Muscles and their myokines

Bente Klarlund Pedersen

Centre of Inflammation and Metabolism, Rigshospitalet – Section 7641, Blegdamsvej 9, DK-2100, Copenhagen, Denmark

bkp@rh.dk

Summary

In the past, the role of physical activity as a life-style modulating factor has been considered as that of a tool to balance energy intake. Although it is important to avoid obesity, physical inactivity should be discussed in a much broader context. There is accumulating epidemiological evidence that a physically active life plays an independent role in the protection against type 2 diabetes, cardiovascular diseases, cancer, dementia and even depression. For most of the last century, researchers sought a link between muscle contraction and humoral changes in the form of an 'exercise factor', which could be released from skeletal muscle during contraction and mediate some of the exercise-induced metabolic changes in other organs such as the liver and the adipose tissue. We have suggested that cytokines or other peptides that are produced, expressed and released by muscle fibres and exert autocrine, paracrine or endocrine effects should be classified as 'myokines'. Given that skeletal muscle is the largest organ in the human body, our discovery that contracting skeletal muscle secretes proteins sets a novel paradigm: skeletal muscle is an endocrine organ producing and releasing myokines, which work in a hormone-like fashion, exerting specific endocrine effects on other organs. Other myokines work *via* paracrine mechanisms, exerting local effects on signalling pathways involved in muscle metabolism. It has been suggested that myokines may contribute to exercise-induced protection against several chronic diseases.

Key words: cytokines, insulin resistance, inflammation, metabolism.

Introduction

Based on current trends, by the year 2020 non-communicable chronic diseases are expected to account for 70% of deaths and 60% of the disease burden (World Health Organisation). In Denmark, physical inactivity is considered the number two actual cause of death (Bronnum-Hansen et al., 2007) and the Centers for Disease Control and Prevention have designated physical inactivity as an actual cause of chronic disease (Mokdad et al., 2004).

On average, physically inactive people have a life span that is 5 years shorter than that of physically active people. Moreover, the expected lifetime without long-standing illness is reduced by approximately 8 years in physically inactive people (Bronnum-Hansen et al., 2007).

The diseasome of physical inactivity

Physical inactivity increases the risk of type 2 diabetes (Tuomilehto et al., 2001), cardiovascular disease (CVD) (Nocon et al., 2008), colon cancer (Wolin et al., 2009), postmenopausal breast cancer (Monninkhof et al., 2007), dementia (Rovio et al., 2005) and depression (Paffenbarger et al., 1994). These are all frequent chronic diseases, associated with an enhanced risk of premature morbidity. It is well established that patients with type 2 diabetes have a markedly increased risk of CVD (Diamant and Tushuizen, 2006), Alzheimer's disease and vascular dementia, and individuals with type 2 diabetes also have a high prevalence of affective illness, including major depression (reviewed in Komulainen et al., 2008). In addition, patients with type 2 diabetes have an elevated risk of developing colon and breast cancer, as well as pancreatic, liver and endometrial cancer (Richardson and Pollack, 2005). We have recently suggested that type 2 diabetes, CVD, colon cancer, breast cancer, dementia and depression constitute a cluster of diseases, which defines a 'diseasome of physical inactivity' (Pedersen, 2009b) (Fig. 1).

The diseasome of physical inactivity represents diseases with highly different phenotypical presentations, but that share important pathogenetic mechanisms. Clearly, independently of body mass index (BMI), physical inactivity is a risk factor for all-cause mortality (Pedersen, 2007a). It is a striking feature that chronic systemic inflammation is associated with physical inactivity independent of obesity (Fischer et al., 2007). I suggest that physical inactivity leads to the accumulation of visceral fat and consequently the activation of a network of inflammatory pathways, which promote the development of insulin resistance, atherosclerosis, neurodegeneration and tumour growth, and thereby the development of the diseases belonging to the diseasome of physical inactivity (Fig. 2).

Association between physical inactivity and abdominal adiposity; what is known?

Several studies have shown a J-shaped association between BMI and mortality, suggesting that both a high and a low BMI are associated with premature death. It appears that the risk observed at a low BMI is more closely linked with low fat-free mass (an estimate of muscle mass) than low fat mass (Heitmann and Frederiksen, 2009). Thus, it appears that little muscle mass is associated with increased abdominal adiposity.

Abdominal adiposity is associated with CVD (Haffner, 2007), type 2 diabetes (Bays, 2009), dementia (Whitmer et al., 2008), colon cancer (Giovannucci, 2007) and breast cancer (Xue and Michels, 2007), as well as all-cause mortality independent of BMI, even in people with a normal body mass (Pischon et al., 2008). Thus, the health consequences of abdominal adiposity and physical inactivity are similar and both physical inactivity (Pedersen and Febbraio, 2008) and abdominal adiposity (Yudkin, 2007) are associated with persistent systemic low-grade inflammation (Festa et al., 2002; Handschin and Spiegelman, 2008).
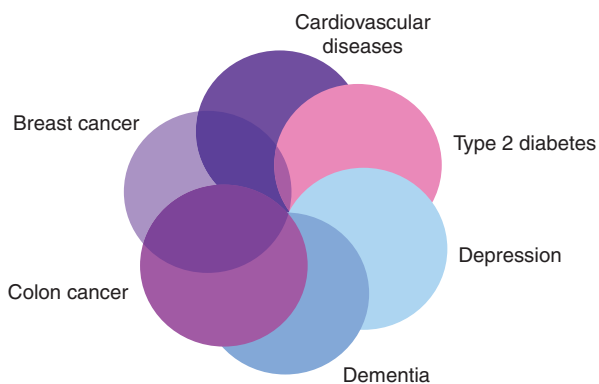
Fig. 1. Type 2 diabetes, cardiovascular disease, colon cancer, postmenopausal breast cancer, dementia and depression constitute a cluster of diseases, which can be identified as 'the diseasome of physical inactivity'.

Evidence exists that visceral fat is more inflamed than subcutaneous fat and constitutes an important source of systemic inflammation (Yudkin, 2007), and an association has been established between physical inactivity and low-grade systemic inflammation in healthy, young individuals (Petersen and Pedersen, 2005). These findings would be compatible with the notion that physical inactivity causes accumulation of visceral fat, which precedes chronic systemic inflammation. Chronic inflammation promotes the development of insulin resistance, atherosclerosis, neurodegeneration and tumour growth (Handschin and Spiegelman, 2008), and thereby the development of the diseases belonging to the diseasome of physical inactivity.

It is also well known that physical inactivity and poor physical fitness, independently of BMI, are associated with an increased risk of CVD, type 2 diabetes, dementia and premature mortality from all causes (Pedersen, 2009b). Thus, the health consequences of abdominal adiposity and physical inactivity are similar and support our overall idea that physical inactivity may be an important and independent player in the development of abdominal adiposity.

We recently conducted a study of physical inactivity that certainly points at a direct link between physical inactivity and accumulation of visceral fat. A group of young healthy men decreased their daily stepping for 2 weeks to 1500 steps from the range of around 10,000 recommended for adults. The 2 week period induced a 7% decline in $\dot{V}_{O2max}$ (ml min^{-1}; cardiovascular fitness) and a markedly impaired glucose tolerance as well as attenuation of postprandial lipid metabolism. The intervention was associated with a 7% increase in intra-abdominal fat mass, measured by magnetic resonance (MR)-scanning, without a change in total fat mass, while total fat-free mass and BMI decreased (Olsen et al., 2008) (Fig. 3). A follow-up study revealed that the volunteers developed a marked decline in peripheral insulin sensitivity accompanied by a decrease in the insulin-stimulated ratio of pAkt^{Thr308}/total Akt after step reduction, without an effect on hepatic endogenous glucose production (Rasmussen et al., 2010).

Association between physical inactivity and impaired cognitive function; what is known?

The ability of the brain to adapt to a new situation or environment, or the consequences of an injury is often referred to as brain plasticity (Hillman et al., 2008). Physical exercise, as indexed by cardiovascular fitness, is a factor that strongly affects brain plasticity.

In rodents, physical exercise improves memory function and structural parameters such as synapse density, neuronal complexity and hippocampal neurogenesis (Eadie et al., 2005; Stranahan et al., 2007; van Praag et al., 1999; Wu et al., 2008).

In the injured brain, exercise induces neuroprotection in animal models of stroke (Hayes et al., 2008), traumatic brain injury (Griesbach et al., 2007) and Parkinson's disease (Yoon et al., 2007). Voluntary running significantly restores the neural stem cell pool, hippocampal neurogenesis and behavioural deficits following a clinically relevant, moderate dose of irradiation (Naylor et al., 2008). These experimental studies indicate the importance of physical exercise for cognitive performance.

Positive cognitive effects of exercise have also been demonstrated in humans. Higher levels of cardiovascular fitness are associated with increased hippocampal volume as well as better memory function (Erickson et al., 2009). Moreover, a recent study measured hippocampus size in response to 12 weeks of aerobic training in patients with schizophrenia and in healthy controls. Following exercise training, relative hippocampal volume increased significantly in patients (12%) and healthy subjects (16%), with no change in the non-exercise group of patients (–1%) (Pajonk et al., 2010).

