day 7 ($P<0.001$; Fig. 1H).

BA treated pigs exhibit increased expression of PHGDH and PEPCK-M in skeletal muscle undergoing accelerated growth. These enzymes are known to regulate flux of glucose carbons into biosynthetic pathways for the generation of anabolic intermediates (Vincent et al., 2015), particularly in cancer cells. We are now exploring the role of PHGDH and PEPCK-M in modulating a muscle anabolic response.

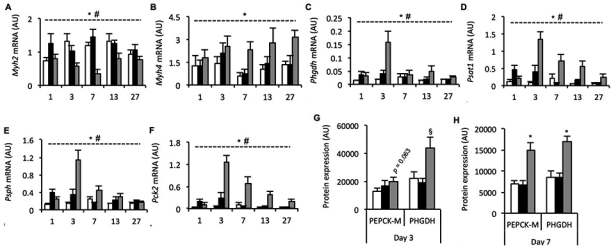


Fig. 1. Effects of beta-adrenergic agonist (BA) and Growth Hormone (GH) treatment for 1, 3, 7, 13 or 27 days on gene and protein expression in LD muscle (relative to control cohort (C)). A-F show mRNA expression of A. MyHC IIA (*myh2*); B. MyHC IIB (*myh4*); C. *Phgdh*; D. *Psat1*; E. *Psph*; and F. *Pck2*. G and H show protein expression of PEPCK-M and PHGDH in LD muscle on days 3 and 7 respectively. * P<0.001 for treatment. # P<0.001 for treatment-time interaction. § P<0.05 for treatment.

Conesa A et al. (2006) *Bioinformatics* **22**, 1096–1102.

Vincent EE et al. (2015) *Molecular Cell* **60**, 195–207.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C06

The influence of sex on the skeletal muscle gene expression response to sprint interval exercise

L.E. Skelly[1], M.J. MacInnis[1], A. Safdar[2], M. Akhtar[2], M.A. Tarnopolsky[1,2] and M.J. Gibala[1]

[1]Kinesiology, McMaster University, Hamilton, ON, Canada and [2]Pediatrics and Medicine, McMaster University, Hamilton, ON, Canada

Men have been reported to experience greater increases in muscle protein synthesis and mitochondrial biogenesis, and indices of glycemic control, after several weeks of sprint interval training (SIT) as compared to women. The potential impact of sex on the skeletal muscle response to an acute session of SIT has not been previously examined and could provide a potential mechanistic framework to explain potential divergent training adaptations. The present study examined the acute response of genes involved in skeletal muscle metabolism and structural remodelling to a single session of SIT in men and women matched for initial fitness [n = 8/8, age = 23±4/22±3 y, peak aerobic capacity (VO_2peak) = 45±6/45±10 ml/kg fat

free mass/min]. None of the women were using oral contraceptives and all were tested in the mid-follicular phase of their menstrual cycles (day 9±2). Subjects completed a session of SIT consisting of 3 x 20-sec all-out cycling efforts against a resistance of 5% body mass, interspersed with 2 min of recovery. Biopsies from m. vastus lateralis were obtained before and immediately and 3 h after exercise. Gene expression was determined using real-time quantitative polymerase chain reaction and analyzed using a two-factor ANOVA (sex, time). Women had higher expression of hexokinase II (*HK2*) and lower expression of forkhead box O3 (*FOXO3*) compared to men (main effects for sex, $p<0.05$). Exercise increased the mRNA expression of *FOXO3*, *HK2*, hormone sensitive lipase (*LIPE*), muscle ring-finger protein-1 (*TRIM63*), myogenic differentiation 1 (*MYOD1*), peroxisome proliferator-activated receptor γ coactivator 1α (*PPARGC1A*), pyruvate dehydrogenase kinase isozyme 4 (*PDK4*) and vascular endothelial growth factor A (*VEGFA*) at 3 h vs rest (all main effects, $p<0.05$). Women had lower expression of glucose transporter 4 (*SLC2A4*) at rest but showed an exercise-induced increase in the expression of *SLC2A4* at 3 h. Exercise also increased the expression of lipoprotein lipase (*LPL*) after 3 h of recovery in women only ($p<0.05$); whereas, the exercise-induced increase in the expression of Atrogin-1 (*FBXO32*) was greater in men vs women ($p<0.05$). There was no effect of exercise or sex on the expression of glycogen synthase kinase-3α (*GSK3A*) or insulin-like growth factor (*IGF1*) ($p>0.05$). In summary, the gene expression response to an acute bout of SIT was generally similar between men and women. The metabolic basis for reported sex-based differences in specific training responses remains to be elucidated. These data also demonstrate that as little as 1 min of intense intermittent exercise alters the expression of genes involved in skeletal muscle metabolism and structural remodeling.

Supported by NSERC, Canada.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C07

Impact of intravenous iron on systolic pulmonary arterial pressure and exercise capacity in older individuals

H. Cheng, M.C. Frise, M.K. Curtis, N.K. Bart, N. Petousi, P. Robbins and K.L. Dorrington

Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK

OBJECTIVES: An increase with age in pulmonary arterial pressure (PAP) during exercise has been shown, but the extent to which this limits exercise capacity in older people remains unclear. One possibility is that exercise-induced hypoxic pulmonary vasoconstriction (HPV) can limit exercise capacity. It is known that intravenous iron blunts HPV. The aim of this study was to examine the hypothesis that intravenous iron abrogates increased PAP during exercise in older individuals. Furthermore, we assessed whether this would have an impact on maximal exercise capacity.

METHODS: Thirty-two volunteers aged between 50 and 80 years with no or mild cardiovascular or respiratory diseases were studied in a double-blind, block-randomized, placebo-controlled protocol with 5 follow-ups. Sixteen volunteers received a 50-ml intravenous iron injection (15 mg/kg; maximum dose: 1 g) as ferric carboxymaltose. The other 16 volunteers were given a 50-ml saline injection as placebo. Systolic pulmonary artery pressure (SPAP), stroke volume (SV) and cardiac output (CO) and were assessed during rest and light exercise (a rise in heart rate of 30 bpm) by Doppler echocardiography. Maximal exercise capacity was determined by peak oxygen consumption (VO2peak) and peak work rate on a cycle ergometer. Heart rate (HR) and oxyhaemoglobin saturation (SpO_2) were also measured during exercise. Haemoglobin (Hgb), erythropoietin (EPO), serum iron, ferritin, soluble transferrin receptor (sTfR) concentrations were tracked from blood samples. All volunteers completed the exercise assessments and gave blood samples before the injection, and at 4 hours, 23 hours, 7 days, 4 weeks and 8 weeks.
RESULTS: A difference in serum iron concentration between groups was present after the injection and persisted for 7 days. Ferritin and sTfR concentrations rose and decreased respectively in the iron group at 23 hours after the injection and these changes persisted for the following 8 weeks. A fall in EPO concentration following iron injection was observed at 23 hours and 7 days. In contrast, Hgb concentration did not show a difference between groups throughout the study.
Linear mixed-effects modelling demonstrated that prior iron infusion attenuated the rise in SPAP in response to exercise for a period of at least 8 weeks, the end point of the study ($p = 0.002$). There were no significant differences between groups demonstrated in SV and CO throughout the follow-ups. VO2peak and peak work rate, as well as HR and SpO_2 at the peak exercise level, showed no differences between groups in the study.
CONCLUSIONS: Our findings demonstrate that SPAP during exercise in elderly people is reduced by iron infusion. However, decreasing SPAP during exercise by intravenous iron injection does not affect exercise capacity in this group.

This study was funded by The Dunhill Medical Trust.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C08

Vagal tone and exercise capacity

A. Machhada[1], G. Ackland[1], S. Kasparov[2] and A.V. Gourine[1]

[1]Neuroscience, Physiology and Pharmacology, University College London, London, UK and [2]School of Physiology and Pharmacology, University of Bristol, Bristol, UK

Background: Higher baroreflex sensitivity, enhanced high frequency component of heart rate variability and a faster heart rate recovery with cessation of exercise

in elite athletes suggests plasticity in the central nervous mechanisms that control the heart. This experimental study was designed to directly test the hypothesis that the strength of parasympathetic tone determines exercise capacity. We hypothesised that vagal withdrawal should decrease and vagal recruitment should enhance exercise capacity. We targeted vagal preganglionic neurones of the dorsal motor nucleus of the vagus nerve (DVMN) which provide functional innervation of the left cardiac ventricle (Machhada et al., 2015; 2016).

Methods: In male Sprague-Dawley rats (380-420 g), DVMN neurones were transduced to express an inhibitory G_i-protein-coupled *Drosophila* allatostatin receptor (AlstR) (n=8) or green fluorescent protein (GFP) as a control (n=8). Application of the insect peptide ligand allatostatin (5 μl) produces rapid, selective inhibition of targeted neurones. A pharmacological study investigated the role of muscarinic and neuronal nitric oxide-mediated mechanisms using systemic treatment with atropine methyl nitrate (2 mg/kg, i.p., n=5) or selective neuronal NO synthase inhibitor 7-nitroindazole (7-NI) (30 mg/kg, i.p., n=8). For optogenetic activation, DVMN neurones were targeted to express an optogenetic construct ChiEF (n=9) or control transgene GFP (n=10) and stimulated with blue laser light (445 nm, 10 ms pulses, 15 Hz, 15 min). Exercise capacity was determined using a treadmill with a shock grid set at the minimum of 0.1 mA. Rats were preselected for their compliance after a three day recruitment protocol and randomized. The experimental protocol involved starting speeds of 20-30 cm/s over 5 min after 15 min acclimatisation. Speeds were then raised in 5 cm/s increments every 5 min until the hind limbs made grid contact four times within a 2 min period. The calculated work (Joules, J) was used as an index of exercise capacity.

Results: Acute inhibition of the DVMN neurones by allatostatin resulted in a dramatic reduction in exercise capacity (8±2 vs 202±27 J; $p<0.0001$; ANOVA). In rats given atropine and vehicle no significant differences in exercise capacity were observed (113±20 vs 112±22 J, $p=0.9$; t-test). Systemic administration of 7-NI was associated with a significant reduction in exercise capacity (33±19 vs 129±19 J, $p=0.0002$; t-test), as did 4 h of atropine treatment (63±12 vs 116±20 J, $p=0.0019$; t-test). Rats expressing ChIEF by the DVMN neurones displayed a significantly higher exercise capacity 4 days following optogenetic stimulation (94±11 vs 47±6 J; $p=0.002$; ANOVA). Improvements were similar to that observed in naïve rats trained to exhaustion over the same period (105±16 vs 47±6 J in rats expressing GFP; $p<0.0001$; ANOVA).

Conclusion: These results suggest that the strength of parasympathetic tone determines exercise capacity.

Machhada A, Ang R, Ackland GL, Ninkina N, Buchman VL, Lythgoe MF, Trapp S, Tinker A, Marina N & Gourine AV. (2015). Control of ventricular excitability by neurons of the dorsal motor nucleus of the vagus nerve. *Heart Rhythm* 12, 2285–93.

Machhada A, Marina N, Stuckey DJ, Lythgoe MF, & Gourine AV. (2016). Origins of the vagal drive controlling left ventricular contractility. *The Journal of Physiology* In Press.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C09

c-Met affects the number of type 2 fibres in mouse soleus

A. Lionikas[1], G. Riboni Verri[1], M.N. Podaru[1], N. George[1], S.I. Wenham[1], P.N. Hamond[1], J. Nicod[2] and D.A. Blizard[3]

[1]School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, UK, [2]Wellcome Trust Centre for Human Genetics, Oxford, UK and [3]Department of Biobehavioral Health, The Pennsylvania State University, University Park, PA, USA

Skeletal muscle is characterized by extensive individual variability; muscle mass can range between 35 and 50 kg in healthy young males (Wolfe 2006). This variability is a function of the difference in the cross-sectional area (CSA) of muscle fibres and/or their number; the latter can also differ 2-fold (MacDougall et al. 1984). Approximately half of the variability in muscle properties is due to poorly understood genetic factors (Silventoinen et al. 2008). Elucidation of the underlying genetics will help understand the mechanisms limiting athletic performance and adaptation to training.

The laboratory mouse also exhibits extensive variability in muscle properties (Lionikas et al. 2013) providing a useful research model. We employed a genome wide association study (GWAS) strategy to search for genes affecting muscle mass in CFW outbred mice. Mass (wet weight) of 4 selected muscles varied 2-fold in a population of ≈2,000 CFW mice. DNA for genotyping was extracted from tail biopsies collected after sacrifice. Twenty two quantitative trait loci (QTL) affecting this variability were mapped in the GWAS analysis (Nicod et al. submitted). A QTL on mouse chromosome 6 harboured only one gene which coded for c-Met protein. An Arg968Cys polymorphism present in CFW mice is likely to affect function of the protein and hence is a plausible basis for the QTL.

The aim of the present study was to examine the effect of the Arg968Cys polymorphism on the properties of muscle fibres in selected CFW carriers of the Arg (n=26) or Cys (n=25) allele. The number and CSA of type 1 and type 2 fibres have been assessed in soleus muscle following ATPase staining. A 2-way ANOVA (sex (28 males, 23 females) and allele (Cys or Arg)) was used for statistical analysis.

The main finding was that carriers of the Arg allele exhibit a significant increase ($p<0.001$) in type 2 fibres compared to those carrying Cys. The number of type 1 fibres was not allele-dependent ($p=0.64$). As the result, proportion of type 1 fibres is greater ($p<0.01$) in the Cys allele carriers.

It has been known that c-Met plays a critical role in embryonic development of skeletal muscle. The present study reveals that variants of this gene can contribute to the differences in muscle mass observed *in vivo*. Furthermore, it indicates that the effect is primarily caused by the influence on the type 2 fibres. Fibre-type specific effects of c-Met help understand the role of genetic variability on muscle properties relevant to performance in athletic events and adaptation to training.

Exploration of the remaining QTLs will further expand our understanding of the role of genetic mechanisms in determining muscle mass and function.

Lionikas A et al. (2013) J Anat. 223(3):289-96.

MacDougall JD et al. (1984) J Appl Physiol Respir Environ Exerc Physiol. 57(5):1399-403.

Nicod J et al (Manuscript under consideration) Nat Genet.

Wolfe RR. Am J Clin Nutr (2006) 84:475-82.

Silventoinen K et al. (2008) Genet Epidemiol. 32(4):341-9.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C10

Regulation of skeletal muscle mass and metabolism by the vitamin D receptor: evidence derived from pre-clinical in vivo studies

J.J. Bass[1], N.J. Szewczyk[1], D.J. Wilkinson[1], K. Smith[1], M. Cleasby[2] and P.J. Atherton[1]

[1]The University of Nottingham, Derby, UK and [2]Dept. of Comparative Biomedical Sciences, Royal Veterinary College, London, UK

Vitamin-D (VitD) is proposed to have actions upon skeletal muscle, with supplementation in athletes being shown to improve muscle function, enhance muscle fibre cross sectional area (1), and mitochondrial respiration in human skeletal muscle cells (2). Further to this, higher serum VitD levels have been shown to immediately increase post-exercise, and are associated with improved muscle recovery (3). Additionally, epidemiological studies have linked low serum VitD to impaired skeletal muscle mass/function, and metabolic dysfunction. VitD is known to auto-regulate and act through the vitamin-D receptor (VDR), with VDR expression confirmed in muscle (4). Moreover, in response to resistance exercise, expression of the VDR has been shown to increase in skeletal muscle (5) and this been linked to muscle regeneration and remodeling (4). However, there is currently no defined mechanistic link between VitD and muscle mass/metabolism. In this study, we hypothesized that the VDR has a mechanistic role within the regulation of skeletal muscle mass and metabolism.

To probe the mechanistic and muscle-cell autonomous role of the VDR, *Tibialis anterior* (TA) muscle of Wistar rats were electroporated (under 2.5% isofluorane) to constitutively over-express (VDR-OE) or under-express (VDR-KD) VDR by cDNA and shRNA lenti-viral mediated transfection; contralateral legs were sham treated to act as internal controls. All rats were given carprofen (50 mg/kg) after electroporation and humanely killed before muscles harvested for analysis. VDR-OE yielded myofibre hypertrophy (cross-sectional area (CSA) +17±7%, $P<0.05$) and increased protein content (+57±12%, $P<0.01$) compared to contralateral controls. VDR-OE increased both myofibrillar (+44%±12%,$P<0.05$) and sarcoplasmic

(+60%±20%, *P*<0.01) protein synthesis, with corresponding increases in multiple anabolic signalling protein activities and abundances e.g. mTOR (+93%±30%, *P*<0.05), RPS6 (+71%±20%, *P*<0.05) and 4E-BP1 (+57%±21%, *P*<0.05). Increases in gene expression of several ribosomal proteins namely, RPS11 (+43%±21%, *P*<0.05), RPS13 (+54%±36%, *P*<0.05) and RPS28 (+79%±24%, *P*<0.05) were shown within VDR-OE muscles compared to controls, matching observed increases in total RNA content (+38%±11%, *P*<0.01, normalised to protein content). Parallel experiments of VDR-KD revealed contrasting effects to VDR-OE: reductions in myofibre CSA (~8±2%, *P*<0.001) and protein content (~28±16%, *P*<0.05) compared to sham controls in tandem to markers of increasing autophagy, e.g. protein expression of Cathepsin L (+72±29%, *P*<0.05) and LC3B-II (+84±43%, *P*<0.05).

The VDR plays a positive role in skeletal muscle mass and metabolism, increasing anabolic capacity, resulting in hypertrophy; in contrast, loss of VDR induces atrophy by up-regulation of autophagy. Our findings are the first to define mechanistic links between the VDR and modulation of *in vivo* muscle mass and metabolism, perhaps explaining effects of VitD deficiency and supplementation.

Agergaard J, Trøstrup J, Uth J, Iversen JV, Boesen A, Andersen JL, et al. (2015) Nutrition & Metabolism;12(1):32.

Ryan ZC, Craig TA, Folmes CD, Wang X, Lanza IR, Schaible NS, et al. (2015) J Biol Chem.;25:jbc.M115.684399

Barker T, Henriksen V, Martins T, Hill H, Kjeldsberg C, Schneider E, et al.(2013). Nutrients. 5(4):1253–75.

Srikuea R, Zhang X, Park-Sarge O-K, Esser K a. (2012) Am J Physiol Cell Physiol. Aug;303(4):C396–405.

Makanae Y, Ogasawara R, Sato K, Takamura Y, Matsutani K, Kido K, et al. (2015) Exp Physiol. Oct 1;100(10):1168–76

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C11

A molecular signature linked to calcium signalling is predictive of exercise training-induced changes in insulin sensitivity

P.K. Davidsen[1], M.A. Sarzynski[2,4], J.M. Herbert[1], P. Antczak[1], M.K. Hesselink[3], P. Schrauwen[3], T.K. Rice[5], S. Weisnagel[6], R.N. Bergman[7], D.C. Rao[5], S. Ghosh[8], C. Bouchard[2] and F. Falciani[1]

[1]Centre for Computational Biology and Modelling, Institute for Integrative Biology, University of Liverpool, Liverpool, UK, [2]Human Genomics Laboratory, Pennington Biomedical Research Center, Baton Rouge, LA, USA, [3]Departments of Human Biology and Human Movement Sciences, Maastricht University Centre, Maastricht, Netherlands, [4]Department of Exercise Science, Arnold School of Public Health, University of South Carolina, Columbia, SC, USA, [5]Division of Biostatistics, Washington University School of Medicine, St Louis, MO, USA, [6]Diabetes Research Unit, CHU de Québec, Université Laval, Quebec, QC, Canada, [7]Diabetes and Obesity Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA and [8]Duke-National University of Singapore Graduate Medical School, Singapore, Singapore

Background: Despite good adherence to supervised endurance training, a significant percentage of individuals (up to ~30%) shows no change in peripheral insulin sensitivity (S_I) and some even demonstrate an adverse response [1]. The molecular mechanisms underlying this heterogeneous ability to improve S_I through regular exercise are currently not well understood, but are likely to include a substantial genetic component [2].
Objective: to produce a molecular classifier that predicts S_I training response guided by a multi-omics analysis framework.
Methods: Peripheral S_I was measured [intravenous glucose tolerance test] before and after a standardized 20-week endurance training programme [3 times/wk] in 478 healthy Caucasians from the HERITAGE Family Study [3]. Illumina HumanCNV370-Quad v3.0 BeadChips were genotyped. Affymetrix U133+2 arrays were used to quantitate gene expression levels from baseline limb muscle biopsies of a subset of participants (N=52).
Results: The functional GWAS analysis identified several calcium signalling-related pathways associated with S_I improvement ('Cardiac muscle contraction' being the most enriched; FDR<0.001). Noteworthy, multiple SNPs in close proximity to genes in the calcium signalling pathway were nominally associated with basal mRNA abundance ($p<0.01$). Furthermore, the mRNA expression levels of the calcium signalling pathway as a whole were differentially expressed at baseline between individuals with a high and low potential for improving their S_I.
We next reasoned that calcium signalling might regulate candidate transcription factors (TFs) of this pathway as a result of SNP variants affecting gene expression. Interestingly, the gene targets of the MEF2 TF family was positively associated with

dS_I (FDR<0.001), implying that individuals exhibiting high responsiveness of S_I to training have an overall higher basal expression of genes co-regulated by MEF2. siRNA has previously been used on differentiated C2C12s to define the global transcriptional signature associated with MEF2 knockdown [4]. Genes down-regulated by knockdown of the MEF2A isoform (n=828 genes), which overall relate to 'muscle function', was highly enriched amongst the most positively associated genes to dS_I in HERITAGE (FDR<0.001). Such in vitro validation prompted us to ask whether a robust multivariate regression model could be developed linking the basal mRNA abundance of MEF2A interacting gene targets to dS_I on a continuous scale. The most predictive model (*HDAC4*, *CAMK2D*, *CAMK2G*) was able to explain nearly half (48%) of the variance of dS_I in the HERITAGE sample. Importantly, the response predictor was successfully validated in an independent training cohort [5]. Conclusion: Combining data from genomics and transcriptomics analyses helped identify an RNA expression signature in resting muscle that is predictive of dSI.

Boule NG et al. (2005). Diabetes Care 28:108-14

Bouchard C (2012). Exp Physiol 97:347:52

Knowler WC (2002). N Engl J Med 346:393:403

Wales S et al. (2014). Nucleic Acids Res 42:11349:62

Meex RCR (2010). Diabetes 59:572-9

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C12

Blunted cumulative muscle protein synthesis and ribosomal biogenesis underlie age-related attenuation of resistance exercise-induced skeletal muscle hypertrophy

M. Brook, D.J. Wilkinson, B.E. Phillips, W.K. Mitchell, N.J. Szewczyk, P.L. Greenhaff, K. Smith and P.J. Atherton

University of Nottingham, Derby, UK

Resistance exercise training (RET) is effective at increasing skeletal muscle strength and mass, attributes desired for optimal athletic performance and mitigating muscle wasting disorders e.g sarcopenia. Skeletal muscle hypertrophy is underpinned by cumulative post-exercise increases in muscle protein synthesis (MPS) driven by acute increases in translational efficiency (e.g. mTOR-signaling) and a chronic increase in synthetic capacity (ribosomal biogenesis). However, RET-induced hypertrophy[1], acute RET-induced MPS[2] and ribosomal gene expression[3], are blunted in older (O) vs. younger (Y) individuals. Here, we tested the hypothesis that age-related "anabolic resistance" is reflected in chronic deficits in MPS (using D_2O approaches) and ribosomal biogenesis. Ten young (Y: 23±1y) and ten older (O: 69±3y) men undertook 6-wks unilateral-RET (6×8 reps, 75%-1RM 3.wk^{-1}). *Vastus Lateralis* muscle thickness (MT), architecture,

maximal voluntary contraction (MVC) and 1-repetition maximum (1-RM) were assessed regularly with DXA at baseline (0-wks) and completion (6-wks). After bilateral baseline muscle biopsies, subjects consumed 150ml D_2O then 50ml.wk^{-1} with biopsies at 3/6-wk 60-90 min post-RET to temporally quantify MPS, mRNA/protein targets (qRT-PCR and immunoblotting /ELISA) and total RNA/DNA concentrations. After 6-wks RET, 1-RM increased in Y (+35±4% P<0.01) and O (+25±3% P<0.01), yet MVC increased in Y (70º +29±6% P<0.01) but not O (+8±3% P=NS). Similarly, quadriceps mass increased at 6-wks in Y only (Y: +4±1% P<0.01 vs. O: +1±0.3% P=0.3). This was consistent with blunted increases in MT (Y: +8±1 and +11±2%, P<0.01 vs. O: +2.6 ±1 and +3.5 ±2%, P=0.08 at 3 and 6-wks, respectively). Basal MPS did not differ between age groups (Y: 1.35±0.1%.d^{-1} vs. O: 1.39±0.1%.d^{-1}). In contrast, reflecting early hypertrophy, MPS increased in Y but not O after 3-wks RET (Y: 1.61±0.1%.d^{-1} P<0.01 vs. O: 1.50±0.09%.d^{-1} P=0.1). Markers of ribosomal biogenesis and translational capacity increased only in Y ug RNA/ug DNA at 3 (Y: 0.47±0.05 to 0.62±0.05 P<0.01 vs. O: 0.53±0.05 to 0.56±0.04) and 6-wks (Y: 0.64±0.03 P<0.01 vs O: 0.57±0.04). Similarly, only Y displayed acute exercise induced C-MYC mRNA/protein and p70S6K1^{Thr389} phosphorylation; in addition O exhibited attenuated basal testosterone (Y: 3.6±0.2 ng/ml vs. O: 2.6±0.2 ng/ml P<0.05), IGF-1 (Y: 155.1± 16 ng/ml vs. O: 84.2±8 ng/ml P<0.01) but not myostatin concentrations. Finally, following a bout of RET (60-90 min), serum testosterone increased only in Y (post-RE: 3.93±0.2 ng/ml P<0.05). RET-induced muscle hypertrophy was blunted in O, likely the result of cumulative deficits in MPS and translational efficiency, ribosomal biogenesis and anabolic capacity, and unfavorable anabolic hormone profiles. Age-related anabolic resistance is thus multi-factorial.

Phillips, B. *et al.* (2012). *J. Appl. Physiol.* **112,** 347–53

Kumar, V. *et al.* (2009). *J. Physiol.* **587,** 211–7

Stec, M. J., Mayhew, D. L. & Bamman, M. M. (2015). *J. Appl. Physiol.* jap.00489.2015.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C13

Age associated motor unit loss is not attenuated by high levels of lifelong exercise

M. Piasecki[1], A. Ireland[1], D. Stashuk[1], A. Hamilton-Wright[2], D. Jones[1] and J. McPhee[1]

[1]Manchester Metropolitan University, Manchester, UK and [2]Mount Allison University, Sackville, NB, Canada

The loss of muscle mass and strength with advancing old age is associated with, and possibly preceded by, loss of motor neurons and remodelling of the surviving motor units (Piasecki et al, 2015). The suggestion that tibialis anterior motor unit numbers can be preserved into old age by maintaining regular and intense exercise (Power *et al.*, 2010) is appealing, but it remains to be shown whether any

other muscles experience similar benefits. To investigate this, intramuscular electromyography (iEMG) and surface EMG (sEMG) were used to estimate motor unit numbers in the vastus lateralis (VL) of 21 young men (mean age 25±5y), 20 healthy older men (mean age 71±6y) and 12 male masters athletes who had trained and competed for most of their adult lives (MA; mean age 70±5y).
The proximal and distal motor points of the VL were identified by percutaneous electrical stimulation. Surface EMG was recorded over each motor point and iEMG was simultaneously recorded adjacent to the sEMG in 12 locations of different depths within the muscle during isometric knee extension held at 25% of maximal voluntary contraction. A compound muscle action potential (CMAP) was generated by maximal percutaneous stimulation of the femoral nerve. Decomposition-enhanced spike-triggered averaging was used to obtain an average MU potential (MUP) area, and a corresponding surface-based MUP (sMUP) area. A motor unit number estimate (MUNE) was calculated at each motor point based on the CMAP divided by the average sMUP. A further estimate of motor unit numbers (intramuscular MUNE; iMUNE) was made by dividing the VL cross sectional area (CSA), measured from magnetic resonance imaging (Figure 1) by the mean MUP size.
The results show that the young had the largest VL, with no difference between the Old and MA. Compared with the young, the Old and MA had 29% ($p = 0.026$) and 36% ($p = 0.011$) lower MUNE values, respectively, and 46% ($p < 0.0005$) and 50% ($p < 0.0005$), respectively, lower iMUNE values compared to the young. The Old and MA did not differ in either of the estimates of motor unit numbers.
These results confirm the substantial neuromuscular deterioration that occurs in VL during healthy ageing and indicates that high levels of life-long exercise do not prevent the decline.

Neuromuscular characteristics of the VL in Young, Old and Master Athletes.

	Young (n=21)	Old (n=20)	MA (n=12)
iMUNE	36.9 (9.4)	19.8 (6.5)***	18.5 (6.4)***
MUNE	369 (135)	265 (123)*	235 (92)*

Data are shown as mean (SD). Significant differences are shown as: *p<0.05; **p<0.01 ***p<0.0005.

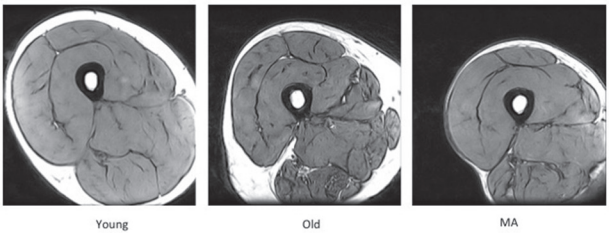


Figure 1. Vastus lateralis images of young, old and master athletes. Obtained from the mid-thigh using MRI.

Piasecki et al (2015), *J Physiol*. In Press

Power et al (2010). *MSSE*, 42, 1644–1650.

