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The anti-inflammatory effect of exercise: its role in diabetes and cardiovascular disease control

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Abstract

Chronic low-grade systemic inflammation is a feature of chronic diseases such as cardiovascular disease and type 2 diabetes. Regular exercise offers protection against all-cause mortality, primarily by protection against atherosclerosis and insulin resistance and there is evidence that physical training is effective as a treatment in patients with chronic heart diseases and type 2 diabetes.

Regular exercise induces anti-inflammatory actions. During exercise, IL-6 (interleukin-6) is produced by muscle fibres. IL-6 stimulates the appearance in the circulation of other anti-inflammatory cytokines such as IL-1ra (interleukin-1 receptor antagonist) and IL-10 (interleukin-10) and inhibits the production of the pro-inflammatory cytokine TNF-α (tumour necrosis factor-α). In addition, IL-6 enhances lipid turnover, stimulating lipolysis as well as fat oxidation. It is suggested that regular exercise induces suppression of TNF-α and thereby offers protection against TNF-α-induced insulin resistance. Recently, IL-6 was introduced as the first myokine, defined as a cytokine, that is produced

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and released by contracting skeletal muscle fibres, exerting its effects in other organs of the body. Myokines may be involved in mediating the beneficial health effects against chronic diseases associated with low-grade inflammation such as diabetes and cardiovascular diseases.

Introduction

Cardiovascular disease and type 2 diabetes are not only leading causes of death and illness in developed countries, but these chronic diseases are becoming the dominating health problem worldwide [1]. Regular exercise offers protection against all-cause mortality, primarily by protection against atherosclerosis and type 2 diabetes [2]. In addition, physical training is effective in the treatment of patients with ischaemic heart disease and type 2 diabetes [3].

Over the past decade, there has been an increasing focus on the role of inflammation in the pathogenesis of atherosclerosis [4]. Further, inflammation has been suggested to be a key factor in insulin resistance [5]. Low-grade chronic inflammation is reflected by increased systemic levels of some cytokines [6] as well as CRP (C-reactive protein). Several reports investigating various markers of inflammation have confirmed an association between low-grade systemic inflammation on one hand and atherosclerosis and type 2 diabetes on the other [7]. Recent findings demonstrate that physical activity induces an increase in the systemic levels of a number of cytokines with anti-inflammatory properties [8]. Skeletal muscle has recently been identified as an endocrine organ, that produces and releases cytokines (termed myokines) [8–11].

Given that skeletal muscle is the largest organ in the human body, the discovery that contracting muscle is a cytokine producing organ opens a new paradigm: skeletal muscle is an endocrine organ that by contraction stimulates the production and release of cytokines, which can influence metabolism and modify cytokine production in tissue and organs (Figure 1).

This chapter reviews the evidence for physical training as a means to treat cardiovascular disease, insulin resistance and type 2 diabetes and discusses to what extent anti-inflammatory activity induced by regular exercise may exert the beneficial health effects in these disorders.

Clinical evidence for physical training in coronary heart disease, insulin resistance and type 2 diabetes

Coronary heart disease

The evidence for a beneficial effect of physical training in patients with coronary heart disease is strong. Physical training improves survival and is believed to have direct effects on the pathogenesis of the disease [3]. A meta-analysis was published in 2004 [12] based on 48 randomized controlled trials and 8940 patients. The patients were typically randomized at the time of

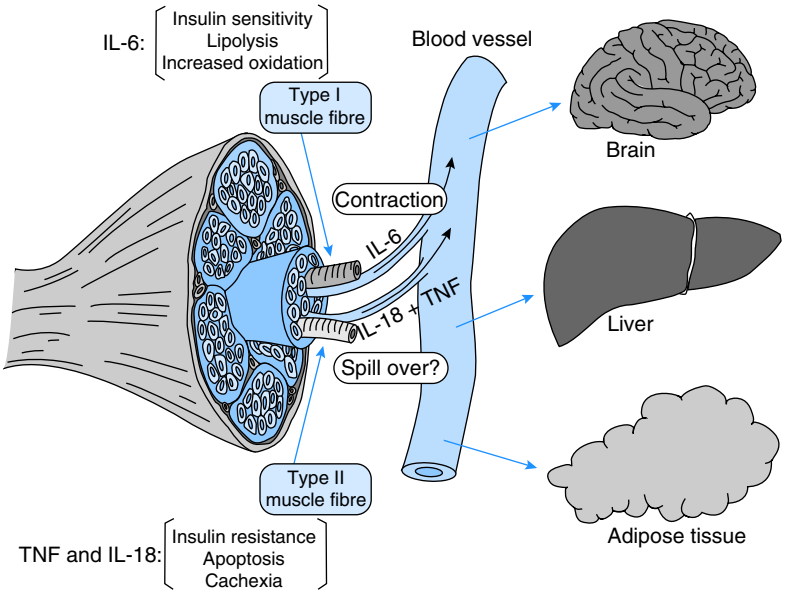

Figure 1. Skeletal muscle is an endocrine organ, that expresses and releases cytokines (also termed myokines) into the circulation and potentially influences metabolism and the inflammatory status in tissue and organs