Several meta-analyses demonstrate a positive association between cardiovascular (or 'aerobic') fitness and cognitive performance in elderly subjects (Angevaren et al., 2008; Colcombe and Kramer, 2003; Etnier et al., 2006; Heyn et al., 2004). Physical activity during midlife appears to protect against dementia or cognitive decline and to improve cognitive performance in old adults with memory impairment (Rovio et al., 2005; Sun et al., 2010; Etgen et al., 2010a; Etgen et al., 2010b; Liu-Ambrose et al., 2010; Andel et al., 2008; Lautenschlager et al., 2008). At the other end of the age spectrum, physical activity and academic achievement display a positive correlation, as indicated by meta-analysis of smaller cohort studies of school children (Sibley and Etnier, 2003). However, the relationship between physical exercise and neurocognitive function in young adults is less clear. Acutely, physical exercise seems to have little effect on memory and cognition; executive function processes involved in working memory remain unaltered, although aspects of delayed long-term memory do improve (Coles and Tomporowski, 2008; Tomporowski, 2003). Long-term physical exercise appears to have a slight effect on reaction time in young people (Sherwood and Selder, 1979). Based on a small sample size, an 8 week training programme resulted in improved reaction time and executive function (Hansen et al., 2004; Hascelik et al., 1989). Previous studies have found changes in brain parenchyma following exercise. This has been demonstrated especially in the hippocampus, where exercise-induced increases in cerebral blood volume (CBV) have been shown to be associated with neurogenesis and vascularisation (Pajonk et al., 2010; Pereira et al., 2007). An association between cardiorespiratory fitness and performance in a cognitive attention task has also been demonstrated (Colcombe et al., 2004).

Diseasome of physical inactivity – summary

There are indications in the literature that physical inactivity is an independent cause of abdominal adiposity and a contributing factor to cognitive decline. It is possible that physical inactivity leads to the accumulation of visceral fat and consequently the activation of a network of inflammatory pathways, which not only promote the development of insulin resistance and atherosclerosis but also lead to neurodegeneration and thereby the development of cognitive impairment (Pedersen, 2009b).

The myokine concept

Our global hypothesis is that myokines play a role in protection against the diseasome of physical inactivity. One decade ago we identified a humoral factor (a cytokine) that was produced and released from contracting muscle cells and appeared to have major metabolic effects. Given that skeletal muscle is the largest organ in the human body, our discovery of contracting muscle as a cytokine-producing organ revealed a whole new paradigm: skeletal muscle is an endocrine organ, which by secretion of hormone-like factors may influence metabolism in tissues and organs. In continuation, we suggested that cytokines and other peptides that are produced, expressed and released by muscle fibres and exert autocrine, paracrine or endocrine effects should be classified as 'myokines' (Pedersen and Febbraio, 2008).

Adipose tissue has been regarded as the major source of cytokines (adipokines) – however, the finding that muscles produce and release cytokines (myokines) suggests that working skeletal muscle in addition to adipose tissue may be a major source of secreted molecules. Myokines provide a conceptual basis to explain how muscles communicate to other organs. Thus, our overall idea is that contracting skeletal muscles release myokines, which work in a hormone-like fashion, exerting specific endocrine effects on other organs or which work locally *via* paracrine mechanisms.

For half a century, researchers sought a link between muscle contraction and changes in peripheral organs in the form of an 'exercise factor', which could be released from skeletal muscle during contraction and mediate some of the exercise-induced metabolic changes in other organs such as the liver and the adipose tissue. The idea that signalling pathways from contracting muscles to other organs are not solely mediated *via* the nervous system was supported by findings from electrical stimulation of paralysed muscles in spinal cord-injured patients (Kjaer et al., 1996).

It was obvious that one or more muscle-derived humoral factors existed. For lack of more exact knowledge, these humoral factors were called the 'work stimulus' or the 'work factor'. In this context, our identification of muscle as a cytokine-producing organ was a breakthrough. In the year 2000, it became clear that contracting human skeletal muscle releases significant amounts of interleukin (IL)-6 into the circulation during prolonged single-limb exercise (Steensberg et al., 2000). Research during subsequent years highlighted the fact that muscle-derived IL-6 is an important player in metabolism (Pedersen and Febbraio, 2008). Today, it appears that skeletal muscle has the capacity to express several myokines (Pedersen and Febbraio, 2008). Thus, although the idea of an exercise factor can be traced back many years, our identification of muscle as a myokine-producing organ opens a whole new field of research.

IL-6 – the myokine prototype
Exercise and plasma-IL-6

The increase in the plasma concentration of IL-6 during exercise has been a consistent finding (Pedersen and Febbraio, 2008). This is followed by the appearance of IL-1 receptor antagonist (IL-1ra) and the anti-inflammatory cytokine IL-10. Concentrations of the chemokines, IL-8, macrophage inflammatory protein 1α (MIP-1α) and MIP-1β are elevated after strenuous exercise. Of note, the cytokine response to exercise and sepsis differs with regard to tumour necrosis factor (TNF)-α. Thus, the cytokine response to exercise is not preceded by an increase in plasma TNF-α. Even though there may be a moderate increase in the systemic concentration of these cytokines, the underlying fact is that the appearance of IL-6 in the circulation is by far the most marked
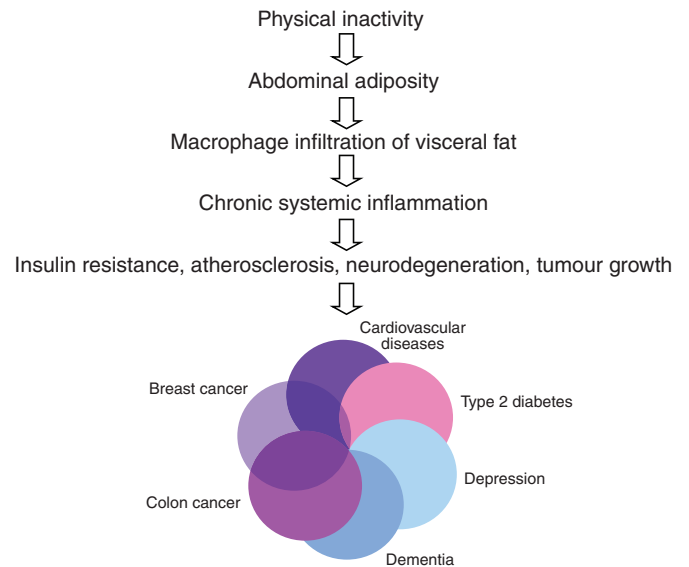

Fig. 2. Hypothesis: physical inactivity leads to the accumulation of visceral fat and consequently to the activation of a network of inflammatory pathways, which promote the development of insulin resistance, atherosclerosis, neurodegeneration and tumour growth, leading to the development of "the diseasome of physical inactivity".

and precedes that of the other cytokines (Pedersen and Febbraio, 2008).

It appears that, unlike IL-6 signalling in macrophages, which seems to be dependent upon the activation of the nuclear factor κ light chain enhancer of activated B cells (NFκB) signalling pathway, intramuscular IL-6 expression is regulated by a network of signalling cascades that among other pathways are likely to involve crosstalk between the Ca^{2+}/nuclear factor of activated T-cells (NFAT) and glycogen/p38 mitogen-activated protein kinase (MAPK) pathways. Thus, when IL-6 is produced by macrophages, it leads to an inflammatory response, whereas muscle cells produce and release IL-6 without activating classical pro-inflammatory pathways. The fact that IL-6 can sometimes act as a pro-inflammatory and sometimes as an anti-inflammatory agent appears to be more dependent on the environment (muscle *versus* immune cell) than on whether IL-6 is activated in an acute or chronic fashion (Pedersen and Febbraio, 2008).

Exercise-induced plasma IL-6 concentrations increase in an almost exponential manner. The peak IL-6 level is reached at the end of the exercise or shortly thereafter, followed by a rapid decrease towards pre-exercise levels. The basal plasma IL-6 concentration may increase up to 100-fold after exercise (Pedersen and Fischer, 2007). As IL-6 is a classical inflammatory cytokine it was initially thought that the IL-6 response was related to muscle damage. However, it has become evident that muscle damage is not required in order to increase plasma IL-6 during exercise. Rather, eccentric exercise may result in a delayed peak and a slower decrease of plasma IL-6 during recovery (Pedersen and Febbraio, 2008).

Contracting skeletal muscle *per se* is the main source of the IL-6 in the circulation in response to exercise. In resting human skeletal muscle, the IL-6 mRNA content is very low, while small amounts of IL-6 protein, predominantly in type I fibres, may be detected using sensitive immunohistochemical methods (Plomgaard et al., 2005). We found that an increase in the IL-6 mRNA content is detectable in the contracting skeletal muscle after 30 min of exercise,
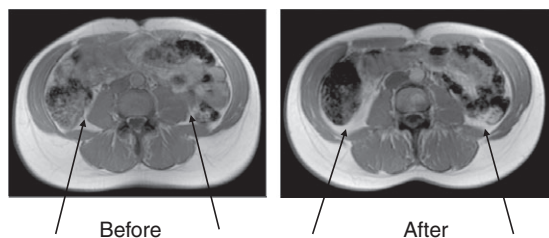
Fig. 3. Magnetic resonance (MR) scan demonstrating visceral fat mass before and after 14 days of reduced stepping and 1.2 kg of mass loss.

and up to 100-fold increases in the IL-6 mRNA content may be present at the end of the exercise bout (Keller et al., 2001; Steensberg et al., 2002). By obtaining arterial–femoral venous differences over an exercising leg, we found that exercising limbs released IL-6. In an attempt to determine which cells produce the IL-6, Keller and colleagues isolated nuclei from muscle biopsies obtained before, during and after exercise (Keller et al., 2001). Using RT-PCR, these authors demonstrated that the nuclear transcription rate for IL-6 increases rapidly and markedly after the onset of exercise. This suggested that a factor associated with contraction increases IL-6 transcription rate, probably in the nuclei from myocytes, given the observation that IL-6 protein is expressed within muscle fibres (Malm et al., 2000). Further evidence that contracting muscle fibres themselves are a source of IL-6 mRNA and protein has been obtained by analysis of biopsies from human vastus lateralis using in situ hybridisation and immunohistochemistry (Hiscock et al., 2004; Penkowa et al., 2003). Contracting skeletal muscles may account for most of the IL-6 found in the circulation, although other studies have demonstrated that skeletal muscle is not the sole source of exercise-induced IL-6. Other sources of IL-6 include connective tissue (Langberg et al., 2002), the brain (Nybo et al., 2002) and adipose tissue (Keller et al., 2003; Lyngso et al., 2002).