This work was supported by funding from UK Medical Research Council (MR/K025252/1).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C14

Greater untwisting rate response to hypoxia and re-oxygenation in sprint athletes

E.J. Stöhr, T. Samuel and S. Cooke

Physiology and health, Cardiff Metropolitan University, Cardiff, UK

Despite an increased aerobic exercise capacity and a greater maximal cardiac output, endurance athletes (ENDURANCE) also have a reduced hyperaemic perfusion of the heart (Heinonen *et al.*, 2008). The mechanisms – and importantly the physiological implications – for this phenomenon are not known and we speculated that ENDURANCE may have a reduced diastolic heart muscle function related to the aerobic nature of their training, and that this would therefore be 1) exacerbated during conditions of low O_2 availability and 2) absent in individuals with superior anaerobic buffering capacity. To test our hypothesis and its underlying mechanisms, we compared 'aerobic' ENDURANCE with 'anaerobic' sprint athletes (SPRINT) during exercise in normoxia and hypoxia (FiO_2 = 12%), the latter being a strong stimulus for increased myocardial perfusion (Duncker *et al.*, 2014). Additionally, the immediate re-oxygenation responses were studied. We measured heart rate (HR), blood pressure (BP) and used echocardiography to quantify conventional left ventricular (LV) function and systolic LV twist and diastolic LV untwisting rate, an essential component of normal *in vivo* LV relaxation (Wang *et al.*, 2007; Opdahl *et al.*, 2012). During exercise in both normoxia and hypoxia, BP, HR and cardiac index increased similarly between the three goups. However, ENDURANCE seemed to rely more on aerobic energy metabolism as reflected by greater O_2 consumption and reduced lactate production in normoxia and hypoxia, whilst arterial desaturation was exacerbated in hypoxia as previously reported (Woorons *et al.*, 2007). Conversely, SPRINT desaturated less and had a more specific metabolic response, showing a similar O_2 consumption to ENDURANCE in normoxia, but (similar to UNTRAINED) a lower O_2 consumption during hypoxia (**Figure 1a**). In agreement with our hypothesis, these general physiological responses were accompanied by the greatest LV untwisting rate in SPRINT and the lowest LV untwisting rate in ENDURANCE (**Figure 1b**). Immediately following hypoxia, SPRINT and UNTRAINED increased their LV untwisting rate while it was unchanged in ENDURANCE (**Figure 1c**). From these preliminary data, we conclude that endurance athletes appear to have a lower LV diastolic response during exercise in normoxia and hypoxia compared with sprint athletes. Moreover, endurance athletes do not augment their diastolic

LV function following hypoxia. These data suggest that the type of exercise training may influence the cardiac response to hypoxia and re-oxygenation, which may have important implications for the cardiac responses to ischaemic events.

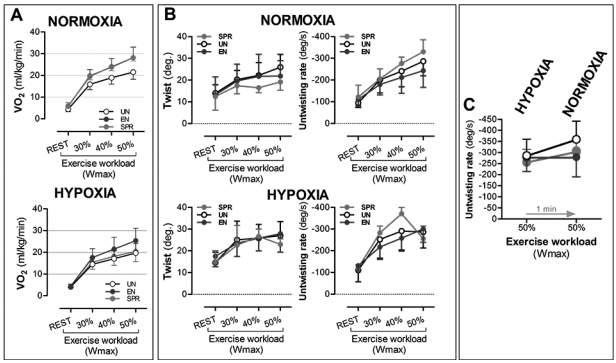


Figure 1. a: O_2 consumption (VO_2) in SPRINT (SPR), ENDURANCE (EN) and UNTRAINED (UN). **b**: LV twist and untwisting rate in normoxia and hypoxia. **c**: Post-hypoxic differences.

Duncker DJ, Koller A, Merkus D & Canty JM, Jr. (2014). Regulation of Coronary Blood Flow in Health and Ischemic Heart Disease. *Prog Cardiovasc Dis* **57,** 409–422.

Heinonen I, Nesterov SV, Liukko K, Kemppainen J, Nagren K, Luotolahti M, Virsu P, Oikonen V, Nuutila P, Kujala UM, Kainulainen H, Boushel R, Knuuti J & Kalliokoski KK. (2008). Myocardial blood flow and adenosine A2A receptor density in endurance athletes and untrained men. *J Physiol* **586,** 5193–5202.

Opdahl A, Remme EW, Helle-Valle T, Edvardsen T & Smiseth OA. (2012). Myocardial Relaxation, Restoring Forces, and Early-Diastolic Load are Independent Determinants of Left Ventricular Untwisting Rate. *Circulation* **126,** 1441–1451.

Wang J, Khoury DS, Yue Y, Torre-Amione G & Nagueh SF. (2007). Left ventricular untwisting rate by speckle tracking echocardiography. *Circulation* **116,** 2580–2586.

Woorons X, Mollard P, Pichon A, Lamberto C, Duvallet A & Richalet JP. (2007). Moderate exercise in hypoxia induces a greater arterial desaturation in trained than untrained men. *Scand J Med Sci Sports* **17,** 431–436.

The authors thank the research participants for their commitment and efforts.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C15

Motor unit recruitment during sustained fatiguing contractions with blood flow occlusion

J. McPhee, M. Piasecki, A. Ireland and D. Jones

Manchester Met University, Manchester, UK

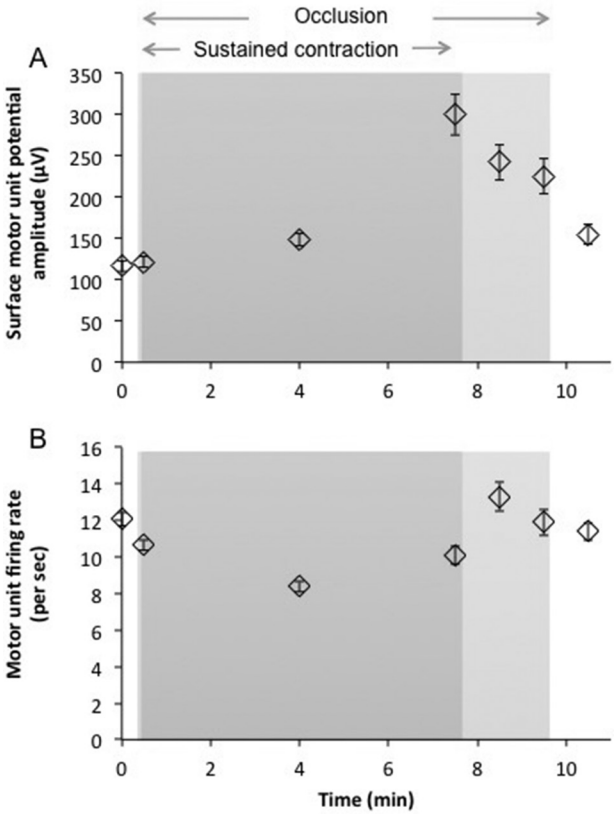
Muscle blood flow can be constrained by external pressure or by internal pressure generated during isometric contractions. The loss of blood supply leads to metabolic fatigue but during submaximal contractions there are a number of ways in which the loss of performance can be compensated for, such as motor unit (MU) rotation and recruitment, or increasing firing frequency. We have examined recruitment and MU firing frequency during a sustained isometric contraction where blood flow was occluded.

Twelve young men sat with their right leg extended and foot strapped into a dynamometer for measuring isometric dorsiflexions. Motor unit potential (MUP) amplitude and firing frequency were measured with an intramuscular concentric needle and the surface-representation (sMUP) determined using 'spike triggered averaging'. Participants held a 25% maximal voluntary contraction (MVC) for 15 sec before a thigh cuff was inflated to occlude leg blood flow while subjects matched a target force of 25% MVC for as long as possible. Visual feedback was available throughout. MUs were sampled for 15 sec at the start of the contraction, half way through and at task failure. Subjects then rested, but with the circulation occluded, and made 15 sec voluntary efforts at 25% MVC at 60 sec and at 120 sec post-fatigue. Occlusion was then released, and 15 sec voluntary efforts at 25% MVC were measured after a further 60 sec. Data were analysed using repeated measures ANOVA and are reported as mean (s.e.m).

Occluding the circulation to a fresh muscle had no immediate effect on MU size or firing rates. Half way through the contraction MU size had increased only slightly, but firing rates decreased from 10.7 (0.3) Hz to 8.4 (0.3) Hz. By task failure, larger MUs were recruited and firing rate (10.1 (0.5) Hz) was similar to those of fresh muscle. MU size remained elevated in the recovery period while occlusion was maintained and subjects reported difficulty holding the 25% MVC target for 15 sec. MU size rapidly returned to fresh values once the blood supply was restored.

During the first half of the fatiguing contraction the results are consistent with peripherally-influenced reflex reduction in MUP firing frequency which may help to maintain electrical excitability and adapts to slow contractile properties of the fatiguing MUs, as suggested by Bigland-Ritchie et al for sustained maximal contractions (J Physiol, 379; 451–459). The increase in firing frequency during the second half of the contraction is indicative of the recruitment of larger, faster MUs. The novelty of these findings is that

recruitment of new MUs is a relatively late event, there is no evidence of MU rotation as a strategy for maintaining force and the muscle remains in an apparent fatigued state if blood flow is occluded after the sustained effort.



Dr McPhee is supported by RCUK LLHW (MR/K025252/1)

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C16

The effect of four weeks endurance training and beta$_2$-agonist or placebo on muscle contractile function and leg lean mass in healthy men

N. Krogh[1], J. Onslev[1], J. Bangsbo[2], V. Backer[1,3] and M. Hostrup[1,2,3]

[1]Respiratory Research Unit, Bispebjerg Hospital, Copenhagen, Denmark, [2]Department of Nutrition, Exercise and Sports, University of Copenhagen, Copenhagen, Denmark and [3]IOC Sports Medicine Copenhagen, Copenhagen, Denmark

Chronic beta$_2$-adrenergic stimulation with beta$_2$-agonists has been shown to affect contractile function of skeletal muscle and to induce muscle hypertrophy in animals that underwent endurance training. In humans, on the other hand, no studies have investigated effects of beta$_2$-agonists and endurance training on contractile function and muscle hypertrophy. Furthermore, the doses of beta$_2$-agonists administered to animals exceed that clinically relevant for humans. Thus, the purpose of the present study was to investigate the effect of four weeks of therapeutic inhalation of the beta$_2$-agonist terbutaline on maximal voluntary contraction (MVC) of the quadriceps muscle and leg lean mass following endurance training. Twenty-one healthy men were randomised into either a terbutaline-group (TER, n=12) or a placebo-group (PLA, n=9). Subjects underwent two visits before and after a four-week intervention. At the two visits before and after intervention, subjects' leg lean mass was determined by dual X-ray absorbance and contractile function of the quadriceps muscle was measured during MVC with transcutaneous electrical muscle stimulation. The four-week intervention consisted of supervised endurance training on a training bike three times a week that consisted of 30 min cycling at 80-85% of maximal heart rate interspersed with 30-s of maximal sprinting every 10 min. During the four-week intervention, subjects inhaled either terbutaline (8x0.5 mg) or placebo once daily according to their respective group. Leg lean mass did not change with the intervention in either group, being 25±1 and 25±1 kg before and after the intervention in TER, and 26±1 and 26±1 kg in PLA, respectively (mean±SE). MVC, time-to-peak twitch force and half-relaxation time did not change with intervention in either group (table 1). A significant interaction of group x time ($P \leq 0.05$) was observed for peak twitch force, in which peak twitch force increased by 73 N with the intervention in TER compared to PLA. However, no within-group changes were observed in peak twitch force with the intervention in TER (309 ± 27 vs. 343 ± 27 N) and PLA (336 ± 33 vs. 297 ± 33 N). In conclusion, the present findings indicate that contractile function of the quadriceps muscle and leg lean mass are unaffected by chronic therapeutic

inhalation of beta$_2$-agonists in response to four weeks of endurance training in healthy men.

Contractile function of the quadriceps muscle

	TER		PLA	
	PRE	POST	PRE	POST
MVC (N)	698 ± 47	689 ± 47	677 ± 57	671 ± 57
Time-to-peak twitch force (ms)	64 ± 3	64 ± 3	65 ± 4	62 ± 4
Half relaxation time (ms)	18 ± 2	16 ± 2	18 ± 3	20 ± 3

Values are mean ± SE

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC01

Nutritional supplement use among school level Athletes in Sri Lanka

E.M. Dassanayaka[1] and N. Silva[2]

[1]Bio Medical Sciences, International Institute of Health Sciences Welisara, Kadawatha, Sri Lanka and [2]National Hospital of Sri lanka, Colombo, Sri Lanka

Introduction: Sports supplements use is seen among school athletes in Colombo but their actual practices and recommendations and associated risks are unknown. As well as the knowledge on the products they use is unknown.
Objective: To assess the knowledge & Practices regarding approved and non-approved sports supplements and the knowledge on banned doping agents.
Methods: A descriptive study was done on 130 conveniently selected school athletes among leading schools in Colombo using a self-administered questionnaire.
Results: The sample population included 13.1% female athletes and 73.4% Male athletes. The participants were from ages 15-20. From the majority 62.3% took dietary supplements and 56.9% of the population took supplements without a doctor's recommendation. Only 13.1% would go to a physician to find information on supplements. 68.4% of the sample took information on supplements from unreliable sources.48.5% believes that supplements are right for them out of which 58.7% relied on the supplement label to select the right supplement. 50% believes that energy drinks can improve sports performance. 56.9% agrees that with doping body shape and muscle mass can be increased. 55.4% agrees that doping can cause harm to the user. Also 45% disagrees respecting individuals who drug dope. 2.3% from the sample have taken a banned substance.
Conclusions: The use of nutritional and sports supplements are common among school level athletes, the use of supplement and other doping substances without consultation may be dangerous for Athletes.
Key words: Sports supplements, Drug doping, Performance

Dr. Nishan Silva, Dr. kithsiri Edirisinghe

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC02

The impact of swimming on the prothrombotic state and fibrinolytic activity in a rat model of nonalcoholic fatty liver disease

I. Bin-Jaliah[1], M.A. Dallak[1] and H.F. Sakr[1,2]

[1]Department of Physiology, College of Medicine, King Khalid University, Abha, Saudi Arabia and [2]Department of Medical Physiology, Faculty of Medicine, Mansoura University, Mansoura, Egypt

The consequential positive energy balance of modern lifestyles, frequently characterized by physical inactivity and unhealthy diet intake, is a major cause for insulin resistance and the metabolic syndrome. The liver, as a key metabolic tissue, develops obesity-related complications. Nonalcoholic fatty liver disease (NAFLD) is associated with increased incidence of cerebrovascular and cardiovascular accidents. The aim of the present work was to investigate the effects of swimming exercise on the prothrombotic factors and fibrinolytic activity of the blood in a rat model of NAFLD.

Forty rats were randomly divided into four groups (n=10 for each). Group 1 rats, fed with standard laboratory chow for 15 weeks, were used as control (Con). Group 2 rats (Con+Ex) were fed a standard laboratory chow for 15 weeks and obliged to swimming exercise from the 11th week to the 15th week. Group 2 rats fed a high-cholesterol diet with 10% fructose solution (HCFD) for 15 weeks. Rats in group 4 (HCFD+Ex), fed with high-cholesterol diet with 10% fructose solution (HCFD) for 15 weeks and obliged to swimming exercise from the 11th week to the 15th week. After 15 weeks, serum glucose, insulin, lipogram, plasminogen activator inhibitor-1 (PAI-1), fibrinogen, von Willibrand factor (vW factor), Fibrin degration products (FDPs), endothelin-1 (ET-1), intercellular adhesion molecule (ICAM), and vascular cell adhesion molecule (VCAM) were assayed. Platelet count, bleeding time, clotting time, prothrombin time (PT), activated partial thromboplastin time (aPTT) and adenosine diphosphate (ADP) platelet aggregation were measured. Data are expressed as mean±S.D. and significance (P<0.05) tested with ANOVA.

HCFD fed rats showed a significant increase in systolic blood pressure from 122±5 to 144±8 mmHg, with increased body weight by 21% compared to control rats. They had significantly higher plasma glucose, insulin, lipid profile and HOMA-IR. PAI-1 increased significantly from 36.33±3.52 to 57.04±5.75 ng.ml^{-1} and fibrinogen from 188.34±12.51 to 350.38±26.92 mg.dl^{-1}. HCFD rats had also higher FDPs, vW factor, ET-1, ICAM, VCAM, and platelet aggregation, with shorter bleeding time by 25%, clotting time by 27%, PT by 29% and aPTT by 35% versus control rats. In contrast, swimming exercise significantly decreased the gained body weight by 15%, glucose by 30%, insulin by 35%, and lipid profile compared to HCFD group. In response to exercise PAI-1 decreased to 42.14±4.26 ng.ml^{-1}. Fibrinogen, FDPs, vW factor, ET-1, ICAM, VCAM, platelet aggregation decreased to normal values with normalization of bleeding time, clotting time, PT and aPTT.

It could, therefore, be concluded that NAFLD increases the prothrombotic markers and platelets adhesion and aggregation. Swimming ameliorates the hypercoagulable hypofibrinolytic state induced by HCFD in a rat model of NAFLD.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC03

Effects of creatine supplementation on the airways of youth, elite football players

P. Kippelen[1], A. Simpson[1], S. Horne[1], P. Sharp[2] and R. Sharps[2]

[1]College of Health & Life Sciences, Brunel University London, Uxbridge, UK and [2]Watford Football Club, Watford, UK

Owing to its well-established ergogenic potential, creatine is a highly popular food supplement in sports. As an oral supplement, creatine is currently considered safe and ethical. However, no data exist on the safety of creatine on lung function in humans. This is particularly striking considering that, in animals, creatine has been shown to exacerbate allergic lung inflammation, airway remodelling and bronchial hyper-responsiveness (1). The aim of this project was to evaluate the effects of a standard course of creatine supplementation on the airways of youth, elite athletes. Twenty Football Academy players, aged 16-21 yr, completed a randomised, double-blind, placebo-controlled, parallel-group trial. The creatine group (n=9) ingested 0.3 g/kg/d of creatine monohydrate (CM) for 1wk and 5 g/d for the remaining 7wk, whereas the placebo group (n=11) received the same dosage of maltodextrin. Airway inflammation (assessed by exhaled nitric oxide, FeNO) and bronchial responsiveness (to dry air hyperpnoea) were assessed pre- and post-supplementation. Atopic status was checked at study entry by skin prick testing. There was a trend (P=0.086, Wilcoxon test) for FeNO to increase post-CM supplementation (Table1), especially in those players sensitized to aero-allergens (FeNO increased by >10 ppb in 4 out of 7 atopic players under CM *versus* 0 out of 8 atopic players under placebo). Furthermore, the airways of the players supplemented with CM were slightly, but significantly (P=0.038, Mann-Whitney test) more responsive to dry air after 8wk of supplementation compared to the placebo group (Table1). Based on these findings, we cannot exclude that creatine supplementation increases inflammation of the airways in susceptible (atopic) youth athletes, and thereby, contributes to the high prevalence of asthma in elite sport (2).

Table1. Airway inflammation and bronchial responsiveness in youth, elite football players supplemented with creatine monohydrate (CM) or placebo for 8 weeks

	FeNO (ppb)		Max fall in FEV1 post-EVH (%)	
	Pre	Post	Pre	Post
CM (n=11)	22 (18-81)	34 (16-95) &	6.3 (3.4-14.0)	6.8 (4.7-12.3) *
Placebo (n=9)	21 (17-34)	20 (16-24)	6.8 (2.8-10.1)	4.8 (3.6-5.3)

Values are median (interquartile range; Q1–Q3); FeNO, fractional nitric oxide in exhaled air; Max fall in FEV1 post-EVH; maximal fall in forced expiratory volume in 1 sec following 6 min of eucapnic voluntary hyperpnoea (EVH) of dry air; & P=0.086 compared to pre-CM supplementation (Wilcoxon test); * P=0.038 compared to post-supplementation in the placebo group (Mann-Whitney test)

Vieira RP *et al*. (2007). *Am J Respir Cell Mol Biol* **37**(6), 660–667.

Carlsen K-H *et al.* (2008). *Allergy* **63**(4), 387–403.

This research was supported by a grant from the Union of European Football Associations (UEFA).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC04

Embryonic polarity axes may be responsible for the normalization of tissue function by the interaction between human bilateral parts

M.C. Ou[2,1], D. Ou[3] and C.C. Pang[4]

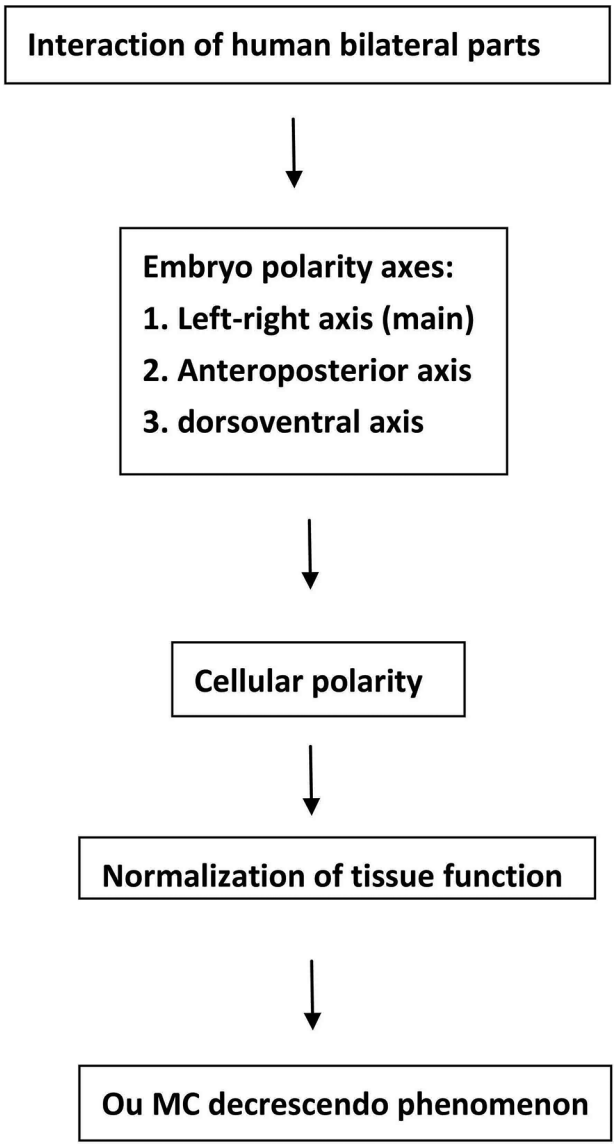
[1]Obstetrics & Gynecology, Taipei Medical College, Taipei City, Taiwan, [2]Obstetrics & Gynecology, Taipei City Hospital, Taipei City, Taiwan, [3]Master of Science in Financial Economics, Oxford University, Oxford, UK and [4]Su Women Hospital, Taipei City, Taiwan

Objective: Ou MC decrescendo phenomenon (OuDP) is produced by placing the contralateral hand over a diseased location to produce a zone under the hand with decreased pain or inflammation [1]. Our objective is to understand the possible mechanism of OuDP.
Methods: OuDP can be induced by the patients themselves or the therapist [1] by placing the contralateral hand directly on the affected area. During 2012-2015, 147 patients with various diseases were treated with OuDP. In these patients, 56 patients was treated with both ipsilateral and contralateral hand to induce OuDP [2-5]. Though complementary therapy is exempt from informed consent by the law in Taiwan (Department Health, ROC, 1993, No.82075656), all patients agreed and provided consent for participation in this study.
Results: The 56 patients treated with contralateral and ipsilateral hand showed that application with contralateral hand resulted in OuDP for all the patients, while the ipsilateral hand resulted in OuDP for only one patient (1/56, 2.0%)—Difference between contralateral and ipsilateral hand to induce OuDP, $P < 0.001$, Paired t test. The OuDP showed effect of remission or cure for the diseases or relief of clinical symptoms in 144 of the 147 patients (98.0%). (Table 1)

Conclusions: The Ou HR appears to be consistently effective for treating a wide variety of diseases, such as infections, inflammation, degenerative diseases, organ dysfunction, and malignant oncologic changes, Embryological development of animals usually patterns along the embryonic axes with regard to the functional features developed during early embryonic life. Recent studies have shown the signaling system of embryonic axes imparts polarization of individual cells leading to normal function. Most human cells and tissue in adults are polarized to have normal functions. OuDP can be effectively induced with the contralateral hand, but not the ipsilateral hand, which implies that the axes of embryonic polarity, especially the left-right axis, are the potential mechanism underlying the OuDP (Figure 1). Linguistic studies have demonstrated that a space–time congruency effect exists from the left to right that is coordinated with the left-right axis of the human body. This indicates there are mutual interactions of the left to right axis between individuals that may result in a physiological response.

Table 1 The efficacy of ipsilateral and contralateral hand to induce Ou MC decrescendo phenomenom (OuDP)

Study	Authors (years)	Patients with OuDP successfully induced/Patients number	
		Ipsilateral hand	Contralateral hand
Reference 1	Ou et al. (2006-9)	0/3	42/42
Reference 3	Ou et al. (2010-11)	0/39	36/39
Reference 3, 4	Ou et al. (2011-14)	0/3	40/40
Reference 5	Ou et al. (2015)	1/11	26/26
Total		1/56	144/147



Ou MC, Pang CC, Ou D, Su CH (2012) The implications of abdominal palpation with Ou MC manipulation for women with acute abdomen. Am J Emerg Med 30:421-425.

Ou, M C, Pang, C C and Ou D (2012) A primitive approach for Ou MC handing remedy with a comparison to the ancient laying on of hands. In: abstract book of the 15th Annual Meeting of the British Infection Association, 2012, pp 9, London.

Ou M C, Ou D and Pang C C(2014) The relation between human bilateral symmetry and disease. Proceedings of The Physiological Society 31: PCA132.

Ou M C, Ou D and Pang C C (2015) The potential mechanism of bilateral human symmetric parts interaction for the degenerative and oncological diseases. Proceedings of The Physiological Society 33: PC08.

Ou M C, Ou D and Pang C C (2015) The Possible Role of Embryonic Polarity Axes for the Normalization of Tissue Function Induced by the Interaction between Human Bilateral Parts. Integrative Cancer Science and Therapeutics (ICST) 3: 329-335.

The authors thank the encouraging and spiritual support from Ms. Ou Yi-Jen.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC05

Are there differences in training protocols and racehorse responses of higher- versus lower-ranked trainers?

K. Mukai[1], H. Ohmura[1], A. Matsui[1], Y. Takahashi[1], H. Miyata[2] and T. Takahashi[1]

[1]Equine Research Institute, Japan Racing Association, Shimotsuke, Japan and [2]Yamaguchi University, Yamaguchi, Japan

There is great interest from both racehorse trainers and scientists in constructing effective training protocols, however, differences in protocols and the responses of horses to them remain poorly described. The purpose of this study was to investigate training programmes of racehorses in Japan to test the hypothesis that higher-ranked trainers exercise horses differently than lower-ranked trainers.
Methods - (1) GPS data loggers on 808 Thoroughbred racehorses from 42 trainers recorded training profiles of each racehorse for approximately one month. (2) Seven well-trained Thoroughbred horses (6 castrated males and 1 female; 488 ± 8 kg) ran on a 6% inclined treadmill to simulate the typical training protocol of higher-ranked (H group, top 20 of 208 trainers; 60% of maximal rate of O_2 consumption (VO_2max), 90 s; 85% VO_2max, 90 s; 110% VO_2max, 60 s) or lower-ranked trainers (L group, bottom 100 of 208 trainers; 60% VO_2max, 90 s; 85% VO_2max, 180 s; 110% VO_2max, 30 s), and arterial blood samples were drawn during the final 10 s of the run. Muscle biopsies were taken from *M. gluteus medius* under local anaesthesia (2% lidocaine, 2 ml/head, s.c.) before, 4 h, and 24 h after the treadmill run, and relative quantitative analysis of mRNA was performed using real-time PCR (3 replicates). Values are means ± SEM. mRNA data were analysed by two-way ANOVA with Tukey's test and the others by paired *t*-test. Statistical significance was set at $P<0.05$.

Results - (1) Training programmes of H were of shorter distance (1137 ± 30 m) than L (1702 ± 29 m) at moderate-intensity (>6.9 and <13.3 m/s) and longer distance (307 ± 12 m) than L (211 ± 12 m) at high-intensity (>13.3 m/s). (2) Despite shorter total run distance in H (H 2279 ± 21; L 2780 ± 26 m), peak plasma lactate concentration (H 22.8 ± 2.0; L 16.1 ± 2.1 mmol/l), VO_2 (H 169 ± 4; L 151 ± 4 ml/(kg×min)) and respiratory exchange ratio (H 1.22 ± 0.02; L 1.13 ± 0.01) in H were higher, and arterial oxygen saturation (H 86.0 ± 0.7; L 89.5 ± 0.8%) and arterial pH (H 7.202 ± 0.018; L 7.275 ± 0.019) in H were lower than in L. Peak heart rate (H 214 ± 3; L 211 ± 4 bpm) and pulmonary arterial temperature (H 41.1 ± 0.3; L 41.1 ± 0.3 °C) did not differ between groups. Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) mRNA increased in both groups 4 h after the treadmill run (H 4.81 ± 0.71; L 2.77 ± 0.58 -fold) with H greater than L. Vascular endothelial growth factor (VEGF) mRNA increased 4 h after the treadmill run in H but not L (H 1.79 ± 0.26; L 1.17 ± 0.29 -fold).
Conclusions - The training programme of H ran less total distance but greater distance at higher intensity than L, presumably providing greater stimulation of aerobic and anaerobic energy pathways than did L. A single training bout with H induced greater adaptations in mitochondrial biogenesis and angiogenesis of skeletal muscle than with L.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC06

Trunk muscle activation in back squat and hack squat at the same relative loads

D. Clark[1,2], M. Lambert[3] and A. Hunter[2]

[1]Fitness Dept., Irish Rugby Football Union, Dublin, Ireland, [2]School of Sport Sciences, University of Stirling, Stirling, UK and [3]Division of Exercise Science and Sports Medicine, Department of Human Biology, University of Cape Town, Cape Town, South Africa

The loaded barbell back squat (BS) (Fig. 1) is an established method for development of strength and power in lower limb (1,2). Trunk muscle activation (TMA) using surface electromyography (sEMG) in BS is novel and clarity on the role of BS in developing dynamic trunk strength and stability is required. BS performed on an unstable surface will result in greater TMA, but compromises the load and therefore primary purpose (1,2). TMA in BS is load sensitive (3) and greater in free barbell version than the more supported Smith machine squat. How does TMA in a more stable squat, hack squat (HS) (Fig. 2), at the same relative, but higher absolute load compare to BS? The centre of gravity of BS system, person and external load, must remain over base of support or feet (4) to prevent failure or injury. In HS the trunk is supported by a 45º angled board and feet are placed anterior to line force (4). Hypothesis, TMA in BS will be greater than HS at the same

relative loads but greater absolute load in HS. Aims of the study: 1) determine max strength in BS and HS, 2) compare TMA in BS and HS, and 3) assess TMA response to load increases in BS and HS. Ethical approval according to Helsinki Declaration (2013) was granted. 3 test sessions (n=10 males): 1) BS and HS 1 rep max (RM) test, 2) EMG test familiarization, 3) EMG tests for 3 reps of BS and HS at 65, 75, 85 and 95% of system mass max (SM). SM = 1RM + (0.886 x body mass) (kg), where 0.886 is body mass minus shanks. Kinematics measured by a linear transducer and sEMG (SENIAM guidelines) for rectus abdominus (RA), external oblique (EO), upper lumbar erector spinae (ULES) and lumbar sacral erector spinae (LSES). Vastus lateralus (VL) sEMG as reference lower limb muscle. sEMG was root mean square (RMS) processed. Mean RMS for each phase of BS and HS at 75, 85 and 95% SM were normalized to mean concentric BS RMS at 65% SM (5). Mean HS 1RM was 28.5 kg (18.24%) greater than BS, hence 4 test loads in HS were significantly higher than BS ($F_{(1, 9)}$ = 19.94 $p<0.01$). Eccentric displacement was 21.5 cm less in HS than BS for 4 test loads. Force was higher in HS than BS at each load and increased with each load in both exercises. BS TMA was greater than HS for all muscles, both phases for all test loads. Difference was significant ($p<0.05$) in 14/24 instances (3 loads x 4 muscles x 2 phases). TMA increased with load in all muscles for both exercises and phases apart from HS LSES in eccentric phase. VL RMS was greater in BS than HS for all loads but only significant in concentric phase. There was a load effect for VL in both exercises. This study demonstrated a greater 1RM for HS vs BS for well-trained cohort. Despite higher absolute tests loads and force in HS, TMA was higher in BS. This study suggests the BS is an effective method of developing trunk strength and that TMA is sensitive to load in both BS (3) and HS.

