Chapter:

The Cellular and Biochemistry of the Anti-Ageing Effect of Exercise and Physical Activity

Dr. Mark Ross[1], Hannah Lithgow[1], Dr. Lawrence Hayes[2], & Professor Geraint Florida-James[1]

[1] School of Applied Sciences, Edinburgh Napier University, Scotland, United Kingdom

[2]Active Ageing Research Group, University of Cumbria, United Kingdom

Abstract (250 words)

Exercise in young adults consistently improves various aspects of physiological and psychological health but we are now realising the potential benefits of exercise with advancing age. Specifically, exercise improves cardiovascular, musculoskeletal, and metabolic health through reductions in oxidative stress, chronic low-grade inflammation and modulating cellular processes within a variety of tissues. In this this chapter we will discuss the effects of acute and chronic exercise on these processes and conditions in an aging population and how manipulating exercise variables can provide different stimuli which can have differing effects on these processes. Additionally we will address how physical inactivity can accelerate aging in tissues by promoting cell senescence and atrophy and, how physical activity and physical inactivity may affect non-communicable disease risk in older adults via cellular processes.

Physical Activity in the Elderly and Non Communicable Disease

Advancing age is associated with increased risk of non-communicable disease (NCD), such as cardiovascular disease (CVD), type 2 diabetes (T2DM), and cancer but to name a few (90). Using mathematical modelling, Lozano et al. (90) suggested that there is a 39% increase in the incidence of deaths attributable to NCD as a direct consequence of the aging population. Healthcare provision, and healthcare insurance costs are significant due to the debilitating effects of such diseases on the human body. Epidemiological evidence strongly suggests that we become more inactive as we age (57), which further increases the risk of NCD incidence, morbidity and mortality in this population (87, 139). Insufficient physical activity in the older population is associated itself with muscle mass loss/atrophy and sarcopenia (45), T2DM (3), CVD (159), and increased risk of infection (89), and is estimated to contribute to $65.7bn worth of healthcare costs per annum worldwide (149), which is equivalent to the gross domestic product of Costa Rica in a single year. In fact, increasing physical activity levels in

the older populations is linked with enhanced cognitive function, physical performance, improved cardiovascular health measures (20), reduced T2DM risk (37), together improving quality of life.

Physical activity and exercise can stimulate a host of changes at the molecular, cellular, and tissue level, which translates to improved physical, as well as psychological health. The following sections will delve into the physiological effects of exercise, and the benefits for the older population, detailing molecular, cellular and tissue-level effects which partly explain the health benefits of exercise and physical activity.

The Aging Cardiovascular System and Physical Activity/Inactivity

The cardiovascular system (CVS) is essential for the delivery of oxygen and nutrients to every cell in the body, the removal of waste products, such as carbon dioxide, lactate and ammonia, and also works to help the immune system fight infection through distributing leukocytes to sites of infection. As we age, various aspects of our cardiovascular system change. Our heart undergoes structural changes, as do our blood vessels, which makes it difficult for the CVS to perform its roles efficiently. Unfortunately, due to aging, we are at a high risk of CVD morbidity and mortality (90), as a result of incidence of stroke, myocardial infarction (MI) and heart failure (HF). Therefore maintaining the health of our CVS is key for longevity.

Aging and Vascular Function: Role of Exercise and Physical Activity

Our blood vessels are to key structures within our body which regulate blood flow to all tissues of the body, and the ability of our vasculature to do so, is termed 'vascular function'. The cells of the inner lining of all blood vessels are the endothelial cells. These cells are crucial in regulating blood flow via producing and releasing vasoactive substances such as nitric oxide (NO) (50). NO subsequently diffuses across to the surrounding vascular smooth muscle cells (VSMCs) and stimulate these cells to relax via Ca^{2+} active re-uptake by the sarcoplasmic reticulum. The relaxation causes a widening of the diameter of the blood vessel, thus allowing increased blood flow to tissues distal to the vessel. This predominantly occurs at the arteriolar level, rather than the artery or capillary level, due to the relative ratio of VSMCs to endothelial cells. We can assess vascular/endothelial function through a technology called 'flow-mediated dilatation', or FMD, which is the use of ultrasound technology to determine changes in vascular diameter (typically the brachial or femoral arteries) in response to an increase in flow after a period of ischaemia or occlusion. The subsequent shear stress after occlusion is removed results in an increase in NO production by the endothelium (28), and so FMD has been validated to be a measure of endothelial, NO-dependent vasodilation (55). Studies to date have found significant relationship between endothelial function/FMD scores and cardiovascular-related mortality, with

poorer scores and lower levels of vasodilation being predictive of earlier mortality (56). Unfortunately, with advancing age, we display significant reductions in endothelial function, as demonstrated in several studies (13, 14, 101, 134, 142). Potential causes include age-related elevations in oxidative stress, which may uncouple endothelial NO synthase (eNOS), which is required for NO production from its precursor, L-arginine. Aged vascular tissue exhibit greater production of superoxide (O_2·-) anions (30, 59, 97) which may contribute to the uncoupling of eNOS. The role of oxidants in the age-related reductions in endothelial function were confirmed in a study by Eskurza et al. (44). In this study, young, old sedentary adults were assessed for vascular function. They confirmed that vascular function was reduced in the older group, but that an acute dose of ascorbic acid (vitamin C, a powerful antioxidant) reversed this effect, so much so that there was no longer a significant difference in vascular function between the two age groups.

Interestingly, the study by Eskurza et al. (44) also included an older, endurance trained group. Vascular function between the young group and the endurance trained older group were not different from one another, indicating a powerful role of exercise and physical activity to prevent or at least attenuate age-related vascular dysfunction. The potential for exercise and physical activity to do this, as indicated by this cross-sectional study, has been confirmed by longitudinal studies in both young (10, 115) and older adults (13, 14).

Cardiovascular Regeneration and Repair with Aging and Exercise

Our bodies have the remarkable ability for endogenous regeneration, through our own stem and progenitor cell network. Stem cells, located in specific tissues, or from the bone marrow, contribute to tissue repair and growth. The walls of our heart contain c-kit$^+$ cardiac stem/progenitor cells (43, 116), which have been shown to differentiate into myocardial cells under stimulation *in vitro* and *in vivo* (43). Aging influences the function of these cardiac stem cells (22), with reduction in stemness of cardiac progenitor cells, impairments in differentiation into myocardial cells, and failure to secrete vital paracrine factors in response to stimulation in animal models (22). Aged mice also display CPCs expressing greater levels of senescent markers such as p27kip1, p53 and p19ARF, and subsequent loss of CPCs due to apoptosis occurred (148). Unfortunately, due to the invasive nature of CPC isolation, characterization and functional assessment, human data are lacking, however rat and mouse models are ideal as whole lifespan effects on such cells can be investigated with relative ease.

Interestingly, exercise training in animals activates c-kit$^+$ and Sca1$^+$ cardiac progenitor cells, which may contribute to left ventricular physiological hypertrophy (163), a response that appears to be dose-dependent (163). Mice that underwent physical training displayed greater number of c-Kit$^+$Lin$^-$ cells than sedentary controls, potentially due to increased survival or increased proliferation of cardiac

resident progenitors (88). It is possible that the increase in cardiac workload leads to increased cellular activation of these CPCs (150), which in turn would support the subsequent physiological hypertrophy observed with exercise training in humans. However, there is a lack of research in this area, and is an exciting area of future work to determine if exercise can be used to stimulate cardiac repair after ischaemic events in patients and in the elderly.

Bone marrow-derived, or tissue-resident endothelial progenitor cells (EPCs) contribute to the regeneration and growth of the vascular endothelium (5, 6). These cells may or may not differentiate into mature endothelial cells, but they do have the ability to secrete pro-angiogenic factors, such as vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8) to support endothelial cell turnover and replication (71). Unfortunately, they circulate in such small numbers, within the region of 0.001-0.01% of all circulating mononuclear cells (21). Despite this, their circulating number has been related to vascular function (18) and mortality risk, with lower progenitor cells associated with impairments in peripheral arterial tonometry and greater risk of mortality and morbidity in humans (110). Several studies have observed lower circulating EPCs in older humans compared to younger counterparts (120, 146), which was independent of other cardiometabolic risk factors (120). EPC function and survival are also affected by aging, with older adults displaying greater number apoptotic EPCs than younger individuals (83), and these cells display functional deficits, such as secretion of pro-angiogenic cytokines and growth factors (82). Together, these data show that aging-associated increased vascular and mortality risk may be partly due to loss of EPC number and/or function. Additionally, Xia et al. (161) treated mouse ischemic hindlimbs with human EPCs from young and old donors. They found that cells from young donors homed to the site of ischemia, and helped to promote vascular repair, and recover blood flow more so than sham delivery. Interestingly, they also found that EPCs from older individuals lacked this ability, and associated this with an inability of these EPCs to migrate *in vitro*, shown to be associated with impaired intracellular CXCR4:JAK-2 signaling (161, 162).

Single bouts of exercise have a remarkable ability to mobilize these progenitor cells from peripheral tissues, such as the bone marrow, into the circulation in the post-exercise recovery period (121, 153), even in older adults, despite an attenuated response (120). The mobilization of such progenitor cells are accompanied by elevations in circulating VEGF (121, 152, 157), granulocyte colony-stimulating factor (G-CSF) (121) and stromal-derived factor-1α (SDF-1α) (152, 157), thought to act as chemoattractive factors. The response of EPCs to acute exercise is both time and intensity-dependent (84). Studies investigating the effect of regular exercise training on circulating EPCs provide mixed results with regards to outcomes. Most (29, 68, 85, 94, 124, 125, 133, 138, 151, 161), but not all studies (91, 146) demonstrate either an improvement in EPC number (due to increased mobilization or enhanced survival) or function with regular exercise training. In an elegant study, Xia et al. (161)

demonstrated that 12 weeks physical exercise training in older populations can restore the age-related impairment in EPC function. The researchers transplanted human EPCs (young and old donors, before and after exercise training) into mice that had undergone femoral artery ligation. Their data concur with their earlier finding that EPCs from older adults displayed reduced neovascularization and ability to recover blood flow in ischemic hindlimb in mice (162), but exercise training resulted in improved vascular repair capability, and recovery of blood flow.