Muscle-derived IL-6, muscle glycogen and carbohydrate ingestion

By manipulating muscle glycogen content, both intramuscular IL-6 mRNA expression (Keller et al., 2001) and protein release (Steensberg et al., 2001) are exacerbated when intramuscular glycogen is compromised, suggesting that IL-6 works as an energy sensor. In addition, a number of studies show that glucose ingestion during exercise attenuates the exercise-induced increase in plasma IL-6 (Pedersen and Febbraio, 2008) and totally inhibits IL-6 release from contracting skeletal muscle in humans (Febbraio et al., 2003).

IL-6 and AMP-activated protein kinase

Transcriptional events play a pivotal role in the metabolic adaptations of skeletal muscle. The expression of genes essential for skeletal muscle glucose and lipid metabolism is tightly coordinated in support of a shift in substrate utilisation. AMP-activated protein kinase (AMPK) regulates skeletal muscle metabolic gene expression programmes in response to changes in energy status (Long and Zierath, 2008). While AMPK may influence the transcription of metabolic genes, AMPK exerts most of its effects via its role as a protein kinase, regulating the activity of key metabolic enzymes by phosphorylation.

Acute treatment of muscle cells with IL-6 increased both basal glucose uptake and translocation of the glucose transporter GLUT4 from intracellular compartments to the plasma membrane (Carey et al., 2006). Moreover, IL-6 increased insulin-stimulated glucose uptake in vitro, while infusion of recombinant human IL-6 into healthy humans during a hyperinsulinaemic, euglycaemic clamp increased the glucose infusion rate without affecting the total suppression of endogenous glucose production (Carey et al., 2006). The effects of IL-6 on glucose uptake in vitro appeared to be mediated by activation of AMPK, as the results were abolished in cells infected with an AMPK dominant negative adenovirus (Carey et al., 2006). Apart from the effects of IL-6 on glucose metabolism, several studies have reported that IL-6 may increase intramyocellular (Bruce and Dyck, 2004; Petersen et al., 2005; Carey et al., 2006) or whole-body (van Hall et al., 2003) fatty acid oxidation. This effect may to some extent be mediated by AMPK (Kahn et al., 2005; Carey et al., 2006). A recent study suggests that IL-6 activates AMPK in skeletal muscle by increasing the concentration of cAMP and, secondarily, the AMP:ATP ratio (Kelly et al., 2009). Work from several groups (Minokoshi et al., 2002; Watt et al., 2006; Steinberg et al., 2003) has demonstrated that leptin may activate AMPK in peripheral tissues such as skeletal muscle. Thus, it appears that IL-6 acutely mediates signalling through the glycoprotein 130 (gp130) receptor and exhibits many 'leptin-like' actions such as activating AMPK and insulin signalling (Steinberg et al., 2009). Although most studies point to an effect of IL-6 on AMPK, Glund and colleagues provided evidence that AMPK-dependent pathways regulate IL-6 release from isolated oxidative skeletal muscle (Glund et al., 2009).

A number of studies both in vitro (Rotter et al., 2003; Lagathu et al., 2003; Senn et al., 2002; Senn et al., 2003) and in rodents in vivo (Klover et al., 2003; Klover et al., 2005; Kim et al., 2004) demonstrate that IL-6 is capable of inducing insulin resistance. In the rodent studies, IL-6 seems to induce insulin resistance via adverse effects on the liver. The IL-6-induced insulin resistance appears to be due to an increase in suppressor of cytokine signalling 3 (SOCS-3) expression (Senn et al., 2003), as SOCS-3 may directly inhibit the insulin receptor (Ueki et al., 2004). However, it is quite clear that in healthy skeletal muscle, and not least in humans, the IL-6-induced activation of AMPK overrides the IL-6-induced activation of SOCS-3. Of note, IL-6 knockout mice develop mature onset obesity and glucose intolerance (Wallenius et al., 2002), supporting the notion that IL-6 may exert beneficial effects on metabolism; however, even this observation is unclear (Di Gregorio et al., 2004).

The anti-inflammatory effects of exercise and IL-6

Systemic low-level inflammation is defined as 2- to 4-fold elevations in circulating levels of pro-inflammatory and anti-inflammatory cytokines, naturally occurring cytokine antagonists, and acute-phase proteins, as well as minor increases in counts of neutrophils and natural killer cells (Bruunsgaard and Pedersen, 2003; Bruunsgaard et al., 2003b; Bruunsgaard et al., 1999). A number of recent of papers have documented that self-reported physical activity or physical performance is correlated inversely with systemic low-level inflammation, suggesting that the anti-inflammatory activity induced by regular exercise may exert some of the beneficial health effects of exercise in patients with chronic diseases (reviewed in Wilund, 2007; Petersen and Pedersen, 2005; Petersen and Pedersen, 2006; Pedersen, 2006).

The fact that the classical pro-inflammatory cytokines TNF-α and IL-1β in general do not increase with exercise, whereas exercise provokes an increase in circulating levels of well-known anti-inflammatory cytokines and cytokine inhibitors such as IL-1ra, IL-10 and sTNF-R (Ostrowski et al., 2000; Ostrowski et al., 1999), suggests that exercise provokes an environment of anti-inflammatory cytokines. Importantly, we showed that rhIL-6 infusion as well as exercise inhibited the endotoxin-induced increase in circulating levels of TNF-α in healthy humans (Starkie et al., 2003). The anti-

inflammatory effects of IL-6 are also demonstrated by the finding that IL-6 stimulates the production of the classical anti-inflammatory cytokines IL-1ra and IL-10 (Steensberg et al., 2003).

IL-6 in a clinical context

It appears that from a rather unexciting existence as a player in the textbook version of the inflammatory response, IL-6 has recently been taken to centre stage in the search for culprits underlying the inflammatory component of the metabolic syndrome (Krook, 2008). The findings suggesting that IL-6 is yet another pro-inflammatory cytokine are in contrast to the numerous studies showing that IL-6 has a beneficial effect on muscle metabolism as reviewed here and elsewhere (Pedersen, 2007b; Glund and Krook, 2008; Pedersen and Febbraio, 2008; Pedersen, 2009b; Pedersen, 2009a).

Chronically elevated IL-6 levels lead to inappropriate hyperinsulinaemia, reduced body mass and impaired insulin-stimulated glucose uptake by the skeletal muscles (Franckhauser et al., 2008). In contrast, IL-6 knockout mice develop obesity and insulin resistance, providing evidence against a causative effect of IL-6 in insulin resistance (Wallenius et al., 2002). Numerous explanations for the two divergent opinions have been put forward, including differences in model systems, chronic *versus* pulsatile exposure, and *in vitro versus in vivo* effects (Glund and Krook, 2008; Pedersen and Febbraio, 2007). Of note, in resting healthy humans, plasma IL-6 is normally about $1–2\,\mathrm{pg\,ml^{-1}}$ or less (Kolb and Mandrup-Poulsen, 2005). Although exercise itself leads to an acute increase in IL-6 production and release by the working muscle, exercise training leads to reduced circulating IL-6 levels and, according to changes in levels of IL-6 receptor expression, to an increase in IL-6 sensitivity in skeletal muscle (Keller et al., 2005). In patients with type 2 diabetes and in elderly people (Pedersen et al., 2003; Bruunsgaard et al., 2003a) circulating levels of IL-6 are about 2- to 3-fold higher than those measured in young and adult healthy individuals. This represents a low but chronic IL-6 exposure, contrasting with the situation in exercise, where IL-6 levels increase acutely up 100-fold. As recently pointed out by Krook (Krook, 2008) the IL-6 levels achieved following electro-transfer by Franckhauser and colleagues (Franckhauser et al., 2008) were around $800\,\mathrm{pg\,ml^{-1}}$, corresponding to many hundredfold higher than both normal and diabetic values, and about 5-fold higher than those seen following strenuous exercise. In fact, concentrations are equivalent to those noted in the context of severe infections.

Recently, White and colleagues (Sadagurski et al., 2010) overexpressed human IL-6 in mice and reported increased insulin sensitivity and prevention of diet-induced obesity. IL-6 transgenic mice fed a high-fat diet were more insulin sensitive and glucose tolerant with reduced body and fat mass, leptin levels and food intake (Sadagurski et al., 2010). Patients with Castleman's disease responded well to IL-6 blocking treatment (Nishimoto et al., 2005); however, patients treated with the IL-6 receptor blocker also displayed an increase in body mass of approximately 4 kg (7%) and marked hypertriglyceridaemia during the treatment period, suggesting the existence of a link between blockage of IL-6 signalling and impaired metabolic homeostasis in humans. In addition, changes in lipid profiles were also seen in patients with rheumatoid arthritis receiving IL-6 blocking treatment (Nishimoto et al., 2004; Maini et al., 2006; Smolen et al., 2008).