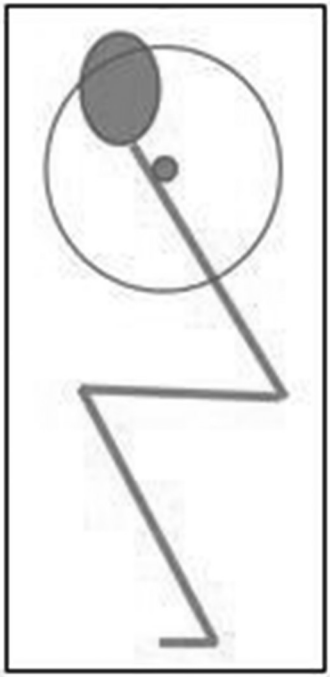


Figure 1. Back squat

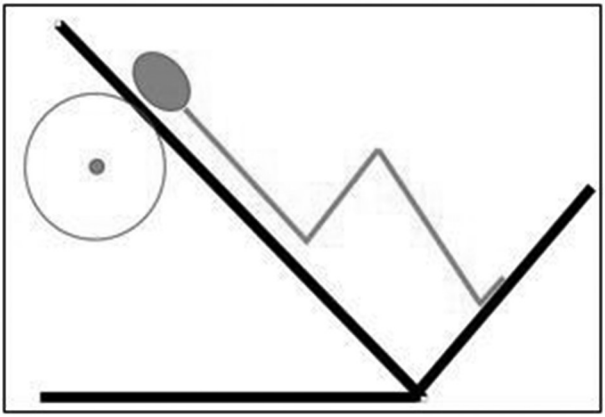


Figure 2. Hack squat

Clark DR *et al.* (2012). *J Strength Cond Res* **26(4),** 1169–78

Schoenfeld BJ. (2010). *J Strength Cond Res* **24(12),** 3497–506

Clark D *et al*. (2015). *Int J Sports Med* (In press), 1–9.

Abelbeck KG. (2002). *J Strength Cond Res* **16(4),** 516–24

Dankaerts W *et al*. (2004). *JElectromyogrKinesiol* **14**, 333–42

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC07

Dietary nitrate supplementation improves mean power during upper body resistive exercise

S. Pavlaki, R. Varnham and R. Ramsbottom

Sport & Health Sciences, Oxford Brookes University, OXFORD, UK

Dietary inorganic nitrate (NO_3^-) supplementation has been shown to improve maximal muscle power and exercise tolerance at high, but not low contraction velocities during lower body exercise (Coggan et al, 2014; Bailey et al, 2015). Supplementation also improves upper body performance (Peeling et al, 2015), however, it remains unclear whether NO_3^- supplementation improves muscle power or exercise tolerance during 'all-out' upper body exercise. We tested the hypotheses that during all-out bench press exercise, acute NO_3^- supplementation would improve performance by: i) increasing total work ii) prolonging time to exhaustion iii) increasing mean power.

Eight men, age 26.4±3.1 years; height 1.79±0.06 m; mass 81.5±12.8 kg (mean±SD), consented to participate in a randomised, double-blind cross-over study, which had University ethical approval and followed the principles laid out by the Declaration of Helsinki. Volunteers responded to poster advertisements and were able to withdraw from the study at any stage without reason. During visits, participants arrived to the laboratory in the morning following an overnight fast. Diet was replicated between trials and foods high in NO_3^- were avoided. Following thorough familiarisation, participants returned to the laboratory on two occasions, separated by a minimum of seven days, during which they were instructed to perform bench press exercise (Smith machine) as fast as they could at 40% 1RM (one repetition maximum), and to continue the protocol to maximal volitional exhaustion. During the two experimental visits participants consumed either beetroot juice (BR; 13 mmol NO_3^-) or placebo (PL; NO_3^- depleted BR) (Beet It Sport, James White Drinks, UK) 2.5 hours before exercise. Blood pressure (BP) was measured using an automated sphygmomanometer (Dinamap 400 ProV) before ingestion, and again before exercise. Bench press performance was assessed from video analysis (30Hz) for time to exhaustion (s), total work (kJ), and mean power (W). Student's t-tests were used to identify differences between BR and PL and a two-way ANOVA for BP (SPSS 22). There was no main effect of supplement on BP (120±8/70±6 vs.119±10/70±6 mmHg, BR vs. PL respectively), and there was no interaction effect for condition over time. There was no difference in time to exhaustion between BR (46.7 ± 6.4) and PL (45.7 ± 5.5 s), however, both total work performed (BR: 8.8 ± 2.5; PL: 8.2 ± 2.5 kJ; $P<0.05$) and mean power (BR: 188 ± 56; PL: 179 ± 58 W; $P<0.01$) were greater with BR compared to PL. The present results show increased total work and mean power with BR during all-out, upper body exercise, without any change in time to exhaustion. The physiological explanation for these findings remains unclear, but may relate to improvements in local muscle oxygen delivery, perfusion or extraction.

Bailey, S. J. Varnham, R. L., DiMenna, F. J., Breese, B. C., Wylie, L. J. and Jones, A. M. (2015) Inorganic nitrate supplementation improves muscle oxygenation, O_2 uptake kinetics, and exercise tolerance at high but not low pedal rates *Journal of Applied Physiology* 118: 1396–1405.

Coggan, A. R., Leibowitz, J. L., A. R., Kadkhodayan, A., Thomas, D. P., Ramamurthy, S., Spearie, C. A., Waller, S. Farmer, M. and Peterson, L. R. (2014) Effect of acute dietary nitrate intake on maximal knee extensor speed and power in healthy men and women *Nitric Oxide* 48: 16–21.

Peeling, P., Cox, G.R., Bullock, N. and Burke, L. M. (2015) Beetroot juice improves on-water 500 m time trial performance, and laboratory-based paddling economy in National and International-level kayak athletes *International Journal of Sport Nutrition and Exercise Metabolism* 25: 278–284.

Volunteers who complied with the strict experimental protocol.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC08

The effects of a 16 week aerobic exercise programme on cognitive function in people living with HIV

A.D. McDermott[1], C. Forde[2], M. Egana[1], J. Gormley[2] and C. Bergin[3]

[1]Physiology, Trinity College Dublin, Dublin, Ireland, [2]Physiotherapy, Trinity College Dublin, Dublin, Ireland and [3]Infectious Diseases, St.James' Hospital, Dublin, Ireland

Since the introduction of highly active antiretroviral therapy there has been a marked increase in the prevalence of HIV associated neurocognitive disorders (HAND). It has been suggested that higher levels of physical fitness are associated with higher levels of cognitive function in people living with HIV (Dufour *et al.* 2011). Furthermore, evidence from other clinical areas suggests that higher levels of physical fitness are associated with improved cognitive function(Erickson *et al* 2011; Suzuki *et al* 2013). This project aimed to investigate whether a 16 week exercise intervention could improve cognitive function in people with HIV. Thirteen participants were recruited from a pre-defined group of patients who had been previously screened for HAND in St. James' Hospital, Dublin. Participants were randomised into two groups: an exercise group (n=6), that completed a 16 week supervised aerobic exercise programme training 2 to 3 times per week, and a control group (n=7) that received no intervention and continued with their routine care. Primary outcomes measured included cognitive function (Montreal Cognitive Assessment (MOCA) and the trail making tests A and B), aerobic fitness (modified Bruce protocol), sleep quality (Pittsburgh Sleep Quality Index; PSQI), metabolic profiles and anthropometrics. Higher levels of moderate physical activity and aerobic fitness were significantly correlated with higher cognitive function at baseline (P=0.04 and P=0.001 respectively). Despite an overall low adherence rate of 60% to the exercise programme, there was a tendency for a numerically larger improvement in short term memory in the exercise group compared to the control group. However, there were no significant improvements in global cognitive scores. In addition, significant improvements were recorded in daytime dysfunction, an important domain of sleep quality, in the exercise group following training compared to the control group (P<0.05). No significant improvements were seen in aerobic fitness or metabolic profiles after the intervention. In conclusion exercise may have beneficial effects on cognitive function and sleep quality in people with HIV. However, further research is warranted with larger sample sizes and adherence rates to allow for a more in depth investigation of the effects of exercise on cognitive function in this population.

Dufour CA, Marquine MJ, Fazeli PL, Henry BL, Ellis RJ, Grant I & Moore DJ. (2013). Physical exercise is associated with less neurocognitive impairment among HIV-infected adults. *J Neurovirol* **19**, 410–417.

Erickson KI, Voss MW, Prakash RS, Basake C,Szabo A, Chaddock L, Kim JS, Heo S, Alves H, White SM, Wojcicki TR, Mailey E, Vieira VJ, Martin AS, Pence BD, Wood JA, McAuley E, & Kramer AF.(2011). Exercise training increases size of hippocampus and improves memory. *PNAS* **7**, 3017–3022.

Suzuki T, Shimada H, Makizako H, Doi T, Yoshida D, Ito K, Shimokata H, Washimi Y, Endo H Kato T. (2013). A randomized controlled trial of multicomponent exercise in older adults with mild cognitive impairment. *PLoS One* **8**, e61483.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC09

Interaction between physiological and mechanical data to clarify elite sprint swimming performance

P. Morouço

Polytechnic Institute of Leiria, Marinha Grande, Portugal

Elite performance evaluation has been mainly conducted through physiological or mechanical approaches separately. However, it is constructive to gather those fields to understand the major factors that explain outstanding performances. That velocity is dependent on the maximal total energy expenditure corrected for body mass and the energy cost (C), being C associated with intra-cyclic variations of the horizontal velocity (dv) of the body. Theoretically, dv are the result of intra-cyclic variations of the horizontal force (dF) of the body. Although higher variations lead to lower performances, it is not known if and how dv and dF are related and their role for very high velocities. 23 elite swimmers were tested (males, 18.6±2.3years of age; 1.79±0.09m of height; 69.9±9.2kg of body mass; 56.7±2.9s of 100m PB). On separate days, all-out 50m front crawl (26.7±1.50s) was performed to calculate dv by a speed-meter cable (Swimsportec, Hildesheim, Germany) attached to the swimmer's hip, and a 30s all-out fully tethered swimming was completed to determine dF by a load-cell system (Globus, Codognè, Italy). Increase in blood lactate concentration (ΔBLa) was measured using a portable analyzer (Lactate Pro, Arkay, Japan). Heart rate was continuously recorded by a HR monitor (RS800CX, Polar Electro Oy, Kempele, Finland). Rate of perceived exertion (RPE) was assessed verbally (REF). SR (Hz) was determined using a portable SR counter (Seiko, Tokyo, Japan). ICC were between 0.94 (0.90–0.98) and 0.98 (0.96–0.99) for the measurements (n=8). Values are means ± S.D., compared by repeated measures. Both dv and dF exhibited similar patterns in the instantaneous curves; with very high different magnitudes (8.8±2.3% vs. 65.0±10.1%, $p<0.05$ respectively). There were no differences in ΔBLa, HR, RPE, or SR within the tests, with a very strong agreement of ΔBLa and SR (average differences were rather low, with limits of agreement (average±1.96 S.D.) ranging from –0.067 to 0.107 for SR and from –1.036 to 1.235 for ΔBLa). Thus, tethering the swimmer did not alter any physiological

or mechanical responses compared with free swimming of similar duration and intensity. dv showed a high but non-linear relationship with swimming mean velocity ($r=-0.78$, $p<0.01$), whereas dF presented a linear relationship ($r=-0.84$, $p<0.001$). Hence, dF should be assessed for elite performance evaluations; higher dF induced a higher dv, leading to lower performances, suggesting that higher variations leads to an increase in C to overcome inertia and drag force. The present study showed that gathering the theoretical hypothesis with experimental data the demands of front crawl elite performance can be explained through a hybrid approach; ie. combining physiological and mechanical knowledge.

Funded by Fundação para a Ciência e a Tecnologia within the project UID/Multi/04044/2013.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC10

Loss of torque complexity during fatiguing submaximal isometric knee extensions in man is slowed by caffeine ingestion

J. Pethick, S. Winter and M. Burnley

School of Sport and Exercise Sciences, University of Kent, Medway, UK

The temporal structure, or complexity, of torque output is thought to reflect the adaptability of motor control and has important implications for system function, with high values endowing greater adaptability in response to alterations in task demand. Neuromuscular fatigue has been demonstrated to reduce torque complexity during repeated isometric knee extension contractions (Pethick *et al*., 2015); however, the mechanism(s) behind this fatigue-induced loss of complexity is not known. We hypothesised that caffeine, an ergogenic aid thought to act primarily through central mechanisms, would attenuate the fatigue-induced loss of torque complexity previously observed. Ten healthy participants performed, on separate days, intermittent isometric submaximal contractions at a target torque of 50% MVC, with a 60% duty factor (6 s contraction, 4 s rest), after having ingested either 6 $mg.kg^{-1}$ caffeine or the same amount of placebo one hour prior to the commencement of the contractions. Torque and surface EMG signals were sampled continuously. Complexity and fractal scaling of torque were quantified by calculating approximate entropy (ApEn) and the detrended fluctuation analysis (DFA) scaling exponent, α. Global, central and peripheral fatigue were quantified using maximal voluntary contractions (MVCs) with femoral nerve stimulation. Values are means $\pm$ SEM, compared by ANOVA and *t*-test. Caffeine ingestion significantly increased time to task failure by 2.4 ± 0.9 mins ($P = 0.019$). Complexity decreased in both trials (decreased ApEn and increased DFA α, both $P < 0.01$), as global, central and peripheral fatigue increased (all $P < 0.01$). However, the rate at which complexity

decreased was significantly lower following caffeine ingestion (ApEn, −0.06 ± 0.01 vs. −0.04 ± 0.01, $P = 0.014$), as were the rates of global (−22.1 ± 5.7 vs. −17.8 ± 4.7 N.m.min^{-1}, $P = 0.011$), central (−5.7 ± 1.3 vs. −3.7 ± 1.1 %.min^{-1}, $P = 0.046$) and peripheral (−8.2 ± 2.1 vs. −6.5 ± 1.6 N.m.min^{-1}, $P = 0.043$) fatigue development. This slower loss of complexity and slower rate of fatigue development, in all its forms, following caffeine ingestion suggests that the mechanisms responsible for the loss of torque complexity and caffeine's ergogenesis are intrinsically linked. However, the slowing of fatigue in all its forms does not allow the identification of a single mechanism responsible for the loss of torque complexity. Instead, the loss of torque complexity could be the expression of an integrated response to neuromuscular fatigue, including both central and peripheral components.

Pethick, J., Winter, S.L. and Burnley, M (2015). Fatigue reduces the complexity of knee extensor torque fluctuations during maximal and submaximal intermittent isometric contractions in man. *Journal of Physiology,* **593, 2085–**2096.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC11

The effects of beetroot Juice and sodium nitrate on muscle damage following eccentric exercise

T. Clifford[1], G. Howatson[1], D. West[2] and E. Stevenson[2]

[1]Northumbria University, Newcastle Upon Tyne, UK and [2]Newcastle University, Newcastle Upon Tyne, UK

Exercise-induced muscle damage (EIMD) is characterized by muscle pain, reduced muscle function and inflammation. It was recently shown that some of these indices can be reduced with acute beetroot juice (BTJ) supplementation (Clifford et al, 2015); however, the active compounds in BTJ responsible for these effects have not been elucidated. More specifically, whether these effects are mediated by nitrate or other phytonutrients (i.e., polyphenols) in BTJ has not been investigated. Thus, the aim of this study was to investigate the effects of BTJ and a nitrate only drink (sodium nitrate; SN) on EIMD. Using a double blind, independent groups design, 30 recreationally active males were randomly assigned to a BTJ (n=10), SN (n=10) or an isocaloric placebo (PLA; n=10) group. The BTJ and SN drinks were matched for nitrate content (~210 mg) and energy content. Drinks were consumed (2 x 250 ml) immediately, 24 and 48 h after performing 100 drop jumps. To assess muscle damage, maximal isometric voluntary contractions (MIVC), countermovement jumps (CMJ), reactive strength index (RSI), pressure-pain threshold (PPT) creatine kinase (CK) and high sensitivity C-reactive protein (hsCRP) were measured pre, immediately post, 24, 48 and 72 h following the drop jumps. Values are mean±SD; statistical analysis was carried out with a mixed model ANOVA. The exercise bout caused a significant decrease in PPT across all groups ($P = 0.001$); however, the decrease was attenuated with BTJ compared to SN and PLA

throughout the 72 h measurement period ($P = 0.043$). PPT had recovered to baseline values in the BTJ group by 72 h (104.3±25.9%) but remained depressed in both the SN (94.1±16.0%) and PLA groups (91.2±19.0%). Muscle function (MIVC, CMJ and RSI) was reduced following exercise by ~15-25% and did not recover to baseline by 72 h in all groups ($P < 0.05$); no group differences were observed ($P > 0.05$). Serum CK increased after exercise and peaked at 24 h post but no group differences were present ($P > 0.05$). hsCRP levels were unaltered by the exercise protocol ($P > 0.05$). These data suggest that BTJ supplementation might be a useful strategy to attenuate muscle pain associated with EIMD, and that any analgesic effects are likely due to phytonutrients in BTJ other than nitrate, or interactions between them. Further research is needed to clarify the potential differing effects of SN and BTJ on neuromuscular recovery.

Clifford T et al. (2015). EJAP, 1–10.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC12

Ketone ester drinks increase blood ketone levels more effectively than ketone salt drinks

B.J. Stubbs, R. Evans, K. Clarke and P.J. Cox

Department of Physiology Anatomy and Genetics, Oxford University, Oxford, UK

Introduction: Ketone bodies (KB) are oxidative fuel substrates and metabolic signals produced in response to starvation or a high fat, low carbohydrate diet. KB may provide a superior fuel source to athletes [1]. We developed a ketone ester (KE) that, when consumed as a drink, rapidly increased circulating KB [2]. Ketone salt (KS) drinks are an alternative to achieve nutritional ketosis [3]. However, the comparative efficacy of KE and KS drinks to raise blood KB is unknown. The aim of this study was to compare blood KB levels after body-weight adjusted, equimolar amounts of KB were consumed in KE and KS drinks.

Methods and Results: Following favourable ethical review, healthy, non-obese volunteers (n = 5) completed a 4-armed randomized, cross-over study. Following an overnight fast, volunteers consumed a weight-adjusted dose of β-hydroxybutyrate (BHB) (low- 1.6 mmol/kg OR high- 3.2 mmol/kg) in KS or KE, artificially flavoured and made up to 300 ml using water. Blood samples were obtained via an IV catheter at baseline (BL) and at regular intervals post-drink. Samples were analyzed for D-BHB. Volunteers completed questionnaires to record any GI/systemic symptoms experienced. D-BHB values are means ± SEM. Symptoms are number reported per 100 possible reports. Repeated measures ANOVA with Tukey Post Hoc corrections were performed. Significance was taken at $p<0.05$.

Consumption of both KE and KS drinks increased the blood levels of D-BHB. Peak D-BHB concentration (D-BHB C_{max}) was significantly greater following the high dose of KE (3.0 ± mM) vs. low dose KE (1.5 ± mM) and vs. both high (1.2 ± mM) and low (0.9 ± mM)

doses of KS. There were no significant differences in D-BHB C_{max} between low dose KE, and low and high doses of KS. D-BHB uptake (AUC) was significantly higher following high dose of KE (417 ± 62 mM.min) vs. all other groups, but there were no differences between low dose KE (166 ± 24 mM.min), and low (117 ± 21 mM.min) and high (170 ± 7 mM.min) doses of KS. GI symptoms reported were significantly higher with high dose of KS (10/100 possible) than low doses of both KS (2.6/100 possible) and KE (3.4/100 possible), however there were no significant differences between the high dose of KE (6.5/100 possible) and all other groups.

Conclusions: Increasing KE dose results in greater D-BHB C_{max} and AUC; however this is not seen following KS drinks. Long term KS consumption may result in clinical complications due to the inorganic ion load in each drink [4], furthermore we saw that high doses of KS cause a greater incidence of GI symptoms. Therefore we conclude that KE drinks are a more effective method to elevate D-BHB in athletes than KS drinks, with fewer acute side effects.

1. Cox P & Clarke K (2014) Acute nutritional ketosis: implications for exercise performance and metabolism. *Extr. Physiol. Med* 3(1):17.

2. Clarke K, *et al.* (2012) Kinetics, safety and tolerability of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate in healthy adult subjects. *Regul. Toxicol. Pharmacol.* 63(3):401-408.

3. Plecko B, *et al.* (2002) Oral beta-hydroxybutyrate supplementation in two patients with hyperinsulinemic hypoglycemia: monitoring of beta-hydroxybutyrate levels in blood and cerebrospinal fluid, and in the brain by in vivo magnetic resonance spectroscopy. *Pediatr Res* 52(2):301-306.

4. Aburto NJ, *et al.* (2013) Effect of lower sodium intake on health: systematic review and meta-analyses. *BMJ* 346.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC13

Muscle mitochondrial dysfunction in Type 2 diabetes likely results from a decline in total mitochondrial DNA copy number

D. Constantin-Teodosiu[1], D. Constantin[1], L.B. Verdijk[2], K. Tsintzas[1], L. van loon[2] and P.L. Greenhaff[1]

[1]Life Sciences, Nottingham University Medical School, Nottingham, UK and [2]Health, Medicine and Life Sciences, Movement Sciences, Maastricht University, Maastricht, Netherlands

Type 2 diabetes (T2D) is associated with excess energy intake and physical inactivity. Intrinsically linked to these events are deficits in muscle total mitochondrial volume and/or function (Kelley *et al*., 2002), but whether this reflects a lower muscle mitochondrial DNA (mtDNA) copy number or simply a muscle deconditioning is debated. We therefore quantified maximal oxygen consumption (VO_{2max}), mtDNA copy number (RQ-PCR) and mitochondrial enzyme activities (GluDH, CS, β-HAD; spectrophotometrically) in muscle biopsies from older age-matched, male

volunteers: healthy trained (57.4 ± 0.9 yrs, *n*=10), healthy sedentary (60.0 ± 2.0 yrs, *n*=10), and T2D patients (58.9 ± 2.0 yrs, *n*=10). Ethical approval was granted before subject's consent was obtained.

	VO_{2max}[a]	ISI[b]	mtDNA	GluDH[c]	CS[c]	β-HAD[c]
Trained	3.8±0.1$^{*\#}$	133±24**	1461±52$^{***\#\#\#}$	9.8±0.9$^{**\#}$	84.4±9.5$^{**\#}$	32.6±3.2$^{*\#}$
Sedentary	3.2±0.2	86±12*	749±35***	6.9±0.4	51.9±7.0	21.3±2.5
T2D	2.9±0.2	48±6	454±59	5.6±0.6	42.6±2.3	20.9±3.6

[a]L min^{-1}; [b]mg L^{2} (mmol mU min)$^{-1}$; [c]mmol min^{-1}mg^{-1} protein, *, **, ***Significantly different from T2D (P<0.05, *P*<0.01 and P<0.001); $^{\#}$, $^{\#\#\#}$Significantly different from sedentary (*P*<0.05, *P*<0.001). All values in text and Table represent mean±SEM. Statistical differences detected using ANOVA.

Except for Insulin Sensitivity Index (ISI), no differences in mitochondrial enzymatic markers were detected between older healthy sedentary and T2D (Table). However, both were markedly different from older trained volunteers. The number of mtDNA copy number in the older trained group was significantly greater than in the older untrained and T2D groups (P<0.001). Furthermore, the mtDNA copy number in the older untrained group was significantly greater than in the T2D group (P<0.001). This suggests that any apparent muscle mitochondrial dysfunction in T2D likely results from a decline in total mtDNA copy number.

Kelley DE et al. (2002). *Diabetes* **51**, 2944–2950.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC14

The effect of type 2 diabetes in muscle deoxygenation during ramp incremental cycling exercise

N. Gildea[1], J. Rocha[1], S. Green[3], D. O'Shea[2] and M. Egana[1]

[1]Physiology, Trinity College Dublin, Dublin, Ireland, [2]Endocrinology, St Columcille's and St Vincent's Hospitals, Dublin, Ireland and [3]School of Science and Health and School of Medicine, University of Western Sydney Australia, Sydney, NSW, Australia

Defects in functional exercise capacity in patients with type 2 diabetes mellitus (T2DM) have been consistently reported, with impairments in maximal exercise performance of ~20% that are independent of obesity, and present in the absence of clinically apparent cardiovascular disease. Whilst the precise mechanisms for this abnormal exercise response remain to be elucidated, both central and peripheral factors have been identified as potential contributors (Green *et al,* 2015). In the present study we tested the hypothesis that T2DM alters the profile of muscle fractional O_2 extraction (estimated using deoxygenated haemoglobin) during incremental cycle exercise. Eight middle-aged participants (6 men, 2 women) with T2DM (46.85±7.58yrs; 31.68±5.76kg/m^2) and eight healthy controls (6 men, 2 women) (42.33±7.56yrs; 30.4±2.22kg/m^2) matched for age and body mass

index respectively, performed a ramp incremental cycling test to exhaustion in an upright position. Exercise was performed initially for 2-min at 10W, followed by 15 W/min (females) or 25 W/min (males) increments on an electrically braked cycle ergometer, with pedal frequency held constant at an individually selected rpm. Pulmonary oxygen uptake (VO_2) was measured on a breath-by-breath basis using an online metabolic system. The rate of muscle deoxygenation (i.e. deoxygenated haemoglobin concentration, Δ[HHb]) profiles of the vastus lateralis (VL) muscle were continuously made with near infrared spectroscopy (NIRS) and analysed with a double linear model. Values are means $\pm$ SD, compared by a paired *t* test. Normalised VO_{2peak} was significantly ($P = 0.047$) reduced in individuals with T2DM compared with their respective non-diabetic counterparts (24.52 ± 4.15 *vs* 29.52 ± 4.99), representing a 17% reduction in peak exercise capacity. The first slope of the double linear regression function used to establish the dynamic adjustment of [HHb], was significantly ($P = 0.038$) larger in participants with T2DM than controls (1.35 ± 0.17 *vs* 1.08 ± 0.35). Such findings are indicative of a greater rate of oxygen extraction for a given increase in VO_2, suggesting that a reduced O_2 delivery is an important underlying cause of exercise intolerance during a maximum graded test in T2DM.

Green S, Egaña M, Baldi C, Lamberts R and Regensteiner J (2015). Cardiovascular Control during Exercise in Type 2 Diabetes Mellitus. *J Diabetes Res* **2015**, 1–11.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC15

Does age affect motor sequence learning ability?

M.K. Fleming, N. Lazarus and D. Newham

Centre of Human and Aerospace Physiological Sciences, King's College London, London, UK

Healthy ageing is typically associated with a decline in cognitive and motor abilities and older adults may show reduced or slower motor sequence learning than younger adults (Boyd et al., 2008; Zimerman et al., 2013). This study aimed to examine the effect of age on an explicit motor sequence learning task. Healthy adult humans (n=25) aged 25-85 years performed a motor sequence learning task with the non-dominant (left) hand requiring movement of a computer mouse from a central square to illuminated targets on a computer monitor. Participants were informed of the presence of a repeated sequence of movements and encouraged to anticipate target appearance. After familiarisation, a sequence of 10 movements was repeated 25 times followed by a random sequence. Onset time (OT) was recorded as the time from target illumination to the cursor leaving the central square. Initial OT (i.e. reaction to target illumination) did not differ between older (>50 years) and younger (< 45 years) adults (t-test, p = 0.38) and there was no correlation between age and initial OT (Pearson correlation, p = 0.13). OT area

under the curve (OT AUC) was significantly better for younger adults than older ($p = 0.04$) and there was a significant moderate correlation between age and OT AUC (Fig. 1A, $p = 0.01$) suggesting reduced rate of learning with age. However, when OT AUC was divided into bandwidths of functional equivalence (Lazarus and Harridge, 2010) using five categories (1 = OT AUC < 10, 2 = 10-15, 3 = 15-20, 4 = 20-24, 5 >24) a spread of ages across each category can be seen (Fig. 1B). There was no difference between younger and older adults for the specificity of sequence learning (OT difference between trained and untrained sequence, $p = 0.38$). These results suggest that motor reactions and sequence learning with the non-dominant hand may not be impaired in healthy older adults and highlights the complexity of the relationship between age and motor function.