The current evidence strongly suggest that exercise has a strong positive benefit for the cardiovascular system in aging populations, through its effects on improving vascular function via increasing NO bioavailability, angiogenesis, and both cardiac (c-kit$^+$ CPC activation and survival) and vascular (improving EPC number and function) repair mechanisms.

Musculoskeletal Health and Function with Healthy Aging

One important change associated with biological aging is our reduced ability to exert force (or torque) around a joint. Age-associated dysfunction of the muscular system, termed *sarcopenia*, is defined as a syndrome characterised by loss of muscle mass and strength. This results in risk of adverse outcomes such as physical disability, inferior quality of life, and mortality (35, 39). Therefore, the European Working Group on Sarcopenia in Older People (EWGSOP) proposed that diagnosis requires evidence of reduced muscle mass, and either low muscle strength, or low physical performance. Recently, it has been observed that reduced muscle power (*dynapenia*) occurs faster than force or mass losses and may be more predictive of functional impairment (95). This is because many tasks of daily living require us to exert force over a short space of time. E.g. when we stand from a chair.

Various interacting tissues, including connective, nervous, skeletal, and muscular, determine measurable force and power *in vivo*. These systems do not operate in isolation, so a holistic view of force production is required. For example, nerve conduction velocity, motor unit recruitment, and firing frequency all influence force via recruitment of muscle, and decline with age (79). However, skeletal muscle is our most important organ for generating force and power, and therefore this section will focus on the biology of aging muscle.

Causes of Age-Associated Muscle Deterioration

Several theories are proposed to explain our reduced muscular capacity with age. Alterations to contractile characteristics, namely decreased twitch speed and a shift in fibre type (from fast to slow)

are observed in the elderly which reduces rate of force development. Decreased anabolic hormone production, increased proinflammatory cytokines, and protein turnover imbalances attenuate the ability of aged muscle to regenerate (which leads to atrophy and therefore reduced muscle mass). Importantly, these mechanisms occurring with age, are exacerbated (or even detected in isolation) by physical inactivity. However, if aging were 'curable' with exercise, masters athletes (older adults who train intensely on a regular basis) and ex-Olympians would be deemed immortal, which is clearly untrue. However, masters athletes do display a younger phenotype than age-matched sedentary counterparts, which results in lower incidence of frailty and dependency. As such, masters athletes may be considered as a model of successful aging (61). This hypothesis is supported by masters athletes presenting greater relative lean mass, and muscle power than sedentary counterparts, thus suggesting chronic exercise (even aerobic in nature) preserves muscle into later life (62). The following sections will discuss how each mechanism may cause muscle deterioration, and how exercise may mediate these mechanisms, with evidence from human studies.

Aging and Reduction in Anabolic Hormones: Influence of Exercise

As we age, less anabolic hormones are released into circulation to interact with muscular receptors, to exert muscle-building effects (131). This theory of muscle aging is supported by cell culture experiments (38), but also administering older adults testosterone and observing increased muscle mass and strength (7, 49, 132). Although supraphysiologic doses of anabolic hormones increase muscle mass, the effect of lifelong exercise or physical fitness on naturally occurring anabolic hormones is unclear. Ari and colleagues (4) reported higher testosterone in masters athletes compared with sedentary counterparts. However, this finding is not ubiquitous (64). Several studies inducting sedentary individuals onto an exercise programme do see an increase in 'anabolic' hormones, which accompanies lean mass gains (63, 66). What is evident however, is that a threshold level of metabolic stress may be required to induce hormone changes, as Khoo et al. (78) noted greater increases in testosterone following high volume, compared to low volume training. Similarly, Herbert et al. (66) reported increased insulin-like growth factor-1 (IGF-1) following high intensity training, but not following low intensity training in previously sedentary older men.

Endocrinology is a complex discipline, with hormones exerting multiple actions, which confounds our ability to draw conclusions about whether hormone changes are to blame for muscle deterioration. For example, IGF-1 may be increased post-exercise compared to pre-exercise, but testosterone, cortisol, myostatin, and growth hormone may not be different, so can we say for sure that the individual is more 'anabolic' than before? Probably not. Similarly, a hormone in circulation may be

increased post-exercise, but unless the hormone is bioavailable (i.e. not bound to a carrier), it cannot exert a cellular effect. The hormone is also reliant upon receptors within muscle to commence a downstream signalling cascade, resulting in transcription and translation of muscle protein. As such, we are some distance from understanding the endocrinology of aging and the effect exercise exerts.

Inflammatory Cytokines: Effect of Aging and Exercise

As we age, we experience increased systemic inflammation. We now know elevated inflammatory cytokines negatively correlate with muscle mass and strength in the elderly. Cytokines are small secreted proteins released by cells, which permit interaction and communication between cells (165). Rodent and cell culture experiments have demonstrated inflammatory cytokines directly impair expression of muscle-specific transcription factors, ultimately inhibiting protein synthesis (104, 140, 147). More evidence on a human level for the *inflamm-aging* hypothesis is provided by Aguirre and colleague (1) who reported significant correlations between knee flexor strength and interleukin-6 (IL-6) and C-reactive protein (CRP), both inflammatory cytokines, in frail, obese, older adults. Furthermore, Mikkelsen et al. (98) measured muscle size and strength, maximal oxygen uptake, but also inflammatory cytokines in old runners, young runners, and age-matched untrained individuals. CRP and IL-6 were higher in older groups, but lower in trained groups compared to untrained groups. It therefore appears age increases inflammation, but exercise may exert an anti-aging effect.

Whilst increased low grade inflammation in the elderly is commonly observed, aging reduces cytokines that contribute to local recruitment of immune cells responsible for muscle remodelling. In other words, age reduces the adaptive response of skeletal muscle to exercise by reducing inflammatory cytokines (58). For example, Hamada et al. (58) observed an increase in systemic inflammation (demonstrated by elevations in CRP), yet lower local exercise-induced inflammation (demonstrated by reduced transcripts for CD18, IL-1β, IL-6, TNF-α, and TGF-β1 in muscle biopsies) in older adults compared to younger adults. To date, the effect of training status on exercise-induced inflammatory cytokine response in older adults is unexamined.

Aging-Associated Effects on Skeletal Muscle Protein Turnover

Regardless of the precise contribution of each of the above factors to muscle deterioration, reduced muscle ultimately results from an imbalance between muscle protein synthesis (MPS) and muscle protein breakdown (MPB). Amino acid-based feeding increased net protein balance, via increased MPS, and reduced MPB (99). Exercise exerts a synergistic effect on MPS and ultimately net protein balance, with resistance exercise most potent (40, 48). MPS in response to amino acids (36, 156) and

resistance exercise (81) is reduced in aged muscle compared to young muscle, and is termed *anabolic resistance* (117). It is worth noting however, that reduced MPS is not always observed in older adults (80, 141). Therefore, blaming chronological age for anabolic resistance, rather than physical inactivity (often associated with advanced age), may have led to classic type I error in cross-section comparison. Breen et al. (17) suggested that inactivity induces anabolic resistance as they observed two weeks' reduced physical activity decreased lean leg mass by ~4%, and postprandial MPS by 26% in ~72 year olds. Furthermore, Symons (141) reported MPS increased in young and old adults to the same extent following resistance exercise and protein ingestion. In the sole study investigating MPS in masters athletes (41), masters and young triathletes completed 30 min downhill running to induce muscle damage, and MPS was lower in the masters triathletes comparing to the young triathletes, which resulted in poorer subsequent cycling performance.

Due to this disruption in net protein balance, older individuals likely require greater protein intake to maintain muscle mass and function (111, 112). This is often difficult as older individuals have a lower appetite, and protein is the most satiating of the macronutrients. Therefore, pragmatic supplementation may be necessary to optimise health (111).

Summary and Practical Applications

Whilst recent advancements in physiological imaging and molecular biology provide insight into the mechanisms underpinning muscle aging, loss of function, and frailty, we are still some way from conclusive evidence to suggest which cause is dominant. What we know, is the above causes occur simultaneously, and are often interlinked. To most of us however, the mechanisms underpinning muscle aging are academic, and the critical issue is our health and independence. Physical inactivity and aging-reduced muscle function increases our likelihood of sarcopenia or dynapenia. Therefore, applied studies that demonstrate improved physical function may have the greatest practical application. For example, Fiatrione and colleagues (46) reported improved strength (175%), lean leg mass (9%), and gait speed (47%) in nonagenarian women following resistance exercise, which demonstrates great muscle plasticity into old age. More recently, high intensity interval training (HIIT) has shown some promise for increasing muscle power in older adults (~65 years) (63, 67, 126). Yet, the efficacy and safety of this model in the old-old (85+ years) is still untested.

In summary, age reduces our capacity to increase muscle strength and size. Yet, by staying active and performing high intensity weight training or power output exercises, we can attenuate the symptoms

of aging, which may result in a younger muscle phenotype. More importantly, having a large amount of muscle (relative to the rest of the population) is a predictor of longevity (https://www.ncbi.nlm.nih.gov/pubmed/24561114).

Vitamin D and Bone Health in Older Adults

Vitamin D status and metabolism is associated with numerous negative skeletal consequences affecting both bone and muscle, such as reduced bone mineral density (BMD), sarcopenia and dynapenia (age-associated loss of muscle strength), osteomalacia (marked softening of bones), and impaired calcium absorption (69). A large proportion of the global population are vitamin D deficient due to not meeting recommended intake guidelines and the climate restricting sufficient dermatological metabolism of vitamin D. The primary source of vitamin D is from direct skin exposure to Ultra Violet B (UVB) rays from the sun initiating the conversion of pre-vitamin D (7-dehydrocholersterol) to vitamin D_3 (cholecalciferol) (70), which is inherently dependent on climate and weather and thus latitude and season. Vitamin D concentrations are inversely linked with advancing age (26), with evidence suggesting that aging affects the cutaneous capacity for the initial metabolic conversion in the vitamin D pathway, and the concentration and expression of subsequent vitamin D metabolites, such as the vitamin D binding protein (DBP) and the vitamin D receptor (VDR) (11). The ligand-activated VDR, expressed in skeletal muscle as well as most other tissues, is a strong mediator of mRNA transcription and thus protein synthesis (12, 130). The expression of VDR and the post-transcriptional regulation of VDR and can be affected by aging (34). As a result, evidence has suggested that a lack of vitamin D in an aging population may affect skeletal muscle mass and strength and thus induce a risk of falls and immobility.