Although high circulating systemic levels of IL-6 appear to exert pro-inflammatory effects, it is also clear that blocking IL-6 signalling leads to mass gain and enhanced blood lipid levels. Although the effects of IL-6 are highly dependent upon the tissue and although both dose and time appear to be determining factors for the
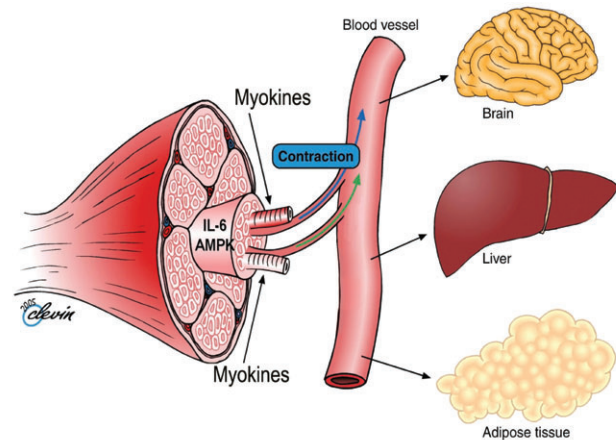

Fig. 4. Biological role of contraction-induced IL-6. Skeletal muscle expresses and releases myokines into the circulation. In response to muscle contractions, both type I and type II muscle fibres express the myokine IL-6, which subsequently exerts its effects both locally within the muscle (e.g. through activation of AMP-activated protein kinase, AMPK) and – when released into the circulation – peripherally in several organs in a hormone-like fashion. Figure reproduced in modified form, with permission (Pedersen and Fischer, 2007).

biological role of exercise, the role of IL-6 in contracting muscle is, as a matter of fact, very clear.

IL6 – summary

In summary, IL-6 is produced and released in response to muscle contractions (Fig. 4). Muscle fibres express the myokine IL-6, which subsequently exerts its effects both locally within the muscle (e.g. through activation of AMPK) and – when released into the circulation – peripherally in several organs in a hormone-like fashion. Specifically in skeletal muscle, IL-6 acts in an autocrine or paracrine manner to signal through a gp130Rβ/IL-6Rα homodimer, resulting in the activation of AMPK and/or phosphatidylinositol 3-kinase (PI3-kinase) to increase glucose uptake and fat oxidation. Furthermore, IL-6 is also known to increase hepatic glucose production during exercise or lipolysis in adipose tissue (Pedersen and Fischer, 2007).

Other myokines

IL-15 – a role in muscle–fat cross-talk?

IL-15 is expressed in human skeletal muscle (Pedersen et al., 2007). It possesses anabolic effects on skeletal muscle *in vitro* and *in vivo* and may also take part in reducing adipose tissue mass (Pedersen et al., 2007). Therefore, IL-15 has been suggested to be involved in muscle–fat crosstalk. Recently, we demonstrated that IL-15 mRNA levels were upregulated in human skeletal muscle following a bout of strength training (Nielsen et al., 2007), suggesting that IL-15 may accumulate within the muscle as a consequence of regular training.

We further demonstrated a negative association in humans between plasma IL-15 concentration and trunk fat mass, but not limb fat mass. In support of this finding, we demonstrated a decrease in visceral fat mass, but not subcutaneous fat mass, when IL-15 was overexpressed in murine muscle (Nielsen et al., 2008).

Quinn and colleagues found that elevated circulating levels of IL-15 in mice resulted in significant reductions in body fat and

increased bone mineral content, without appreciably affecting lean body mass or levels of other cytokines (Quinn et al., 2009). Although this model represents an artificial system, the findings lend some support to the idea that IL-15 secretion from muscle tissue may modulate visceral fat mass specifically *via* an endocrine mechanism.

IL-8 – a role in exercise-induced angiogenesis?

IL-8 is a known chemokine that attracts primarily neutrophils. In addition to its chemokine properties, IL-8 acts as an angiogenic factor. The plasma concentration of IL-8 increases in response to exhaustive exercise such as running, which involves eccentric muscle contractions, whereas we found no increase in plasma IL-8 in relation to concentric exercise (Pedersen and Febbraio, 2008).

However, when measuring the arterio-venous concentration difference across a concentrically exercising limb we detected a small and transient net release of IL-8, which did not result in an increase in the systemic IL-8 plasma concentration (Akerstrom et al., 2005). The fact that high local IL-8 expression occurs in contracting muscle with only a small and transient net release may indicate that muscle-derived IL-8 acts locally and exerts its effect in an autocrine or paracrine fashion (Akerstrom et al., 2005). It is not likely that muscle-derived IL-8 would work as a chemoattractant of neutrophils and macrophages when, in fact, in concentric exercise there is little or no accumulation of neutrophils or macrophages in skeletal muscle. However, a more likely function of muscle-derived IL-8 is to stimulate angiogenesis. It induces its chemotactic effects *via* the chemokine receptor CXCR1, whereas CXCR2, which is expressed by human microvascular endothelial cells, is the receptor responsible for IL-8-induced angiogenesis (Bek et al., 2002; Koch et al., 1992; Norrby, 1996). The expression of the IL-8 receptor CXCR2 is enhanced in human skeletal muscle biopsies after concentric exercise and the increase in CXCR2 protein is seen not only in the muscle fibres but also to a larger extent in the vascular endothelium, suggesting that it may play a role in angiogenesis (Frydelund-Larsen et al., 2007). IL-8 signalling promotes angiogenic responses in endothelial cells, increases proliferation and survival of endothelial and cancer cells, and potentiates the migration of cancer cells, endothelial cells and infiltrating neutrophils at the tumour site. Accordingly, IL-8 expression correlates with the angiogenesis, tumourigenicity and metastasis of tumours in numerous xenograft and orthotopic *in vivo* models (Waugh and Wilson, 2008).

Brain-derived neurotrophic factor – a role in neurobiology and metabolism

Neurotrophins are a family of structurally related growth factors, including brain-derived neurotrophic factor (BDNF), which exert many of their effects on neurons primarily through Trk receptor tyrosine kinases. Of these, BDNF and its receptor TrkB are most widely and abundantly expressed in the brain (Huang and Reichardt, 2001). However, recent studies show that BDNF is also expressed in non-neurogenic tissues, including skeletal muscle (Matthews et al., 2009). BDNF has been shown to regulate neuronal development and to modulate synaptic plasticity (Hofer and Barde, 1988). BDNF plays a key role in regulating survival, growth and maintenance of neurons (Mattson et al., 2004), and BDNF has a bearing on learning and memory (Tyler et al., 2002). However, BDNF has also been identified as a key component of the hypothalamic pathway that controls body mass and energy homeostasis (Wisse and Schwartz, 2003). Most recently, we have shown that BDNF appears to be a major player not only in central

metabolic pathways but also as a regulator of metabolism in skeletal muscle (Matthews et al., 2009).

Hippocampal samples from Alzheimer's disease donors show decreased BDNF expression (Connor et al., 1997) and individuals with Alzheimer's disease have low plasma levels of BDNF (Laske et al., 2005). Also, patients with major depression have lower levels of serum BDNF than normal control subjects (Karege et al., 2002). Other studies suggest that plasma BDNF is a biomarker of impaired memory and general cognitive function in ageing women (Komulainen et al., 2008) and a low circulating BDNF level was recently shown to be an independent and robust biomarker of mortality risk in old women (Krabbe et al., 2009). Interestingly, low levels of circulating BDNF are also found in obese individuals and those with type 2 diabetes (Krabbe et al., 2007). In addition, we have demonstrated that there is a cerebral output of BDNF and that this is inhibited during hyperglycaemic clamp conditions in humans. This last finding may explain the concomitant finding of low circulating levels of BDNF in individuals with type 2 diabetes, and the association between low plasma BDNF and the severity of insulin resistance (Krabbe et al., 2007).

BDNF appears to play a role in both neurobiology and metabolism. Studies have demonstrated that physical exercise may increase circulating BDNF levels in humans (Matthews et al., 1985). To identify whether the brain is a source of BDNF during exercise, eight volunteers rowed for 4h while simultaneous blood samples were obtained from the radial artery and the internal jugular vein. To further identify the putative cerebral region(s) responsible for BDNF release, mouse brains were dissected and analysed for BDNF mRNA expression following treadmill exercise. In humans, a BDNF release from the brain was observed at rest and increased 2- to 3-fold during exercise. Both at rest and during exercise, the brain contributed 70–80% of the circulating BDNF, while this contribution decreased following 1h of recovery. In mice, exercise induced a 3- to 5-fold increase in BDNF mRNA expression in the hippocampus and cortex, peaking 2h after the termination of exercise. These results suggest that the brain is a major but not the sole contributor to circulating BDNF. Moreover, the importance of the cortex and hippocampus as sources of plasma BDNF becomes even more prominent in the response to exercise (Rasmussen et al., 2009).

We further studied whether skeletal muscle would produce BDNF in response to exercise (Matthews et al., 2009) and found that BDNF mRNA and protein expression were increased in human skeletal muscle after exercise. However muscle-derived BDNF appeared not to be released into the circulation. BDNF mRNA and protein expression were increased in muscle cells that were electrically stimulated. Interestingly, BDNF increased phosphorylation of AMPK and acetyl-CoA carboxylase (ACC) and enhanced fat oxidation both *in vitro* and *ex vivo*.

Taken together, these data demonstrate that BDNF is a protein, produced in skeletal muscle cells, that is increased by contraction to enhance fat oxidation in an AMPK-dependent fashion, most probably by acting in an autocrine and/or paracrine manner within skeletal muscle. Hence, BDNF has been identified as a novel contraction-induced protein that may contribute to the multiple health benefits associated with exercise, possibly by enhancing fat oxidation in skeletal muscle. By demonstrating that BDNF is expressed in muscle and has an impact on fat oxidation, we add a new dimension to the pleiotrophic nature of BDNF, which can now be identified as playing a role in neurobiology as well as in both central and peripheral metabolism.