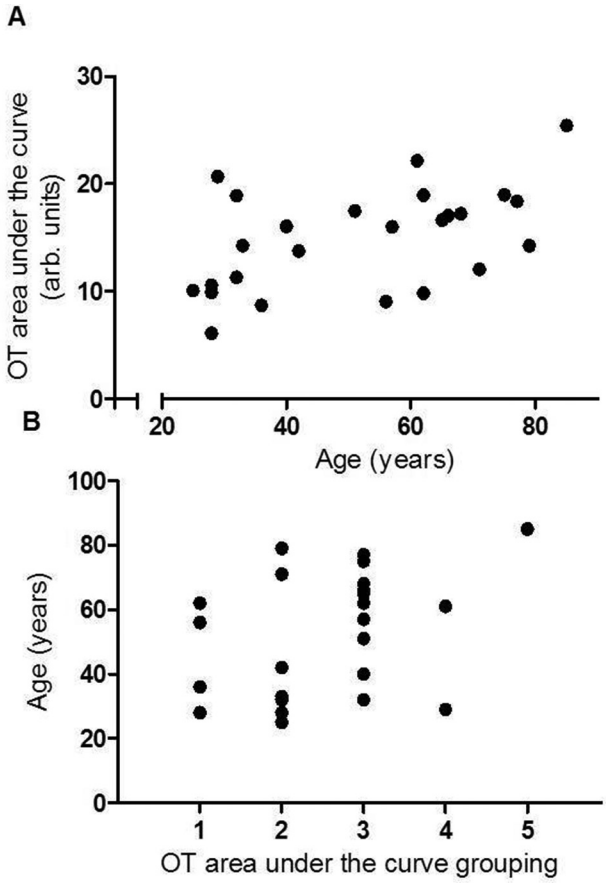


Fig. 1. A. Onset time (OT) area under the curve (arb. units) as a function of age of participant. Values <24 indicate learning of the movement sequence. **B.** Age of participant as a function of OT area under the curve grouping (1= excellent learning: OT AUC <10, 5 = no learning: OT AUC >24), showing a spread of ages for each grouping.

Boyd, L.A., Vidoni, E.D., Siengsukon, C.F., 2008. Multidimensional motor sequence learning is impaired in older but not younger or middle-aged adults. Phys Ther. 88, 351–62.

Lazarus, N.R., Harridge, S.D., 2010. Exercise, physiological function, and the selection of participants for aging research. J Gerontol A Biol Sci Med Sci. 65, 854–7.

Zimerman, M., Nitsch, M., Giraux, P., Gerloff, C., Cohen, L.G., Hummel, F.C., 2013. Neuroenhancement of the aging brain: restoring skill acquisition in old subjects. Ann Neurol. 73, 10–5

Thank you to Roger Woledge for writing the Matlab programme and Tony Christopher and Lindsey Marjoram for technical support.MKF is supported by a King's College London doctoral scholarship and a project grant from the Stroke Association awarded to DJN.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC16

A novel homozygous mutation in the VHL gene in man is associated with exaggerated cardiopulmonary responses to acute hypoxia and limited exercise capacity

F. Formenti[1,2,3], F. Della Ragione[4], P. Robbins[2] and S. Perrotta[5]

[1]Centre of Human and Aerospace Physiological Sciences, King's College London, London, UK, [2]Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK, [3]Nuffield Division of Anaesthetics, University of Oxford, Oxford, UK, [4]Department of Biochemistry, Biophysics and General Pathology, Second University of Naples, Naples, Italy and [5]Dipartimento della Donna, del Bambino e di Chirurgia Generale e Specialistica, Second University of Naples, Naples, Italy

The hypoxia-inducible factor (HIF) transcriptional pathway is fundamental for the regulation of cellular function in hypoxia [1]. Genetic mutations of the HIF pathway favoured Tibetans' adaptation to life at high altitude [2], but other mutations, e.g. in the Von Hippel-Lindau (VHL) gene, can compromise the HIF pathway function, and be associated with polycythaemia, abnormal cardiopulmonary function [3, 4] and metabolism [5]. A patient homozygous for a novel C>A mutation on VHL (Val74Val) presented with lower than normal levels of VHL protein and polycythaemia. We explored this patient's physiological response to acute hypoxia and, separately, to exercise.
Methods - The patient's cardiopulmonary physiology was studied at rest at sea level (baseline) and in a hypoxic chamber at a simulated altitude of about 3,500 m. Respired gases were sampled continuously, and end-tidal partial pressure of carbon dioxide ($ETCO_2$) was kept close to the patient's air-breathing value throughout the protocol. We monitored heart rate, arterial oxygen saturation, ventilation, pulmonary arterial systolic pressure (PASP) and cardiac output, and averaged data over 4 minutes. Results from 15 control participants who took part in comparable studies [3, 4] are presented for comparison.

The patient's exercise capacity was measured with an incremental exercise test on a cycle ergometer, where the workload was increased by 20 W per min until exhaustion (tested twice). We measured venous blood lactate at the end of each workload, and respiratory gases continuously. Data were averaged for each min of exercise, and compared with results from control participants (n=6) [5].
All values presented are mean ± S.D.
Results - Table 1 shows the results from the test of cardiopulmonary responses to acute hypoxia. During air breathing conditions at baseline, the patient's end-tidal partial pressure of oxygen (ETO_2) was high and $ETCO_2$ low. The patient's PASP and cardiac output were elevated at baseline, and showed a marked increase in response to hypoxia.
Figure 1 shows the patient's responses to the exercise test to exhaustion on the cycle ergometer. The patient showed a limited exercise capacity, associated with a low peak value for lactate in venous blood, and a greater than normal increase in ventilation for equivalent work rates. $ETCO_2$ remained low throughout the exercise protocol. Control participants stopped exercising later in the protocol (~240 W; data not shown).
Conclusions - These findings highlight the role of the HIF pathway in regulating human physiology at whole organism level.

	End Tidal O2 (mmHg)	End Tidal CO2 (mmHg)	PASP* (mmHg)	Cardiac Output (l min-1)	Heart Rate (beats min-1)	Ventilation (l min-1)	Saturation (%)
Baseline patient	120 ± 5	25 ± 3	26 ± 2	6.6 ± 0.3	63 ± 9	10 ± 3	97 ± 1
Baseline control (n=15)	100 ± 4	39 ± 3	20 ± 2	4.4 ± 0.7	59 ± 11	11 ± 3	96 ± 3
Hypoxia patient	46 ± 1	28 ± 1	41 ± 2	9.2 ± 0.3	83 ± 7	20 ± 4	86 ± 1
Hypoxia control (n=15)	50 ± 1	39 ± 2	26 ± 3	5.6 ± 1.0	71 ± 11	18 ± 9	84 ± 4

*control n=11

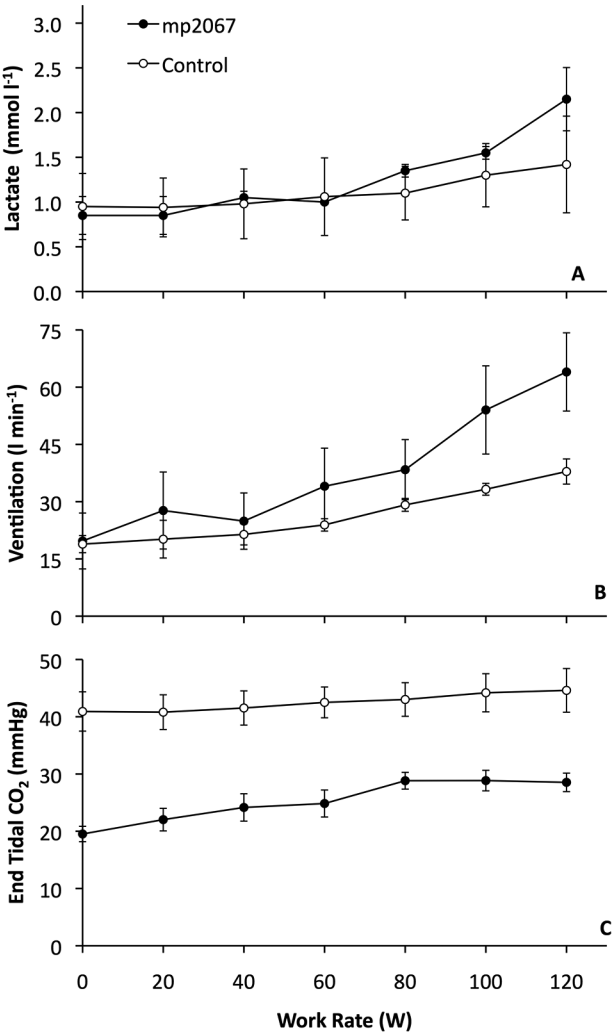


Figure 1

Semenza GL (2012). *Cell* **148**(3), 399–408

Petousi N *et al.* (2014). *J Appl Physiol* **116**(7), 893–904

Smith TG *et al.* (2006). *PLoS Med* **3**(7), p. e290

Formenti F *et al.* (2011). *Faseb J* **25**(6), 2001–11

Formenti F *et al.* (2010). Proc Natl Acad Sci USA **107**(28), 12722–7

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC17

Influence of priming exercise and type 2 diabetes on oxygen uptake and muscle deoxygenation kinetics during submaximal exercise

J. Rocha[1], N. Gildea[1], D. O'Shea[2], S. Green[3] and M. Egana[1]

[1]Physiology, Trinity College Dublin, Dublin, Ireland, [2]Endocrinology, St Columcille's and St Vincent's Hospitals, Dublin, Ireland and [3]School of Science and Health and School of Medicine, University of Western Sydney, Sydney, NSW, Australia

Middle aged and young adults with uncomplicated type 2 diabetes (T2D) show a slowed adjustment of oxidative metabolism during metabolic transitions (i.e. oxygen uptake, VO2 kinetics) due to progressive limitations of both O_2 delivery and utilisation. Priming exercise (PE) has been shown to increase the speed of adjustment of oxidative metabolism during subsequent moderate-intensity step transitions in healthy adults presenting initially slow VO2 kinetics. We tested the hypothesis that PE would increase the speed of the adjustment of the primary phase (tau_p, τ_p) of VO_2 during moderate intensity cycling in T2D and that this would be due to a better matching of O_2 delivery to utilisation. Ten middle-aged participants with uncomplicated T2D (50.7 $\pm$ 9.0 years, 30.4 $\pm$ 5.3 kg/m^2; 7 men / 3 women) and 10 non-diabetic (ND) controls (44.4 $\pm$ 9.6 years, 31.1 $\pm$ 4.1 kg/m^2; 7 men / 3 women) were recruited. Participants completed four bouts of constant-load cycling at 80% of their ventilatory threshold previously established during a ramp incremental test. Two of these constant-load bouts were completed without priming exercise (ModA) and two bouts were undertaken with prior heavy intensity priming exercise (ModB). VO_2 kinetics was calculated from continuously measured breath-by-breath data, while the rate of muscle deoxygenation (i.e., deoxygenated hemoglobin, HHb) and tissue oxygen saturation (i.e., tissue oxygenation index) were continuously measured by Near–infrared spectroscopy (NIRS) at the vastus lateralis muscle. The time constant of the primary phase, $\tau_{p,}$ was significantly slower in T2D, but PE significantly (P<0.05) reduced τ_p in both groups by a similar magnitude (T2D, 48.29 $\pm$ 11.5 vs. 35.9$\pm$13.0 s; ND, 34.0 $\pm$ 9.6 vs. 26.8 $\pm$ 10.5 s). The adjustment of deoxygenated heamoglobin (HHb) did not show any differences between groups but its amplitude was increased after PE (P=0.036). Total tissue oxygenation at baseline and end of exercise was lower in T2D (P<0.05), however, PE increased tissue oxygenation index at baseline (p=0.002) and the delta values at the end of exercise (p=0.001) in both groups. The HHb/VO_2 ratios (20-120s) were reduced after PE (T2D, 1.12 $\pm$ 0.12 vs 1.07 $\pm$ 0.12; ND, 1.03 $\pm$ 0.13 vs 1.00 $\pm$ 0.12; P=0.038). These preliminary data support that in middle-aged adults with T2D, priming exercise prior to moderate-intensity exercise, beneficially affects the speed of adjustment of oxidative metabolism, possibly due to the partial improvement in the transient mismatch of muscle O2 delivery relative to utilisation.

The authors would like to thank all the volunteers for their participation in this study.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC18

Awareness and knowledge of prevention and basic rehabilitation protocols of sports injuries among senior school athletes in Sri Lanka

S.S. Jayalath[1], A. Karunaratne[2], M. Fonseka[1] and N. Silva[3]

[1]Physiotherapy, International Institute of Health Sciences, Welisara, Sri Lanka, [2]Health and life sciences, Coventry University, Coventry, UK and [3]Medical officer, Ministry of Health, Colombo, Sri Lanka

Problem Statement: Sports injuries are gradually increasing all over the world. School athletes are the first stepping stone in improving knowledge and skills in the community, therefore understanding and improving their knowledge is of great importance. Furthermore school athletes are the potential group that will make up the senior professional athletes population in the future. Prevention of injuries and proper first aid helps minimize injuries and reduce further complications. There for evaluation of the existing knowledge and practices of school athletes regarding injuries and their prevention is an essential exercise. This study will help to determine the extent of their knowledge in prevention of injuries and basic first aid.
Objective: To identify the knowledge of prevention and rehabilitation of sports injuries among senior school athletes in Sri Lanka.
Methods: A descriptive cross sectional study on senior school athletes in Colombo was done using a sample of 150 conveniently selected students, using a self-administered questionnaire with 10 close ended questions and analyzed using descriptive and analytical statistics. Ethical clearance was obtained from the Ethics Review Committee of the International Institute of Health Sciences, Sri Lanka.
Results: Out of 150 participants, only 45 students have adequate knowledge of cryotherapy, 51 have basic knowledge of relieving cramps, 69.3% continue stretching though they have pain, 67.3% are unaware of proper bandaging protocols, 39.3% understand splinting and its uses and 26.7% are well informed on the necessity of CPR. Whereas, out of the 150 students, 80 students are aware of emergency wound care, 64% know standard first aid for ankle sprains, 75.3% are aware of the purpose of vapo-coolant spray, and 99.3% perform warmup exercises and 96% practice cool down exercises.
Conclusions: Senior school athletes perform warm up and cool down exercises which is an integral part of injury prevention, but when considering basic first aid and post injury rehabilitation there are many aspects in which majority of the athletes are lacking in knowledge and training. Provision of knowledge on prevention of injuries and complications of injuries would help to upgrade their performance. It is vital to improve their knowledge in these areas to prevent further complications as well. Furthermore by considering all above factors it is necessary to establish a

system to train and improve their knowledge serially. Additionally, policy changes may be needed to address this lack of knowledge in management of these injuries when they occur.

Key words: Senior School Athletes, Sport Injuries, Prevention.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC19

Commonly used activating solutions cause different levels of specific force in chemically skinned human muscle fibres

M. Kalakoutis[1], A. Atkinson[2], J. Ochala[1] and S.D. Harridge[1]

[1]Centre of Human and Aerospace Physiological Sciences, King's College London, London, UK and [2]Centre for Biomolecular Spectroscopy and Randall Division of Cell and Molecular Biophysics, King's College London, London, UK

Specific force (SF) represents a muscle's contractile quality and is the peak isometric force normalised to cross-sectional area (CSA). There is large variance in published SF measurements from human skinned muscle fibres of young, healthy individuals, reduced only in part when methodological differences between studies are accounted for (Kalakoutis et al., 2014). Despite research on the effects of different chemical substrates on the mechanical properties of skinned fibres, experimental solutions used differ between research groups. The aim was to quantify the effect of this methodological difference, with emphasis on SF production. Human Vastus Lateralis muscle fibres (n = 96) were obtained from a biopsy sample following local anaesthesia (2% lidocaine) in one young, healthy, male. Fibres were chemically skinned and exposed to two different activating solutions, A and B, in a random order. SF was measured at 15°C and time to half peak tension (t_{50}) was calculated as an indication of contraction kinetics. Three differences between solutions A and B were the uses of:

1) Imidazole (A) or Tes (B)
2) Glutathione (GL) (B only)
3) Potassium Chloride (KCl) (A) or Potassium Propionate (K-prop) (B).

The impact of each difference on skinned fibre contraction was isolated by making new solutions which differed in only one chemical constituent. A paired t-test assessed significance ($p<0.05$) of mechanical results (mean ± SD). ^{1}H nuclear magnetic resonance spectroscopy (^{1}H NMR) monitored potential formation of new compounds by reaction of Imidazole or Tes, which could affect SF.

A significantly higher SF and shorter t_{50} was measured from the same fibres in solution B (109.8 ± 45.3 kPa; 1.5 ± 0.9s) compared with solution A (75.8 ± 43 kPa; 9.4 ± 4.4s). Isolating the effects of individual chemical components showed SF was 15.4 ± 7.7% higher ($p<0.05$) in a solution containing an optimum concentration of Tes (60mM), not Imidazole (20mM). The t_{50} was shorter ($p<0.05$) in solution

containing GL (2.3 ± 1.1s) compared to without GL (5.8±2.9s) and in a solution containing K-prop (1.5 ± 1.4s) compared with a similar solution containing KCl (4.0 ± 3.0s). ^{1}H NMR spectra corresponded to the compounds expected based on each solution's composition, with no indication of reaction products.
The higher SF elicited in solution B was largely due to the use of Tes instead of Imidazole. The shorter t_{50} in solution B was partly accounted for by a lower Cl^- concentration due to the use of K-prop instead of KCl and by the use of GL. ^{1}H NMR experiments could not measure effects on ionic strength or $[Mg^{2+}]$ so these remain possible mechanisms of the higher SF elicited by solution B.
These findings show that the use of different experimental solutions contributes substantially to the disparity of SF measurements reported by different publications studying human skinned fibres.

Kalakoutis, M., Ochala, J., Harridge, S. & Woledge, R. (2014) Specific force in human chemically skinned single muscle fibres: An evaluation of the variability in published values. Unpublished poster presentation at: Physiology 2014, June 30th – July 2nd 2014, London, United Kingdom.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC20

Effects of training on pulmonary function amongst Sri Lankan national level athletes

K.U. Wijayasiri

sports and exercise medicine unit, colombo south teaching hospital, Dehiwala, Colombo, Sri Lanka, Colombo, Sri Lanka

Introduction - Poor performance of Sri Lankan athletes in the international arena is observed despite regular training. Performance depends on the physical fitness and technical training. Although techniques are addressed, a player's physical fitness is not optimized by the present training programs.
Objective - To determine the status of pulmonary functions amongst Sri Lankan national level athletes in comparison to matched controls.
Methodology - National level athletes (n = 63) engaged in resistance and endurance sports were studied. Baseline data were collected by a questionnaire and clinical examination. Pulmonary functions were assessed by a Vitallograph spirometer. Results were compared with age, height, weight and gender matched controls (n= 63). Data were analyzed using SPSS version 16 statistical package.
Results - Inspiratory function as indicated by the Forced Inspiratory Vital Capacity (FIVC), Forced Vital Capacity (FVC) and Forced Expiratory Volume in 1st second (FEV_1) were significantly higher amongst the athletes ($p< 0.05$). The small air way function as determined by mid stream Forced Expiratory Flow ($FEF_{25\%-75\%}$) of the athletes was similar to the controls ($p>0.05$). The expiratory muscle efficiency as

indicated by Peak Expiratory Flow Rate (PEFR) and FEV1/ FVC ratio was not significantly different between the athletes and the controls ($p > 0.05$).
Discussion - Better training should be associated with an optimal improvement of respiratory function; ie. Increasing the depth of breathing by increasing the Tidal volume and Vital Capacity. In order to achieve this both inspiratory and expiratory capacities have to be increased significantly. The results indicate that the respiratory efficiency of the athletes had not optimally improved with training.
Conclusion - The study concludes that training programs for the athletes must consist of exercise schedules to optimize the strength of respiratory muscles. This will achieve optimal pulmonary function amongst athletes. Improvement of pulmonary function may in turn promote better performance of athletes at competition.
Key words - National athletes, pulmonary function tests, respiratory muscles, exercise training, physical fitness.

George et al, 2014 Evaluation of the effect of exercise on pulmonary function in young healthy adults, International Journal of Biomedical and Advance Research Vol. 5, No 6

Vincent et al, 2012 ACSM's advanced exercise physiology. American College of Sports Medicine, 2nd edition, 242–296

José et al, 2001 Inspiratory Capacity, Dynamic Hyperinflation, Breathlessness, and Exercise Performance during the 6–Minute-Walk Test in Chronic Obstructive Pulmonary Disease. American Journal of Respiratory and Critical Care Medicine, Vol. 163, No. 6

Illi et al, 2012 Effect of Respiratory Muscle Training on Exercise Performance in Healthy Individuals; A Systematic Review and Meta-Analysis. Sports Med; 42 (8): 707–724

Romer et al, 2002, Effects of inspiratory muscle training on time-trial performance in trained cyclists. J Sports Sci 20:547–562

Sport Associations and the Players: Athletics, Badminton, Foot ball, Cricket and Rugby.

All the Academic staff and Non-Academic staff: Department of Physiology, Faculty of Medical Sciences, University of Sri Jayawardenepura,Nugegoda, Sri Lanka.

All the Doctors and other staff: Sports Medicine Unit, Colombo South Teaching Hospital, Kalubowila, Colombo, Sri Lanka.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC21

Body image perception in association with rigid and flexible dieting in athletes and non-athletes

A. Vlahoyiannis, I. Papasotiriou and A. Nifli

Technological Educational Institute of Thessaly, Department of Nutrition & Dietetics, Karditsa, Greece

Body image imposes or justifies eating patterns. Structured eating behaviours have been shown to correlate with BMI (1), eating disorders (1) and weight loss outcome (2). However, their relationship with perception and distortion of body image has not been investigated. The current study included 92 people, 68 males (34 athletes / 34 non-athletes) and 24 females (8 athletes/ 16 non-athletes). We evaluated anthropometric data and estimated body composition through skinfold measurement. Quantitative data of perceived and intentional body image were obtained by self-assessment with Somatomorphic Matrix software (3). The control of dietary restraint was assessed with FC12 and RC16 questionnaires (4). Statistical analysis revealed significant differences for actual and perceived body image in non-athletes. More specifically, women non-athletes were found to overestimate body fat and FFMI ($t(15) = 3.741$, $p = 0.002$, $t(15) = 6.309$, $p = 0000$ respectively), while men non-athletes underestimated FFMI ($t(33) = -2.604$, $p = 0.14$). Flexible and rigid dieting strategies were equally adopted by study subgroups, and correlated significantly ($r(91) = 0.649$, $p < 0.001$). Classification as mainly flexible (score > 5 only in FC12), mainly rigid (score > 7 only in RC16), highly-structured (score > 5 in FC12 and > 7 in RC16) and non-structured (score < 5 in FC12 and < 7 in RC16) dieters and application of one-way ANOVA revealed statistically significant differences for the nonconformities of actual and perceived % body fat ($F(3,88) = 2.583$, $p = 0.058$) and FFMI ($F(3,88) = 6.334$, $p = 0.001$), as well as for the nonconformities of actual and intentional % body fat ($F(3,88) = 3.068$, $p = 0.032$) and intentional FFMI ($F(3,88) = 4.913$, $p = 0.001$). Further application of Hochberg post-hoc test revealed that flexible dieters presented lower discrepancy for actual and perceived % body fat than highly-structured dieters (-3.3442 ± 1.2071 *vs* 1.3406 ± 6.1629, $p = 0.45$ respectively). In addition, in flexible dieters perceived and actual FFMI were closer than in non-structured dieters (-1.2857 ± 2.79966 *vs* 1.3477 ± 2.6448, $p = 0.005$ respectively), and the same was calculated for intentional and actual FFMI (0.8489 ± 3.32429 *vs* 4.2620 ± 3.6, $p = 0.002$ respectively). In contrast, rigid dieters tended to underestimate their FFMI compared to non-structured dieters (-5.1615 ± 3.15271 *vs* 1.3477 ± 2.6446, $p = 0.018$) and to aim to rather low % body fat than non-structured dieters (-18.32 ± 4 *vs* -2.9044 ± 6.8, $p = 0.035$ respectively). Our data suggest that participation in athletic activities may prevent body image distortion, while dietary strategies do associate with more accurate body image perception and body image dissatisfaction.

Stewart TM, Williamson DA, White MA. Rigid vs. flexible dieting: association with eating disorder symptoms in nonobese women. Appetite. 2002;38(1):39-44.

Westenhoefer J, Engel D, Holst C, Lorenz J, Peacock M, Stubbs J, et al. Cognitive and weight-related correlates of flexible and rigid restrained eating behaviour. Eating behaviors. 2013;14(1):69-72.

Gruber A, Pope H, Borowiecki J, Cohane G. The development of the somatomorphic matrix: A bi-axial instrument for measuring body image in men and women. Kinanthropometry VI. 2000:217-31.

Westenhoefer J, Stunkard AJ, Pudel V. Validation of the flexible and rigid control dimensions of dietary restraint. The International journal of eating disorders. 1999;26(1):53-64.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC22

The influence of the Female Athlete Triad on bone quality in elite endurance runners

J. Coulson, A. Ireland, H. Degens and J. McPhee

School of Health care Science, Manchester Metropolitan University, Manchester, UK

Elite female athletes have exceptionally high physical activity levels. The physiological stresses associated with high exercise can disrupt normal homeostatic processes, altering menstrual cycles and energy balance, leading to a condition known as The Female Athlete Triad. The aim of this study was to examine bone and muscle characteristics of female elite-level endurance runners compared with age-matched controls. The study received ethical approval and all participants provided written consent. Controls (C) (n=15), eumenorrheic athletes (EA) (n=15) and amenorrheic athletes (AA) (n=14) completed dual energy X-ray absorptiometry (DEXA) and peripheral quantitative computed tomography (pQCT) scanning, three-day food diary, magnetic resonance imaging and muscle function testing. The amenorrheic athletes had 11.4% greater endochondral circumference of the radial diaphysis than controls. At the radial epiphysis EA had 14% greater total area than C, but the AA and C were similar. At the tibia diaphysis, the EA had a greater total area, cortical area and periosteal circumference than C (13.5,14.2 and 7.06% difference respectively). Similarly, the AA tibia diaphysis total area and periosteal circumference were greater than C (18 and 8.9% difference respectively), although EA had a 13.8% greater cortical thickness than AA at the tibia diaphysis. DEXA results (g/cm^2) highlighted significant differences between C and AA at sites of trunk (0.91±0.16 for C vs 0.82±0.22 for AA), spine (1.05±0.24 vs 0.92±0.33) and lumbar spine (L1-4) (1.19±0.28 vs 1.04±0.33) ($p<0.05$). At the pelvis significant differences were found between C and EA, and between EA and AA (1.11 ±0.25 C, 1.14±0.23 EA and 0.99±0.093 AA; all $p<0.05$). Both the AA and EA had significantly

higher energy intake per day than controls (p=0.008 and p=0.001, respectively), with a trend towards higher calorie intake in EA than AA. The AA consumed significantly less protein than C (p=0.015). The EA consumed significantly more protein (p=0.043) and more fat than C (p=0.024). Muscle size and strength were similar across all groups.
These results show that amenorrheic female athletes can have wider and thinner bones than eumenorrheic athletes and controls and highlight the importance of screening for the Female Athlete Triad.

Acknowledgements: Dr McPhee receives funding support from Life Long health and Wellbeing nr: MR/K025252/1 and MR/K024973/1

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC23

GFP-lentivirus transduced human primary skeletal muscle-derived fibroblasts retain their potential for adipogenic transdifferentiation

O. Jaka[1], C.C. Agley[1,2], F.C. Lewis[1], N. Lazarus[1], G.M. Ellison-Hughes[1] and S.D. Harridge[1]

[1]Centre of Human & Aerospace Physiological Sciences, King's College London, London, UK and [2]Cambridge Stem Cell Institute, University of Cambridge, Cambridge, UK

Fatty degeneration in skeletal muscle is a hallmark of many myopathies, sarcopenia, obesity and type-2 diabetes. It has been shown that human skeletal muscle fibroblasts (but not the myogenic cells) have the potential for transdifferentiation into adipocytes in culture, suggesting that these cells may be the cause of adipocyte accumulation in muscle (1). In order to study the transdifferentiation potential of these cells *in vivo* (xenotransplantation), it is important to genetically label the fibroblasts before transplanting them into the host species and to confirm that they still retain their potential for adipogenic transdifferentiation. In this study we have sought to transduce human primary skeletal muscle fibroblasts with a GFP lentivirus and determine if the transdifferentiation potential of human muscle-derived primary fibroblasts is retained.
Following local anaesthesia (2% lidocaine), a muscle biopsy sample was obtained from the *vastus lateralis* muscle of a healthy, young, female subject (aged 20 years). Following isolation and expansion, cells were purified by immuno-magnetic cell-sorting using CD56 microbeads (2). The CD56-negative fraction (enriched for fibroblasts) was subsequently grown in skeletal muscle growth medium (PromoCell) and transduced with a GFP lentivirus at different doses (1:15, 1:30, 1:40 and 1:100). The transduction efficiency was measured by flow cytometry. For transdifferentiation, GFP-positive fibroblasts were exposed to 300 μM oleic acid and 300 μM palmitic acid complexed to BSA at 15 mg/ml in proliferation medium (1).

The transduction efficiency was measured by flow cytometry 24 hours post-transduction for each GFP-lentiviral dose. The transduction efficiency for each dose was 72% (1:15), 60% (1:30), 50% (1:40) and 30% (1:100). The 1:15 dose appeared to compromise fibroblast viability, so the 1:30 dose was selected for further transductions. Using this dose, the transduction efficiency was 75% five days after transduction. Following treatment with fatty acids for 72 hours, cells were fixed and analysed by immunohistochemistry using antibodies against the adipogenic transcription factors C/EBPα and PPARγ, and Oil Red O which stains lipids.
The results showed that applying fatty acids to GFP-positive fibroblasts resulted in their transdifferentiation into adipocytes as evidenced by a clear accumulation of Oil Red O positive lipid droplets and increased expression of the adipogenic transcription factors C/EBPα and PPARγ compared to the non-fatty acid treated GFP-positive fibroblasts.
This study confirms that GFP lentivirus transduction of human primary skeletal muscle derived fibroblasts does not affect their transdifferentiation potential and can therefore be used to successfully label cells for future *in vivo* experiments.

(1) Agley CC *et al.* (2013). *J Cell Sci* **126**, 5610–25.

(2) Agley CC *et al.* (2015). *J Vis Exp* **12**, 52049.