It is generally accepted that vitamin D in combination with calcium beneficially affects bone health and quality, primarily the readily measured surrogate of bone strength: BMD. During the aging process there is a decline in the intestinal absorption of calcium, which may be predetermined by the bioavailability of the active form of vitamin D ($1,25(OH)_2D_3$) (154), which declines with advancing age. Vitamin D stimulates the production of calcium-binding protein (CBP) in the intestine to facilitate the absorption of calcium. Vitamin D is also a regulator of cell growth and maturation, particularly of osteoblasts (bone cells), and mediates the function of white blood cells such as macrophages and activated T- and B-lymphocytes, which modulate the immune system.

Although there are mixed results on the effect of exercise and vitamin D metabolism in older adults, research has indicated that mechanical stress such as exercise and strength training can alter the expression and action of key vitamin D metabolites and increase skeletal muscle mass and strength

(2, 93). This may be through alterations in vitamin D signalling, which has been found to influence skeletal muscle protein synthesis. Vitamin D seems to have a role on skeletal muscle (23) that is easily manipulated by exercise and physical activity. A lower vitamin D status has been associated with a decline in muscle mass and strength, which becomes increasingly prevalent as age advances. Investigations *in vitro* have reported $1,25(OH)_2D_3$ to stimulate key cellular pathways of muscle growth and differentiation, acting primarily through the action of VDR, to induce myogenesis (24, 51). Currently it is uncertain if the effect of vitamin D on skeletal health is association or causation.

The Elderly Immune System and Changes with Exercise

Immune Cell Senescence and Aging

Human immunosenescence is the canopy term used to refer to the gradual deterioration of the immune system and function attributed to advancing age. The complex process of aging negatively impacts the innate and adaptive immune system and their functional capacity, therefore compromising the ability of the host to elicit an effective immune response to fight (ever-evolving) invading pathogens or prevent the development of a pro-inflammatory environment. The innate and adaptive immune systems are differently affected by aging, whereby innate immunity appears to be better preserved while adaptive immunity exhibits age-dependent depreciation.

Immunological parameters that impact health and mortality, creating the immune risk profile, become exhausted with the aging process. The functionality of the components of the adaptive immune system can become exhausted, specifically the main matured cells involved: bone marrow cells (B cells) and thymus lymphocytes (T cells) and their subsets. The primary lymphocyte subpopulation, $CD3^+$ T cells can be divided into $CD4^+$ and $CD8^+$ subsets, which exhibit helper and cytotoxic functions. In particular, CD8 T cells are affected by age, inducing the development of an inverted CD4:CD8 T cell ratio and thus contributing to immune incompetence.

Thymic Atrophy with Aging

Age-dependent regression of the thymus, thymic atrophy, defined as the loss of thymic mass, induces a decline in the output of naïve T cells. Therefore, as age advances fewer T cells are developed and exported into the vascular pool (86), directly impacting on the peripheral T cell repertoire and altering white blood cell subset diversity, and thus the cells that are circulated to the target tissues.

There is an increase in the proportion of T cells expressing markers associated with senescence delineating T cell subpopulations from naïve T cells (recent thymic products with no proliferative history) to exhausted senescent T cells (not so recent poorly proliferative cells that exhibit severe functional abnormalities). These markers are primarily used to identify T cell subpopulations, but may also be used to provide insight into T cell differentiation, activation, and functional status. The combination of markers can be utilised to define naïve T cells turnover and loss of naïve T cells, assessing proliferative history. Aging can also restrict the T cell receptor (TCR) repertoire. T cell receptors are complex integral membrane proteins that are responsible for recognising antigens that are bound to the major histocompatibility complex (MHC). A diminished TCR pool reduces the capacity for T cells to identify specific bound antigens and illicit a distinct and critical immune response.

Exercise and Immunosenescence

The beneficial effect of exercise became apparent in the early work of David Neiman in the 1990's who demonstrated that individuals who exercise are at less risk of upper respiratory tract infections (URTI), which are a major cause of visits to and treatment from physician. However, there is a hyperbolic relationship between intensity and volume of exercise and the risk of URTIs, suggesting that excessive or too intense exercise can be detrimental to effective immunity by supressing immune function. There are both acute and chronic effects of exercise on immune function. In response to an acute bout of exercise, one of the major changes that occurs is a change in the number of leukocytes (118), with a biphasic response induced. The redeployment of lymphocytes from tissues or the blood vessel wall with exercise consists of an initial increase, known as lymphoctosis, that is followed by a significant transient drop in lymphocyte number, known as lymphocytopenia. Immediately upon cessation of exercise the rise in lymphocyte and neutrophil number usually precedes a reduction to below baseline levels, creating a pocket period of reduced immune protection, known as exercise-induced immunosuppression. Each of the individual cell types respond differently to exercise as they all perform different tasks to achieve sound immune function, however it is the Natural Killer (NK) cells and the cytotoxic T cells that display the largest response (128). Exercise-induced immunosuppression can also be altered by cytokines, the signalling molecules of the immune system. Circulating concentrations of cytokines have numerous responsibilities and roles in the inflammatory profile and protection against pathogens, directly and indirectly. Aging is recognised to strongly affect the redeployment of lymphocytes with particular subsets not mobilised in the bloodstream: although the relative numbers of T cells are similar between young and old, it is the absolute numbers that change. This causes a rise in senescent T cells that are mobilised and thus circulate around the body

unable to play an efficient role in immune function and protection (129). This age-related accumulation of senescent T cells lowers the naïve T cell stock and can increase host infection risk. This is also due to older individuals having less naïve and low differentiated cells in the circulation and peripheral tissues for redeployment (114). Exercise can override the age-related impairments in T cell subset redeployment, specifically $CD8^+$ T cells (135). Aerobic fitness level, achieved through regular exercise, is inversely associated with the proportion of senescent T cells, with the relationship withstanding adjustment for age (136). Regular exercise appears to alleviate the deleterious effect of aging on the immune system.

Programmed cell death, or apoptosis, is an important mechanism in the mediation of the immune response, serving as a key role in the removal of damaged, infected, exhausted or redundant cells. This orchestrated system then allows for alterations in the proportion of cells that make up the bloodstream repertoire of T cells. Acute bouts of exercise have been shown to induce increases in both senescent and naïve T cells, and elevate apoptotic lymphocytes (100). Since aging induces an accumulation of senescent T cells, it is imperative for effective immune function to induce apoptosis in specific cell types, preferentially the older less functional cells, to allow for naïve T cells to be exported into the circulation, favourably altering the bloodstream repertoire. Exercise has been associated with an increase in apoptotic cells, although the mechanisms are not yet well understood. In addition, despite the modality of exercise, there is no evidence to suggest that lymphocyte-apoptosis contributes to exercise-induced lymphocytopenia (127).

Endocrine System, Aging and Physical Activity/Inactivity

Incidence and prevalence of Diabetes Mellitus

Diabetes is a global health problem, costing the national health services millions annually. Diabetes is a serious chronic disease, classified into two types: Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM). The latest prevalence stats from Diabetes UK in 2016, report that almost 3.6 million people suffer from diabetes across the UK, with an additional 1 million likely to have undiagnosed T2DM, based on the Diabetes Prevalence Model 2016. Worldwide there are an expected 450 million people with Diabetes, with the incidence rate on the rise.

The initial pathophysiological events in the development of diabetes are insulin resistance, high blood glucose levels (hyperglycaemia), and impaired beta cell function (76). Beta cells are insulin-producing cells located in the islets of Langerhans in the pancreas. Degeneration of these cells is the main cause

of T1DM. T1DM is defined as insulin-dependent diabetes mellitus and requires medical monitoring and management in order to maintain euglycaemia (normal blood glucose concentration). The immune system attacks the beta cells, seizing the secretion of insulin and exposing the body to a hyperglycaemic state. This result is because insulin is the hormone responsible for the uptake of glucose from the systemic blood flow to the tissues. T2DM is defined as non-insulin-dependent diabetes mellitus and is the more common diagnosis. Although beta cell function may be affected, the autoimmune system does not attack the cells as in T1DM, the cells do not produce enough insulin to maintain euglycaemia. More commonly, T2DM is characterised by the body becoming resistant to the insulin that is secreted, known as insulin resistance or reduced insulin sensitivity. A lack of physical activity and exercise and a poor diet can lead to T2DM (105), suggesting it is a lifestyle-induced disease.

Role of Physical Activity and Exercise on Improving Insulin Sensitivity in Older Adults

Diabetes is very common in adults over the age of 65, with a decrease in insulin sensitivity observed with advancing age. Age-related changes, such as reduced physical activity, changes in diet, and undesirable changes in body composition, i.e. reduced muscle mass and increased fat mass, can affect glucose tolerance. A healthy lifestyle can reverse the detrimental effects on glucose metabolism. Most interventions focus on prevention rather than treatment, as diabetes is difficult to fully reverse. Although, it is known that regular exercise and a physically active lifestyle can help attenuate the usual decline in insulin sensitivity and glucose tolerance that is associated with aging. Older people, including those who are frail and/or weak, have been shown to benefit from endurance and resistance training, which can prevent age-related loss of muscle mass and strength, defined as sarcopenia and dynapenia. Muscle tissue has been identified as a major regulator of glucose homeostasis and tolerance; the more muscle tissue available to uptake glucose, the greater the control over systemic glucose levels. The initial step paramount for cellular glucose utilisation is the transport of glucose across the cell membrane into the matrix of the cell by the action of insulin, thus preventing hypo- and hyperglycaemia. The sensitivity of cells to the action of insulin may thus determine the rate at which glucose is cleared from the circulation. Since muscle tissue stores glucose, primarily as glycogen, the muscle mass has an available supply of glucose to maintain homeostasis in the case of hypoglycaemia. However, only during muscle activity can skeletal muscle glycogen breakdown provide a source of glucose (73), as it converted to lactate and then into blood glucose.