Fibroblast growth factor-21 – an insulin-stimulated myokine

The so-called myomouse developed by Walsh (Walsh, 2009) has contributed to the identification of other new myokines. Substantial increases in muscle fibre hypertrophy, mass and strength occur upon induction of Akt signalling in murine skeletal muscle. The increase in muscle mass caused by myogenic Akt induction results in diminished fat deposition and improvements in whole-body metabolism. Based on these findings Walsh has devised a protocol to identify novel muscle-secreted proteins (myokines) that confer the phenotypic changes brought on by myogenic Akt induction. One of these newly discovered factors, referred to as follistatin-like 1, functions to promote endothelial cell function and stimulates revascularisation in response to ischaemic insult through its ability to activate Akt–eNOS signalling (Ouchi et al., 2008). Using the myomouse model, Walsh and co-workers have further identified that fibroblast growth factor-21 (FGF21) is produced by skeletal muscle (Izumiya et al., 2008). FGF21 is known to be a hormone that is induced in liver by fasting. A recent study has demonstrated that FGF21 induces hepatic expression of peroxisome proliferator-activated receptor-γ coactivator protein-1α (PGC-1α), a key transcriptional regulator of energy homeostasis, and causes corresponding increases in fatty acid oxidation, tricarboxylic acid cycle flux and gluconeogenesis without increasing glycogenolysis. Mice lacking FGF21 fail to fully induce PGC-1α expression in response to a prolonged fast and have impaired gluconeogenesis and ketogenesis (Potthoff et al., 2009). These results reveal an unexpected relationship between FGF21 and PGC-1. However, the precise biological function of muscle-derived FGF21 is not known.

We studied muscular FGF21 expression and plasma FGF21 after acute insulin stimulation in young healthy men during a hyperinsulinaemic–euglycaemic clamp (Hojman et al., 2009). Furthermore, we investigated systemic levels and muscle FGF21 expression in humans with or without insulin resistance and chronically elevated insulin. FGF21 was barely detectable in young healthy men before insulin infusion, but after 3 or 4 h of insulin infusion during a hyperinsulinaemic–euglycaemic clamp, muscular FGF21 expression increased significantly. Plasma FGF21 followed the same pattern. In individuals with chronically elevated insulin, muscular FGF21 expression was associated with hyperinsulinaemia in men, but not in women. In plasma, hyperinsulinaemia and fasting glucose were positively associated with plasma FGF21 while plasma FGF21 correlated negatively with high-density lipoprotein (HDL) cholesterol. Our findings so far suggest that FGF21 is expressed in human skeletal muscle in response to insulin stimulation, indicating that FGF21 is an insulin-regulated myokine (Hojman et al., 2009).

Other myokines – summary

During recent years increased efforts have focused on elucidating the secretory function of the skeletal muscle. It appears that contracting muscle is capable of expressing and secreting various cyokines and peptides. So far, a handful of myokines has been identified. However, we recently published that skeletal muscle is capable of producing several hundred secreted proteins (Henningsen et al., 2010).

Conclusion

The finding that muscles produce myokines creates a paradigm shift and reveals new scientific, technological and scholarly horizons. We are convinced that the characterisation of the biological effects of known and unknown peptides, constituting the muscle secretome, will dominate the coming decade. Moreover, we suggest that myokines may be involved in mediating some of the health effects of regular exercise, in particular chronic diseases associated with low-grade inflammation and impaired metabolism.

References

Akerstrom, T. C., Steensberg, A., Keller, P., Keller, C., Penkowa, M. and Pedersen, B. K. (2005). Exercise induces interleukin-8 expression in human skeletal muscle. *J. Physiol.* **563**, 507-516.

Andel, R., Crowe, M., Pedersen, N. L., Fratiglioni, L., Johansson, B. and Gatz, M. (2008). Physical Exercise at Midlife and Risk of Dementia Three Decades Later: A Population-Based Study of Swedish Twins. *J. Gerontol. A. Biol. Sci. Med. Sci.* **63**, 62-66.

Angevaren, M., Aufdemkampe, G., Verhaar, H. J., Aleman, A. and Vanhees, L. (2008). Physical activity and enhanced fitness to improve cognitive function in older people without known cognitive impairment. *Cochrane Database Syst. Rev.* **16**, CD005381.

Bays, H. E. (2009). "Sick fat," metabolic disease, and atherosclerosis. *Am. J. Med.* **122**, S26-S37.

Bek, E. L., McMillen, M. A., Scott, P., Angus, L. D. and Shaftan, G. W. (2002). The effect of diabetes on endothelin, interleukin-8 and vascular endothelial growth factor-mediated angiogenesis in rats. *Clin. Sci. (Lond.)* **103** Suppl. 48, 424S-429S.

Bronnum-Hansen, H., Juel, K., Davidsen, M. and Sorensen, J. (2007). Impact of selected risk factors on expected lifetime without long-standing, limiting illness in Denmark. *Prev. Med.* **45**, 49-53.

Bruce, C. R. and Dyck, D. J. (2004). Cytokine regulation of skeletal muscle fatty acid metabolism: effect of interleukin-6 and tumor necrosis factor-alpha. *Am. J. Physiol. Endocrinol. Metab.* **287**, E616-E621.

Bruunsgaard, H. and Pedersen, B. K. (2003). Age-related inflammatory cytokines and disease. *Immunol. Allergy Clin. North Am.* **23**, 15-39.

Bruunsgaard, H., Pedersen, A. N., Schroll, M., Skinhoj, P. and Pedersen, B. K. (1999). Impaired production of proinflammatory cytokines in response to lipopolysaccharide (LPS) stimulation in elderly humans. *Clin. Exp. Immunol.* **118**, 235-241.

Bruunsgaard, H., Andersen-Ranberg, K., Hjelmborg, J. B., Pedersen, B. K. and Jeune, B. (2003a). Elevated levels of tumor necrosis factor alpha and mortality in centenarians. *Am. J. Med.* **115**, 278-283.

Bruunsgaard, H., Ladelund, S., Pedersen, A. N., Schroll, M., Jorgensen, T. and Pedersen, B. K. (2003b). Predicting death from tumour necrosis factor-alpha and interleukin-6 in 80-year-old people. *Clin. Exp. Immunol.* **132**, 24-31.

Carey, A. L., Steinberg, G. R., Macaulay, S. L., Thomas, W. G., Holmes, A. G., Ramm, G., Prelovsek, O., Hohnen-Behrens, C., Watt, M. J., James, D. E. et al. (2006). Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. *Diabetes.* **55**, 2688-2697.

Colcombe, S. and Kramer, A. F. (2003). Fitness effects on the cognitive function of older adults: a meta-analytic study. *Psychol. Sci.* **14**, 125-130.

Colcombe, S. J., Kramer, A. F., Erickson, K. I., Scalf, P., McAuley, E., Cohen, N. J., Webb, A., Jerome, G. J., Marquez, D. X. and Elavsky, S. (2004). Cardiovascular fitness, cortical plasticity, and aging. *Proc. Natl. Acad. Sci. USA* **101**, 3316-3321.

Coles, K. and Tomporowski, P. D. (2008). Effects of acute exercise on executive processing, short-term and long-term memory. *J Sports Sci.* **26**, 333-344.

Connor, B., Young, D., Yan, Q., Faull, R. L., Synek, B. and Dragunow, M. (1997). Brain-derived neurotrophic factor is reduced in Alzheimer's disease. *Brain Res. Mol. Brain Res.* **49**, 71-81.

Di Gregorio, G. B., Hensley, L., Lu, T., Ranganathan, G. and Kern, P. A. (2004). Lipid and carbohydrate metabolism in mice with a targeted mutation in the IL-6 gene: absence of development of age-related obesity. *Am. J. Physiol. Endocrinol. Metab.* **287**, E182-E187.

Diamant, M. and Tushuizen, M. E. (2006). The metabolic syndrome and endothelial dysfunction: common highway to type 2 diabetes and CVD. *Curr. Diab. Rep.* **6**, 279-286.

Eadie, B. D., Redila, V. A. and Christie, B. R. (2005). Voluntary exercise alters the cytoarchitecture of the adult dentate gyrus by increasing cellular proliferation, dendritic complexity, and spine density. *J. Comp. Neurol.* **486**, 39-47.

Erickson, K. I., Prakash, R. S., Voss, M. W., Chaddock, L., Hu, L., Morris, K. S., White, S. M., Wojcicki, T. R., McAuley, E. and Kramer, A. F. (2009). Aerobic fitness is associated with hippocampal volume in elderly humans. *Hippocampus* **19**, 1030-1039.

Etgen, T., Bickel, H. and Forstl, H. (2010a). Metabolic and endocrine factors in mild cognitive impairment. *Ageing Res. Rev.* **9**, 280-288.

Etgen, T., Sander, D., Huntgeburth, U., Poppert, H., Forstl, H. and Bickel, H. (2010b). Physical activity and incident cognitive impairment in elderly persons: the INVADE study. *Arch. Intern. Med.* **170**, 186-193.

Etnier, J. L., Nowell, P. M., Landers, D. M. and Sibley, B. A. (2006). A meta-regression to examine the relationship between aerobic fitness and cognitive performance. *Brain Res. Rev.* **52**, 119-130.

Febbraio, M. A., Steensberg, A., Keller, C., Starkie, R. L., Krustrup, P., Ott, P., Secher, N. H. and Pedersen, B. K. (2003). Glucose ingestion attenuates interleukin-6 release from contracting skeletal muscle in humans. *J. Physiol.* **549**, 607-612.

Festa, A., D'Agostino, R., Jr, Tracy, R. P. and Haffner, S. M. (2002). Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes* **51**, 1131-1137.

Fischer, C. P., Berntsen, A., Perstrup, L. B., Eskildsen, P. and Pedersen, B. K. (2007). Plasma levels of interleukin-6 and C-reactive protein are associated with physical inactivity independent of obesity. *Scand. J. Med. Sci. Sports* **17**, 580-587.

Franckhauser, S., Elias, I., Rotter, S., V, Ferre, T., Nagaev, I., Andersson, C. X., Agudo, J., Ruberte, J., Bosch, F. and Smith, U. (2008). Overexpression of Il6 leads to hyperinsulinaemia, liver inflammation and reduced body weight in mice. *Diabetologia.* **51**, 1306-1316.