This work was funded by the BBSRC.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC24

Acute effects of respiratory warm-up on exercise-induced bronchoconstriction and exercise performance

P.A. Eichenberger[1], T.A. Scherer[2] and C.M. Spengler[1,3]

[1]Exercise Physiology Lab, Institute of Human Movement Sciences and Sport, ETH Zurich, Zurich, Switzerland, [2]LungenZentrum Hirslanden, Zurich, Switzerland and [3]Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland

Short bouts of whole-body warm-up exercise were shown to protect airways by attenuating bronchoconstriction in response to subsequent intense exercise in people with exercise-induced bronchoconstriction (EIB). Whether isolated respiratory warm-up offers similar refractoriness and whether this refractoriness translates into improved exercise performance is unknown. Thus, the aim of this study was to investigate whether 1) respiratory warm-up by normocapnic hyperpnea with partial rebreathing attenuates EIB severity during subsequent exercise and, 2) whether the suggested attenuation leads to improved exercise performance.
Nine subjects (6 females, 3 males; age: 25±5 years; forced expiratory volume in 1s [FEV_1]: 104±15% predicted) with a history of mild EIB and a ≥10% decrease in FEV_1 after a control 8-min exercise challenge (ECh) took part in this study. They

were tested in 4 different conditions: Exercise after 1) no warm-up (NWU) or after 10min of respiratory warm-up at either 50% (WU50) or 70% (WU70) of maximal voluntary ventilation, or at variable intensity (30s-80%, 45s-30%, etc; WU80/30). Each warm-up was followed by an 8-min cycling ECh with dry air, followed – after 30min - by constant-load cycling to exhaustion (CL) at similar intensity and air condition. Lung function was measured at baseline, 0, 5, 10 and 15min after NWU/WU, and 5, 10, 15, 20, 25 and 30min after the ECh. Values are means±SD and compared by repeated-measures ANOVA.
The maximal decrease in FEV_1 after WU did not differ between conditions and never reached ≥10%. The maximal decrease in FEV_1 after the ECh was -14.9±3.6% in NWU which was significantly attenuated after WU50 (-9.3±5.0%), WU70 (-7.2±5.0%), and WU80/30 (-8.6±7.5%), with no difference between warm-up conditions ($p>0.05$). Workload and ventilation during ECh did not differ between conditions, suggesting that the ventilatory stimulus to the bronchial system was similar. In NWU, FEV_1 immediately before CL was still significantly reduced compared to baseline and WU-conditions (all $p<0.05$). This did, however, not translate into significant improvements in times to exhaustion and did not affect ventilation and gas exchange during CL (all $p>0.05$).
These data indicate that intense respiratory warm-up carried out before whole-body exercise can attenuate EIB severity and improve recovery, even in the absence of significant airway narrowing acutely after warm-up. The lack of improvements in exercise performance might be due to a stronger bronchoprotection induced by the ECh, masking improvements observed with prior respiratory warm-up.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC25

The effect of carbohydrate mouth rinsing on fencing performance and cognitive function following a fatigue inducing simulated bout of fencing

L. Bottoms[1], G. Rowlatt[2] and R. Buscombe[2]

[1]University of Hertfordshire, College Lane, UK and [2]University of East London, Stratford, UK

The ergogenic effect of carbohydrate (CHO) ingestion both pre and during endurance sport has been well documented (Shabort et al, 1999). Carter et al. (2004) were the first to have subsequently established a performance effect of CHO independent of blood glycogen levels signalling a potential key role of the central nervous system. To investigate this phenomenon they employed a protocol whereby participants rinsed a CHO solution in their mouth before spitting it out, referred to as carbohydrate mouth rinsing (CMR). CMR has subsequently been shown to improve high intensity exercise lasting between 30 and 60 minutes (Jeukendrup et al, 2013), however to date there exists no tests of CMR in relation to completion of an intermittent sports specific testing protocol. The present study sought to

investigate the impact of CMR on cognitive and sports specific performance after a period of fatigue induced fencing. Twelve participants who were all regularly competing in national level fencing competitions and training a minimum of once per week volunteered to participate in the study (31.2±14.3 years; 81.4±16.5 kgs). On two separate occasions in a randomised cross over design, the participants undertook a standardised 10 minute sport specific warm up. The participants completed a Stroop and lunge test (measuring number of lunges and hits on target) pre and post execution of a previously validated fatigue inducing fencing protocol. During the fatiguing protocol the participants mouth rinsed between simulated fights 25ml of either a tasteless 6.7% maltodextrin solution (MALT) or 25ml of water (PLAC). Heart rate and perceived exertion (RPE) were measured throughout the fatiguing exercise protocol and blood lactate and glucose were measured pre and post exercise. A series of two-way repeated ANOVA's were conducted with the various cognitive and physiological outcomes serving as dependent measures. The results demonstrated no interaction between pre and post and trial for the number of lunges ($P>0.05$), however there was an interaction for lunge accuracy ($P<0.05$), with accuracy improving post fatiguing exercise in the MALT trial (Table 1). There was also a tendency for RPE to be lower during the MALT trial compared to the PLAC ($P=0.08$). In conclusion, this study provides evidence for a positive effect of CMR on accuracy in a sports specific task. The RPE data is in line with the hypothesised role that CMR plays in mediating central processing in the perception of exertion after fatiguing exercise. CMR may be a suitable alternative to ingestion of CHO pre and during competitive sports performance.

Table 1: Mean (±SD) lunge accuracy pre and post fatiguing exercise for both trials

	Pre Protocol (%)	Post Protocol (%)
PLA	82.1 (±8.8)	78.8 (±6.4)
MALT	81.2 (±8.3)	87.6 (±9.4)

Carter, J.M. *et al.* (2004). *Medicine and Science in Sports and Exercise,* **36,** 2107–2111.

Jeukendrup, A.E. *et al.* (2013). *Sports Science Exchange,* **26**(118), 1–8.

Schabort, E.J. *et al.* (1999). *Medicine and Science in Sports and Exercise,* **31**, 464–471.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC26

Longitudinal changes in body composition of inter-county Gaelic athletic association hurlers measured by dual-energy x-ray absorptiometry

R. Davies, W. McCormack, C. Toomey and P. Jakeman

Physical Education and Sport Sciences, University of Limerick, Limerick, Ireland

The development of the high performance athlete involves cyclical periods of training and competition. Accurate and precise measurement of body composition

forms a central role in the development of the athlete or team yet longitudinal change in body composition is rarely reported. In this study we report the longitudinal change in body composition of elite, inter-county hurlers within season and over four sequential seasons. Whole body and segmental body compositional analysis was measured by dual energy x-ray absorptiometry (DXA) on 66 senior, male, outfield, inter-county hurlers, mean age 23.7 [95% CI, 22.9 : 24.5] y in the off-season period in September (BASAL). Further measurements were obtained at approximately 3 month intervals pre-competition (PRE), within-competition (IN) and in the off-season (OFF) of the following year. In addition, 11 players were followed across four consecutive seasons. Data are reported as the mean [95% CI]. Repeated measures ANOVA was used to assess change over time. Statistical significance was detected at 0.05 α-level. Estimates of effect size were calculated separately, η^2 pertains to change over time while R^2 is used to represent the effect between composite variables and constituents, i.e. Δ fat mass and Δ body mass.
No change for body mass was observed from BASAL to PRE (0.21 kg [-0.77 : 0.39], -0.2% [-0.8 : 0.4], $p = 1.000$, $\eta^2 = 0.007$). A moderate reduction in body mass occurred from PRE to IN (-0.78 kg [-1.21 : -0.35], -0.9% [-1.4 : -0.4], $p = 0.002$, $\eta^2 = 0.169$) but increased from IN to OFF (1.46 kg [0.98 : 1.94], 1.7% [1.2 : 2.3], $p < 0.001$, $\eta^2 = 0.369$). No change was observed between BASAL and OFF (0.47 kg [-0.09 : 1.04], 0.61% [-0.06 : 1.28], $p = 0.219$, $\eta^2 = 0.049$). A concurrent increase in lean mass (1.04 kg [0.64 : 1.43], equivalent to 1.6% [1.0 : 2.2], $p < 0.001$, $\eta^2 = 0.307$), and decrease in fat mass (-1.26 kg [-1.64 : -0.87] equivalent to -8.1% [-10.8 : -5.5], $p < 0.001$, $\eta^2 = 0.365$), was observed BASAL to PRE. This was followed by the restoration of fat mass in the period IN to OFF (1.73 kg [1.33 : 2.12] or 12.4% [9.5 : 15.7], $p < 0.001$, $\eta^2 = 0.54$), with the trunk acting as the primary region of change (1.07 kg [0.84 : 1.30], $R^2 = 0.93$). Longitutinally, over four years, an overall decrease in body mass was observed (-2.05 kg [-3.37 : -0.73], -2.4% [-3.9 : -0.8], $p = 0.006$, $\eta^2 = 0.546$). In contrast the accrual of lean mass during his period was 1.3 % [0.7 : 2.0] or 0.88 kg [0.44 : 1.32] per annum, with similar within-season fluctuations in fat mass.
In conclusion, cyclical change in body composition occur within and between-season that belie change in body mass. Whilst cycling of fat mass persisted over consecutive seasons, the linear increase in lean tissue mass was a robust observation considered optimal for enhanced physical performance.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC27

Astrocyte activation in response to disease alters cerebrovascular function: implication for metabolic changes and perfusion stress in ageing

S. Serres[1,2], M. Sarmiento Soto[2], J. Larkin[2], C. Martin[2,3], A. Khrapitchev[2], V. Economopoulos[2], G. Bonvento[4], C. Escartin[4] and N. Sibson[2]

[1]School of Life Sciences, University of Nottingham, Nottingham, UK, [2]CRUK/MRC Oxford Institute for Radiation Oncology, University of Oxford, Oxford, UK, [3]Psychology, University of Sheffield, Sheffield, UK and [4]MIRCen CEA Saclay, Fontenay-aux-roses, UK

Background: Neurovascular coupling, or functional hyperaemia, serves to match local cerebral blood flow (CBF) to regional neuronal energy use ensuring normal functioning of the brain[1]. This is thought to be accomplished by astrocytes that form a physical bridge between neurons and blood vessels[2]. However, in response to disease, astrocytes become activated and this may have significant consequences for cerebrovascular function. The aim of this study was to determine the effects of astrocyte activation on brain vasculature using *in vivo* magnetic resonance imaging (MRI), laser speckle contrast imaging (LSCI) and histology.

Methods: Male rats (N=16) were anesthetized with 2% isoflurane and injected intracortically with either (i) a lentivirus expressing ciliary neurotrophic factor (Lv-CNTF; N=7) known to switch astrocytic phenotype to an activated state, or (ii) a self-inactivated lentivirus expressing LacZ (Lv-LacZ; N=9). 6 weeks later, animals were anaesthetised with 2% isoflurane, tracheotomised and artificially ventilated. The left femoral artery was cannulated for monitoring mean arterial blood pressure (MABP), blood gases (PaCO2 and PaO2) and pH. Animals underwent MRI to measure basal CBF and LSCI to measure the CBF response, to both electrical stimulation of the whisker pad and hypercapnic (CO_2) challenge, under 1.2% isoflurane in 70%N_2/30%O_2, and maintained at ~ 37°C. Animals were transcardially perfusion-fixed under terminal anaesthesia and histology was performed post-mortem to detect molecular and cellular markers associated with astrocyte activation. All data are given as mean ± SEM and compared by paired *t*-test.

Results: CBF responses to whisker-pad (9.7±2.0 vs 19.6± 3.3%; injected vs non-injected; $p<0.05$) and hypercapnic (44.7±7.3 vs 68.1±1.0%; injected vs non-injected; $p<0.01$) challenges were significantly reduced in the CNTF-Lv animals. Similarly basal CBF was significantly reduced (49.0±10.5 vs 59.9±10.0ml/100g/min; injected vs non-injected; $p<0.05$) and correlated closely with the area of astrocyte activation ($p<0.05$; $r^2=0.3$). Histologically, astrocyte activation was associated with changes in the microvascular network. The use of hypoxic probe pimonidazole revealed hypoxia during astrocyte activation, and thus potential metabolic changes. No changes were observed in the LacZ-Lv animals.

Conclusion and future directions: These findings suggest that metabolic and vascular changes associated with astrocyte activation may suppress neurovascular coupling, and thus alters normal functioning of the brain. In Nottingham, we aim to use cutting-edge imaging methods (e.g. Dynamic Nuclear Polarisation ^{13}C magnetic

resonance spectroscopy) to detect specific metabolic changes associated with astrocyte activation and chronic perfusion stress in ageing that could be modulated by nutrition and exercise.

1. Attwell D, Buchan AM, Charpak S, Lauritzen M, Macvicar BA, Newman EA. Glial and neuronal control of brain blood flow. *Nature* **468**, 232–243 (2010).

2. Howarth C. The contribution of astrocytes to the regulation of cerebral blood flow. *Front Neurosci* **8**, 103 (2014).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC28

The effect of regularly performing a single supramaximal cycle sprint on maximal aerobic capacity in sedentary men and women

R.S. Metcalfe[1] and N.B. Vollaard[2]

[1]School of Sport, Ulster University, Derry, UK and [2]Department for Health, University of Bath, Bath, UK

Supramaximal sprint interval training (SIT) provides a potent stimulus for improving maximal aerobic capacity (VO_2max), which is a strong marker for both endurance performance and future cardiovascular health and premature mortality. Cycling based SIT typically involves six or more 'all-out' 30-s Wingate sprints per training session, yet we have recently demonstrated that similar improvements in VO_2max can be achieved with as few as two 20-s sprints (1). This suggests that the volume of sprint exercise has limited influence on subsequent adaptations in VO_2max. In this study, we aimed to examine whether a single 20-s 'all-out' cycle-sprint per training session can provide a sufficient stimulus for improving VO_2max. Thirty sedentary participants (10 men / 20 women; mean±SD age 24±6 y, BMI 22.6±4.0 kg/m^2, VO_2max 33.2±7.1 mL/kg/min) were randomised to a training group (n=16) or a no-intervention control group (n=14). Training involved three exercise sessions per week for four weeks, consisting of a single 20-s Wingate sprint (no warm-up or cool-down). VO_2max was determined prior to training and three days following the final training session. Mean VO_2max did not significantly change in the training group (2.15±0.62 vs. 2.22±0.64 L/min) or the control group (2.07±0.69 vs. 2.08±0.68 L/min; effect of time: P=0.17; group x time interaction effect: P=0.26). In conclusion, although we have previously demonstrated that regularly performing two repeated cycle-sprints provides a sufficient training stimulus for a robust increase in VO_2max, our present study suggests that this is not the case when training sessions are limited to a single 20-s sprint.

Metcalfe RS, Babraj JA, Fawkner SG, Vollaard NB. Towards the minimal amount of exercise for improving metabolic health: beneficial effects of reduced-exertion high-intensity interval training. Eur J Appl Physiol. 2012;112(7):2767-75.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC29

The content of full-length and truncated isoforms of PGC-1a in trained human skeletal muscles after low and high intensity endurance exercise

E. Lysenko, D.V. Popov, T. Vepkhvadze and O. Vinogradova

SSC RF Institute of biomedical problems RAS, Moscow, Russian Federation

It is known that full-length PGC-1α (FL-PGC-1a) in contrast to N-truncated (NT-PGC-1a) protein has a short half-life. Taking into consideration that the content of PGC-1a could influence further adaptive changes in muscle the aim of our study was to investigate the effect of ubiquitin-proteasome system activation after high intensity endurance exercises on PGC-1a content.

Nine healthy endurance-trained (VO_2max 61 ml/min/kg) subjects performed two continuous 70 min bicycle sessions on separate occasions: with low (L; 50% VO_2max) and high (H; 70% VO_2max) intensity. Before exercise, and 2 minutes, 4 and 8 hours after it biopsy samples from the *m. vastus lateralis* were taken under local anaesthesia (2 mL 2% lidocaine) by a microbiopsy technique. mRNA expression level, total and phosphorylated protein content were evaluated using q-PCR and Western blot. All data are normalized to reference gene or protein and submitted as median and interquartile range. The study was approved by the Human Ethics Committee of the Institute of Biomedical Problems (Moscow, Russia).

Immediately after exercise increase in phosphorylation level of AMPK substrate – ACC^{Ser79} in both sessions was observed (from 0.09(0.05-0.18) to 0.24(0.11-0.72) in L-session; from 0.08(0.04-0.30) to 0.52(0.19-1.06) in H-session). *PGC-1a* mRNA expression level after 4 hours of recovery also increased in a load-dependent manner (from 0.15(0.10-0.20) to 0.37(0.29-0.45) in L session; from 0.14(0.10-0.16) to 0,67(0.45-0.84) in H session). Phosphorylation of $FOXO1^{Ser256}$ increased in L session immediately after the exrcise (from 1.31(0.81-2.10) to 1.46(1.08-3.50)), and decreased after 4 hours recovery (from 1.88(0.73-2.31) to 0.87(0.26-2.43)) in H session. *MuRF1* expression decreased only immediately after L session (from 0.22(0.13-0.30) to 0.14(0.11-0.17)), whereas *Atrogin-1* expression decreased in both groups: in L session after 8 hours from 1.38(0.87-1.83) to 0.67(0.61-1.45); and in H session after 4 hours from 1.38(0.77-1.68) to 0.80(0.70-0.90) and after 8 hours to 0.83(0.71-1.26)). Content of FL-PGC-1a was unchanged in L session, and had a tendency to decrease in H session immediately after the exercise (from 1.00(0.60-2.00) to 0.60(0.45-1.45); P=0.06). Whereas the content of NT-PGC-1a increased in L session (from 0.76(0.38-1.57) to 1.08(0.72-1.74)) and had a tendency to increase in H session (from 1.06(0.42-1.34) to 1.45(0.40-3.68), P=0.06).

Thus the high intensity endurance exercise in comparison with the low intensity exercise results in ubiquitin-proteasome system activation. As the stability of FL-PGC-1a is low, this result may indicate that its content decreases after high intensity endurance exercise.

This work was supported by the Russian Science Foundation (grant 14-15-00768).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC30

Chronic probiotic supplementation and its effects on ehsp72 concentration following a desert-based ultramarathon

L. Taylor[1], C. Suckling[2], J. Roberts[2], B. Chrismas[3] and H. Marshall[4]

[1]Athlete Health and Performance Research Centre, Aspetar Orthopaedic and Sports Medicine Hospital, Doha, Qatar, [2]Department of Life Sciences, Anglia Ruskin University, Cambridge, UK, [3]College of Arts and Sciences, Qatar University, Doha, Qatar and [4]Sport Science and Physical Activity Research, University of Bedfordshire, Bedford, UK

Extracellular heat shock protein 72 (eHsp72) concentration has been shown to increase in response to exercise and/or environmental stress within humans. Ultra-endurance athletic events expose participants, including elite athletes, to a prolonged duration of exercise, often in challenging environmental conditions including extreme heat [e.g. Marathon des Sables (MDS)]. Such conditions increase risk of exertional heat illness and related pathophysiology's, including cellular damage. Probiotic and glutamine supplementation have been shown to increase eHsp72 concentration, which may subsequently lead to a reduction in cellular damage and offer a role of cellular protection, particularly to the gut, and may ultimately improve performance and gut health, in events like the MDS. The eHsp72 response to probiotic supplementation and ultra-endurance events in MDS like events has received little attention and thus research is required to understand how the heat shock response can aid cellular protection (and perhaps performance) during such events. The present study explored chronic probiotic supplementation on the eHsp72 response to the MDS. Thirty-two (6 female) competitors (age 41 yrs; range 23-53 yrs, height 1.75 ± 0.08 m, body mass 77.05 ± 12.00 kg) were randomly allocated to receive probiotic (PRO), probiotic and glutamine (PGLn), or no (CON) supplementation for 12 wk prior to the MDS. Blood samples via venepuncture were collected: i) 12 wk (baseline), ii) 7 d (pre-race) prior to departure for the MDS; iii) post-race (within 6-8 h of race completion) and iv) 7 d post-race. The MDS 2015 consisted of 7 d of consecutive stages across the Sahara Desert, Morocco, with a total distance of 249.4 km (average temperature ~ 38°C). Plasma eHsp72 concentration was determined via ELISA and expressed as percentage change from baseline. Mean post-race eHsp72 concentration was significantly increased ($p < 0.05$) by 124% from baseline, however there was no significant effect of group on eHsp72 concentration at any time ($p > 0.05$). PRO and PGLn did not alter the eHsp72 response to the MDS. However, this result could be attributed, in part, to experimental limitations. Firstly, post-race data collection was delayed (samples obtained 6-8 h post-race completion) due to the unique logistical challenges associated with the MDS and thus a true zenith in eHsp72 concentration within and between

groups may not have been obtained. Secondly, again due to logistical challenges, pre and post discrete stage blood samples were not obtained; consequently within and between stage nadir and zeniths of eHsp72 concentrations, and the influence of PRO and PGLn upon this, were not provided by the present data. Nevertheless, PRO and PGLn supplementation did not enhance, or acquiesce, the eHsp72 response to the MDS relative to the experimental limitations provided.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC31

Does dancing in old age afford neuromuscular protection?

M.V. Narici[1], K. Rehfeld[2], N. Müller[3], D. Rankin[1] and A. Hökelmann[2]

[1]MRC-ARUK Centre for Musculoskeletal Ageing Research, Faculty of Medicine, University of Nottingham, Derby, UK, [2]Institut für Sportwissenschaft, Otto- von- Guericke-Universität Magdeburg, Magdeburg, Germany and [3]Helmhholtz Associat, German Centre for Neurodegenerative Diseases (DZNE), Magdeburg, Germany

Motor neuron degeneration, denervation, loss of structural and functional integrity of the neuromuscular junction (NMJ) and loss of motor units (MUs), markedly contribute to the age-related decline in muscle mass (sarcopenia) (Deschenes, 2011). Evidence of NMJ degeneration in sarcopenic individuals is now available from serum measurements of c-terminal peptide agrin fragment (CAF), a breakdown product of the heparan sulphate proteoglycan agrin, released after NMJ damage (Hettwer et al. 2013). Interestingly, aerobic exercise in senile rats seems to protect against denervation and NMJ degeneration (Valdez et al. 2010) and in humans, no decline in MUs has been found in muscles of master runners (Power et al. 2010). Hence the present study aimed to investigate whether an aerobic activity such as dancing could have neuroprotective effects when compared to conventional gym exercise training. Thirty-seven older individuals (aged 71.6±3.5 yr) were recruited (18 female and 19 male) and randomly assigned either to a Dance Group (DG, 9 female, 10 male) or to a Gym Exercise Group (GEG, 9 female, 9 male). Both interventions took place twice a week, lasted 90 minutes each, for a period of six months. DG training consisted of Line, Jazz, Rock 'n' Roll, Latin-American and Square dances. GEG training consisted of endurance, strength-endurance and flexibility training. For both DG and GEG, each set of exercises/dances, lasted 20 minutes. Blood samples were collected before and after the intervention to measure CAF levels in serum using a commercially available Elisa kit (NTCAF ELISA, Neurotune AG, Schlieren, Switzerland). The data were compared to those of reference populations of older sarcopenic and young controls (Hettwer et al. 2013). Values are means ± S.D., compared by paired or unpaired Student's t-Test, as appropriate. Since no significant differences were found between CAF values of male and female participants of both groups, values were pooled together. Pre-training, CAF values of the DG

(202.9±66.3 pM) and GEG (228.5±70.5 pM) groups were respectively 1.9 and 2.2-fold higher than those of the young reference population and were statistically not different from the aged-matched elderly controls (214.1±118.2 pM). However, after the 6-month intervention period, CAF levels decreased by 15% ($P<0.001$) in DG, (pre 202.6±66 pM post 172.1±50.2 pM) while no changes were found in the GEG (pre 228.5±70.5pM, post 219.7±60.0 pM, n.s.). The present findings suggest a reduction of neuromuscular degeneration in older humans as a result of a six-month recreational dancing intervention. Instead, general fitness training based on strength, endurance and flexibility exercises does not seem to produce these benefits. It is not clear how dancing affords this protection but this could be due to a reduction of oxidative stress, inflammation and/or improved neurotrophin levels (Gonzalez-Freire et al. 2014).

Deschenes MR (2011). *Curr Aging Sci* **4**, 209–220.

Hettwer S et al. (2013). *Exp Gerontol* **48**, 69–75.

Valdez G et al. (2010). *Proc Natl Acad Sci* **107**, 14863–14868.

Power GA et al. (2010). *Med Sci Sports Exerc* **42**,1644-1650.

Gonzalez-Freire M (2014). *Front Aging Neurosci* **6**; 208.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC32

Effect of a short duration high intensity/low volume resistance training on skeletal mRNA in young healthy subjects

A. Paoli[1], T. Moro[1], L. Monaco[2], F. Naro[2] and C. Reggiani[1]

[1]Department of Biomedical Sciences, University of Padova, Padova, Italy and [2]DAHFMO Unit of Histology and Medical Embryology, Sapienza University, Roma, Italy

Introduction

Resistance training may be carried out via different methods that have been shown to have differing effects on muscle metabolism and signalling pathways. As a matter of fact a resistance training program is a composite of several important variables including that may affect physiological outcomes. On the other way also an high intensity interval exercise performed on cycloergometer has been demonstrated to influence some metabolic pathway as PGC-1α. Thus, the aim of our study was to analyse mRNA response to a single bout of high-intensity resistance training (HIRT), traditional resistance training (TRT) and high-intensity interval training (HIT).

Methods

12 healthy subjects performed in two different moments and with different legs HIRT and TRT protocol. HIRT consisted in 2 sets of 6/2/2 reps with incomplete rest between (20") sets while TRT consisted of 4 sets x15 reps with 1'15" of rest between sets. HIT was performed on a cycloergometer as follow: 30" of all out with 4' of rest, repeated for 4 times. Biopsies from the vastus lateralis were taken one week before training sessions (pre), immediately after (T0), 6 hours after (T6) and 24 hours after (T24) training. The following genes, related to hypertrophy, metabolism, autophagy and inflammation, was analysed by RT-qPCR: IGF-1, IGF-14a, MGF, myostatin, STARS, PGC-1α, PGC-1α-4, Atrogin, Beclin, IL6, myogenin
Results
Our data showed that HIRT seems to influence in a greater extent the gene linked to mechanical deformation (MGF) and STARS, whilst TRT seems influence STARS and IGF-1. HIT influenced IGF-14a, Beclin, IL6, myogenin, PGC-1α and myostatin.
Discussion
Our results suggest that different kind of exercise may influence different early genes after exercise. An high resistance training (HIRT) affects mechanical-related factors whilst a more traditional, long duration resistance training (TRT) seems to influence the IGF-1 pathway. The HIT exercise increases in a significant manner PGC-1α but also muscle atrophy related genes as atrogin, beclin and myostatin.

Paoli A. Am J Physiol Endocrinol Metab. 2012 302: E387-E387.

Paoli A et al. J Transl Med. 2012 Nov 24(10):237

Paoli A et al. Clin Physiol Funct Imag 2015 Jan;35(1):1-6

Gibala MJ et al. J Physiol. 2012 590(5):1077-1084

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC33

Identifying exercise-sensitive sirtuin 1 networks in skeletal muscle

B. Stocks[1], S. LaBarge[2], J. Dent[1], L. Hamilton[3], S. Schenk[2] and A. Philp[1]

[1]School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Birmingham, UK, [2]University of California, San Diego, San Diego, CA, USA and [3]University of Stirling, Stirling, UK

Endurance performance relies upon efficient production of ATP in skeletal muscle via mitochondrial oxidative phosphorylation. As such, skeletal muscle mitochondrial biogenesis is a predominant mechanism by which endurance training improves performance. From a molecular perspective, various molecules interact to regulate mitochondrial content and function in skeletal muscle. Sirtuin 1 (SIRT1), a NAD+-dependent deacetylase, has been proposed to play an intimate role in mitochondrial biogenesis and is sensitive to metabolic fluctuations

invoked by endurance exercise. However, despite considerable research attention, relatively little is known regarding exercise-induced adaption mediated by SIRT1 in skeletal muscle. Therefore, to determine SIRT1-deactylase dependent signalling in skeletal muscle, SIRT1 muscle-specific knockout mice (mKO) and control wild-type (WT) littermates underwent acute treadmill running in the fasted state (60 minutes at intervals of 5-25 m/min @ 10° gradient). Following endurance performance, mice were sacrificed immediately post exercise and one and three hours-post exercise (n=6/group). Immunoblotting techniques were used to determine protein content and post-translational modification of the purported SIRT1 targets p53, CREB and AMPK. Despite loss of SIRT1 activity in the mKO group, we observed comparable phosphorylation of p53, CREB and AMPK post exercise in both mKO and WT mice, suggesting that factors in addition to SIRT1 regulate the phosphorylation and presumed activity of these targets following endurance exercise.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC34

Biomechanics and the cardiorespiratory responses to self-selected running speed in simulated altered gravities – a case study

P. Carvil[1], T. Russomano[1,2], R. Baptista[2], V. Jain[1], K. Lindsay[1], T. Subasinghe[1], J. Waldie[3] and D.A. Green[1]

[1]Centre for Human and Aerospace Physiological Sciences, King's College London, London, UK, [2]Microgravity Centre, School of Engineering, PUCRS University, Porto Alegre, Brazil and [3]RMIT University, Melbourne, VIC, Australia

Introduction: The human body has evolved in Earth's gravity (1Gz), effecting physiological development and daily locomotion. When exposed to extended periods of microgravity, physiological de-conditioning ensues, despite exercise being implemented to counter deconditioning. With further exploratory missions planned away from Earth's 1Gz environment, more comprehensive countermeasures are required to protect the human body. The SkinSuit provides a potential integrative countermeasure against microgravity deconditioning by imparting a cumulative axial loading regime, however little research exists on the effects of reloading subjects in altered gravities. A range of 'gravity loading' was created to study the response to running, at self-selected speed.
Methods: A healthy male (72kg; 1.70m; 26yr) volunteered for the study which received local ethical approval by PUCRS Research Ethics Committee. After familiarisation and a five minute resting state, the subject performed five minutes of running at a self-selected running pace on a treadmill at three randomised gravities, once with and once without the SkinSuit. In addition to normal 1Gz

running, bodyweight suspension was used to unload the subject to simulate Martian (0.38Gz) and Lunar (0.16Gz) gravities; when worn the SkinSuit provided approximately an additional 0.8Gz of axial loading, thus creating a range of 'gravity loading' (0.16-1.8Gz). Respiratory responses, heart rate (HR), gait kinematics and ratings of subjective comfort (1-10), were reported for rest and the final minute of each run.

Results: Respiratory responses were positively, linearly associated with gravity loading (r=0.94), with minute ventilation increasing from rest by 10.9 $l.min^{-1}$ when running at normal 1Gz without the SkinSuit, to 31.3 $l.min^{-1}$ when running at 1Gz with the addition of the Skinsuit (+0.8Gz). HR was not associated with loading, but was associated with self-selected running speed. In reduced simulated gravities i.e. Lunar, a change of gait was observed with a shift to 'progressive jumping'. Greater need for movement control (4/10 vs.1/10) was reported when wearing the SkinSuit.