acute myocardial infarction or up to six weeks thereafter. The exercise training was predominantly aerobic, but varied considerably as regards frequency, intensity and duration. Exercise-based cardiac rehabilitation reduced all-cause mortality by 20% (OR 0.80; 95% CI 0.68–0.93). Exercise-based cardiac rehabilitation reduced cardiac mortality by 26% (OR 0.74; 95% CI 0.61–0.96). Exercise-based cardiac rehabilitation in addition reduced total cholesterol, triglyceride levels and systolic blood pressure. More patients in the exercise-based cardiac rehabilitation group ceased smoking (OR 0.64; 95% CI 0.50–0.83). There was no effect on non-fatal myocardial infarction. In summary, exercise has pronounced health outcome effects in patients with cardiac diseases.

Insulin resistance

Few studies have examined the isolated effect of training on the prevention of diabetes in patients with impaired glucose tolerance, but there is good evidence for a beneficial effect of combined physical training and dietary modification. Two randomized controlled trials including people with impaired glucose tolerance have found that lifestyle modification protects against the development of type 2 diabetes. A Finnish trial randomized 522 overweight middle-aged people with impaired glucose tolerance to a physical training combined with diet group or to a control group and followed them for 3.2 years [13]. The risk of type 2 diabetes was reduced by

58% in the intervention group. The effect was greatest in the patients who made the greatest lifestyle modification. An American trial randomized 3234 people with impaired glucose tolerance to either treatment with metformin, lifestyle modification entailing dietary change and at least 150 min of physical exercise weekly, or placebo, and followed them for 2.8 years [14]. The lifestyle modification reduced the risk of type 2 diabetes by 58%. The reduction was thus the same as in the Finnish trial [13], whereas treatment with metformin only reduced the risk of diabetes by 31%. It is not possible to determine the isolated effect of exercise in these trials [13,14], in which the intervention was combined exercise and diet. In summary, there is strong evidence that exercise protects against development of type 2 diabetes in patients with insulin resistance.

Type 2 diabetes

The beneficial effect of training in patients with type 2 diabetes is very well documented, and there is international consensus that physical training comprises one of the three cornerstones of the treatment of diabetes together with diet and medicine. A meta-analysis published in 2001 examined the effect of at least eight weeks of physical training on glycaemic control [15]. The meta-analysis included 14 controlled clinical trials encompassing a total of 504 patients. Twelve of the trials examined the effect of aerobic training [mean (S.D.); 3.4 (0.9) times/week for 18 (15) weeks], whilst two examined the effect of strength conditioning [mean (S.D.); 10 (0.7) exercises, 2.5 (0.7) sets, 13 (0.7) repetitions, 2.5 (0.4) times/week for 15 (10) weeks]. No differences could be identified between the effect of aerobic training and strength conditioning. Neither could any dose–response effect be demonstrated relative to either the intensity or the duration of training. Post-intervention, HbA1c (haemoglobin A1c) was lower in the exercise groups than in the control groups (7.65% versus 8.31%; weighted mean difference, 0.66%; $P<0.001$). In comparison, intensive glycaemic control with metformin reduced HbA1c by 0.6%, whereas it reduced the risk of diabetes-related complications by 32% and the risk of diabetes-related mortality by 42% [16]. A meta-analysis encompassing 95 783 non-diabetic individuals showed that cardiovascular morbidity is strongly correlated to fasting blood glucose [17]. The effect of physical training on HbA1c is thus clinically relevant and there is evidence to support exercise recommendations in patients with type 2 diabetes.

The players in chronic low-grade inflammation and its link with chronic diseases

The local inflammatory response is accompanied by a systemic response known as the acute phase response [8]. This response includes the production of a large number of hepatocyte-derived acute phase proteins, such as CRP and can be mimicked by the injection of the cytokines TNF-α (tumour necrosis

factor-α), IL-1β (interleukin-1β) and IL-6 (interleukin-6) into laboratory animals or humans. The initial cytokines in the cytokine cascade are (named in order): TNF -α, IL-1β, IL-6, IL-1ra (interleukin-1 receptor antagonist) and sTNF-R (soluble TNF-α-receptors). IL-1ra inhibits IL-1 signal transduction, and sTNF-R represents the naturally occurring inhibitors of TNF-α. In response to an acute infection or trauma, the cytokines and cytokine inhibitors may increase 3- to 4-fold and decrease after recovery. Chronic low-grade systemic inflammation has been introduced as a term for conditions in which a 2- to 3-fold increase in the systemic concentrations of TNF-α, IL-1, IL-6, IL-1ra, sTNF-R and CRP is reflected. In the latter case, the stimuli for the cytokine production are not known, but the likely origin of TNF-α in chronic low-grade systemic inflammation is mainly the adipose tissue.