Frydelund-Larsen, L., Penkowa, M., Akerstrom, T., Zankari, A., Nielsen, S. and Pedersen, B. K. (2007). Exercise induces interleukin-8 receptor (CXCR2) expression in human skeletal muscle. *Exp. Physiol.* **92**, 233-240.

Giovannucci, E. (2007). Metabolic syndrome, hyperinsulinemia, and colon cancer: a review. *Am. J. Clin. Nutr.* **86**, s836-s842.

Glund, S. and Krook, A. (2008). Role of interleukin-6 signalling in glucose and lipid metabolism. *Acta Physiol. (Oxf).* **192**, 37-48.

Glund, S., Treebak, J. T., Long, Y. C., Barres, R., Viollet, B., Wojtaszewski, J. F. and Zierath, J. R. (2009). Role of adenosine 5′-monophosphate-activated protein kinase in interleukin-6 release from isolated mouse skeletal muscle. *Endocrinology* **150**, 600-606.

Griesbach, G. S., Gomez-Pinilla, F. and Hovda, D. A. (2007). Time window for voluntary exercise-induced increases in hippocampal neuroplasticity molecules after traumatic brain injury is severity dependent. *J. Neurotrauma* **24**, 1161-1171.

Haffner, S. M. (2007). Abdominal adiposity and cardiometabolic risk: do we have all the answers? *Am. J. Med.* **120**, S10-S16.

Handschin, C. and Spiegelman, B. M. (2008). The role of exercise and PGC1[alpha] in inflammation and chronic disease. *Nature* **454**, 463-469.

Hansen, A. L., Johnsen, B. H., Sollers, J. J., III Stenvik, K. and Thayer, J. F. (2004). Heart rate variability and its relation to prefrontal cognitive function: the effects of training and detraining. *Eur. J. Appl. Physiol.* **93**, 263-272.

Hascelik, Z., Basgoze, O., Turker, K., Narman, S. and Ozker, R. (1989). The effects of physical training on physical fitness tests and auditory and visual reaction times of volleyball players. *J Sports Med. Phys. Fitness* **29**, 234-239.

Hayes, K., Sprague, S., Guo, M., Davis, W., Friedman, A., Kumar, A., Jimenez, D. F. and Ding, Y. (2008). Forced, not voluntary, exercise effectively induces neuroprotection in stroke. *Acta Neuropathol.* **115**, 289-296.

Heitmann, B. L. and Frederiksen, P. (2009). Thigh circumference and risk of heart disease and premature death: prospective cohort study. *BMJ.* 339:b3292. doi: 10. 1136/bmj. b3292.

Henningsen, J., Rigbolt, K. T., Blagoev, B., Pedersen, B. K. and Kratchmarova, I. (2010). Dynamics of the skeletal muscle secretome during myoblast differentiation. *Mol. Cell. Proteomics* **9**, 2482-2496.

Heyn, P., Abreu, B. C. and Ottenbacher, K. J. (2004). The effects of exercise training on elderly persons with cognitive impairment and dementia: a meta-analysis. *Arch. Phys. Med. Rehabil.* **85**, 1694-1704.

Hillman, C. H., Erickson, K. I. and Kramer, A. F. (2008). Be smart, exercise your heart: exercise effects on brain and cognition. *Nat. Rev. Neurosci.* **9**, 58-65.

Hiscock, N., Chan, M. H., Bisucci, T., Darby, I. A. and Febbraio, M. A. (2004). Skeletal myocytes are a source of interleukin-6 mRNA expression and protein release during contraction: evidence of fiber type specificity. *FASEB J.* **18**, 992-994.

Hofer, M. M. and Barde, Y. A. (1988). Brain-derived neurotrophic factor prevents neuronal death in vivo. *Nature* **331**, 261-262.

Hojman, P., Pedersen, M., Nielsen, A. R., Krogh-Madsen, R., Yfanti, C., Akerstrom, T., Nielsen, S. and Pedersen, B. K. (2009). Fibroblast growth factor-21 is induced in human skeletal muscles by hyperinsulinemia. *Diabetes* **58**, 2797-2801.

Huang, E. J. and Reichardt, L. F. (2001). Neurotrophins: roles in neuronal development and function. *Annu. Rev. Neurosci.* **24**, 677-736.

Izumiya, Y., Bina, H. A., Ouchi, N., Akasaki, Y., Kharitonenkov, A. and Walsh, K. (2008). FGF21 is an Akt-regulated myokine. *FEBS Lett.* **582**, 3805-3810.

Kahn, B. B., Alquier, T., Carling, D. and Hardie, D. G. (2005). AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metab.* **1**, 15-25.

Karege, F., Perret, G., Bondolfi, G., Schwald, M., Bertschy, G. and Aubry, J. M. (2002). Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry Res.* **109**, 143-148.

Keller, C., Steensberg, A., Pilegaard, H., Osada, T., Saltin, B., Pedersen, B. K. and Neufer, P. D. (2001). Transcriptional activation of the IL-6 gene in human contracting skeletal muscle: influence of muscle glycogen content. *FASEB J.* **15**, 2748-2750.

Keller, C., Keller, P., Marshall-Gradisnik, S. M. and Pedersen, B. K. (2003). IL-6 gene expression in human adipose tissue in response to exercise-effect of carbohydrate ingestion. *J. Physiol.* **550**, 927-931.

Keller, C., Steensberg, A., Hansen, A. K., Fischer, C. P., Plomgaard, P. and Pedersen, B. K. (2005). The effect of exercise, training, and glycogen availability on IL-6 receptor expression in human skeletal muscle. *J. Appl. Physiol.* **99**, 2075-2079.

Kelly, M., Gauthier, M. S., Saha, A. K. and Ruderman, N. B. (2009). Activation of AMP-activated protein kinase (AMPK) by Interleukin-6 in rat skeletal muscle:

Association with changes in cAMP, energy state, and endogenous fuel mobilization. *Diabetes.* **58**, 1953-1960.

Kim, H. J., Higashimori, T., Park, S. Y., Choi, H., Dong, J., Kim, Y. J., Noh, H. L., Cho, Y. R., Cline, G., Kim, Y. B. et al. (2004). Differential effects of interleukin-6 and -10 on skeletal muscle and liver insulin action in vivo. *Diabetes.* **53**, 1060-1067.

Kjaer, M., Pollack, S. F., Mohr, T., Weiss, H., Gleim, G. W., Bach, F. W., Nicolaisen, T., Galbo, H. and Ragnarsson, K. T. (1996). Regulation of glucose turnover and hormonal responses during electrical cycling in tetraplegic humans. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **271**, R191-R199.

Klover, P. J., Zimmers, T. A., Koniaris, L. G. and Mooney, R. A. (2003). Chronic exposure to interleukin-6 causes hepatic insulin resistance in mice. *Diabetes* **52**, 2784-2789.

Klover, P. J., Clementi, A. H. and Mooney, R. A. (2005). Interleukin-6 depletion selectively improves hepatic insulin action in obesity. *Endocrinology* **146**, 3417-3427.

Koch, A. E., Polverini, P. J., Kunkel, S. L., Harlow, L. A., DiPietro, L. A., Elner, V. M., Elner, S. G. and Strieter, R. M. (1992). Interleukin-8 as a macrophage-derived mediator of angiogenesis. *Science* **258**, 1798-1801.

Kolb, H. and Mandrup-Poulsen, T. (2005). An immune origin of type 2 diabetes? *Diabetologia* **48**, 1038-1050.

Komulainen, P., Pedersen, M., Hanninen, T., Bruunsgaard, H., Lakka, T. A., Kivipelto, M., Hassinen, M., Rauramaa, T. H., Pedersen, B. K. and Rauramaa, R. (2008). BDNF is a novel marker of cognitive function in ageing women: The DR's EXTRA Study. *Neurobiol. Learn. Mem.* **90**, 596-603.

Krabbe, K. S., Nielsen, A. R., Krogh-Madsen, R., Plomgaard, P., Rasmussen, P., Erikstrup, C., Fischer, C. P., Lindegaard, B., Petersen, A. M., Taudorf, S. et al. (2007). Brain-derived neurotrophic factor (BDNF) and type 2 diabetes. *Diabetologia.* **50**, 431-438.

Krabbe, K. S., Mortensen, E. L., Avlund, K., Pedersen, A. N., Pedersen, B. K., Jorgensen, T. and Bruunsgaard, H. (2009). Brain-Derived Neurotrophic Factor Predicts Mortality Risk in Older Women. *J Am. Geriatr. Soc.* **57**, 1447-1452.

Krook, A. (2008). IL-6 and metabolism-new evidence and new questions. *Diabetologia* **51**, 1097-1099.

Lagathu, C., Bastard, J. P., Auclair, M., Maachi, M., Capeau, J. and Caron, M. (2003). Chronic interleukin-6 (IL-6) treatment increased IL-6 secretion and induced insulin resistance in adipocyte: prevention by rosiglitazone. *Biochem. Biophys. Res. Commun.* **311**, 372-379.

Langberg, H., Olesen, J. L., Gemmer, C. and Kjaer, M. (2002). Substantial elevation of interleukin 6 concentration in peritendinous tissue, in contrast to muscle, following prolonged exercise in humans. *J. Physiol.* **542**, 985-990.

Laske, C., Stransky, E., Leyhe, T., Eschweiler, G. W., Wittorf, A., Richartz, E., Bartels, M., Buchkremer, G. and Schott, K. (2005). Stage-dependent BDNF serum concentrations in Alzheimer's disease. *J. Neural Transm.* **113**, 1217-1224.

Lautenschlager, N. T., Cox, K. L., Flicker, L., Foster, J. K., van Bockxmeer, F. M., Xiao, J., Greenop, K. R. and Almeida, O. P. (2008). Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease: a randomized trial. *JAMA* **300**, 1027-1037.