In addition, exercise training and maintaining a physically active lifestyle can be beneficial in promoting loss of excess abdominal or visceral adiposity that accumulates with an energy imbalance. In turn, this can result in alleviating insulin resistance (52). Obesity is associated with a low-grade

chronic inflammatory response, resulting from the secretion and activation of some pro-inflammatory cytokines/adipokines and respective pathways (137). Adipocytes exhibit properties shared by immune cells, mainly pro-inflammatory cytokine production, such as IL-6, tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP), which can influence insulin production. Therefore, if older adults perform regular exercise and maintains a healthy body composition, the daily control over blood glucose levels may prevent the onset of a chronic hyperglycaemic state.

In addition to altering body composition, exercise can also influence insulin sensitivity on a cellular basis (65). Exercise upregulates the demand on hepatic and skeletal muscle metabolism to provide fuel for the mechanical stress induced. During exercise, the production, regulation, and uptake of glucose is mediated by the glucose transporter type 4 (GLUT4) through insulin-controlled pathways (109), with excess glucose contributing to glycogen stores if not metabolically required. Insulin secretion is inhibited during exercise and thus the body relies on hepatic and skeletal muscle tissue cells being sufficiently sensitive to insulin to maintain glucose homeostasis. Regular exercise can improve the efficiency of this mechanism, and as a result can improve insulin sensitivity of cells. Continuing to exercise throughout the age span, particularly in older adult or elderly ages, can delay the onset or reduce the risk of insulin resistance (54) and thus diabetes.

Oxidative Stress- An Aging Problem, An Exercise Solution?

Free radicals, and reactive oxygen species (ROS) can be generated within the body by various metabolic pathways and enzymes, such as mitochondrial complexes in the electron transport chain (ETC), cytochrome P450, xanthine oxidase and nicotinamide dinucleotide phosphate (NADPH) oxidase (102). Oxidative stress occurs when free radical or ROS production exceeds the body's antioxidant capacity, leading to unchecked effects of these reactive molecules and compounds on tissues, such as DNA modifications, damage to lipids, proteins and other macromolecules. The accumulation of oxidative stress has been purported to lead to the aging associated tissue dysfunction. This 'free radical theory of aging' (60) hypothesizes that this elevated exposure to oxidative stress damages macromolecules, impairing antioxidant and repair mechanisms which leads to the deleterious effects on tissues (122). Indeed aging is associated with elevated levels of oxidative stress in various tissues in the body such as skeletal muscle (9), the heart, brain (103) and the vascular tree (92). Specifically, advanced age is linked with defective mitochondria which itself results from reduction in cytochrome C oxidase activity (103). This mitochondrial dysfunction leads to greater escape of generated electrons which can stimulate oxidative damage. Oxidative stress may play a role in processes such as inflammation (31), sarcopenia (72), insulin resistance (106). Whilst there is plethora of evidence to

show that lowering oxidative stress promotes tissue function (44, 142, 145), there is some evidence to challenge the free radical theory of aging, with studies showing that increasing antioxidant capacity in mice fails to extend lifespan (27), indicating that lowering oxidative stress may promote tissue function without affecting longevity.

Exercise and physical activity modulates some of the deleterious side-effects of aging, and is known to be protective against oxidative stress-associated conditions, including CVD, diabetes (37), and cancer (25). However, acute exercise, due to the elevated oxygen consumption ($\dot{V}O_2$), there is an enhanced leakage of superoxide ($O_2^{\cdot-}$) from the ETC (158), leading to an imbalance between ROS production and antioxidant capacity. This overproduction of $O_2^{\cdot-}$ though, acts as an important redox signal for regular exercise-induced adaptations (33, 96, 160). Several studies in human aging populations report reductions in plasma or urine markers of oxidative stress with endurance training or regular aerobic exercise (e.g. Thiobarbituric Acid Reactive Substances; TBARS, lipid peroxidation, $O_2^{\cdot-}$) (53, 74, 77) or an improvement in antioxidant capacity (upregulation of antioxidant enzymes, such as superoxide dismutase; SOD, and catalase) (42, 75, 143). Resistance exercise may also confer some benefits, with some studies reporting positive effects on oxidative stress biomarkers and antioxidant capacity (15, 16, 108, 155). However there is some contrasting evidence to show lack of efficacy of exercise training to modulate some oxidative stress biomarkers (107). These differences lie due to variety of biomarkers of oxidative stress and damage, as well as antioxidant capacity, and as yet, due to the rapid appearance and subsequent disappearance of ROS and free radicals, measurement is difficult, and often requires downstream markers (32).

Physical inactivity itself promotes the elevation of basal ROS and oxidative stress (8, 113). Animal models of physical inactivity show that skeletal muscle from immobilized limbs in mice produce higher levels of $O_2^{\cdot-}$ and hydrogen peroxide (H_2O_2) than mobilized limbs (19, 144, 164). In cross-sectional studies comparing active vs. inactive animals, lipid peroxidation and protein damage levels in skeletal muscle are elevated in sedentary vs. active rodent models (47, 119). In humans, one study showed that 2 weeks of unilateral limb immobilization in old men resulted in greater H_2O_2 production and mitochondrial leakage than the mobilized limb, however this returned to normal after a period of exercise training, suggesting that exercise may be able to counteract the pro-oxidant effect of inactivity. Further studies show that inactive older individuals display greater levels of oxidative stress biomarkers than trained age-matched controls (123). Together, these animal and human models of inactivity show that sedentary behaviours promotes localised ROS production, which may have significant effects on tissue function, compromising health of older individuals. Considering the positive effect of regular physical aerobic and/or resistance exercise, physical activity should be

promoted to counteract the negative effects of both aging and inactivity has on production of free radicals and downregulation of antioxidant enzymes in this susceptible population.

Future Directions

The exact 'dose' of exercise to promote healthy aging and longevity is still unknown, and unlikely to be described in the near future due to the varying effects that manipulating the time, intensity and frequency of exercise has on our cells and tissues. However, what is known is that exercise acts as a powerful, health-promoting, stimulus. Its ability to positively benefit a wide variety of cells, tissues and organs means it can be regarded as a potent anti-aging therapeutic intervention. The strong evidence available shows that physical activity and exercise can reduce NCD risk, improve cardiovascular, immune and muscle function, leading to improved quality of life in our ever increasing aging population.

References

1. **Aguirre LE, Jan IZ, Fowler K, Waters DL, Villareal DT, and Armamento-Villareal R.** Testosterone and Adipokines are Determinants of Physical Performance, Strength, and Aerobic Fitness in Frail, Obese, Older Adults. *International Journal of Endocrinology* 2014, 2014.

2. **Aly YE, Abdou AS, Rashad MM, and Nassef MM.** Effect of exercise on serum vitamin D and tissue vitamin D receptors in experimentally induced type 2 Diabetes Mellitus. *J Adv Res* 7: 671-679, 2016.

3. **Amati F, Dubé JJ, Coen PM, Stefanovic-Racic M, Toledo FGS, and Goodpaster BH.** Physical Inactivity and Obesity Underlie the Insulin Resistance of Aging. *Diabetes Care* 32: 1547-1549, 2009.

4. **Ari Z, Kutlu N, Uyanik BS, Taneli F, Buyukyazi G, and Tavli T.** Serum testosterone, growth hormone, and insulin-like growth factor-1 levels, mental reaction time, and maximal aerobic exercise in sedentary and long-term physically trained elderly males. *The International journal of neuroscience* 114: 623-637, 2004.

5. **Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M, and Isner JM.** Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circulation Research* 85: 221-228, 1999.

6. **Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, and Isner JM.** Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275: 964-966, 1997.

7. **Atkinson RA, Srinivas-Shankar U, Roberts SA, Connolly MJ, Adams JE, Oldham JA, Wu FC, Seynnes OR, Stewart CE, Maganaris CN, and Narici MV.** Effects of testosterone on skeletal muscle architecture in intermediate-frail and frail elderly men. *The journals of gerontology Series A, Biological sciences and medical sciences* 65: 1215-1219, 2010.

8. **Bar-Shai M, Carmeli E, Ljubuncic P, and Reznick AZ.** Exercise and immobilization in aging animals: The involvement of oxidative stress and NF-κB activation. *Free Radical Biology and Medicine* 44: 202-214, 2008.

9. **Bejma J and Ji LL.** Aging and acute exercise enhance free radical generation in rat skeletal muscle. *Journal of Applied Physiology* 87: 465-470, 1999.

10. **Birk GK, Dawson EA, Atkinson C, Haynes A, Cable NT, Thijssen DHJ, and Green DJ.** Brachial artery adaptation to lower limb exercise training: role of shear stress. *Journal of Applied Physiology* 112: 1653-1658, 2012.

11. **Bischoff-Ferrari HA, Borchers M, Gudat F, Durmuller U, Stahelin HB, and Dick W.** Vitamin D receptor expression in human muscle tissue decreases with age. *J Bone Miner Res* 19: 265-269, 2004.

12. **Bischoff HA, Borchers M, Gudat F, Duermueller U, Theiler R, Stahelin HB, and Dick W.** In situ detection of 1,25-dihydroxyvitamin D3 receptor in human skeletal muscle tissue. *Histochem J* 33: 19-24, 2001.

13. **Black MA, Cable NT, Thijssen DHJ, and Green DJ.** Impact of age, sex, and exercise on brachial artery flow-mediated dilatation. *Am J Physiol Heart Circ Physiol* 297: H1109-H1116, 2009.