The link between inflammation, insulin resistance and atherosclerosis

Ageing is associated with increased resting plasma levels of TNF-α, IL-6, IL-1ra, sTNF-R and CRP [18]. High levels of TNF-α are associated with dementia and atherosclerosis [19]. Also, elevated levels of circulating IL-6 are associated with several disorders. Increased levels of both TNF-α and IL-6 are observed in obese individuals, in smokers and in patients with type 2 diabetes mellitus. Plasma concentrations of IL-6 have been shown to predict all-cause mortality as well as cardiovascular mortality. Furthermore, plasma concentrations of IL-6 and TNF-α have been shown to predict the risk of myocardial infarction in several studies, and the CRP level is shown to be a stronger predictor of cardiovascular events than the low density lipoprotein cholesterol level.

Mounting evidence suggests that TNF-α plays a direct role in the metabolic syndrome [20]. Patients with diabetes demonstrate high mRNA and protein expression of TNF-α in skeletal muscle and increased TNF-α levels in plasma and it is likely that adipose tissue, which produces TNF-α, is the main source of the circulating TNF-α. Mounting evidence points to an effect of TNF-α on insulin signalling. TNF-α impairs insulin-stimulated rates of glucose storage in cultured human muscle cells and impairs insulin mediated glucose uptake in rats. Obese mice with a gene knockout of TNF-α are protected from insulin resistance and inhibition of TNF-α with an anti-TNF-α antibody treatment improves the insulin sensitivity in the insulin resistance rat model. *In vitro* studies demonstrate that TNF-α has direct inhibitory effects on insulin signalling. Recently, it was demonstrated that TNF-α infusion in healthy humans induces insulin resistance in skeletal muscle, without an effect on endogenous glucose production. TNF-α directly impaired glucose uptake and metabolism by altering insulin signal transduction. These data provide a direct molecular link between low-grade systemic inflammation and insulin resistance [20]. It has also been proposed that TNF-α indirectly causes insulin resistance *in vivo*

by increasing the release of NEFAs (non-esterified fatty acids) from adipose tissue. TNF-α increases lipolysis in human, rat and 3T3-L1 adipocytes. Recently, it was found that TNF-α had no effect on muscle fatty acid oxidation, but increased fatty acid incorporation into diacylglycerol, which may be involved in the development of TNF-α-induced insulin resistance in skeletal muscle.

Recent evidence suggests that TNF-α plays a key role in linking insulin resistance to vascular disease. Several downstream mediators and signalling pathways seem to provide the crosstalk between inflammatory and metabolic signalling. These include the discovery of JNK (c-Jun N-terminal kinase) and IκK (IκB kinase) as critical regulators of insulin action activated by TNF-α [21]. In human TNF-α infusion studies, TNF-α increases phosphorylation of the p70 S6 kinase, extracellular signal-regulated kinase-1/2 and JNK, concomitant with increased serine and reduced tyrosine phosphorylation of insulin receptor substrate-1. These signalling effects are associated with impaired phosphorylation of Akt substrate 160, the most proximal step identified in the insulin signalling cascade regulating GLUT4 translocation and glucose uptake [22] (Figure 2).

With regard to IL-6, its role in insulin resistance is highly controversial. In humans, circulating IL-6 levels may or may not be associated with insulin resistance [23]. Infusion of recombinant human (rh) IL-6 into resting

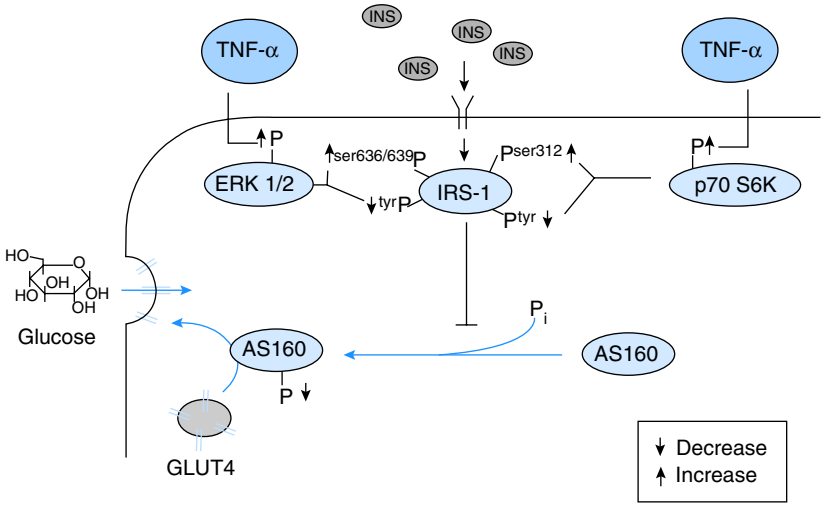

Figure 2. TNF-α represents a molecular link between low-grade systemic inflammation and the metabolic syndrome