Liu-Ambrose, T., Nagamatsu, L. S., Graf, P., Beattie, B. L., Ashe, M. C. and Handy, T. C. (2010). Resistance training and executive functions: a 12-month randomized controlled trial. *Arch. Intern. Med.* **170**, 170-178.

Long, Y. C. and Zierath, J. R. (2008). Influence of AMP-activated protein kinase and calcineurin on metabolic networks in skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* **295**, E545-E552.

Lyngso, D., Simonsen, L. and Bulow, J. (2002). Interleukin-6 production in human subcutaneous abdominal adipose tissue: the effect of exercise. *J. Physiol.* **543**, 373-378.

Maini, R. N., Taylor, P. C., Szechinski, J., Pavelka, K., Broll, J., Balint, G., Emery, P., Raemen, F., Petersen, J., Smolen, J. et al. (2006). Double-blind randomized controlled clinical trial of the interleukin-6 receptor antagonist, tocilizumab, in European patients with rheumatoid arthritis who had an incomplete response to methotrexate. *Arthritis Rheum.* **54**, 2817-2829.

Malm, C., Nyberg, P., Engstrom, M., Sjodin, B., Lenkei, R., Ekblom, B. and IL (2000). Immunological changes in human skeletal muscle and blood after eccentric exercise and multiple biopsies. *J. Physiol* **529**, 243-262.

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F. and Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**, 412-419.

Matthews, V. B., Astrom, M. B., Chan, M. H., Bruce, C. R., Krabbe, K. S., Prelovsek, O., Akerstrom, T., Yfanti, C., Broholm, C., Mortensen, O. H. et al. (2009). Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase. *Diabetologia* **52**, 1409-1418.

Mattson, M. P., Maudsley, S. and Martin, B. (2004). BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. *Trends Neurosci.* **27**, 589-594.

Minokoshi, Y., Kim, Y. B., Peroni, O. D., Fryer, L. G., Muller, C., Carling, D. and Kahn, B. B. (2002). Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* **415**, 339-343.

Mokdad, A. H., Marks, J. S., Stroup, D. F. and Gerberding, J. L. (2004). Actual causes of death in the United States, 2000. *JAMA* **291**, 1238-1245.

Monninkhof, E. M., Elias, S. G., Vlems, F. A., van, d. T., I, Schuit, A. J., Voskuil, D. W. and van Leeuwen, F. E. (2007). Physical activity and breast cancer: a systematic review. *Epidemiology* **18**, 137-157.

Naylor, A. S., Bull, C., Nilsson, M. K., Zhu, C., Bjork-Eriksson, T., Eriksson, P. S., Blomgren, K. and Kuhn, H. G. (2008). Voluntary running rescues adult hippocampal neurogenesis after irradiation of the young mouse brain. *Proc. Natl. Acad. Sci. USA* **105**, 14632-14637.

Nielsen, A. R., Mounier, R., Plomgaard, P., Mortensen, O. H., Penkowa, M., Speerschneider, T., Pilegaard, H. and Pedersen, B. K. (2007). Expression of interleukin-15 in human skeletal muscle effect of exercise and muscle fibre type composition. *J. Physiol.* **584**, 305-312.

Nielsen, A. R., Hojman, P., Erikstrup, C., Fischer, C. P., Plomgaard, P., Mounier, R., Mortensen, O. H., Broholm, C., Taudorf, S., Krogh-Madsen, R. et al. (2008). Association between IL-15 and obesity: IL-15 as a potential regulator of fat mass. *J. Clin. Endocrinol. Metab.* **93**, 4486-4493.

Nishimoto, N., Yoshizaki, K., Miyasaka, N., Yamamoto, K., Kawai, S., Takeuchi, T., Hashimoto, J., Azuma, J. and Kishimoto, T. (2004). Treatment of rheumatoid arthritis with humanized anti-interleukin-6 receptor antibody: a multicenter, double-blind, placebo-controlled trial. *Arthritis Rheum.* **50**, 1761-1769.

Nishimoto, N., Kanakura, Y., Aozasa, K., Johkoh, T., Nakamura, M., Nakano, S., Nakano, N., Ikeda, Y., Sasaki, T., Nishioka, K. et al. (2005). Humanized anti-interleukin-6 receptor antibody treatment of multicentric Castleman disease. *Blood.* **106**, 2627-2632.

Nocon, M., Hiemann, T., Muller-Riemenschneider, F., Thalau, F., Roll, S. and Willich, S. N. (2008). Association of physical activity with all-cause and cardiovascular mortality: a systematic review and meta-analysis. *Eur. J. Cardiovasc. Prev. Rehabil.* **15**, 239-246.

Norrby, K. (1996). Interleukin-8 and de novo mammalian angiogenesis. *Cell Prolif.* **29**, 315-323.

Nybo, L., Nielsen, B., Pedersen, B. K., Moller, K. and Secher, N. H. (2002). Interleukin-6 release from the human brain during prolonged exercise. *J. Physiol.* **542**, 991-995.

Olsen, R. H., Krogh-Madsen, R., Thomsen, C., Booth, F. W. and Pedersen, B. K. (2008). Metabolic responses to reduced daily steps in healthy nonexercising men. *JAMA* **299**, 1261-1263.

Ostrowski, K., Rohde, T., Asp, S., Schjerling, P. and Pedersen, B. K. (1999). Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *J. Physiol.* **515**, 287-291.

Ostrowski, K, Schjerling, P. and Pedersen, BK (2000). Physical activity and plasma interleukin-6 in humans-effect of intensity of exercise. *Eur. J. Appl. Physiol.* **83**, 512-515.

Ouchi, N., Oshima, Y., Ohashi, K., Higuchi, A., Ikegami, C., Izumiya, Y. and Walsh, K. (2008). Follistatin-like 1, a secreted muscle protein, promotes endothelial cell function and revascularization in ischemic tissue through a nitric-oxide synthase-dependent mechanism. *J. Biol. Chem.* **283**, 32802-32811.

Paffenbarger, R. S., Jr, Lee, I. M. and Leung, R. (1994). Physical activity and personal characteristics associated with depression and suicide in American college men. *Acta Psychiatr. Scand. Suppl.* **377**, 16-22.

Pajonk, F. G., Wobrock, T., Gruber, O., Scherk, H., Berner, D., Kaizl, I., Kierer, A., Muller, S., Oest, M., Meyer, T. et al. (2010). Hippocampal plasticity in response to exercise in schizophrenia. *Arch. Gen. Psychiatry* **67**, 133-143.

Pedersen, B. K. (2006). The anti-inflammatory effect of exercise: its role in diabetes and cardiovascular disease control. *Essays Biochem.* **42**, 105-117.

Pedersen, B. K. (2007a). Body mass index-independent effect of fitness and physical activity for all-cause mortality. *Scand. J. Med. Sci. Sports.* **17**, 196-204.

Pedersen, B. K. (2007b). IL-6 signalling in exercise and disease. *Biochem. Soc. Trans.* **35**, 1295-1297.

Pedersen, B. K. (2009a). Edward F. Adolph distinguished lecture: muscle as an endocrine organ: IL-6 and other myokines. *J. Appl. Physiol.* **107**, 1006-1014.

Pedersen, B. K. (2009b). The Diseasome of Physical Inactivity and the role of myokines in muscle-fat cross talk. *J. Physiol.* **587**, 5559-5568.

Pedersen, B. K. and Febbraio, M. A. (2007). Point: Interleukin-6 does have a beneficial role in insulin sensitivity and glucose homeostasis. *J. Appl. Physiol.* **102**, 814-816.

Pedersen, B. K. and Febbraio, M. A. (2008). Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol. Rev.* **88**, 1379-1406.

Pedersen, B. K. and Fischer, C. P. (2007). Beneficial health effects of exercise-the role of IL-6 as a myokine. *Trends Pharmacol. Sci.* **28**, 152-156.

Pedersen, B. K., Akerstrom, T. C., Nielsen, A. R. and Fischer, C. P. (2007). Role of myokines in exercise and metabolism. *J. Appl. Physiol.* **103(3)**, 1093-1090.

Pedersen, M., Bruunsgaard, H., Weis, N., Hendel, H. W., Andreassen, B. U., Eldrup, E., Dela, F. and Pedersen, B. K. (2003). Circulating levels of TNF-alpha and IL-6-relation to truncal fat mass and muscle mass in healthy elderly individuals and in patients with type-2 diabetes. *Mech. Ageing Dev.* **124**, 495-502.

Penkowa, M., Keller, C., Keller, P., Jauffred, S. and Pedersen, B. K. (2003). Immunohistochemical detection of interleukin-6 in human skeletal muscle fibers following exercise. *FASEB J.* **17**, 2166-2168.

Pereira, A. C., Huddleston, D. E., Brickman, A. M., Sosunov, A. A., Hen, R., McKhann, G. M., Sloan, R., Gage, F. H., Brown, T. R. and Small, S. A. (2007). An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. *Proc. Natl. Acad. Sci. USA* **104**, 5638-5643.

Petersen, A. M. and Pedersen, B. K. (2005). The anti-inflammatory effect of exercise. *J. Appl. Physiol.* **98**, 1154-1162.

Petersen, A. M. and Pedersen, B. K. (2006). The role of IL-6 in mediating the anti-inflammatory effects of exercise. *J. Physiol. Pharmacol.* **57 Suppl. 10**, 43-51.

Petersen, E. W., Carey, A. L., Sacchetti, M., Steinberg, G. R., Macaulay, S. L., Febbraio, M. A. and Pedersen, B. K. (2005). Acute IL-6 treatment increases fatty acid turnover in elderly humans *in vivo* and in tissue culture *in vitro*: evidence that IL-6 acts independently of lipolytic hormones. *Am J. Physiol.* **288**, E155-E162.