14. **Black MA, Green DJ, and Cable NT.** Exercise prevents age-related decline in nitric-oxide-mediated vasodilator function in cutaneous microvessels. *J Physiol* 586: 3511-3524, 2008.

15. **BLOOMER RJ, SCHILLING BK, KARLAGE RE, LEDOUX MS, PFEIFFER RF, and CALLEGARI J.** Effect of Resistance Training on Blood Oxidative Stress in Parkinson Disease. *Medicine & Science in Sports & Exercise* 40: 1385-1389, 2008.

16. **Bobeuf F, Labonte M, Dionne IJ, and Khalil A.** Combined effect of antioxidant supplementation and resistance training on oxidative stress markers, muscle and body composition in an elderly population. *The journal of nutrition, health & aging* 15: 883-889, 2011.

17. **Breen L, Stokes KA, Churchward-Venne TA, Moore DR, Baker SK, Smith K, Atherton PJ, and Phillips SM.** Two weeks of reduced activity decreases leg lean mass and induces "anabolic resistance" of myofibrillar protein synthesis in healthy elderly. *The Journal of clinical endocrinology and metabolism* 98: 2604-2612, 2013.

18. **Bruyndonckx L, Hoymans VY, Frederix G, De Guchtenaere A, Franckx H, Vissers DK, Vrints CJ, Ramet J, and Conraads VM.** Endothelial progenitor cells and endothelial microparticles are independent predictors of endothelial function. *The Journal of Pediatrics* 165: 300-305, 2014.

19. **Cannavino J, Brocca L, Sandri M, Bottinelli R, and Pellegrino MA.** PGC1-α over-expression prevents metabolic alterations and soleus muscle atrophy in hindlimb unloaded mice. *The Journal of Physiology* 592: 4575-4589, 2014.

20. **Carlsson AC, Arnlov J, Sundstrom J, Michaelsson K, Byberg L, and Lind L.** Physical activity, obesity and risk of cardiovascular disease in middle-aged men during a median of 30 years of follow-up. *Eur J Prev Cardiol* 23: 359-365, 2016.

21. **Case J, Mead LE, Bessler WK, Prater D, White HA, Saadatzadeh MR, Bhavsar JR, Yoder MC, Haneline LS, and Ingram DA.** Human CD34+AC133+VEGFR-2+ cells are not endothelial progenitor cells but distinct, primitive hematopoietic progenitors. *Exp Hematol* 35: 1109-1118, 2007.

22. **Castaldi A, Dodia RM, Orogo AM, Zambrano CM, Najor RH, Gustafsson ÅB, Heller Brown J, and Purcell NH.** Decline in cellular function of aged mouse c-kit+ cardiac progenitor cells. *The Journal of Physiology*: n/a-n/a.

23. **Ceglia L and Harris SS.** Vitamin D and its role in skeletal muscle. *Calcif Tissue Int* 92: 151-162, 2013.

24. **Ceglia L, Niramitmahapanya S, da Silva Morais M, Rivas DA, Harris SS, Bischoff-Ferrari H, Fielding RA, and Dawson-Hughes B.** A randomized study on the effect of vitamin D(3) supplementation on skeletal muscle morphology and vitamin D receptor concentration in older women. *J Clin Endocrinol Metab* 98: E1927-1935, 2013.

25. **Celis-Morales CA, Lyall DM, Welsh P, Anderson J, Steell L, Guo Y, Maldonado R, Mackay DF, Pell JP, Sattar N, and Gill JMR.** Association between active commuting and incident cardiovascular disease, cancer, and mortality: prospective cohort study. *BMJ* 357: j1456, 2017.

26. **Chapuy MC, Durr F, and Chapuy P.** Age-related changes in parathyroid hormone and 25 hydroxycholecalciferol levels. *J Gerontol* 38: 19-22, 1983.

27. **Chen X, Liang H, Van Remmen H, Vijg J, and Richardson A.** Catalase transgenic mice: characterization and sensitivity to oxidative stress. *Archives of Biochemistry and Biophysics* 422: 197-210, 2004.

28. **Chistiakov DA, Orekhov AN, and Bobryshev YV.** Effects of shear stress on endothelial cells: go with the flow. *Acta Physiol (Oxf)* 219: 382-408, 2017.

29. **Choi J, Moon K, Jung S, Kim J, Choi S, Kim DY, Chu C, and Kwon S.** Regular exercise training increases the number of endothelial progenitor cells and decreases homocysteine levels in healthy peripheral blood. *Korean Journal of Physiology and Pharmacology* 18: 163-168, 2014.

30. **Chrissobolis S and Faraci FM.** The role of oxidative stress and NADPH oxidase in cerebrovascular disease. *Trends in Molecular Medicine* 14: 495-502, 2008.

31. **Chung HY, Cesari M, Anton S, Marzetti E, Giovannini S, Seo AY, Carter C, Yu BP, and Leeuwenburgh C.** Molecular inflammation: Underpinnings of aging and age-related diseases. *Ageing Research Reviews* 8: 18-30, 2009.

32. **Cobley JN, Close GL, Bailey DM, and Davison GW.** Exercise redox biochemistry: Conceptual, methodological and technical recommendations. *Redox Biol* 12: 540-548, 2017.

33. **Cobley JN, McHardy H, Morton JP, Nikolaidis MG, and Close GL.** Influence of vitamin C and vitamin E on redox signaling: Implications for exercise adaptations. *Free Radic Biol Med* 84: 65-76, 2015.

34. **Coleman LA, Mishina M, Thompson M, Spencer SM, Reber AJ, Davis WG, Cheng PY, Belongia EA, Talbot HK, Sundaram ME, Griffin MR, Shay DK, and Sambhara S.** Age, serum 25-hydroxyvitamin D and vitamin D receptor (VDR) expression and function in peripheral blood mononuclear cells. *Oncotarget*, 2016.

35. **Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, Martin FC, Michel JP, Rolland Y, Schneider SM, Topinkova E, Vandewoude M, and Zamboni M.** Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age and ageing* 39: 412-423, 2010.

36. **Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, Wackerhage H, Taylor PM, and Rennie MJ.** Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 19: 422-424, 2005.

37. **de Souto Barreto P, Cesari M, Andrieu S, Vellas B, and Rolland Y.** Physical Activity and Incident Chronic Diseases: A Longitudinal Observational Study in 16 European Countries. *Am J Prev Med* 52: 373-378, 2017.

38. **Deane CS, Hughes DC, Sculthorpe N, Lewis MP, Stewart CE, and Sharples AP.** Impaired hypertrophy in myoblasts is improved with testosterone administration. *The Journal of steroid biochemistry and molecular biology* 138: 152-161, 2013.

39. **Delmonico MJ, Harris TB, Lee JS, Visser M, Nevitt M, Kritchevsky SB, Tylavsky FA, and Newman AB.** Alternative definitions of sarcopenia, lower extremity performance, and functional impairment with aging in older men and women. *Journal of the American Geriatrics Society* 55: 769-774, 2007.

40. **Devries MC, Breen L, Von Allmen M, MacDonald MJ, Moore DR, Offord EA, Horcajada MN, Breuille D, and Phillips SM.** Low-load resistance training during step-reduction attenuates declines in muscle mass and strength and enhances anabolic sensitivity in older men. *Physiological reports* 3, 2015.

41. **Doering TM, Jenkins DG, Reaburn PR, Borges NR, Hohmann E, and Phillips SM.** Lower Integrated Muscle Protein Synthesis in Masters Compared with Younger Athletes. *Medicine and science in sports and exercise* 48: 1613-1618, 2016.

42. **Done AJ and Traustadottir T.** Aerobic exercise increases resistance to oxidative stress in sedentary older middle-aged adults. A pilot study. *Age (Dordr)* 38: 505-512, 2016.

43. **Ellison Georgina M, Vicinanza C, Smith Andrew J, Aquila I, Leone A, Waring Cheryl D, Henning Beverley J, Stirparo Giuliano G, Papait R, Scarfò M, Agosti V, Viglietto G, Condorelli G, Indolfi C, Ottolenghi S, Torella D, and Nadal-Ginard B.** Adult c-kitpos Cardiac Stem Cells Are Necessary and Sufficient for Functional Cardiac Regeneration and Repair. *Cell* 154: 827-842, 2013.

44. **Eskurza I, Monahan KD, Robinson JA, and Seals DR.** Effect of acute and chronic ascorbic acid on flow-mediated dilatation with sedentary and physically active human ageing. *The Journal of Physiology* 556: 315-324, 2004.

45. **Evans WJ.** Skeletal muscle loss: cachexia, sarcopenia, and inactivity. *The American Journal of Clinical Nutrition* 91: 1123S-1127S, 2010.

46. **Fiatarone MA, Marks EC, Ryan ND, Meredith CN, Lipsitz LA, and Evans WJ.** High-intensity strength training in nonagenarians. Effects on skeletal muscle. *Jama* 263: 3029-3034, 1990.

47. **Figueiredo PA, Powers SK, Ferreira RM, Amado F, Appell HJ, and Duarte JA.** Impact of Lifelong Sedentary Behavior on Mitochondrial Function of Mice Skeletal Muscle. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 64A: 927-939, 2009.

48. **Francaux M, Demeulder B, Naslain D, Fortin R, Lutz O, Caty G, and Deldicque L.** Aging Reduces the Activation of the mTORC1 Pathway after Resistance Exercise and Protein Intake in Human Skeletal Muscle: Potential Role of REDD1 and Impaired Anabolic Sensitivity. *Nutrients* 8, 2016.

49. **Frederiksen L, Hojlund K, Hougaard DM, Brixen K, and Andersen M.** Testosterone therapy increased muscle mass and lipid oxidation in aging men. *Age (Dordrecht, Netherlands)* 34: 145-156, 2012.

50. **Furchgott R and Zawadzki J.** The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288: 373-376, 1980.

51. **Garcia LA, King KK, Ferrini MG, Norris KC, and Artaza JN.** 1,25(OH)2vitamin D3 stimulates myogenic differentiation by inhibiting cell proliferation and modulating the expression of promyogenic growth factors and myostatin in C2C12 skeletal muscle cells. *Endocrinology* 152: 2976-2986, 2011.