Conclusions: In this case study a linear, positive, trend between 'gravity loading' and respiratory response was observed. When the SkinSuit (0.8Gz) was combined with the Lunar simulation (0.16Gz), approximating Earth's 1Gz, the respiratory responses were nearly identical, indicating the potential role of gravity loading on metabolic cost.

The authors would like to thank the European Space Agencies' Space Medicine Office for the loan of the skinsuit.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC35

Extended field of view ultrasound compared to MRI in the assessment of changes in skeletal muscle cross-sectional area and volume

M.V. Franchi[1], J.I. Quinlan[1], L. Philbrooks[1], A. Hale[2], B.E. Phillips[1], P.L. Greenhaff[3], N.J. Szewczyk[1], P.J. Atherton[1], S. Francis[2], K. Smith[1] and M.V. Narici[1]

[1]MRC-ARUK Centre of Excellence for Musculoskeletal Ageing Research, Division of Medical Sciences & Graduate Entry Medicine, School of Medicine, University of Nottingham, Derby, UK, [2]MRC-ARUK Centre of Excellence for Musculoskeletal Ageing Research, Sir Peter Mansfield Imaging Centre, University of Nottingham, Nottingham, University of Nottingham, NOTTINGHAM, UK and [3]MRC-ARUK Centre of Excellence for Musculoskeletal Ageing Research, School of Life Sciences, University of Nottingham, QMC, University of Nottingham, NOTTINGHAM, UK

The assessment of changes in skeletal muscle mass is an important requirement, as several medical conditions involve severe muscle loss (cachexia, sarcopenia) (Heymsfield *et al.* 2014). Furthermore, accurate measurement of anatomical cross sectional area (ACSA) and muscle volume enable the estimation of

hypertrophy produced by different regimes of overloading (Narici *et al.* 1996, 2003). Computer Tomography (CT), Dual-Energy X-Ray absorptiometry (DXA) and Magnetic Resonance Imaging (MRI) are gold-standard techniques used in clinical settings for the evaluation of muscle mass: yet these techniques are expensive and often not easily accessible. Recently, the use of extended field of view (EFOV) ultrasound has been advocated as a reliable alternative for assessing vastus lateralis and quadriceps ACSA (Ahtiainen *et al.* 2010, Noorkoiv *et al.* 2010). However, the validity of the EFOV technique as compared to MRI in the assessment of regional and total muscle hypertrophy in response to chronic overloading regimes has not yet been investigated. The present study aims to determine whether EFOV is an accurate and reliable method for measuring quadriceps femoris (QF) and vastus lateralis (VL) muscle ACSA and total volume, as well as its ability to detect changes thereof during long term overloading regimes. Muscle mass of nine recreationally active males (8 young, 25 ±6 years old and 1 elderly, 65 years old), taking part in an 8-wk resistance training study, was assessed by EFOV US and MRI at 0, 4 and 8 week time points. ACSA was measured at 10, 20, 30, 40, 50 and 60% of femur length (FL) (from the medial patella border to the greater trochanter process) by EFOV US and compared with MRI. Pearson correlation and linear regression between the two techniques showed strong correlation between ACSA at mid regions (40, 50, 60%) of the QF 50 and 60% FL (R^2= 0.95 and 0.90, respectively) and the VL (50% and 60% LF (R^2= 0.91 and 0.89 respectively). Additionally, the two techniques showed good agreement when the average difference (D) in ACSA was assessed at these specific locations for quadriceps (D = 5.7 and 5.6% respectively) and for VL (D = 18.2 and 17.9 respectively). Furthermore, QF volume and VL volume (up to 60% of FL) showed a strong correlation (R^2= 0.85 and 0.89 respectively), as did measurements for whole QF volume (R^2= 0.92) (D= 9.3, 20.7 and 11.4% respectively). Despite a systematic underestimation by US, a strong correlation existed between EFOV US and MRI for the values of ACSA and of VOL measured at the three time points (0, 4 and 8 wks). Thus, these results show that EFOV US is a valid and reliable technique to quantify muscle ACSA and volume, and represents a useful tool for the assessment of regional and total changes in skeletal muscle mass.

Heymsfield *et al.* (2014), *J Cachexia Sarcopenia Muscle* **5:9–18**

Narici *et al.* (1996), *J Physiol* **496 (Pt 1):287-97**

Narici *et al.* (2003), *Journal of Applied Physiology* **95, 2229–2234**

Ahtiainen *et al.* (2010), *European Journal of Applied Physiology* **108, 273–279**

Noorkoiv *et al.* (2010), *European Journal of Applied Physiology* **109, 631–639**

Biotechnology and Biological Science Research Council (BBSRC) U.K.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC36

Do changes in neuromuscular activation contribute to the quadriceps femoris angle-torque relationship?

M.B. Lanza, T.G. Balshaw and J.P. Folland

School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, UK

Introduction: Joint position and muscle length are well known to influence torque production and the angle-torque relationship has been described for many muscle groups including Quadriceps Femoris (Q). However, contrasting reports exist as to whether neuromuscular activation changes with joint position and contributes to the Q angle-torque relationship (Kubo et al. 2004; Kooistra et al. 2007). Therefore, the aim of this study was to investigate Q neuromuscular activation, using surface electromyography (EMG) and the interpolated twitch technique (ITT) at four knee joint angles (25°, 50°, 80° and 105°; 0° = full knee extension).
Method: Thirteen healthy males (21 ± 2 years; 1.78 ± 0.07 m; 73 ± 5 kg) completed two familiarization and two identical experimental sessions. Test sessions involved isometric torque and EMG (6 sites, 2 electrodes on each of the superficial quadriceps; Delsys Trigno, Boston, MA) recordings of the right leg (Q) at each knee joint angle during the following knee extension tasks: maximum voluntary contractions (MVCs); electrically evoked twitch and doublet contractions (via femoral nerve stimulation, DS7AH, Digitmer, UK); maximal and submaximal voluntary contractions with superimposed doublets. Recordings at each angle were done in a counterbalanced order across the two sessions. Measurements included maximum voluntary torque, maximal M-wave peak-to-peak amplitude (Mmax P-P), absolute EMG root-mean-square amplitude at MVT (EMG_{MVT}), EMG_{MVT} normalised to Mmax P-P ($EMG_{MVT\text{-}PP}$). In addition doublet stimulation at rest and superimposed during sub-maximal and maximal contractions was used to calculate neuromuscular activation (ACT) at MVT.
Results: Maximal voluntary torque (MVT) was significantly influenced by joint angle with MVT greater (Bonferroni, $P \leq 0.001$) for the mid-range positions (50°, 256 ± 34 Nm; 80°, 273 ± 37 Nm) than the most extended (25°, 136 ± 33 Nm) and flexed (106° 218 ± 36 Nm) positions. ACT was lower at the most extended positions (25° 83 ± 9%; 50° 83 ± 7%) compared to the most flexed positions (106° 94 ± 3%; 80° 95 ± 3%; Bonferroni, $P \leq 0.029$). Absolute EMG at MVT (EMG_{MVT}) showed no significant difference between angle positions (ANOVA, P=0.246), although $EMG_{MVT\text{-}PP}$ showed a tendency to be lower at 25° (7.03 ± 1.80%) than 80° (8.24 ± 2.18%; Bonferroni, P=0.087).
Conclusion: ACT, calculated with the ITT, showed differences between angle positions that appears to contribute to the angle-torque relationship. In contrast, the similarity in absolute and normalized EMG_{MVT} across the measured knee joint

angles suggested similar neural drive across the measured joint angles. Based on these contrary findings it remains ambiguous if Q neuromuscular activation changes with knee joint angle

Kooistra, RD et al. (2007). *EJAP* **309P**

Kubo, K. et al., (2004) EJAP **349P**

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC37

The effect of gravity-loading - via the Mk VI Gravity-Loading Countermeasure Skinsuit - upon maximal aerobic exercise (VO_{2max})

J. Attias[1], J. Scott[2], T. Russomano[1] and D.A. Green[1]

[1]Centre of Human and Aerospace Physiological Sciences, King's College London, London, UK and [2]Space Medicine Office, Cologne, Germany

Introduction: Physiological de-conditioning associated with spaceflight and disuse environments is a major concern. The Gravity-Loading Countermeasure Skinsuit (GLCS) attempts to recreate gravity via incremental increases in z-axis fibre tension along the body's longitudinal axis, in a manner analogous to Earth's gravity. Conceptually, the Mk VI can be utilised by a spaceflight crew during exercise countermeasures and sleep. In order to evaluate whether the GLCS has scope as a countermeasure garment, it was necessary to determine its effect upon the cardiorespiratory responses to maximal aerobic exercise.
Methods: In two separate randomised visits, eight male subjects (29.6±5.6 yrs; 177.1±6.8 cm and 74.2±7.1 kg) completed a cycle ergometer maximal oxygen uptake (VO_{2max}) test (Bruce protocol) with stepwise increments of 50W every 3 minutes, whilst wearing either a custom-fabricated Mk VI GLCS or loose fitting clothing (GYM). Cardiorespiratory parameters (breath-by-breath; H_R, V_{tex}, FR, TI/TTOT, V_E, RER and VCO_2), and subjective comfort, body control and rating of perceived exertion (RPE) were measured continuously and analysed at rest, 75% VO_{2max} and at VO_{2max}. Student's t-test for paired data and Wilcoxon test was used to analyse physiological (± SEM) and subjective data (±95% confidence intervals).
Results: V_{Tex} was decreased ($p=0.021$) in GLCS vs. GYM at 75% VO_{2max}, though no other cardiorespiratory parameter was different between attires at rest, 75% VO_{2max} or VO_{2max}. Absolute VO_{2max} and the wattage required to achieve it were not different between GYM and GLCS (55.35ml/kg/min^{-1} vs. 54.09ml/kg/min^{-1} & 275W vs. 268.75W respectively). Furthermore, there was no difference in anaerobic threshold – determined by the elicited VO_2 at the point of anaerobic threshold detection – between GYM and GLCS. However, total work product (KiloWatts [KW]) was 12.6% lower in the GLCS (148.1 KW ± 16.9 vs. 132KW ± 15.8 in GYM; $p=0.001$). Movement discomfort ($p=0.02$) and body control ($p=0.02$) - both with

scales of 0-10 where 0 is least discomfort and most control - were increased in the GLCS at rest albeit remaining moderate, but were no different at VO_{2max}, whereas RPE and thermal comfort were unaffected throughout.
Discussion: The MK VI GLCS did not significantly affect $VO_{2max,}$ maximal wattage or cardiorespiratory responses at VO_{2max}, but did reduce the total work performed. These data suggest that the GLCS does not inhibit oxygen uptake during maximal exercise, but may reduce the duration of work required to achieve a given physiological output. This may have application to as a countermeasure for astronauts and as a rehabilitation tool for a number of populations.

EPSRC (UK)

Space Medicine Office (Germany)

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC38

Do muscle-tendon unit and patellar tendon stiffness influence explosive strength in man?

G. Massey[1,2], T.G. Balshaw[1,2], T. Maden-Wilkinson[1], N. Tillin[3] and J.P. Folland[1,2]

[1]Loughborough University, Loughborough, UK, [2]Arthritis Research UK, Centre for Sport, Exercise and Osteoarthritis, Loughborough, UK and [3]University of Roehampton, Roehampton, UK

Introduction: Stiffer connective tissues (muscle-aponeurosis and tendon) theoretically facilitate the rate of force transmission from the contractile apparatus to the skeleton and thus the capability of the muscle-tendon unit (MTU) to rapidly develop torque (i.e. explosive strength, ES). However, an association between in vivo tissue stiffness (k) and ES remains unsubstantiated. Recent work suggests MTU k may not discriminate inter-individual differences in ES (1). Whether tendon k separately influences ES is unexplored. Purpose: To examine the relationship between absolute and relative tissue k (both MTU and tendon) and ES.
Methods: Following familiarisation, 52 healthy untrained males (18-30 yrs) completed duplicate test sessions. Isometric knee extension contractions were performed (~110° knee angle) with a strain gauge perpendicular to the tibia sampling external force. Maximal voluntary torque (MVT) was assessed before a series of explosive voluntary and involuntary (supra-maximal octets [8 pulses at 300Hz] electrically evoked via femoral nerve stimulation) contractions. Measures of ES were time to absolute and relative torque levels (50 Nm & 25% MVT increments), and sequential time periods (e.g. t25-50% MVT). Constant loading-rate ramp contractions were performed with simultaneous force and ultrasound recordings of both vastus lateralis aponeurosis and patellar tendon (PT) elongation to derive MTU

and PT k. Absolute (N/mm) and relative (to MVT and resting tissue length) k were measured over identical torque ranges as the ES measures. Pearson correlations tested relationships between tissue k and ES.
Results: Absolute MTU and PT k were unrelated to absolute voluntary or involuntary ES measures (-0.243>r< -0.002, 0.092<P<-0.99). Relative PT k was also unrelated to any voluntary or involuntary ES measure (-0.246>r< -0.004, 0.085<P<–0.976). In contrast, relative MTU k was related to some measures of relative voluntary ES: t25-50% (r=-0.411, P=0.003), t50-75% (r=-0.314, P=0.028) and t75% (r=-0.298, P=0.038). Relative MTU k was related to involuntary t25-50% (r=-0.389, P=0.006) with a tendency to relate to involuntary t50% (r=-0.273, P=0.06).
Conclusions: Absolute and relative PT k were not associated with ES, and this was also the case for absolute MTU k. Relative MTU k was however associated with relative measures of voluntary and involuntary ES. These results suggest a differential influence of tissue components (muscle-aponeurosis vs tendon) on relative ES. However the overriding influence of maximum strength and/or tissue length seemingly negate any relationship between absolute MTU k and ES.

(1) Hannah and Folland (2015). Muscle-tendon unit stiffness does not independently affect voluntary explosive force production or muscle intrinsic contractile properties. Appl. Physiol. Nutr. Metab. 40: 87–95.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC39

The effects of a polyphenol drink on oxidative stress markers and exercise performance in trained cyclists: a single-blind crossover study

M. Soden and S. Aldred

School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Birmingham, UK

Supplementation with a polyphenol supplement has been shown to reduce inflammation, oxidative stress, skeletal muscle damage, and improve isometric strength 24 hours after exhaustive and fatiguing exercise protocols. However, previous studies have not examined the effects on exercise performance within a simulated performance setting. The aim of the present study was to investigate the effects of Cherry Active (CA), a drink containing polyphenol compounds, on markers of oxidative stress and exercise performance in trained cyclists. Eight trained male cyclists (29.1 $\pm$ 5.3 yrs) were assigned to an experimental condition (30mls of CA), or a placebo (PLA) condition, in a counterbalanced fashion, with a 10 day washout period between trials. Drinks were consumed twice a day for 8 days and within 60 minutes of the time-trial (TT). Participants undertook a strenuous cycling bout, consisting of 25 minutes at 60%W_{max}, 40 minutes at 80%W_{max}, and 95%W_{max} until

voluntary fatigue (visit 2). This was followed 24h later by a simulated 30 minute cycling TT (visit 3). Blood samples were obtained in all visits; before, during, and after exercise; to identify changes in oxidative stress markers. A repeated measures ANOVA was used to determine significnat differences between conditons, all values are means ± standard error. Markers of oxidative stress malonaldialdehyde (MDA), protein carbonyls (PC), or total antioxidant capacity (ferric reducing ability of plasma (FRAP)) were not different between the CA and PLA groups. FRAP increased in response to exercise during both trials (Pre visit 2: 508μM ± 18 to Post visit 2: 613μM ± 21, $p < .05$; Pre visit 3: 552μM ± 14 to Post visit 3: 603μM ± 22, $p > .05$). MDA showed a small increase over the two trials (Pre visit 2: 10.4μM ± .56 to Fatigue at 95%W_{max} 11.4μM ± .47, $p > .05$; Pre visit 3: 9.9μM ± .52 to Post visit 3: 11.7μM ± .86, $p > .05$) and there was a tendency for PC to decrease during the TT (Pre visit 3: 1.3 nm/mg ± .14 to Post visit 3: 1.1nm/mg ± .12, $p > .05$).TT finishing time was not different between CA and PLA (CA: 1605 secs ± 111; PLA: 1609 secs ± 115) nor was power output (CA: 292W ± 34.8; PLA: 292W ± 40.1). These findings indicate 8 days of CA supplementation did not attenuate oxidative stress, nor augment antioxidant defences in trained cyclists compared to PLA. Furthermore, CA did not enhance recovery 24 hours after completing a strenuous cycling protocol or provide an ergogenic effect in a cycling TT performance.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC40

Examining the role of mTOR localisation in human skeletal muscle responses to protein and carbohydrate ingestion.

J. Dent[1], Z. Song[1], M. McLeod[1], L. Breen[1], L. Hamilton[2] and A. Philp[1]

[1]School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Birmingham, UK and [2]University of Stirling, Stirling, UK

The mechanistic target of rapamycin (mTOR) is a key regulator of protein synthesis in skeletal muscle particularly sensitive to nutrient availability. It is not fully understood how carbohydrate and amino acid availability results in activation of mTOR or the downstream propagation of intracellular signals resulting in cell growth in human skeletal muscle. Recently, it has emerged that mTOR localisation to the surface of the lysosome, and translocation to the cell membrane in its active form may be critical for its nutrient-stimulated downstream effects. We utilized novel biochemical approaches to study mTOR localization and protein complex formation in human skeletal muscle in the fasted state and 1h and 3h post consumption of a protein-carbohydrate beverage (Gatorade Recover®, Gatorade, IL, USA) providing 20/44/1g of protein/carbohydrate/fat. In contrast to cell and rodent studies, we observed mTOR to interact with the lysosomal marker LAMP2 in basal conditions

and mTOR/LAMP2 complexes translocating to the cell periphery following nutrient stimulation. This redistribution of mTOR coincided with increased mTOR activity as assessed via kinase activity and immunoblotting of proximal targets of mTOR. Collectively our results provide information about the nutrient-sensing mechanisms regulating the activation of mTOR in skeletal muscle, suggesting that mTOR cellular localisation may be fundamentally important for the initiation of the protein synthetic response following nutrient stimulation in human skeletal muscle. It is anticipated that further understanding of these mechanisms will optimize our ability to combine the two potent activators of skeletal muscle protein synthesis - resistance exercise and nutrition - to maximize the growth response in humans.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC41

Effects of regular aerobic training on peripheral vascular activity in young healthy males

N. Potocnik, K. cankar, Z. melik and M. strucl

Medical Faclty, Insitute of Physiology, Ljubljana, Slovenia

Physical activity is known to have beneficial effects on prevention of cardiovascular disease. The regular aerobic exercise is associated with higher central arterial compliance, but its effect on peripheral arterial compliance is controversial. We aimed to test the hypotheses that regular aerobic training provokes beneficial changes in peripheral vascular activity at rest. Further we aimed to determine the influence of different autonomic stimuli (0.1Hz breathing and mental arithmetic stress) on peripheral vascular reactivity in physically trained young healthy adults compared to their sedentary peers.

Experiments were performed on 21 males, 19-24 years old (12 physically trained, VO_2max = 40±3 ml/kg/min - group A, 11 sedentary controls, VO_2max = 33±4 ml/kg/min - group B). Written informed consent was obtained from each and the study was conducted in accordance with the Helsinki Declaration. VO_2max was determined directly on a separate day using cycloergometry (Quark CPET, Cosmed). On the testing day ECG, arterial blood pressure (Finapres, Ohmeda) and peripheral artery compliance on the finger artery at rest, 3 minutes during 0.1Hz breathing and 3 min during mental arithmetic challenge were measured. A noninvasive method based on the comparison of the arterial pressure and arterial volume of finger artery was used to measure peripheral artery compliance evaluated as compliance index (CI).

Our results revealed elevated CI in group A compared to group B (3.42±0.30 and 1.28±0.31, p=0.004) at rest, but no significant differences in CI between groups during both physiological stimuli. CI decreased during 0.1Hz breathing (1.53 ±0.20, p=0.003) and mental stress (0.87 ±0.13, p=0.002) in group A, but only during

mental stress in group B (0.59 ±0.12, p=0.03). There were no differences in heart rate (p=0.08 at rest, 0.12 at 0.1Hz breathing and 0.34 at stress test), systolic and diastolic blood pressure between groups, at rest or during autonomic stimuli. A linear correlation between CI and VO_2max was found (p<0.001) at rest, during 0.1Hz breathing (p=0.017) and in mental stress (p=0.007).
Regular aerobic training increases peripheral arterial compliance in healthy subjects. Surprisingly the increase was not found during 0.1Hz breathing and mental arithmetic. Our findings indicate that peripheral and not central autonomic mechanisms govern peripheral arterial properties in young healthy males.

Tanaka H et al. (2000). *Circulation* 102(11):1270-5.

Parnell MM, Holst DP, Kaye DM. (2002) *Clin Sci (Lond)* 102(1):1-7.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC42

AMPK does not play a requisite role in regulation of *PGC-1*α gene expression via alternative promotor in endurance-trained human skeletal muscle

D.V. Popov[1,2], E. Lysenko[1,2], A.D. Butkov[1], T. Vepkhvadze[1] and O. Vinogradova[1,2]

[1]Laboratory of exercise physiology, Institute of Biomedical problems of Russian Academy of Sciences, Moscow, Russian Federation and [2]Faculty of Fundamental Medicine, M.V.Lomonosov Moscow State University, Moscow, Russian Federation

The *PGC-1*α is a master regulator of mitochondrial biogenesis in skeletal muscle. The expression of *PGC-1*α*-b* mRNA via the alternative promoter plays an important role in exercise-dependent expression of *PGC-1*α gene. The goal of the study was to investigate a role of AMPK in regulation of *PGC-1*α gene expression via the alternative promoter in endurance-trained skeletal muscle. We investigated activation of AMPK and *PGC-1*α gene expression via the alternative promoter before and after acute endurance exercise, with or without administration of a single dose of metformin, well known AMPK activator. In order to improve metformin delivery to skeletal muscle, the exercise bouts were performed when the level of metformin in the blood was near maximal. On the other hand, to avoid exercise-induced activation of AMPK, we used low-intensity exercise.
The study was approved by the Human Ethics Committee of the IBMP RAS and complied with the guidelines set forth in the Declaration of Helsinki. 9 amateur endurance-trained athletes Vo_{2max} 56 (53–62) ml/min/kg] participated in this study. Each participant swallowed 2 g of metformin or placebo before exercise (45 min, 40% Vo_{2max}). Biopsies from the vastus lateralis muscle were taken prior to and at 2 min, 4 h, and 8 h after exercise. Data are expressed as median and interquartile range. The Wilcoxon test was used to compare repeated measurements at level of significance $P \leq 0.05$.

In the experimental trial the plasma concentration of metformin prior to and just before termination of exercise was high (~ 1000 mg/l). The exercise intensity was low, and therefore did not induce lactate accumulation in the blood in the placebo trial. The phosphorylation level of $AMPK^{Thr172}$ did not change at 2 min after the exercise in either the metformin or placebo trials. However, $ACC^{Ser79/222}$ (the substrate of AMPK, i.e. an endogenous marker of AMPK activity) showed a 2.6-fold ($P < 0.01$) increase in phosphorylation level at 2 min after exercise in the metformin trial only. Post-exercise expression of *PGC-1α* gene via both the alternative and canonical promoters did not differ between trials.

Lack of a metformin-induced increase in *PGC-1α* gene expression via the canonical promoter under increased AMPK activity is in accordance with recent findings showing that the canonical promoter in endurance-trained human skeletal muscle is constitutively expressed, and that its expression does not depend on the intensity of exercise or intensity-dependent activation of AMPK (Popov *et al.*, 2015). Lack of a metformin-induced increase in *PGC-1α* gene expression via the alternative promoter does not confirm a role of AMPK in regulation of *PGC-1α* gene expression in endurance-trained human skeletal muscle.

Popov, D.V., Lysenko, E.A., Vepkhvadze, T.F., Kurochkina, N.S., Maknovskii, P.A., & Vinogradova, O.L. (2015). Promoter-specific regulation of *PPARGC1A* gene expression in human skeletal muscle. *J Mol Endocrinol* **55**, 159–168.

This work was supported by the Russian Science Foundation (grant 14-15-00768).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC43

The lymphocyte secretome from young adults enhances skeletal muscle regeneration, but effects are attenuated in the secretome of older adults

S. Al-Dabbagh[1], J. McPhee[1], C. Stewart[2], C. Murgatroyd[1] and N. Al-Shanti[1]

[1]School of Healthcare Science, Healthcare Science Research Centre, Manchester, UK and [2]Research Institute for Sport & Exercise Sciences, Liverpool, UK

Older people experience skeletal muscle wasting, in part due to impaired proliferative capacity of quiescent skeletal muscle satellite cells which can be reversed by exposure to young blood. To investigate the role of immune cells, we isolated lymphocytes from whole blood of young and older healthy volunteers and cultured them with, or without, anti-CD3/CD28 activators to induce release of cytokines, interleukins and growth factors into the media. The secreted proteins were used to prepare conditioned media that was subsequently used to culture C2C12 myoblasts. The conditioned media from activated young lymphocytes increased the rate of proliferation of myoblasts by ~3-fold (P<0.005) and caused an approximate 4-fold (P<0.005) increase in migration compared with non-activated lymphocyte control media. These responses

were characterized by minimal myotube formation (2%), a low fusion index (5%), low myosin heavy chain content and substantial migration. In contrast, myoblasts treated with conditioned media from activated old lymphocytes exhibited a high degree of differentiation, evident as elongated, multi-nucleated myotube formation that was comparable to control conditions, thus showing no effect on proliferation, or migration of the activated lymphocytes from old. These results indicate that young lymphocytes secretions induce muscle regeneration by enhancing muscle cell proliferation and migration, whereas secreted proteins from lymphocytes of older people may contribute to the attenuated skeletal muscle satellite cell proliferation and migration.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC44

The impact of 8-month training preparation for an Ironman distance triathlon immune response in recreational athletes

A.V. Tanner[1], J. Roberts[2] and R. Lancaster[3]

[1]Life and Medical Sciences, University of Hertfordshire, Hatfield, UK, [2]Faculty of Science & Technology, Anglia Ruskin University, Cambridge, UK and [3]University of Bedfordshire, Bedford, UK

The popularity of extreme endurance events has grown over the past decade and more recreationally trained athletes are undertaking extreme events, such as long distance triathlons. However, few studies have investigated the effect of high volume triathlon training on illness risk and a high training load has been indicated as a predictor of increased risk of URTI symptoms in athletes (Gleeson et al., 2013). 61 recreational athletes (following an 8-month training plan to prepare for an Iron-distance triathlon) (IMM) and 37 recreationally active controls (CON) completed the study. At months 0, 2, 4, and 6 participants undertook an incremental exercise test to assess maximal oxygen consumption (VO_{2max}). For six months leading up to the race, participants completed bi-monthly nutrition and sleep diaries and weekly illness symptom and training diaries. A subset of 12 IMM and 12 CON were studied for the 8-month period before the race and provided a resting saliva sample, for analysis of secretory IgA (s-IgA) and salivary lysozyme (S-Lys) and completed diaries for a further two month period. 55 participants completed the Ironman distance triathlon in an average time of 12:58:03 $\pm$ 1:19:28. There was no significant difference in VO2max between IMM and CON at baseline (45.9 $\pm$ 6.8 $mL.^{-1}kg.^{-1}min^{-1}$ vs 46.2 $\pm$ 7.2$mL.^{-1}kg.^{-1}min^{-1}$ respectively). There was a significant increase in VO_{2max} in IMM between 0 and 2 months (7%, $p<0.05$); however, no further significant changes. VO_{2max} did not change in CON. There was no significant difference in incidence of URTI or symptom score between months 0 and 6 in IMM or CON. There was no difference in incidence of URTI between IMM and CON, except a significantly higher symptom score in IMM at month 6 ($p=<0.05$). In the subset, in IMM, S-IgA secretion was significantly higher at month 8 compared to

baseline and saliva flow rate was significantly higher at month 8 compared to month 4 ($p<0.05$). There was no significant change in S-IgA concentration or S-Lys concentration or secretion. The increase in VO_{2max} during the first two months of initiating triathlon training indicates adaptations occurred quickly; however after month 2 increasing training load did not affect VO_{2max}. There was minimal difference in illness episodes scores or URTI episodes between time points or groups, this may reflect the moderate and progressive training load undertaken by IMM; which did not increase the risk of URTI. Furthermore, the increase in S-IgA secretion in the IMM group was likely to be due to changes in flow rate. Overall, it appears that 8- months of training for a long distance triathlon has no detrimental effect on the immune response in recreational athletes, but further analysis is required to examine nutritional and sleep factors.

GLEESON, M., BISHOP, N., OLIVEIRA, M. & TAULER, P. 2013. Influence of training load on upper respiratory tract infection incidence and antigen-stimulated cytokine production. *Scandinavian Journal of Medicine & Science in Sports,* 23, 451–457.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC45

Potential urinary biomarkers of systemic responses towards muscular exercise

A. Lyons, R. Carpenter, J. Culpan and O. Corcoran

School of Health, Sport and Bioscience, University of East London, Stratford, London, UK

The biochemical basis of muscle function during stress imposed by isokinetic exercise has mainly focused on measuring muscle proteins (CK and myoglobin) and lactate present in the blood plasma, whereas the range of low-molecular weight metabolites during muscular stress has received limited attention. This study investigated the effect of concentric isokinetic exercise of the knee extensor and flexor muscles (functional to standing, walking and running), at 80% and 40%MVC intensities of equal workloads, on urine and blood serum matrices. A hypothesis-free approach was employed to mathematically determine, by chemometrics, which untargeted metabolites measured by ^{1}H NMR spectroscopy are altered in response to muscular exercise of this nature.

Urinary citrate, glycine and hippurate exhibited a decrease following isokinetic exercise, whereas trimethylamine *N*-oxide increased. According to the literature, this pattern has previously been associated with kidney stress on the renal papilla and tubules from the filtration of myoglobin protein from the blood which accumulates as a result of leakage from the muscle membrane during stress or injury. Thus, these urinary biomolecular markers may reflect renal filtration of muscle proteins present in the blood following exercise and may be indirectly representative of isokinetic exercise-induced muscular stress. The serum matrix was less sensitive to exercise-induced change than urine in this isokinetic intervention study, since the homeostasis of blood is maintained by the renal system.