Pischon, T., Boeing, H., Hoffmann, K., Bergmann, M., Schulze, M. B., Overvad, K., van der Schouw, Y. T., Spencer, E., Moons, K. G. M., Tjonneland, A. et al. (2008). General and Abdominal Adiposity and Risk of Death in Europe. *N. Engl. J. Med.* **359**, 2105-2120.

Plomgaard, P., Penkowa, M. and Pedersen, B. K. (2005). Fiber type specific expression of TNF-alpha, IL-6 and IL-18 in human skeletal muscles. *Exerc. Immunol. Rev.* **11**, 53-63.

Potthoff, M. J., Inagaki, T., Satapati, S., Ding, X., He, T., Goetz, R., Mohammadi, M., Finck, B. N., Mangelsdorf, D. J., Kliewer, S. A. et al. (2009). FGF21 induces PGC-1alpha and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. *Proc. Natl. Acad. Sci. USA* **106**, 10853-10858.

Quinn, L. S., Anderson, B. G., Strait-Bodey, L., Stroud, A. M. and Argiles, J. M. (2009). Oversecretion of interleukin-15 from skeletal muscle reduces adiposity. *Am. J. Physiol. Endocrinol. Metab.* **296**, E191-E202.

Rasmussen, P., Brassard, P., Adser, H., Pedersen, M. V., Leick, L., Hart, E., Secher, N. H., Pedersen, B. K. and Pilegaard, H. (2009). Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Exp. Physiol.* **94**, 1062-1069.

Rasmussen, P., Nielsen, J., Overgaard, M., Krogh-Madsen, R., Gjedde, A., Secher, N. H. and Petersen, N. C. (2010). Reduced muscle activation during exercise related to brain oxygenation and metabolism in humans. *J. Physiol.* **588**, 1985-1995.

Richardson, L. C. and Pollack, L. A. (2005). Therapy insight: Influence of type 2 diabetes on the development, treatment and outcomes of cancer. *Nat. Clin. Pract. Oncol.* **2**, 48-53.

Rotter, V., Nagaev, I. and Smith, U. (2003). Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. *J. Biol. Chem.* **278**, 45777-45784.

Rovio, S., Kareholt, I., Helkala, E. L., Viitanen, M., Winblad, B., Tuomilehto, J., Soininen, H., Nissinen, A. and Kivipelto, M. (2005). Leisure-time physical activity at midlife and the risk of dementia and Alzheimer's disease. *Lancet Neurol.* **4**, 705-711.

Sadagurski, M., Norquay, L., Farhang, J., D'Aquino, K., Copps, K. and White, M. F. (2010). Human IL6 enhances leptin action in mice. *Diabetologia.* **53**, 525-535.

Senn, J. J., Klover, P. J., Nowak, I. A. and Mooney, R. A. (2002). Interleukin-6 induces cellular insulin resistance in hepatocytes. *Diabetes* **51**, 3391-3399.

Senn, J. J., Klover, P. J., Nowak, I. A., Zimmers, T. A., Koniaris, L. G., Furlanetto, R. W. and Mooney, R. A. (2003). Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. *J. Biol. Chem.* **278**, 13740-13746.

Sherwood, D. E. and Selder, D. J. (1979). Cardiorespiratory health, reaction time and aging. *Med. Sci. Sports* **11**, 186-189.

Sibley, B. A. and Etnier, J. (2003). The relationship between physical activity and cognition in children: a meta-analysis. *Pediatr. Exerc. Sci.* **15**, 243-256.

Smolen, J. S., Beaulieu, A., Rubbert-Roth, A., Ramos-Remus, C., Rovensky, J., Alecock, E., Woodworth, T. and Alten, R. (2008). Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial. *Lancet* **371**, 987-997.

Starkie, R., Ostrowski, S. R., Jauffred, S., Febbraio, M. and Pedersen, B. K. (2003). Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans. *FASEB J.* **17**, 884-886.

Steensberg, A., van, H. G., Osada, T., Sacchetti, M., Saltin, B. and Klarlund, P. B. (2000). Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J. Physiol.* **529 Pt 1:237-42.** , 237-242.

Steensberg, A., Febbraio, M. A., Osada, T., Schjerling, P., van, H. G., Saltin, B. and Pedersen, B. K. (2001). Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. *J. Physiol.* **537**, 633-639.

Steensberg, A., Keller, C., Starkie, R. L., Osada, T., Febbraio, M. A. and Pedersen, B. K. (2002). IL-6 and TNF-alpha expression in, and release from, contracting human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* **283**, E1272-E1278.

Steensberg, A., Fischer, C. P., Keller, C., Moller, K. and Pedersen, B. K. (2003). IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *Am. J. Physiol. Endocrinol. Metab.* **285**, E433-E437.

Steinberg, G. R., Rush, J. W. and Dyck, D. J. (2003). AMPK expression and phosphorylation are increased in rodent muscle after chronic leptin treatment. *Am. J. Physiol. Endocrinol. Metab.* **284**, E648-E654.

Steinberg, G. R., Watt, M. J. and Febbraio, M. A. (2009). Cytokine regulation of AMPK signalling. *Front. Biosci.* **14:1902-16.** , 1902-1916.

Stranahan, A. M., Khalil, D. and Gould, E. (2007). Running induces widespread structural alterations in the hippocampus and entorhinal cortex. *Hippocampus.* **17**, 1017-1022.

Sun, Q., Townsend, M. K., Okereke, O. I., Franco, O. H., Hu, F. B. and Grodstein, F. (2010). Physical activity at midlife in relation to successful survival in women at age 70 years or older. *Arch. Intern. Med.* **170**, 194-201.

Tomporowski, P. D. (2003). Effects of acute bouts of exercise on cognition. *Acta Psychol. (Amst.)* **112**, 297-324.

Tuomilehto, J., Lindstrom, J., Eriksson, J. G., Valle, T. T., Hamalainen, H., Ilanne-Parikka, P., Keinanen-Kiukaanniemi, S., Laakso, M., Louheranta, A., Rastas, M. et al. (2001). Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N. Engl. J. Med.* **344**, 1343-1350.

Tyler, W. J., Alonso, M., Bramham, C. R. and Pozzo-Miller, L. D. (2002). From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. *Learn. Mem.* **9**, 224-237.

Ueki, K., Kondo, T. and Kahn, C. R. (2004). Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. *Mol. Cell. Biol.* **24**, 5434-5446.

van Hall, G., Steensberg, A., Sacchetti, M., Fischer, C., Keller, C., Schjerling, P., Hiscock, N., Moller, K., Saltin, B., Febbraio, M. A. et al. (2003). Interleukin-6 stimulates lipolysis and fat oxidation in humans. *J. Clin. Endocrinol. Metab.* **88**, 3005-3010.

van Praag, H., Christie, B. R., Sejnowski, T. J. and Gage, F. H. (1999). Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc. Natl. Acad. Sci. USA* **96**, 13427-13431.

Wallenius, V., Wallenius, K., Ahren, B., Rudling, M., Carlsten, H., Dickson, S. L., Ohlsson, C. and Jansson, J. O. (2002). Interleukin-6-deficient mice develop mature-onset obesity. *Nat. Med.* **8**, 75-79.

Walsh, K. (2009). Adipokines, myokines and cardiovascular disease. *Circ. J.* **73**, 13-18.

Watt, M. J., Dzamko, N., Thomas, W. G., Rose-John, S., Ernst, M., Carling, D., Kemp, B. E., Febbraio, M. A. and Steinberg, G. R. (2006). CNTF reverses obesity-induced insulin resistance by activating skeletal muscle AMPK. *Nat. Med.* **12**, 541-548.

Waugh, D. J. and Wilson, C. (2008). The interleukin-8 pathway in cancer. *Clin. Cancer Res.* **14**, 6735-6741.

Whitmer, R. A., Gustafson, D. R., Barrett-Connor, E., Haan, M. N., Gunderson, E. P. and Yaffe, K. (2008). Central obesity and increased risk of dementia more than three decades later. *Neurology.* **71**, 1057-1064.

Wilund, K. R. (2007). Is the anti-inflammatory effect of regular exercise responsible for reduced cardiovascular disease? *Clin. Sci. (Lond).* **112**, 543-555.

Wisse, B. E. and Schwartz, M. W. (2003). The skinny on neurotrophins. *Nat. Neurosci.* **6**, 655-656.

Wolin, K. Y., Yan, Y., Colditz, G. A. and Lee, I. M. (2009). Physical activity and colon cancer prevention: a meta-analysis. *Br. J. Cancer* **100**, 611-616.

Wu, C. W., Chang, Y. T., Yu, L., Chen, H. I., Jen, C. J., Wu, S. Y., Lo, C. P. and Kuo, Y. M. (2008). Exercise enhances the proliferation of neural stem cells and neurite growth and survival of neuronal progenitor cells in dentate gyrus of middle-aged mice. *J. Appl. Physiol.* **105**, 1585-1594.

Xue, F. and Michels, K. B. (2007). Diabetes, metabolic syndrome, and breast cancer: a review of the current evidence. *Am. J. Clin. Nutr.* **86**, s823-s835.

Yoon, M. C., Shin, M. S., Kim, T. S., Kim, B. K., Ko, I. G., Sung, Y. H., Kim, S. E., Lee, H. H., Kim, Y. P. and Kim, C. J. (2007). Treadmill exercise suppresses nigrostriatal dopaminergic neuronal loss in 6-hydroxydopamine-induced Parkinson's rats. *Neurosci. Lett.* **423**, 12-17.

Yudkin, J. S. (2007). Inflammation, obesity, and the metabolic syndrome. *Horm. Metab. Res.* **39**, 707-709.