52. **Gastaldelli A, Miyazaki Y, Pettiti M, Matsuda M, Mahankali S, Santini E, DeFronzo RA, and Ferrannini E.** Metabolic effects of visceral fat accumulation in type 2 diabetes. *J Clin Endocrinol Metab* 87: 5098-5103, 2002.

53. **Ghosh S, Lertwattanarak R, Lefort N, Molina-Carrion M, Joya-Galeana J, Bowen BP, Garduno-Garcia JdJ, Abdul-Ghani M, Richardson A, DeFronzo RA, Mandarino L, Van Remmen H, and Musi N.** Reduction in Reactive Oxygen Species Production by Mitochondria From Elderly Subjects With Normal and Impaired Glucose Tolerance. *Diabetes* 60: 2051-2060, 2011.

54. **Goodyear LJ and Kahn BB.** Exercise, glucose transport, and insulin sensitivity. *Annu Rev Med* 49: 235-261, 1998.

55. **Green D.** Point: Flow-mediated dilation does reflect nitric oxide-mediated endothelial function. 99: 1233-1234, 2005.

56. **Green DJ, Jones H, Thijssen D, Cable NT, and Atkinson G.** Flow-mediated dilation and cardiovascular event prediction. *Hypertension* 57: 363-369, 2011.

57. **Hall KS, Cohen HJ, Pieper CF, Fillenbaum GG, Kraus WE, Huffman KM, Cornish MA, Shiloh A, Flynn C, Sloane R, Newby LK, and Morey MC.** Physical Performance Across the Adult Life Span: Correlates With Age and Physical Activity. *The Journals of Gerontology: Series A* 72: 572-578, 2017.

58. **Hamada K, Vannier E, Sacheck JM, Witsell AL, and Roubenoff R.** Senescence of human skeletal muscle impairs the local inflammatory cytokine response to acute eccentric exercise. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 19: 264-266, 2005.

59. **Hamilton CA, Brosnan MJ, McIntyre M, Graham D, and Dominiczak AF.** Superoxide excess in hypertension and aging: a common cause of endothelial dysfunction. *Hypertension* 37: 529-534, 2001.

60. **Harman D.** Aging: A Theory Based on Free Radical and Radiation Chemistry. *Journal of Gerontology* 11: 298-300, 1956.

61. **Hawkins SA, Wiswell RA, and Marcell TJ.** Exercise and the master athlete--a model of successful aging? *The journals of gerontology Series A, Biological sciences and medical sciences* 58: 1009-1011, 2003.

62. **Hayes LD, Grace FM, Sculthorpe N, Herbert P, Kilduff LP, and Baker JS.** Does chronic exercise attenuate age-related physiological decline in males? *Research in sports medicine (Print)* 21: 343-354, 2013.

63. **Hayes LD, Herbert P, Sculthorpe NF, and Grace FM.** Exercise training improves free testosterone in lifelong sedentary aging men. *Endocrine connections* 6: 306-310, 2017.

64. **Hayes LD, Sculthorpe N, Herbert P, Baker JS, Hullin DA, Kilduff LP, and Grace FM.** Resting steroid hormone concentrations in lifetime exercisers and lifetime sedentary males. *The aging male : the official journal of the International Society for the Study of the Aging Male* 18: 22-26, 2015.

65. **Henriksen EJ.** Invited review: Effects of acute exercise and exercise training on insulin resistance. *J Appl Physiol (1985)* 93: 788-796, 2002.

66. **Herbert P, Hayes LD, Sculthorpe N, and Grace FM.** High-intensity interval training (HIIT) increases insulin-like growth factor-I (IGF-I) in sedentary aging men but not masters' athletes: an observational study. *The aging male : the official journal of the International Society for the Study of the Aging Male* 20: 54-59, 2017.

67. **Herbert P, Hayes LD, Sculthorpe NF, and Grace FM.** HIIT produces increases in muscle power and free testosterone in male masters athletes. *Endocrine connections* 6: 430-436, 2017.

68. **Hoetzer GL, Van Guilder GP, Irmiger HM, Keith RS, Stauffer BL, and DeSouza CA.** Aging, exercise, and endothelial progenitor cell clonogenic and migratory capacity in men. *J Appl Physiol* 102: 847-852, 2007.

69. **Holick MF.** Vitamin D deficiency. *N Engl J Med* 357: 266-281, 2007.

70. **Holick MF and Chen TC.** Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr* 87: 1080S-1086S, 2008.

71. **Hur J, Yoon C-H, Kim H-S, Choi J-H, Kang H-J, Hwang K-K, Oh B-H, Lee M-M, and Park Y-B.** Characterization of two types of endothelial progenitor cells and their different contributions to neovasculogenesis. *Arterioscler Thromb Vasc Biol* 24: 288-293, 2004.

72. **Jackson MJ.** Reactive oxygen species in sarcopenia: Should we focus on excess oxidative damage or defective redox signalling? *Molecular Aspects of Medicine*.

73. **Jensen J, Rustad PI, Kolnes AJ, and Lai YC.** The role of skeletal muscle glycogen breakdown for regulation of insulin sensitivity by exercise. *Front Physiol* 2, 2011.

74. **Jessup JV, Horne C, Yarandi H, and Quindry J.** The Effects of Endurance Exercise and Vitamin E on Oxidative Stress in the Elderly. *Biological Research For Nursing* 5: 47-55, 2003.

75. **Johnson ML, Irving BA, Lanza IR, Vendelbo MH, Konopka AR, Robinson MM, Henderson GC, Klaus KA, Morse DM, Heppelmann C, Bergen IIIHR, Dasari S, Schimke JM, Jakaitis DR, and Nair KS.** Differential Effect of Endurance Training on Mitochondrial Protein Damage, Degradation, and Acetylation in the Context of Aging. *The Journals of Gerontology: Series A* 70: 1386-1393, 2015.

76. **Kahn SE.** The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. *Diabetologia* 46: 3-19, 2003.

77. **Karolkiewicz J, Michalak E, Pospieszna B, Deskur-Śmielecka E, Nowak A, and Pilaczyńska-Szcześniak Ł.** Response of oxidative stress markers and antioxidant parameters to an 8-week aerobic physical activity program in healthy, postmenopausal women. *Archives of Gerontology and Geriatrics* 49: e67-e71, 2009.

78. **Khoo J, Tian HH, Tan B, Chew K, Ng CS, Leong D, Teo RC, and Chen RY.** Comparing effects of low- and high-volume moderate-intensity exercise on sexual function and testosterone in obese men. *The journal of sexual medicine* 10: 1823-1832, 2013.

79. **Kommalage M and Gunawardena S.** Influence of age, gender, and sidedness on ulnar nerve conduction. *Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society* 30: 98-101, 2013.

80. **Koopman R, Walrand S, Beelen M, Gijsen AP, Kies AK, Boirie Y, Saris WH, and van Loon LJ.** Dietary protein digestion and absorption rates and the subsequent postprandial muscle protein

synthetic response do not differ between young and elderly men. *The Journal of nutrition* 139: 1707-1713, 2009.

81. **Kumar V, Selby A, Rankin D, Patel R, Atherton P, Hildebrandt W, Williams J, Smith K, Seynnes O, Hiscock N, and Rennie MJ.** Age-related differences in the dose-response relationship of muscle protein synthesis to resistance exercise in young and old men. *The Journal of physiology* 587: 211-217, 2009.

82. **Kushner E, Van Guilder G, MacEneaney O, Greiner J, Cech J, Stauffer B, and DeSouza C.** Ageing and endothelial progenitor cell release of proangiogenic cytokines. *Age and Ageing* 39: 268-272, 2010.

83. **Kushner EJ, MacEneaney OJ, Weil BR, Greiner JJ, Stauffer BL, and DeSouza CA.** Aging is associated with a proapoptotic endothelial progenitor cell phenotype. *J Vasc Res* 48: 408-414, 2011.

84. **Laufs U, Urhausen A, Werner N, Scharhag J, Heitz A, Kissner G, Böhm M, Kindermann W, and Nickenig G.** Running exercise of different duration and intensity: effect on endothelial progenitor cells in healthy subjects. *European Journal of Cardiovascular Prevention & Rehabilitation* 12: 407-414, 2005.

85. **Laufs U, Werner N, Link A, Endres M, Wassmann S, Jürgens K, Miche E, Böhm M, and Nickenig G.** Physical training increases endothelial progenitor cells, inhibits neointima formation, and enhances angiogenesis. *Circ* 109: 220-226, 2004.

86. **Lazuardi L, Jenewein B, Wolf AM, Pfister G, Tzankov A, and Grubeck-Loebenstein B.** Age-related loss of naive T cells and dysregulation of T-cell/B-cell interactions in human lymph nodes. *Immunology* 114: 37-43, 2005.

87. **Lee I-M, Shiroma EJ, Lobelo F, Puska P, Blair SN, and Katzmarzyk PT.** Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *The Lancet* 380: 219-229, 2012.

88. **Leite CF, Lopes CS, Alves AC, Fuzaro CSC, Silva MV, Oliveira LFd, Garcia LP, Farnesi TS, Cuba MBd, Rocha LB, Rodrigues V, Oliveira CJFd, and Dias da Silva VJ.** Endogenous resident c-Kit cardiac stem cells increase in mice with an exercise-induced, physiologically hypertrophied heart. *Stem Cell Research* 15: 151-164, 2015.

89. **Leveille SG, Gray S, LaCroix AZ, Ferrucci L, Black DJ, and Guralnik JM.** Physical Inactivity and Smoking Increase Risk for Serious Infections in Older Women. *Journal of the American Geriatrics Society* 48: 1582-1588, 2000.

90. **Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, AlMazroa MA, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Abdulhak AB, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FGR, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo J-P, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, et al.** Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 380: 2095-2128, 2012.