Further work is needed to cross-validate conventional assays used to measure biomarkers of muscular stress with the metabolomics platform to confirm that urinary trimethylamine *N*-oxide, citrate, glycine and hippurate are indirect biomolecular markers of muscular exercise. In sports medicine, NMR-urinalysis may provide diagnostic and prognostic information on injured and recovering athletes to determine whether or not they are fit to play, and predict risk of injury based on their current state of health.

Medicines Research Group, School of Health, Sport and Bioscience, University of East London.

School of Biological and Chemical Sciences, Queen Mary University London.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC46

Changes in Frequency-Domain indices after a High Intensity Interval Training acute intervention to reduce music performance anxiety

C. Blasco-Lafarga[1], P. Monteagudo-Chiner[1], N. Blasco-Lafarga[2], A. Cordellat[1], R. Sanchis-Sanchis[1], A. Roldán[1], A. Jiménez-Muñoz[1], A. Berruga-García[2] and G. Sanchis-Soler[1]

[1]Physical Education and Sport Department, University of Valencia, Valencia, Spain and [2]Generalitat Valenciana, Primary Health Centre in Lliria-Hospital area, Lliria, Spain

The sympathetic branch arouses to sustain the adaptation to stressors, although faster parasympathetic adjustments are responsible of immediate adaptive changes (e.g. anxiety control before a demanding concert). Sometimes this vagal control fails, like in Music Performance Anxiety (MPA) (1). Recent studies showed a decrease in the High Frequency index (HF) (2), a vagal index under the indirect modulatory control of the cortical-amygdala neurocircuitry, in the basis of emotion regulation (3). These studies also reflect an increase in the LF/HF (Low Frequency/High Frequency ratio) with unclear results for the former LF (2). On the other hand, the High Intensity Interval Training (HIIT) has been associated to a vagal improvement and a reduction of the sympathetic arousal in long-term interventions (4), but little is Known about short acute proposals. This study aims to analyze the changes in the frequency indices of HRV after one week of HIIT in a group of young musicians facing a demanding concert. 12 healthy male wind-instrumentalists (23±4.88y; 78.86±11.46kg) performed two concerts in one week. 48h after the first concert they underwent a graded cycling test until voluntary exhaustion (15-W increase per 1-min, Tacx flow ergometer, Tacx, Wassenaar, Netherlands) for high intensity familiarization and medical supervision. 48h later, musicians conducted 2-to-4 30-s cycling all-out Wingates, interspersed with 4 min of recovery (187.90±12.25 bpm; 11.70±3.38 mmol/L^{-1} lactate; 9.60±1.07 RPE on Borg 10 scale). 5 min of a 10-min Heart Rate recording in sitting-position (Polar

rx800), were retained twice for HRV analysis (Kubios, 2.1): fasting in the morning, in baseline condition (BS), and immediately before the concert (BC). Wilcoxon test showed BC pre-post training changes in LF/HF ratio ($p<0.05$; 3.05±2.75 vs 1.68±1.29 ms^2), and a slight trend toward a significant improvement in HF ($p=0.07$; 1119.50±1356.96 vs 2097.94±2868.36 ms^2), with no significant differences for LF (1870.15±2386.33 vs 2154.34±2148.79 ms^2). The improvement was no significant in BS (LF/HF: 2.76±3.56 vs 1.51±1.07 ms^2; HF: 2872.67±3324.60 vs 3308.11±3641.26 ms^2; LF: 3444.06±3523.09 vs 3212.49±2824.80 ms^2). Despite the short time of the intervention and the reduced sample, our results support the hypothesis that the parasympathetic reactivation and the better vagal balance following HIIT (5) might be helpful in the control of MPA. LF/HF ratio and HF, which were susceptible to extraordinary stressful events (2), are also susceptible to benefit from acute HIIT interventions. New studies will show if longer recovery following exercise might show BS vagal reactivation. Lack of time among professional musicians might suggest that HIIT is a short-term efficient solution for MPA.

Wells R, Outhred T, Heathers JAJ, Quintana DS, Kemp AH. Matter Over Mind: A Randomised-Controlled Trial of Single-Session Biofeedback Training on Performance Anxiety and Heart Rate Variability in Musicians. PloS one. 2012;7(10):e46597.

Williamon A, Aufegger L, Wasley D, Looney D, Mandic DP. Complexity of physiological responses decreases in high-stress musical performance. Journal of The Royal Society Interface. 2013;10(89):20130719.

Cristea IA, Valenza G, Scilingo EP, Szentágotai Tătar A, Gentili C, David D. Autonomic effects of cognitive reappraisal and acceptance in social anxiety: Evidence for common and distinct pathways for parasympathetic reactivity. Journal of anxiety disorders. 2014;28(8):795-803.

Heydari M, Boutcher YN, Boutcher SH. The effects of high-intensity intermittent exercise training on cardiovascular response to mental and physical challenge. International Journal of Psychophysiology. 2013;87(2):141-6.

Stanley J, Peake JM, Buchheit M. Cardiac parasympathetic reactivation following exercise: implications for training prescription. Sports Medicine. 2013;43(12):1259-77.

Thanks to La primitiva, L'Amistat & La Paz (Musicians); Quart & Siete Aguas Town-Halls.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC47

Static and dynamic postural changes after a mountain ultra-marathon of 80 Km and 5500 d+

G. Marcolin[1], A. Grainer[1], C. Reggiani[1], P. Bisiacchi[2], G. Cona[2], N. Petrone[3] and A. Paoli[1]

[1]Department of Biomedical Sciences, University of Padova, Padova, Italy, [2]Department of General Psychology, University of Padova, Padova, Italy and [3]Department of Industrial Engineering, University of Padova, Padova, Italy

The study aimed to investigate the effect of fatigue on static and dynamic postural control after finishing a mountain ultra-marathon race. Twelve male athletes participated at the study. Postural stability was assessed before and immediately after the race. Bipodalic standing balance was measured on a dynamometric platform with eyes opened (OE) and closed (CE). Dynamic test was performed with OE on an instrumented plate which allowed medio-lateral oscillations. Stabilometric data were affected by fatigue in the OE condition, concerning sway path (p=0.0006), sway area (p=0.0006), area of the confidence ellipse (p=0.0016), maximal AP (p=0.0017) and ML (p=0.0039) oscillations.

In the CE condition the sway path (p=0.0334), the maximal ML oscillations (p=0.0161) and the area of the confident ellipse (p=0.0180) were also negatively influenced. Stabilogram diffusion analysis showed in the OE condition an increase of short-term diffusion coefficients considering the anterior-posterior direction (*Dfys;* p=0.0023*)* and the combination of the two directions *(Dfr*^{2}*s;* p=0.0032*)*. Equally, long term diffusion coefficients increased considering the anterior-posterior direction (*Dfyl;* p=0.0093*)* and the combination of the two (*Dfr*^{2}*l;* p=0.0086*)*. In CE condition greater values were detected for medio-lateral (*Dfxl;* p=0.033*),* anterior-posterior (*Dfyl;* p=0.0459*)* and the combination of the two (*Dfr*^{2}*l;* p=0.0048). Dynamic test showed an increase of the time spent with the edges of the plate on the floor (p=0.0152). These results suggest a more marked involvement of cognitive resources to monitor postural stability after fatiguing. This caused a worsening in the automatic task (quiet standing) and a positive compensation in the less automatic task (dynamic test).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC48

The effects of selective breeding and endurance training on the maximum aerobic exercise metabolism in bank voles

M. Dzialo, E.T. Sadowska and P. Koteja

Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland

Problem: It is known that nature (genetic factors) and nurture (environmental factors) influence physiological performance traits, such as the aerobic capacity. An intriguing question is whether and to what extent a genetically determined superior performance is mediated by an increased propensity to the effects of training.
Purpose: The aim of this study was to check whether long-term artificial selection (20 generations) for increased maximum rate of aerobic metabolism achieved during voluntary exercise (swimming; VO2swim), which resulted in about 50% increase of VO2swim, improved also the effect of training of aerobic performance traits in bank voles (Myodes glareolus).
Methods: Males from four selected (A) and four unselected, control (C) lines (32 individuals from each type of lines) were randomly assigned to two groups (at the age of 51-56 days). In one group the animals were subject to interval training three times a week for 8-weeks. Each training session consisted of ten 2.0 min cycles, and each cycle comprised three phases: 0.5 min of "active rest" (running at 1km/hour), 0.5 min of speeding up, and 1 min running at sub-maximal speed (2.5-4.1 km/h; chosen individually, based on preliminary trials). Animals from the second group were sedentary, but were accustomed to the treadmill. At the beginning and the end of experiment measurements of the maximal rate of forced-running exercise metabolism (VO2max) and endurance running distance (ERD) were performed. All the protocols were approved by the Local Ethical Committee in Kraków (decision 66/2012).
Results: Analysis of covariance showed that training had no effect on body mass ($F_{1,6}=1.18$, $P=0.32$), but at the end of experiment voles from the A lines had a higher body mass than those from C lines (adjusted least-square means (LSM)±SE: A: 26.4±0.4 g, C: 22.7±0.7 g; $F_{1,6}=8.05$, $P=0.03$). Selection resulted in increased post-training VO2max (mass-adjusted LSM±SE, A-lines: 5.1±0.1 ml O2/min; C-lines: 4.1±0.1 ml O2/min, $F_{1,6}=37.13$, $P<0.01$) and a higher ERD (A-lines: 1824±180 m, C-lines: 1206±180 m, $F_{1,6}=7.71$, $P=0.03$). However, training had increased only endurance (trained: 1938±176 m, sedentary: 1163±170 m; $F_{1,6}=18.40$, $P<0.01$), without changing level of VO2max in either type of lines ($F_{1,6}=0.30$, $P=0.61$).The selection × training interaction was not significant for any of the traits.
Conclusion: This experiment showed that selection (nature) was effective in increasing the aerobic performance traits, but did not increase effect of training (nurture). Interestingly, the results revealed that ERD has been improved despite the fact that VO2max remained unchanged. This suggest that endurance running in bank vole may not be limited by VO2max.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC49

The influence of dehydration on long-term heat acclimation and temperate exercise

R. Neal, H. Massey, M.J. Tipton and J. Corbett

Sport and Exercise Science, University of Portsmouth, Portsmouth, UK

The ergogenic potential of heat is contentious[1]. Long-term (≥ 10 day) heat acclimation (HA) has been shown to be ergogenic under cool ambient conditions with mechanisms underpinning this including increased maximal oxygen uptake, possibly mediated by plasma volume (PV) expansion and an increased maximal cardiac output [2], as well as reduced physiological strain through improved thermoregulation [3]. Recently, short-term (5 day) HA with restricted fluid intake has been shown to augment PV expansion and accelerate HA relative to euhydrated HA[4]; performance improvements in the heat have been documented in trained men following this regime[5]. This study examined the effect of dehydration on long-term HA and exercise performance in a temperate environment.

A within-participant, balanced cross-over design with a three-month wash-out period was employed. Each participant completed both control (euhydrated HA [HAEu]) and intervention (HA with permissive dehydration [HADe]) conditions; achieved by completely restricting fluid intake in the isothermic strain HA sessions; a total of 1.75 L of fluid was provided during each control session. All procedures adhered to ethical standards of the Human Tissues Act and the University's Ethics Committee and with the Declaration of Helsinki. Eight males (Mean[SD] age 21[3] years; maximal oxygen uptake [VO_{2max}]: 55.1[7.1] mL.kg^{-1}·min^{-1}; peak power output [PPO]: 338[46] W; training: 10[3] hours.week^{-1}) undertook a long-term HA programme (T_{amb}= 40 °C, 50% rh) with a euhydrated Heat Stress Test (HST) (60 mins cycling at 35% PPO, preceding, mid-way though, immediately following and one week after HA (HA consisted of eight isothermic strain sessions: 90 mins. day^{-1}, target rectal temperature [T_{re}] of 38.5-38.7 °C). A graded exercise test (GXT) for determination of blood lactate threshold (LT), VO_{2max} and PPO was performed in a temperate environment (22 °C, 50% rh) pre- and post-HA.

HA induced adaptation when exercising in the heat (Table 1), although there were no differences in the extent of HA between HAEu and HADe, except for the maintenance (Post- to Decay-HA) of a greater Δ blood volume in the HAEu condition (7.0[5.6%]) compared to a return to baseline from Post- to Decay-HA in the HADe condition ($P<0.05$). HAEu and HADe reduced thermal and cardiovascular strain similarly in a temperate environment following long-term HA and an improvement in oxygen pulse was also observed ($P<0.05$). Performance trials in a temperate environment suggest that PPO was improved following long term HA but did not differ between conditions and VO_{2max} and LT were unaltered.

HA induced favourable thermal, thermoregulatory, physiological and cardiovascular responses to exercise in hot and temperate environments in trained men.

However, the rate of HA was not improved with dehydration as an additional stimulus and the ergogenic benefits of HA were unsupported.

Table 1 Effect of heat acclimation (HA) on physiological and perceptual indices during exercise in the heat (40 °C, 50% r.h.) midway through (Mid-HA), immediately after (Post-HA) and one week following (Decay-HA) a 10-day HA programme with and without permissive dehydration. Bonferroni corrected repeated measures analysis of variance and post-hoc tests were used to determine statistical significance, indicated here by a directional arrow, when P≤0.05.

HST variable	Mid-HA	Post-HA	Decay-HA
Thermal strain	↓	↓	↓
Cardiovascular strain	↓	↓	↓
Physiological Strain Index	↔	↓	↓
Sweating	↑	↑	↔
Perceptual strain	↔	↓	↓

[1] Minson, C. & Cotter J.; Nybo L. & Lundby, C (2015). CrossTalk proposal: Heat acclimatization does improve performance in a cool condition. *J Physiol.*

[2]Lorenzo, S., Halliwill, J. R., Sawka, M. N. & Minson, C. T. (2010). Heat acclimation improves exercise performance. *J Appl Physiol.* 109: 1140–1147.

[3]Corbett, J., Neal, R. A., Lunt, H. C. & Tipton, M. J. (2014). Adaptation to heat and exercise performance under cooler conditions: a new hot topic. *Sport Med.* 44(10): 1323–31.

[4]Garrett, A. T., Goosens, N. G., Rehrer N. J., Patterson, M. J., Harrison, J., Sammut, I. & Cotter, J. D. (2014). Short-term heat acclimation is effective and may be enhanced rather than impaired by dehydration. *Am J Hum Bio.* 26: 311–320.

[5]Garrett, A. T., Creasy, R., Rehrer, N. J., Patterson, M. J. & Cotter, J. (2012). Effectiveness of short-term heat acclimation for highly trained athletes. *Eur J Appl Physiol.* 112: 1827–1837.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC50

Lifelong exercise is associated with greater age-related motor unit remodelling

A. Ireland[1], M. Piasecki[1], J. Coulson[1], A. Hamilton-Wright[2], D. Stashuk[3], D. Jones[1] and J. McPhee[1]

[1]School of Healthcare Science, Manchester Metropolitan University, Manchester, UK, [2]School of Mathematics and Computer Science, Mount Allison University, Sackville, NB, Canada and [3]Department of Systems Design Engineering, University of Waterloo, Waterloo, ON, Canada

In vastus lateralis (VL), around ~40% of motor units (MUs) are lost by age 70 even in active older men, whilst remaining MUs are around 25% larger due to collateral reinnervation of orphaned fibres (Piasecki et al. 2015). This remodelling is accompanied by reduced stability (indicated by increased MU potential (MUP) variability, or 'jiggle') and firing rate in remaining units. There is some evidence that exercise is effective in attenuating MU loss (Power et al., 2010), although it is unknown whether remodelling is affected. Therefore, intramuscular electromyographic signals were recorded at proximal and distal motor points of (VL) in 26 young males (age 25±5y), 22 old males (71±5y) and 15 Master athletes (MA, 70±6 yrs) during contractions at 25% of knee extension maximum voluntary contraction (MVC).

Data were analysed using decomposition-based quantitative electromyography (DQEMG) to identify characteristics of individual MUPs, resulting in 698, 627 and 414 detected MUPs in young, old and MA respectively. Jiggle was assessed following application of a 'near fibre' method to examine only contributions from fibres located very close to the electrode, thereby reducing artefact or attenuation. Group effects were assessed using one-way ANOVA, with Bonferroni-corrected posthoc tests used to locate group differences. Firing rates were normally distributed and are reported as mean(SD). MUP size and jiggle were not normally distributed; they were log transformed prior to analysis and are presented as median(IQR). Median MUP size was 62% and 18% larger in MA than young and old respectively, whilst old median MUP size was 37% larger than young (Table 1, all $P<0.001$). Jiggle and firing rate did not differ between old and MA (both $P>0.5$), but values in both groups were higher and lower respectively than in young (all $P<0.05$). Regular exercise does not prevent age-associated MU remodelling. Conversely, remodelling is more pronounced in highly active older males but this is not accompanied by greater slowing of MUs or MU instability above that observed in healthy ageing. Exercise participation may improve effectiveness of reinnervation following MU loss, contributing to preservation of muscle size and strength in older age.

Table 1

Variable	Young	Old	Master Athlete
Area (μV.ms)	1108 (690-1656)	1335(947-1993)**	1660(1117-2595)**†
Jiggle (%)	23.9(19.7-29.6)	26.4(20.7-33.4)**	25.6(20.5-33.6)*
Firing rate (Hz)	9.6(2.2)	8.7(2.2)**	8.7(2.5)**

Table 1. Motor unit potential (MUP) characteristics separated by group. Asterisks indicate significant difference from young males: *P < 0.05, **P<0.001. Dagger indicates significant difference from old males: †P < 0.001.

Piasecki M *et al*. (2015). *J Physiol* (In Press).

Power GA *et al*. (2010). Med Sci Sports Exerc, 42, 1644–50.

This work was supported by funding from UK Medical Research Council (MR/K025252/1).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC51

Post exercise hypotension after interval and continuous exercise

Z.L. Incledon, M.G. Hughes, B. Chant and J. Whitaker

Cardiff School Of Sport, Cardiff Metropolitan University, Cardiff, UK

Exercise training has been shown to mediate acute and chronic decreases in blood pressure. Post exercise hypotension (PEH) has been deemed clinically significant, as it can be utilised by individuals with hypertension to lower BP for up to 24 hours.

PEH has also been implicated in blood volume changes (Hayes *et al*., 2000). The current exercise guidelines for hypertension are 30 minutes of continuous low-moderate exercise five times a week. There has been a recent increase of reports in the literature supporting positive health and performance benefits following periods of high intensity exercise training (Gibala *et al.,* 2012). However, the literature lacks clarity about whether PEH is affected by exercise intensity. Work by Hecksteden *et al.* (2013) implied that there was a relationship between PEH and the chronic BP reduction upon completion of a training programme. Therefore, PEH may give a prediction into the likely hypotensive benefits following a training programme. With this in mind the aim of this study was to compare the PEH response following acute high intensity interval training (HIT), modified sprint interval training (mSIT) and continuous endurance training (ET) sessions.

The acute blood pressure response was compared between a HIT session (4x4 minute at 90% VO_{2max}, with 3 minutes recovery at 50% VO_{2max}) similar to that used by Helgerud *et al.* (2007), a mSIT session (60 seconds at 100% of VO_{2max} interspersed with 75 seconds at 50% of VO_{2max}) previously used by Little *et al*. (2010) and a continuous training session (CT) (30 minutes at 50% VO_{2max}). Blood pressure was measured in 12 active normotensive participants (Age: 21 $\pm$ 1.7 years, Mass: 79.6 $\pm$ 14.7 kg, VO_{2max}: 45.2 $\pm$ 7.5 ml/kg/min) before, during and in the 60 minutes following exercise. Changes in plasma volume were also predicted.

In the 1-hr post exercise the reduction in systolic blood pressure was significantly greater following HIT (10.4mm Hg, $p < 0.05$) compared to mSIT (5.3mm Hg) and ET (1.6mm Hg). No differences were found in diastolic blood pressure during recovery ($P > 0.05$). There was no significant difference in percentage plasma volume change between the three intensities ($P > 0.05$).

The results of this study show that exercise intensity has an effect on the PEH response, with HIT eliciting a significantly larger drop in BP than mSIT and ET. HIT decreased SBP by 9mm Hg more than ET, considering that a 2mm Hg difference in BP can be deemed clinically significant, current exercise recommendations for hypertension should expand to include HIT. Future study should focus on hypertensive individuals in a carefully monitored clinical setting.

Gibala M *et al.* (2012). Physiological Adaptations to Low-Volume, High-Intensity Interval Training In Health and Disease. *The Journal of Physiology,* 590.5, 1077–1084.

Hayes P *et al.* (2000). Importance of Post-Exercise Hypotension in Plasma Volume Restoration. Acta *Physiologica Scandinavica,* 169, 115–124.

Hecksteden A *et al.* (2013). Association Between Post-exercise Hypotension and Long-Term Training-Induced Blood Pressure Reduction: A Pilot Study. *Clinical Journal of Sports Medicine,* 23, 58–63.

Helgerud J *et al.* (2007), Aerobic High-Intensity Intervals Improve VO2max More Than Moderate Training. *Medicine and Science in Sports and Exercise,* 39, (4), 665–671.

Little J *et al.* (2010). A Practical Model of Low-Volume High-Intensity Interval Training Induces Mitochondrial Biogenesis in Human Skeletal Muscle: Potential Mechanisms. *Journal of Physiology,* 588, (6), 1011–1022.

I would like to thank Dr. Michael Hughes for his excellent support, guidance and mentoring throughout all of my studies. I would also like to thank Ben Chant and Jazmine Whitaker, this data was collected as group and without their hard work would not have been possible.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC52

Post exercise hypotension after high intensity interval exercise - comparison of upper and lower body exercise

M.G. Hughes, C. Williams and P. Smith

Cardiff School of Sport, Cardiff Metropolitan University, Cardiff, UK

The recovery from exercise leads to 'post-exercise hypotension' (PEH) which can persist for some hours, thus providing potential benefits for managing hypertension. Most PEH research has used lower body cycling, however in some populations this may not be a viable mode of exercise and greater knowledge about the comparative responses between exercise modes may provide insight into the mechanisms underpinning PEH. The few studies which have compared PEH in upper and lower body activities have either used activities unsuited for training (maximal tests)[1] or have failed to control for the relative exercise intensity (i.e., $\%VO_2max$) between the two modes of exercise.[2] Therefore the aim of this study was to examine the blood pressure response after arm crank (ARM) ergometry and conventional leg cycling (LEG). In keeping with the developing interest in high-intensity interval exercise, the LEG-ARM comparison was made using the interval training model of Wisloff et al. (3).

After ethical approval, nine young, recreationally active, normotensive participants (6 male, 3 female; age 20.2 ± 1.2 years; maximal oxygen consumption 46 ± 12 ml/kg/min; baseline blood pressure (systolic / diastolic) 129 ± 12mmHg / 80 ± 10 mmHg) volunteered for the study. Maximal exercise tests were carried out each using ARM and LEG ergometry. Subsequently, intensities were derived for interval sessions of 4 x (4min at 90-95% HRpeak: 3 min at 70% HRpeak) based on the mode-specific peak heart rate. Manual blood pressure, obtained pre-exercise (baseline) and then for 60-min during recovery (15 min intervals), was the primary outcome and statistical comparisons were made using 2-way repeated measures ANOVA.

Mean VO_2 (2.34 ± 0.57 vs. 1.70 ± 0.42 l/min; $P<0.001$) and power (237 ± 68 vs. 115 ± 37W; $P<0.001$) were higher during LEG than ARM intervals, while blood lactate (5.6 ± 1.8 vs 4.7± 0.9 mM; $P=0.132$) was not different. Mean systolic blood pressure (SBP) was reduced in recovery ($P=0.019$) by 10 ± 1 mmHg (LEG) and 7 ± 1 mmHg (ARM) with no difference between exercise modes ($P=0.423$) and no (time*mode interaction) (Figure 1). Diastolic blood pressure was not reduced post-exercise following either mode of exercise.

With the current procedures, both exercise modes reduce SBP during the 60-min recovery, highlighting the efficacy of interval exercise using either ARM or LEG as a potential stimulus for blood pressure management. The similar PEH, in the face of significantly different power and VO_2, may support the likely importance of local factors in the regulation of PEH.

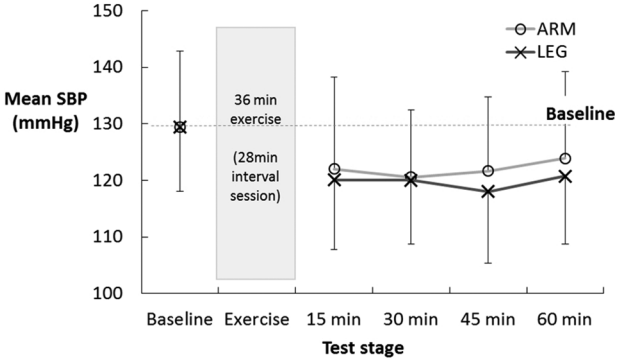


Figure 1. Systolic blood pressure (SBP) after a high intensity interval session using arm and leg cycling.

Almeida WS *et al*. *Science & Sports*. 2010;25:74-80.

MacDonald JR *et al*. *Journal of Human Hypertension*. 2000;14:310-20.

Wisloff U *et al*. (2009). *Exercise and Sports Science Reviews*. 139 - 146.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC53

Sex differences in human eccentric hamstring strength and Biceps Femoris long head architecture

T. Maden-Wilkinson[1], R. Moody[1], F. Behan[1,2], T.G. Balshaw[1,2] and J.P. Folland[1,2]

[1]School of Sports, Exercise and Health Sciences, Loughborough University, Loughborough, UK and [2]Arthritis Research UK, Loughborough University, Loughborough, UK

Hamstring strain injuries (HSI) are the most prevalent non-contact injury in many sports with the *Biceps Femoris long head* (BFlh) reported as being the most commonly injured muscle. Reported incidence rates among professional male athletes are considerably higher than among professional female athletes (1). Eccentric strength and shorter muscle fascicle length (FL) are known risk factors for HSI (2,3), but whether these contribute to the sex difference in injury risk is unknown. This study aimed to examine the sex difference in eccentric strength and FL.

Thirty recreationally active participants (15 males; 23 ± 3 yrs, 75 ±10 kg, 1.79 ± 0.07 m; 15 females; 21 ± 1 yrs, 62 ± 7 kg, 1.68 ± 0.07 m) with no previous history of hamstring strength training were assessed for maximal eccentric strength using a Nordic hamstring rig. Participants were positioned with strain gauges attached 2cm above both lateral malleoli. Following a structured warm up, participants were instructed to control their fall until they could no longer resist the increasing gravitational moment of the exercise and fall to the floor. This break point angle was quantified using video recordings synchronized with the force trace (0º= full extension) and analysed manually using video analysis software (Kinovea, France Ver. 0.8.15). Participants performed 3-5 maximal contractions with visual and verbal feedback. Maximal eccentric torque was calculated by the mean of the maximal force produced at both legs, and normalised relative to lever length and body mass ($Nm.kg^{-1}$). B-mode ultrasound using a 92mm probe was performed to quantify muscle thickness (MT), pennation angle (PA) and muscle fascicle length (FL) of the *BFlh* at 50% muscle length.

Relative maximal eccentric torque produced during the NHE was significantly greater in Males (1.55 ± 0.25 $Nm.kg^{-1}$) than Females (0.95 ± 0.18 $Nm.kg^{-1}$) ($p<0.001$) with the break point angle significantly lower in males (47 ± 11°) than Females (58 ± 11°) ($p<0.01$), In terms of muscle architecture of the BFlh, males had significantly greater MT (Males: 28.9 ± 6.3 mm; Females: 22.5 ± 4.4mm, $p<0.01$) and a steeper PA (Males: 20.0 ± 5.5°; Females: 15.7 ± 2.6°, $p=0.011$), however there was no significant difference between males and females for muscle FL (Males: 89.1 ± 20.8mm; Females: 89.8 ±19.5mm, $p=0.919$). There was a weak relationship between break point angle and relative force in both sexes ($r=0.23$-0.28, $p<0.05$). These data suggest that the sex differences in HSI injury prevalence could partly be attributed to differences in muscle architecture and eccentric hamstring strength

Cross et al., (2013) Am J Sports Med, 41(4), 742–748

Opar et al., (2015) Med Sci Sports Exercise, 47(4) 857–65

Timmins et al., (2015) Br J Sports Med, In Press

The authors wish to thank the participants of the study

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC54

Active GSK3 and a functional β-catenin-TCF4 transcriptional complex are necessary for the differentiation of human myogenic progenitor cells

C.C. Agley[1,2], F.C. Lewis[1], O. Jaka[1], C. Velloso[1], N. Lazarus[1], G.M. Ellison-Hughes[1] and S.D. Harridge[1]

[1]Centre of Human & Aerospace Physiological Sciences, King's College London, London, UK and [2]Wellcome Trust - Medical Research Council Cambridge Stem Cell Institute, University of Cambridge, Cambridge, UK

Canonical Wnt-β-catenin signalling is essential for skeletal muscle myogenesis during development, but its role in adult human skeletal muscle repair and regeneration remains unknown. Binding of Wnt ligands at the cell surface disables the capacity of GSK3 to phosphorylate β-catenin thus preventing its degradation and stimulating its interactions with TCF transcription factors. Here we manipulated a number of the molecular players in the Wnt signalling cascade in adult primary human skeletal muscle progenitors to assess their role in differentiation. Muscle biopsy samples were obtained from the vastus lateralis of healthy young male subjects (25.5±(*SD*) 3.1 years) following local anaesthesia (2% lidocaine). Muscle-derived cells were isolated and expanded in culture for 7 days after which $CD56^{pos}$ myogenic cells were immunomagnetically purified (Agley *et al.* 2013). Myogenic cells were either maintained in growth medium or stimulated to differentiate in serum-free conditions. In addition to studying β-catenin expression levels and localisation during differentiation, the following were applied in order to manipulate Wnt signalling: (i) pharmacological GSK inhibition, (ii) Lentiviral overexpression of constitutively active β-catenin (EβC) or (iii) dominant negative TCF4 (dnTCF4). Cells were analysed using western blotting, immunocytochemistry and qRT-PCR. Although detectable in growth medium, active β-catenin was mainly cytoplasmic. Under serum-free differentiating conditions active β-catenin stained strongly in the nucleus of differentiated MHC^{pos} myotubes. Inhibition of GSK via BIO (5μM) increased active-β-catenin (Control: 45.8±8.0 (AU); BIO: 105.2±4.0 (AU); $P<0.05$), but severely blunted the normal differentiation response with marked and significant reductions in fusion index (Control: 73.1±3.7 %; BIO: 5.5±4.0%; $P<0.001$), individual myotube size and myogenin expression. Two further structurally diverse GSK inhibitors (CHIR-99021 and LiCl) produced very similar effects. EβC gave a milder phenotype of reduced fusion and myotube size although myogenin and MHC were still expressed. Contrastingly, loss of β-catenin-dependent TCF-driven transactivation (dnTCF4) entirely prevented fusion of myogenic precursors and myogenin expression was absent. Discrepancies between GSK3 inhibition and β-catenin overexpression reveal that active GSK3 is essential for myogenic fusion and differentiation with roles which likely extend beyond the regulation of β-catenin stability alone. Although greatly increasing nuclear β-catenin decreases myogenic cell fusion, disruption of its transcriptional co-activator role completely abolishes differentiation. Together these data show that active GSK3 and a functional β-catenin-TCF4 transcriptional complex are necessary for the differentiation of adult human myogenic progenitor cells.