91. **Luk T-H, Dai Y-L, Siu C-W, Yiu K-H, Chan H-T, Lee SWL, Li S-W, Fong B, Wong W-K, Tam S, Lau C-P, and Tse H-F.** Effect of exercise training on vascular endothelial function in patients with stable coronary artery disease: a randomized controlled trial. *Eur J Prev Cardiol* 19: 830-839, 2012.

92. **Luttrell M, Seawright J, Wilson E, and Woodman C.** Effect of age and exercise training on protein:protein interactions among eNOS and its regulatory proteins in rat aortas. *European Journal of Applied Physiology* 113: 2761-2768, 2013.

93. **Makanae Y, Ogasawara R, Sato K, Takamura Y, Matsutani K, Kido K, Shiozawa N, Nakazato K, and Fujita S.** Acute bout of resistance exercise increases vitamin D receptor protein expression in rat skeletal muscle. *Exp Physiol* 100: 1168-1176, 2015.

94. **Manfredini F, Rigolin GM, Malagoni AM, Catizone L, Mandini S, Sofritti O, Mauro E, Soffritti S, Boari B, Cuneo A, Zamboni P, and Manfredini R.** Exercise training and endothelial progenitor cells in haemodialysis patients. *Journal of International Medical Research* 37: 534-540, 2009.

95. **Manini TM and Clark BC.** Dynapenia and aging: an update. *The journals of gerontology Series A, Biological sciences and medical sciences* 67: 28-40, 2012.

96. **Margaritelis NV, Theodorou AA, Paschalis V, Veskoukis AS, Dipla K, Zafeiridis A, Panayiotou G, Vrabas IS, Kyparos A, and Nikolaidis MG.** Adaptations to endurance training depend on exercise-induced oxidative stress: exploiting redox inter-individual variability. *Acta Physiologica*: n/a-n/a.

97. **Mayhan WG, Arrick DM, Sharpe GM, and Sun H.** Age-related alterations in reactivity of cerebral arterioles: role of oxidative stress. *Microcirculation* 15: 225-236, 2008.

98. **Mikkelsen UR, Couppe C, Karlsen A, Grosset JF, Schjerling P, Mackey AL, Klausen HH, Magnusson SP, and Kjaer M.** Life-long endurance exercise in humans: circulating levels of inflammatory markers and leg muscle size. *Mechanisms of ageing and development* 134: 531-540, 2013.

99. **Moore DR, Churchward-Venne TA, Witard O, Breen L, Burd NA, Tipton KD, and Phillips SM.** Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men. *The journals of gerontology Series A, Biological sciences and medical sciences* 70: 57-62, 2015.

100. **Mooren FC and Kruger K.** Apoptotic lymphocytes induce progenitor cell mobilization after exercise. *J Appl Physiol (1985)* 119: 135-139, 2015.

101. **Muller-Delp JM.** Aging-induced adaptations of microvascular reactivity. *Microcirculation* 13: 301-314, 2006.

102. **Murphy Michael P.** How mitochondria produce reactive oxygen species. *Biochemical Journal* 417: 1-13, 2009.

103. **Navarro A, Gomez C, López-Cepero JM, and Boveris A.** Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 286: R505-R511, 2004.

104. **Otis JS, Niccoli S, Hawdon N, Sarvas JL, Frye MA, Chicco AJ, and Lees SJ.** Pro-inflammatory mediation of myoblast proliferation. *PloS one* 9: e92363, 2014.

105. **Pan XR, Li GW, Hu YH, Wang JX, Yang WY, An ZX, Hu ZX, Lin J, Xiao JZ, Cao HB, Liu PA, Jiang XG, Jiang YY, Wang JP, Zheng H, Zhang H, Bennett PH, and Howard BV.** Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. *Diabetes Care* 20: 537-544, 1997.

106. **Paneni F, Costantino S, and Cosentino F.** Role of oxidative stress in endothelial insulin resistance. *World J Diabetes* 6: 326-332, 2015.

107. **Parise G, Brose AN, and Tarnopolsky MA.** Resistance exercise training decreases oxidative damage to DNA and increases cytochrome oxidase activity in older adults. *Experimental Gerontology* 40: 173-180, 2005.

108. **Parise G, Phillips SM, Kaczor JJ, and Tarnopolsky MA.** Antioxidant enzyme activity is up-regulated after unilateral resistance exercise training in older adults. *Free Radical Biology and Medicine* 39: 289-295, 2005.

109. **Park DR, Park KH, Kim BJ, Yoon CS, and Kim UH.** Exercise Ameliorates Insulin Resistance via Ca2+ Signals Distinct from Those of Insulin for GLUT4 Translocation in Skeletal Muscles. *Diabetes* 19, 2014.

110. **Patel RS, Li Q, Ghasemzadeh N, Eapen DJ, Moss LD, Janjua AU, Manocha P, Kassem HA, Veledar E, Samady H, Taylor WR, Zafari AM, Sperling L, Vaccarino V, Waller EK, and Quyyumi AA.** Circulating CD34+ progenitor cells and risk of mortality in a population with coronary artery disease. *Circ Res* 116: 289-297, 2015.

111. **Phillips SM.** Nutritional supplements in support of resistance exercise to counter age-related sarcopenia. *Advances in nutrition (Bethesda, Md)* 6: 452-460, 2015.

112. **Phillips SM, Chevalier S, and Leidy HJ.** Protein "requirements" beyond the RDA: implications for optimizing health. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme* 41: 565-572, 2016.

113. **Pierre N, Appriou Z, Gratas-Delamarche A, and Derbre F.** From physical inactivity to immobilization: Dissecting the role of oxidative stress in skeletal muscle insulin resistance and atrophy. *Free Radic Biol Med* 98: 197-207, 2016.

114. **Provinciali M, Moresi R, Donnini A, and Lisa RM.** Reference values for CD4+ and CD8+ T lymphocytes with naive or memory phenotype and their association with mortality in the elderly. *Gerontology* 55: 314-321, 2009.

115. **Rakobowchuk M, Tanguay S, Burgomaster KA, Howarth KR, Gibala MJ, and MacDonald MJ.** Sprint interval and traditional endurance training induce similar improvements in peripheral arterial stiffness and flow-mediated dilation in healthy humans. *Am J Physiol Regul Integr Comp Physiol* 295: R236-R242, 2008.

116. **Renko O, Tolonen A-M, Rysä J, Magga J, Mustonen E, Ruskoaho H, and Serpi R.** SDF1 gradient associates with the distribution of c-Kit+ cardiac cells in the heart. *Scientific Reports* 8: 1160, 2018.

117. **Rennie MJ.** Anabolic resistance: the effects of aging, sexual dimorphism, and immobilization on human muscle protein turnover. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme* 34: 377-381, 2009.

118. **Robson PJ, Blannin AK, Walsh NP, Castell LM, and Gleeson M.** Effects of exercise intensity, duration and recovery on in vitro neutrophil function in male athletes. *Int J Sports Med* 20: 128-135, 1999.

119. **Rosa EF, Silva AC, Ihara SSM, Mora OA, Aboulafia J, and Nouailhetas VLA.** Habitual exercise program protects murine intestinal, skeletal, and cardiac muscles against aging. *Journal of Applied Physiology* 99: 1569-1575, 2005.

120. **Ross MD, Malone EM, Simpson R, Cranston I, Ingram L, Wright GP, Chambers G, and Florida-James GD.** Lower Resting and Exercise-Induced Circulating Angiogenic Progenitors and Angiogenic T-Cells in Older Men. *American Journal of Physiology - Heart and Circulatory Physiology*, 2017.

121. **Ross MD, Wekesa AL, Phelan JP, and Harrison M.** Resistance exercise increases endothelial progenitor cells and angiogenic factors. *Med Sci Sports Exerc* 46: 16-23, 2014.

122. **Sallam N and Laher I.** Exercise Modulates Oxidative Stress and Inflammation in Aging and Cardiovascular Diseases. *Oxid Med Cell Longev* 2016: 7239639, 2016.

123. **Santos-Parker JR, Strahler TR, Vorwald VM, Pierce GL, and Seals DR.** Habitual aerobic exercise does not protect against micro- or macrovascular endothelial dysfunction in healthy estrogen-deficient postmenopausal women. *J Appl Physiol (1985)* 122: 11-19, 2017.

124. **Sarto P, Balducci E, Balconi G, Fiordaliso F, Merlo L, Tuzzato G, Pappagallo GL, Frigato N, Zanocco A, Forestieri C, Azzarello G, Mazzucco A, Valenti MT, Alborino F, Noventa D, Vinante O, Pascotto P, Sartore S, Dejana E, and Latini R.** Effects of exercise training on endothelial progenitor cells in patients with chronic heart failure. *Journal of Cardiac Failure* 13: 701-708, 2007.

125. **Schlager O, Giurgea A, Schuhfried O, Seidinger D, Hammer A, Gröger M, Fialka-Moser V, Gschwandtner M, Koppensteiner R, and Steiner S.** Exercise training increases endothelial progenitor cells and decreases asymmetric dimethylarginine in peripheral arterial disease: A randomized controlled trial. *Atherosclerosis* 217: 240-248, 2011.

126. **Sculthorpe N, Herbert P, and Grace FM.** Low-Frequency High-Intensity Interval Training is an Effective Method to Improve Muscle Power in Lifelong Sedentary Aging Men: A Randomized Controlled Trial. *Journal of the American Geriatrics Society* 63: 2412-2413, 2015.

127. **Simpson RJ, Florida-James GD, Whyte GP, Black JR, Ross JA, and Guy K.** Apoptosis does not contribute to the blood lymphocytopenia observed after intensive and downhill treadmill running in humans. *Res Sports Med* 15: 157-174, 2007.

128. **Simpson RJ, Florida-James GD, Whyte GP, and Guy K.** The effects of intensive, moderate and downhill treadmill running on human blood lymphocytes expressing the adhesion/activation molecules CD54 (ICAM-1), CD18 (beta2 integrin) and CD53. *Eur J Appl Physiol* 97: 109-121, 2006.

129. **Simpson RJ, Lowder TW, Spielmann G, Bigley AB, LaVoy EC, and Kunz H.** Exercise and the aging immune system. *Ageing Res Rev* 11: 404-420, 2012.