Agley, C.C, Rowlerson, A., Velloso, C.P. Lazarus, NR., & Harridge, S.D.R. (2013) Human skeletal muscle fibroblasts, but not myogenic cells, readily undergo adipogenic differentiation. *Journal of Cell Science* 126: 5610–25

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC55

Neural and morphological contributions to the individual changes in explosive and maximal strength following a 12-week training intervention period

T.G. Balshaw[1,2], G. Massey[1,2], T. Maden-Wilkinson[1], N. Tillin[3] and J.P. Folland[1,2]

[1]School of Sport, Exercise, & Health Sciences, Loughborough University, Loughborough, UK, [2]Arthritis Research UK Centre for Sport, Exercise and Osteoarthritis, Loughborough, UK and [3]University of Roehampton, London, UK

Whilst group level changes in neural drive and muscle hypertrophy have been widely reported after strength training (Tillin et al., 2011, 2012; Erskine et al., 2012), the contributions of these adaptations to individual changes in strength are poorly understood. The purpose of this study was to assess the contribution of underlying physiological adaptations (neural, intrinsic contractile properties, muscle size and architecture) to the functional changes in explosive and maximal strength following training.

Thirty-five healthy young males completed explosive strength training (EST, n=12), conventional strength training (CST, n=13), or control (CON, n=10) for 12 wks. Training involved 4 x 10 knee extension repetitions (x3/wk): contracting "as fast and hard as possible" for ~1 s (EST); or gradually increasing to 75% of maximal voluntary torque (MVT) before holding for 3 s (CST). Functional and physiological changes were measured pre and post. Knee extension torque (T) and quadriceps EMG were measured during maximum voluntary (MVT and $EMG_{@MVT}$) as well as explosive voluntary ($EMG_{0\text{-}50,\ 0\text{-}100,\ 0\text{-}150}$) and evoked contractions (T at 50 ms increments, $T_{50,\ 100,}$ and $_{150}$). Quadriceps muscle volume ($Q_{VOL,}$ via MRI), pennation angle and fascicle length (via ultrasound recordings) were also determined. Pearson's product moment bivariate correlations and, when multiple predictor variables were correlated with the outcome, stepwise multiple linear regressions were calculated between strength changes (ΔMVT, ΔT_{50}, ΔT_{100}, ΔT_{150}) and the changes in physiological predictor variables.

ΔMVT was correlated with $\Delta EMG_{@MVT}$ ($r=0.553$, $P=0.001$) and ΔQ_{VOL} ($r=0.608$, $P<0.001$) but none of the other predictor variables ($r\leq0.216$, $P\geq0.213$). ΔT_{50} and ΔT_{100} were only correlated with $\Delta EMG_{0\text{-}50}$ ($r=0.730$, $P<0.001$) and $\Delta EMG_{0\text{-}100}$ ($r=0.561$, $P<0.001$), respectively. ΔT_{150} was correlated with $\Delta EMG_{0\text{-}150}$ ($r=0.667$, $P<0.001$) and ΔMVT ($r=0.550$, $P=0.001$). Stepwise regression with ΔQ_{VOL} (37%) and $\Delta EMG_{@MVT}$ (17%) explained a combined 54% of the variance in the ΔMVT. $\Delta EMG_{0\text{-}150}$ (44%) and ΔMVT (10%) in combination explained 54% of the variation in ΔT_{150}.

In conclusion, improvements in MVT were explained primarily by ΔQ_{VOL}, with a smaller contribution from changes in neural drive. In contrast, changes in early phase explosive

torque production (0-100 ms) were explained exclusively by changes in neural drive. Changes in late phase explosive force production were also largely explained by changes in neural drive but with a contribution from changes in MVT.

Erskine, R.M., Fletcher, G., Hanson, B. and Folland, J.P., 2012. Whey protein does not enhance the adaptations to elbow flexor resistance training. *Medicine & Science in Sports & Exercise*, *44*(9), pp. 1791–800

Tillin, N.A., Pain, M.T. and Folland, J.P., 2011. Short term unilateral resistance training affects the agonist–antagonist but not the force–agonist activation relationship. *Muscle & nerve*, *43*(3), pp.375-384.

Tillin, N.A., Pain, M.T. and Folland, J.P., 2012. Short term training for explosive strength causes neural and mechanical adaptations. *Experimental physiology*, *97*(5), pp.630-641.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC56

The relationship between Patellar tendon stiffness, rate of torque development and maximum isometric torque

J. Quinlan[1], M.V. Franchi[1], N.J. Szewczyk[1], B.E. Phillips[1], P.L. Greenhaff[1], P.J. Atherton[1], K. Smith[1], C. Maganaris[2] and M.V. Narici[1]

[1]University of Nottingham, Derby, UK and [2]Liverpool John Moores University, Liverpool, UK

Tendons are viscoelastic anatomical links between muscles and bones; Their primary role is the transmission of contractile force onto a joint, to enable movement. To fulfill this role, tendons must be sufficiently stiff. Mechanical stiffness is important when considering the role of either tendon or aponeurosis on the mechanical output of muscle contraction (1). It has previous been shown that the Vastus Lateralis aponeurosis stiffness is positively correlated to the rate of torque development (RTD) (2), however it is also important to consider the in series patellar tendon, since it is this tendon which is distally attached to the tibia, thus enabling knee extension movement. In addition it is known that tendons adapt to the mechanical loading they habitually undergo, by becoming stiffer or more compliant when chronic loading increases or decreases, respectively (1). The research has investigated the relationship between: 1) patellar tendon stiffness and rate knee extension torque development ,(RTD) and 2): patellar tendon stiffness and knee extension maximal voluntary contraction (MVC).

Eight healthy young males (24±5yrs, 174±4cm, 72.38±10kg) were recruited for an 8 week training study. Baseline measures of patellar tendon stiffness derived from combining dynamometry and ultrasound scanning in vivo, knee extension MVC and RTD were obtained at 90° knee joint angle. RTD was assessed at 3 time variables 0 - 50ms, 0 - 200ms and 0 - 2/3 maximal force ($F_{2/3}$). Pearson's correlation was applied to the variables with a significance level of $P<0.05$. Patellar tendon stiffness, assessed at both maximal force and the highest common force to all participants, was significantly correlated to RTD obtained at 0-50ms ($r^2=0.75$ and 0.58 respectively). However,

this correlation was absent at either of the later time points, 0-200ms or 0-$F_{2/3}$, when compared to patellar tendon stiffness at maximal or highest common force. Nonetheless, patellar tendon stiffness at maximal force and highest common force was also significantly correlated to MVC (r^2=0.70 and 0.69 respectively).
The above results indicate that tendon stiffness is an important contributing factor in the early stages of force transmission (50ms), which indicates that after the first 50ms as the tendon is pulled by the muscle, the tendon becomes an effective mechanical link for contractile force transmission and joint movement. Additionally, the high correlation between tendon stiffness and MVC indicates that differences in tendon stiffness largely reflect the differences in the maximum tensile force applied by the in-series muscle, despite maximum muscle forces not being applied often in daily activities. It is of interest to establish whether the proportionality between stiffness and MVC also applies between tendon and muscle dimensions.

Magnusson SP et al. J Physiol. 2008;586:71–81.

Bojsen-Møller J et al. J Appl Physiol 2005;99(3):986–94.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC57

Chronic beta$_2$-adrenergic stimulation induces oxidative-to-glycolytic and slow-to-fast twitch transition of skeletal muscle and attenuates training-induced increases in rate of Ca2+ uptake of the sarcoplasmic reticulum following 11 weeks of resistance training in active young men

S. Jessen[1], A. Kalsen[1], M. Kreiberg[1], J. Onslev[1], N. Ørtenblad[2], V. Backer[1], J. Bangsbo[3] and M. Hostrup[1]

[1]Department of Respiratory Research, Bispebjerg University Hospital, Copenhagen, Denmark, [2]Department of Sports Science and Biomechanics, University of Southern Denmark, Odense, Denmark and [3]Department of Nutrition, Exercise and Sports, Section of Integrated Physiology, University of Copenhagen, Copenhagen, Denmark

Chronic stimulation of β_2-adrenoceptors increases Ca2+-handling, and induces shifts myosin heavy chain(MHC) I to MHCII isoform and from an oxidative to a glycolytic phenotype in animals. However, no study has investigated the additive effect of β_2-adrenoceptor stimulation and resistance training in humans. In the present study we investigated the effect of 11 weeks of resistance training alone or in combination with β_2-adrenergic stimulation on sarcoplasmic reticulum (SR) Ca^{2+} handling and MHC isoform distribution, as well as on oxidative and glycolytic enzymatic activity of skeletal muscle in young men. Twenty-six trained men were randomized to either placebo (PLA) or oral salbutamol (4×4 mg×d^{-1}, SAL). Subjects completed a supervised 11-week resistance training intervention three times pr. week. Before and after the intervention, MHC isoform distribution, SR Ca^{2+} handling, and maximal activity of CS, HAD, PFK, LDH, and CK were measured in biopsies

collected from the vastus lateralis muscle. In SAL, MHCIIa isoforms increased ($P \leq 0.05$) by 6±5% (mean±95%CI) with the intervention, whereas MHCI isoforms decreased ($P \leq 0.05$) by 6±6%. MHC isoform distribution did not change with the intervention in PLA. Rate of Ca^{2+} release increased ($P \leq 0.05$) in both groups with the intervention (SAL: 2.6±0.5 to 2.8±0.5 $Ca^{2+} \times min^{-1}(g \times protein^{-1})$; PLA: 2.5±0.5 to 2.8±0.5 $Ca^{2+} \times min^{-1}(g \times protein^{-1})$). In SAL, time constant of Ca^{2+} uptake was unchanged with the intervention (-6%), whereas it was 14% lower ($P \leq 0.01$) after the intervention in PLA compared with before. In SAL, maximal activity of CS decreased ($P \leq 0.01$) with the intervention (32±3 to 29±3 $\mu mol \times g\ dw^{-1} \times min^{-1}$), whereas no change was observed in PLA (29±3 to 30±3 $\mu mol \times g\ dw^{-1} \times min^{-1}$). Likewise, maximal activity of HAD decreased ($P \leq 0.001$) with the intervention in SAL (24±2 to 20±2 $\mu mol \times g\ dw^{-1} \times min^{-1}$), while no change was observed in PLA (22±2 to 22±2 $\mu mol \times g\ dw^{-1} \times min^{-1}$). Maximal activity of LDH increased ($P \leq 0.001$) with the intervention in SAL (463±83 to 524±83 $\mu mol \times g\ dw^{-1} \times min^{-1}$), whereas no change was observed in PLA (515±83 to 535±83 $\mu mol \times g\ dw^{-1} \times min^{-1}$). Maximal activity of PFK and CK did not change with intervention in either group. In conclusion, β_2-adrenergic stimulation induces muscle fiber type transition towards a fast twitch glycolytic phenotype following resistance training. Furthermore, β_2-adrenergic stimulation attenuates resistance training-induced increases in Ca^{2+} uptake function.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC58

Real-time monitoring of driver's autonomic regulation in formula-one racing

P.O. Julu[1] and G. Zenios[2]

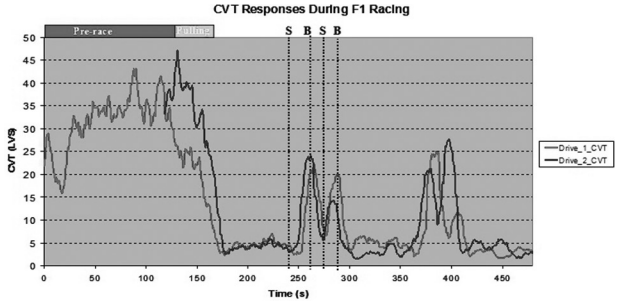
[1]Cardivascular Research Centre, Queen Mary University of London, London, UK and [2]Extreme Biometrics, Tunbridge Wells, UK

Every second is valuable in formula-one (F1) racing, so it requires real-time monitoring of performance. While the mechanical aspects of the race can be monitored, there is currently no appropriate method for monitoring brain controls of human functions during racing. We have investigated the role of cardiac vagal tone (CVT) in F1 racing because of the importance of central parasympathetic restraint during both mental and physical challenges.

We asked a Red Bull Racing F1 driver to volunteer as a subject to be monitored during practice in a simulator equipped with all aspects of F1 racing except the moments of inertia forces (G-force). He was wearing the full racing suite with helmet, but we added the Neurozoid wireless electrocardiogram (ECG) sensors placed in a conformation of modified Einthoven Lead II position (Neurozoid, Extreme Biometrics, Tunbridge Wells, Sussex, UK). The Neurozoid fed the ECG to a cloud-based processor via a wireless mobile device for the measurement of CVT in real-time using the NeuroScope method previously described in details (Julu et al., 2003). The CVT is measured in clinically validated, atropine-derived units of

the Linear Vagal Scale (LVS, Julu 1992) and data is sent back from the cloud-based processor to the telemetry desk of the race Engineer in real-time. The driver carried out two drives of ten laps each separated by 30 minutes of rest period in a simulated Yas Marina race circuit in Abu Dhabi. This race tract is notorious for its long high-speed straights ending with sharp bends requiring intense and heavy braking. The pre-race CVT remained above 30 LVS units at the start of both drives, but was quickly withdrawn to below 5 LVS units when the driver pulled from the pit to the race track (Fig.1). The normal range of resting supine CVT in sedentary non-athletes is 5-10 LVS units (Mckechnie et al., 2002). The CVT remained below 5 units during racing except when driving on the long high-speed straights when it recovered briefly to levels above 15 LVS units only to be quickly withdrawn again to levels below 10 LVS units during the sharp bends at the end of the straights (Fig.1). The CVT responses were consistent during the two drives (Fig.1).

Real-time changes in CVT can be monitored continuously during F1 racing and it consistently matched the task being performed by our F1 volunteer driver, where intense engagements caused vagal withdrawals to the extents that reflected the speed and intensity of the tasks being performed. The pre-race CVT level in the driver was nearly threefold that in sedentary people. We propose that the extent of CVT withdrawal can be used as a physiological measure of engagement and or perceived difficulty of a task in F1 racing. This can be interpreted as a measure of the perceived stress while performing a particular task.



Two examples of cardiac vagal tone (CVT) responses during formula-one (F1) racing involving driving through long straight tracks (S) at full throttle and ending in a sharp bends (B) that require heavy braking and concentration. Note the rapid withdrawal of CVT as the car pulls off at the start of the race and the recovery of CVT during the straight parts of the track. This recovery is halted at sharp bends by sudden withdrawal of CVT and the recovery resumes at another segment of a long straight track. This is consistently repeated in all segments of the racetrack with long straights ending with sharp bends.

Julu, P.O.O. (1 992). *J. Auton. Pharmacal.* **12,** 109–115

Julu, P. O. O., Cooper V. L., Hansen S. & Hainsworth R. (2003) *J Physiol.* **549.1.** 299–311

Mckechnie, P.S., Hamilton, R.M. & Macfarlane, P.W (2002). *International Journal of Bioelectromagnetism.* **4(2), 309–**310

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC59

The effect of chronic beta$_2$-administration on exercise-induced increase in maximal oxygen uptake

J. Onslev[1,2], N. Krogh[1,2], V. Backer[1], J. Bangsbo[2] and M. Hostrup[1,2]

[1]Department of Respiratory Research, Bispebjerg Hospital, København NV, Denmark and [2]Department of nutrition, exercise and sports, August Krogh Institute, Copenhagen, Denmark

Chronic high-dose beta$_2$-agonist administration has been shown to decrease skeletal muscle citrate synthase and cytochrome oxidase C activity in both endurance trained and sedentary rats, which could have deleterious effects on the oxidative capacity. Indeed, this effect has later been connected to a diminished exercise performance measured by time to exhaustion on a treadmill. Furthermore, a shift in fibre type towards a more glycolytic phenotype in response to chronic beta$_2$-agonist administration has been demonstrated, both indicating increased dependence on anaerobic metabolism of the skeletal muscle cells. Whether or not this is also the case in humans and at therapeutic doses is unknown.

The effect of chronic beta2-agonist on exercise performance and oxidative capacity was examined in a 4-week randomized, double-blinded, controlled study. Twenty one subjects was included in an active group (TER, n = 12) and a placebo group (PLA, n = 9). Throughout 4 weeks of endurance training TER received 8 x 0.5 mg terbutaline by inhalation daily. Before and after the intervention the maximal oxygen consumption (VO_2-max) was measured in an incremental cycling test. Furthermore, time to exhaustion (TTE) was assessed in a relative resistance cycling test corresponding to 120% of VO_2-max (120%-VO_2-max-test).

A significant ($P \leq 0.01$) interaction effect of group*time was observed for VO_2-max. VO_2-max was higher in PLA than in TER (4153±133 vs. 3990±154 ml/min) after the intervention compared with before the intervention (3904±128 vs. 3965±149 ml/min). Furthermore, a significant ($P \leq 0.001$) within-group time effect was seen in PLA on VO_2-max (3904±128 vs. 4153±133 ml/min), whereas the VO_2-max remained similar for TER (3965±149 vs. 3990±154 ml/min). Also, during the VO_2-max-test the incremental peak power output (iPPO) was increased by 19±13W ($P \leq 0.01$) and 38±15W ($P \leq 0.001$) with the intervention in TER and PLA resulting a significant ($P \leq 0.05$) interaction effect (20±19W). Lastly, the results from the 120%-VO_2-max-test revealed a significant ($P \leq 0.05$) interaction on group*time on TTE, where TTE was greater in TER than in PLA after the intervention (165,9±21 vs. 131,4±18 s) than before the intervention (127,8±17 vs. 146,1±19 ml/min). This is accompanied by a significant ($P \leq 0.05$) within-group time effect in TER (127,8±17 vs. 165,9±21 ml/min).

We conclude that therapeutic doses of inhaled terbutaline blunts the endurance training-induced increase in VO_2-max. Furthermore, the increase in iPPO in TER unaccompanied by a corresponding increase in VO_2-max points towards an increased anaerobic energy contribution. Also, this conclusion is supported by the

120%-VO_2-max-test, which shows an increased TTE in TER. Overall, results indicate that the muscle cells decreases its oxidative capacity while increasing its anaerobic energy contribution going towards a more glycolytic phenotype.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC60

Expiratory muscle fatigue following upper-body exercise in healthy humans

N.B. Tiller[1,2], I.G. Campbell[3] and L.M. Romer[1]

[1]Division of Sport, Health and Exercise Sciences, Brunel University London, London, UK, [2]Academy of Sport and Physical Activity, Sheffield Hallam University, Sheffield, UK and [3]School of Life and Medical Sciences, University of Hertfordshire, Hertfordshire, UK

Introduction: The diaphragm and abdominal muscles exhibit contractile fatigue in response to sustained, high-intensity, whole-body exercise. We hypothesized that upper-body exercise would increase the contribution of the thoracic muscles to the control of trunk stability, and leave the diaphragm and abdominal muscles susceptible to contractile fatigue. Methods: Seven healthy, physically active men (peak upper-body O_2 uptake [VO_{2peak}], 31.9 ± 5.3 $mlkg^{-1}min^{-1}$; mean ± SD) performed arm-crank exercise to the limit of tolerance at work rates equivalent to 30% (Tlim; 24.5 ± 5.8 min) and 60% (Tlim; 9.8 ± 1.8 min) of the difference between gas exchange threshold and VO_{2peak} (i.e. heavy and severe intensities). Diaphragm and abdominal muscle fatigue were assessed by measuring the change from baseline in potentiated transdiaphragmatic and gastric twitch pressures ($P_{di,tw}$ and $P_{ga,tw}$) in response to cervical and thoracic magnetic stimulation, respectively. Results: Tidal transdiaphragmatic pressures were elevated during heavy and severe exercise (33 ± 11 vs. 53 ± 13 cmH_2O, p = 0.002), due to equivalent changes in gastric and oesophageal pressure. There was limited evidence of diaphragm fatigue following either trial. However, 5 of 7 participants exhibited >15% reduction in $P_{ga,tw}$ at 5-15 min after severe exercise, with a mean reduction of 22 ± 18% (p = 0.038) and moderate effect size (h^2 = 0.36); values had partially returned to baseline at 25-35 min after exercise (–15 ± 15%; p = 0.066). Conclusions: We present preliminary evidence that the abdominal muscles (but not the diaphragm) fatigue in response to sustained, high-intensity upper-body exercise in healthy, physically active men. Since upper-body exercise induced submaximal cardiorespiratory stress, the fatigue observed was likely due to additional (non-respiratory) loading of the thoracic complex.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC61

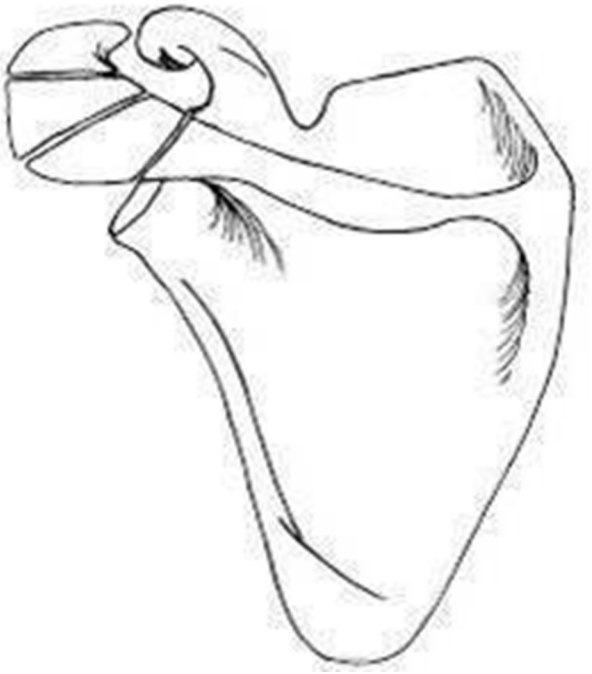
Training with os acromiale: How blood flow restriction training supported the rehab process in sprint kayak

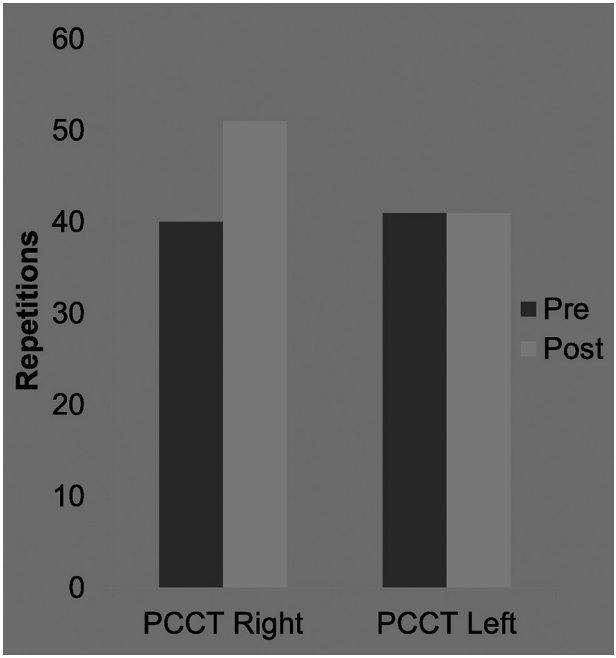
J. Coe and M. Thompson

EIS, Manchester, UK

Background: Os Acromiale is a failure of fusion of the acromial process, seen in around 8% of the population (Kurts et al. 2006). The os acromiale has been implicated as a risk factor for the development of impingement syndrome, when an os acromiale is unstable; the downward pull of the deltoid reduces subacromial space. As a result traditional strength training can be disturbed.
There is a growing body of literature surrounding blood flow restriction resistance exercise (BFRRE) demonstrating rapid gains in muscle cross sectional area (CSA) and functional strength (Neilsen et al. 2012) similar or even greater extent (Takarada et al. 2002) as seen with heavy-load resistance training. Generally thought to be the results of multiple local and systemic factors (Widegren 2000). BFRRE offers a unique training modality due to its relatively low load (≥50% 1RM) reducing the stress on surrounding joint and bone structures.
Purpose: The primary aim of the study was to investigate if blood flow restricted exercise is an effective strategy to increase posterior cuff capacity and CSA around the shoulder girdle when range of motion and training intensity is limited.
Participant: 21year old female, weight 73.4kg, height 173.8cm. Informed consent and BFRRE risk assessment was completed prior to intervention.
Methods:Intervention: 6weeks of unilateral (affected arm only) BFRRE. In total, 18 sessions were completed in a periodised design. The first block consisted of 9 (3sessions per week on non-consecutive days) sessions of half range bench pull at 30% 1RM and reverse fly, 4 sets to failure with 60s rest. The second block consisted of half range press ups and supine pull, 4 sets to failure with 60s rest. The first session of each block was an introductory session of 1 set of 20reps and 3 sets of 15reps. The occlusion cuff was placed at the proximal as high up the arm as possible and inflated to 100mmHg. Between blocks one and two, a one week 'flush out' was included.
Measures: Pre and post the intervention anthropometry was assessed according to ISAK along with the posterior cuff capacity test (Moore, Uhl & Kibler 2013).
Results: The anthropometry results demonstrate an increase in bicep girth from 32.1cm pre to 33.0cm post (+2.7%) and a 0.7cm increase in shoulder girth (0.6%). With no change in body mass (73.4kg vs 73.1kg). Total volume load during the first block increased from 144reps to 169reps (14%) in bench pull and 65reps to 94reps (30%) supine pull during the second block. A meaningful 21% increase in posterior cuff capacity (40reps Vs 51reps) was observed.

Practical Implications: With those who are unable to complete traditional resistance exercise, BFRRE was an effective strategy to induce muscle hypertrophy and endurance. Changes in posterior cuff capacity suggest local muscular endurance changes proximal to the cuff and therefore potential implications for rehabilitation when access to specific musculature is limited.





Fahs, CA., Loenneke, JP., Rossow, LM., Thiebaud, RS and Bemben, MG. (2012). Methodological considerations for blood flow restricted resistance exercise. Journal of Trainology 1:14-22.

Widegren U, Ryder JW and Zierath JR. (2001). Mitogen-activated protein kinase signal transduction in skeletal muscle: effects of exercise and muscle contraction. Acta Physiol Scand 172: 227–238.

Takarade, Y., Sato, Y and Ishii, N. (2002). Effects of resistance exercise combined with vascular occlusion on muscle function in athletes. Eur J Appl Physiol. 86:308–314.

Moore, SD., Uhl, TL and Kibler B. (2013). Improvements in Shoulder Endurance Following a Baseball-Specific Strengthening Program in High School Baseball Players. Sports Health. 5:233-238.

Kurtz CA, Humble BJ, Rodosky MW, Sekiya JK. (2006). Symptomatic Os Acromiale. Journal of the American Academy of Orthopaedic Surgeons. 14:12-19.

British Canoeing

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC62

Does the menstrual cycle affect temperature sensitivity of muscle force?

J. Bieles

King's College London, London, UK

Maximum voluntary force (MVF) exerted by thumb adduction (TA) declines with skin cooling, with greater decrements in post- menopausal vs. pre-menopausal women[1]. TA MVF is affected by the menstrual cycle, with increases of ~10% during the follicular stage when oestrogen levels rise[2], although similar effects were not observed in handgrip strength[3]. Intrinsic hand and forearm muscles contribute to handgrip strength, which declines when both are cooled[4]. However, the effect of hand cooling alone has not been reported. The aim of this study was to determine the effect of hand cooling on TA and handgrip strength during the menstrual cycle. We hypothesised that: i) hand cooling would reduce TA MVF to a greater extent than handgrip; ii) cooling effects are greater when oestrogen levels are lower.
17 healthy women (18-42 yrs) with regular menstrual bleeds not using hormonal contraception, volunteered to attend twice: 7-10 days after the start of the menstrual cycle when oestrogen levels are high, and after the luteinizing hormone peak, when oestrogen levels are low. The hand was cooled and warmed (skin temperatures 12-40^{o}C) in a water bath. TA MVF and maximal handgrip strength were measured at 4-minute intervals and normalised to the subject's mean at skin temperatures >35^{o}C. A 2-way rANOVA was used to determine the effect of temperature and menstrual status. Post-hoc t-tests were performed across temperature bins.
A-priori power calculation suggests a sample size of 40 is required to establish an effect of menstrual cycle upon the effect of cooling on strength. These preliminary data show pre-ovulation TA MVF is higher than post-ovulation ($p=0.017$). At skin temperatures <20^{o}C MVF is lower than when >20^{o}C ($p=0.001$). Handgrip strength at skin temperatures <25^{o}C is lower than those >25^{o}C, pre- and post-ovulation ($p<0.001$) irrespective of menstrual phase. There was no interaction between temperature and menstrual cycle for handgrip strength or TA MVF.
The decline in handgrip strength with hand cooling occurred at a higher temperature (25^{o}C) than for TA MVF (20^{o}C). This is despite the fact that forearm muscles were not directly cooled, suggesting mechanisms other than local muscle cooling contribute to the decline in handgrip strength. TA MVF, but not handgrip, was lower post-ovulation suggesting an influence of female hormonal status may have been observed when the muscles were cooled directly. Further data is required to determine if there is an interaction between temperature and menstrual cycle phase on force.

Bieles JS, et al., Eur J Appl Physiol, 2012. 112(3): p. 1117–1122.

Phillips SK, et al., Clin Sci, 1993. 84(1): p. 95–8.

de Jonge XAKJ, et al., J Physiol, 2001. 530(1): p161-166.

Chi, et al., Int J Ind Ergonom, 2012. 42(1): p. 113–121.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

V

W

Z