130. **Simpson RU, Thomas GA, and Arnold AJ.** Identification of 1,25-dihydroxyvitamin D3 receptors and activities in muscle. *J Biol Chem* 260: 8882-8891, 1985.

131. **Sipila S, Narici M, Kjaer M, Pollanen E, Atkinson RA, Hansen M, and Kovanen V.** Sex hormones and skeletal muscle weakness. *Biogerontology* 14: 231-245, 2013.

132. **Smith GI, Yoshino J, Reeds DN, Bradley D, Burrows RE, Heisey HD, Moseley AC, and Mittendorfer B.** Testosterone and progesterone, but not estradiol, stimulate muscle protein

synthesis in postmenopausal women. *The Journal of clinical endocrinology and metabolism* 99: 256-265, 2014.

133. **Sonnenschein K, Horváth T, Mueller M, Markowski A, Siegmund T, Jacob C, Drexler H, and Landmesser U.** Exercise training improves in vivo endothelial repair capacity of early endothelial progenitor cells in subjects with metabolic syndrome. *European Journal of Cardiovascular Prevention & Rehabilitation* 18: 406-414, 2011.

134. **Soucy KG, Ryoo S, Benjo A, Lim HK, Gupta G, Sohi JS, Elser J, Aon MA, Nyhan D, Shoukas AA, and Berkowitz DE.** Impaired shear stress-induced nitric oxide production through decreased NOS phosphorylation contributes to age-related vascular stiffness. *J Appl Physiol* 101: 1751-1759, 2006.

135. **Spielmann G, Bollard CM, Bigley AB, Hanley PJ, Blaney JW, LaVoy EC, Pircher H, and Simpson RJ.** The effects of age and latent cytomegalovirus infection on the redeployment of CD8+ T cell subsets in response to acute exercise in humans. *Brain Behav Immun* 39: 142-151, 2014.

136. **Spielmann G, McFarlin BK, O'Connor DP, Smith PJ, Pircher H, and Simpson RJ.** Aerobic fitness is associated with lower proportions of senescent blood T-cells in man. *Brain Behav Immun* 25: 1521-1529, 2011.

137. **Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, Ristow M, Boeing H, and Pfeiffer AF.** Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Diabetes* 52: 812-817, 2003.

138. **Steiner S, Niessner A, Ziegler S, Richter B, Seidinger D, Pleiner J, Penka M, Wolzt M, Huber K, Wojta J, Minar E, and Kopp CW.** Endurance training increases the number of endothelial progenitor cells in patients with cardiovascular risk and coronary artery disease. *Atherosclerosis* 181: 305-310, 2005.

139. **Stenholm S, Koster A, Valkeinen H, Patel KV, Bandinelli S, Guralnik JM, and Ferrucci L.** Association of Physical Activity History With Physical Function and Mortality in Old Age. *J Gerontol A Biol Sci Med Sci* 71: 496-501, 2016.

140. **Strle K, Broussard SR, McCusker RH, Shen WH, Johnson RW, Freund GG, Dantzer R, and Kelley KW.** Proinflammatory cytokine impairment of insulin-like growth factor I-induced protein synthesis in skeletal muscle myoblasts requires ceramide. *Endocrinology* 145: 4592-4602, 2004.

141. **Symons TB, Sheffield-Moore M, Mamerow MM, Wolfe RR, and Paddon-Jones D.** The anabolic response to resistance exercise and a protein-rich meal is not diminished by age. *The journal of nutrition, health & aging* 15: 376-381, 2011.

142. **Taddei S, Virdis A, Ghiadoni L, Salvetti G, Bernini G, Magagna A, and Salvetti A.** Age-related reduction of NO availability and oxidative stress in humans. *Hypertension* 38: 274-279, 2001.

143. **Takahashi M, Miyashita M, Kawanishi N, Park J-H, Hayashida H, Kim H-S, Nakamura Y, Sakamoto S, and Suzuki K.** Low-volume exercise training attenuates oxidative stress and neutrophils activation in older adults. *European Journal of Applied Physiology* 113: 1117-1126, 2013.

144. **Talbert EE, Smuder AJ, Min K, Kwon OS, Szeto HH, and Powers SK.** Immobilization-induced activation of key proteolytic systems in skeletal muscles is prevented by a mitochondria-targeted antioxidant. *Journal of Applied Physiology* 115: 529-538, 2013.

145. **Tatchum-Talom R and Martin DS.** Tempol improves vascular function in the mesenteric vascular bed of senescent rats. *Canadian Journal of Physiology and Pharmacology* 82: 200-207, 2004.

146. **Thijssen DHJ, Vos JB, Verseyden C, Van Zonneveld AJ, Smits P, Sweep FCGJ, Hopman MTE, and De Boer HC.** Haematopoietic stem cells and endothelial progenitor cells in healthy men: effect of aging and training. *Aging Cell* 5: 495-503, 2006.

147. **Tidball JG.** Regulation of muscle growth and regeneration by the immune system. *Nature reviews Immunology* 17: 165-178, 2017.

148. **Torella D, Rota M, Nurzynska D, Musso E, Monsen A, Shiraishi I, Zias E, Walsh K, Rosenzweig A, Sussman MA, Urbanek K, Nadal-Ginard B, Kajstura J, Anversa P, and Leri A.** Cardiac Stem Cell and Myocyte Aging, Heart Failure, and Insulin-Like Growth Factor-1 Overexpression. *Circulation Research* 94: 514-524, 2004.

149. **Torjesen I.** Global cost of physical inactivity is estimated at $67.5bn a year. *BMJ* 354: i4187, 2016.

150. **Urbanek K, Quaini F, Tasca G, Torella D, Castaldo C, Nadal-Ginard B, Leri A, Kajstura J, Quaini E, and Anversa P.** Intense myocyte formation from cardiac stem cells in human cardiac hypertrophy. *Proceedings of the National Academy of Sciences* 100: 10440-10445, 2003.

151. **Van Craenenbroeck E, Hoymans V, Beckers P, Possemiers N, Wuyts K, Paelinck B, Vrints C, and Conraads V.** Exercise training improves function of circulating angiogenic cells in patients with chronic heart failure. *Basic Research in Cardiology* 105: 665-676, 2010.

152. **Van Craenenbroeck EM, Beckers PJ, Possemiers NM, Wuyts K, Frederix G, Hoymans VY, Wuyts F, Paelinck BP, Vrints CJ, and Conraads VM.** Exercise acutely reverses dysfunction of circulating angiogenic cells in chronic heart failure. *Eur Heart J* 31: 1924-1934, 2010.

153. **Van Craenenbroeck EMF, Vrints CJ, Haine SE, Vermeulen K, Goovaerts I, Van Tendeloo VFI, Hoymans VY, and Conraads VMA.** A maximal exercise bout increases the number of circulating CD34+/KDR+ endothelial progenitor cells in healthy subjects. Relation with lipid profile. *J Appl Physiol* 104: 1006-1013, 2008.

154. **Veldurthy V, Wei R, Oz L, Dhawan P, Jeon YH, and Christakos S.** Vitamin D, calcium homeostasis and aging. *Bone Res* 4: 16041, 2016.

155. **Vincent HK, Bourguignon C, and Vincent KR.** Resistance Training Lowers Exercise-Induced Oxidative Stress and Homocysteine Levels in Overweight and Obese Older Adults. *Obesity* 14: 1921-1930, 2006.

156. **Volpi E, Mittendorfer B, Rasmussen BB, and Wolfe RR.** The response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced hyperinsulinemia is impaired in the elderly. *The Journal of clinical endocrinology and metabolism* 85: 4481-4490, 2000.

157. **Wang J-S, Lee M-Y, Lien H-Y, and Weng T-P.** Hypoxic exercise training improves cardiac/muscular hemodynamics and is associated with modulated circulating progenitor cells in sedentary men. *International Journal of Cardiology* 170: 315-323, 2014.

158. **Wang P, Li CG, Qi Z, Cui D, and Ding S.** Acute Exercise Induced Mitochondrial H(2)O(2) Production in Mouse Skeletal Muscle: Association with p(66Shc) and FOXO3a Signaling and Antioxidant Enzymes. *Oxidative Medicine and Cellular Longevity* 2015: 536456, 2015.

159. **Wannamethee SG, Shaper AG, and Walker M.** Changes in physical activity, mortality, and incidence of coronary heart disease in older men. *The Lancet* 351: 1603-1608, 1998.

160. **Webb R, Hughes MG, Thomas AW, and Morris K.** The Ability of Exercise-Associated Oxidative Stress to Trigger Redox-Sensitive Signalling Responses. *Antioxidants (Basel)* 6: 63, 2017.

161. **Xia W-H, Li J, Su C, Yang Z, Chen L, Wu F, Zhang Y-Y, Yu B-B, Qiu Y-X, Wang S-M, and Tao J.** Physical exercise attenuates age-associated reduction in endothelium-reparative capacity of endothelial progenitor cells by increasing CXCR4/JAK-2 signaling in healthy men. *Aging Cell* 11: 111-119, 2012.

162. **Xia WH, Yang Z, Xu SY, Chen L, Zhang XY, Li J, Liu X, Qiu YX, Shuai XT, and Tao J.** Age-related decline in reendothelialization capacity of human endothelial progenitor cells is restored by shear stress. *Hypertension* 59: 1225-1231, 2012.

163. **Xiao J, Xu T, Li J, Lv D, Chen P, Zhou Q, and Xu J.** Exercise-induced physiological hypertrophy initiates activation of cardiac progenitor cells. *Int J Clin Exp Pathol* 7: 663-669, 2014.

164. **Xu X, Chen C-n, Arriaga EA, and Thompson LV.** Asymmetric superoxide release inside and outside the mitochondria in skeletal muscle under conditions of aging and disuse. *Journal of Applied Physiology* 109: 1133-1139, 2010.

165. **Zhang JM and An J.** Cytokines, Inflammation and Pain. *International anesthesiology clinics* 45: 27-37, 2007.