TNF-α infusion to humans increases phosphorylation of p70 S6 kinase, ERK-1/2 (extracellular signal-regulated kinase-1/2) and JNK, concomitant with increased serine and reduced tyrosine phosphorylation of IRS (insulin receptor substrate)-1. These signalling effects are associated with impaired phosphorylation of AS160 (Akt substrate 160), the most proximal step identified in the insulin signalling cascade regulating GLUT4 translocation and glucose uptake. Thus excessive concentrations of TNF-α negatively regulate insulin signalling and whole-body glucose uptake in humans.

healthy humans does not impair whole body, lower limb or subcutaneous adipose tissue glucose uptake or EGP (endogenous glucose production), although IL-6 contributes to the contraction-induced increase in EGP. When diabetes patients were given an rhIL-6 infusion, plasma concentrations of insulin decreased to levels comparable with that in age and BMI (body mass index)-matched healthy controls, indicating that the IL-6 enhanced insulin sensitivity. *In vitro* studies demonstrate that IL-6 can induce insulin resistance in isolated 3T3-L1 adipocytes and in mice. However, the IL-6 dose applied in the latter studies was supraphysiological, and is therefore probably not relevant to human physiology. Interestingly, IL-6 knockout mice develop impaired glucose tolerance that is reverted by IL-6 [24]. Thus accumulating data suggest that IL-6 enhances glucose uptake in myocytes.

AMPK (AMP-activated protein kinase) activity stimulates a variety of processes that increases ATP generation including fatty acid oxidation and glucose transport in skeletal muscle [25]. Incubation with IL-6 increases the phosphorylation of AMPK (an indicator of its activation) and that of its target molecule, ACC (acetyl-CoA carboxylase) in skeletal muscles. In addition, AMPK activity and ACC levels are very low in IL-6 knockout mice, suggesting a role of IL-6 in the regulation of AMPK activity. These data suggest that IL-6 activation of AMPK is dependent on the presence of IL-6 [26].

A number of studies indicate that IL-6 enhances lipolysis [27–31], as well as fat oxidation [30]. Consistent with this idea, Wallenius et al. [24] demonstrated that IL-6 deficient mice developed mature-onset obesity and insulin resistance. In addition, when the mice were treated with IL-6, there was a significant decrease in body fat mass in the IL-6 knockout, but not in the wild-type mice. To determine whether physiological concentrations of IL-6 affected lipid metabolism, our group administered physiological concentrations of rhIL-6 to healthy young and elderly humans as well as patients with type 2 diabetes [30,32]. The latter studies identified IL-6 as a potent modulator of fat metabolism in humans, increasing lipolysis as well as fat oxidation without causing hypertriacylglycerolaemia.

Of note, whereas it is known that both TNF-α and IL-6 induce lipolysis, only IL-6 appears to induce fat oxidation [23]. High levels of IL-6 and TNF-α in patients with the metabolic syndrome is associated with truncal fat mass and both TNF-α and IL-6 are produced in adipose tissue. Given the different biological profiles of TNF-α and IL-6 and given that TNF-α can trigger IL-6 release, one theory holds that it is TNF-α derived from adipose tissue that actually is the 'driver' behind insulin resistance and cardiovascular diseases and that locally produced TNF-α causes increased systemic levels of IL-6.

The cytokine response to exercise

In sepsis and experimental models of sepsis, the cytokine cascade consists of (named in order): TNF-α, IL-1β, IL-6, IL-1ra, sTNF-R and IL-10

(interleukin-10) [33]. The first two cytokines in the cytokine cascade are TNF-α and IL-1β, which are produced locally. These cytokines are usually referred to as pro-inflammatory cytokines. TNF-α and IL-1β stimulate the production of IL-6, which has been classified as both a pro- and an anti-inflammatory cytokine. The cytokine response to exercise differs from that elicited by severe infections [34–37]. The fact that the classical pro-inflammatory cytokines, TNF-α and IL-1β, in general do not increase with exercise indicates that the cytokine cascade induced by exercise markedly differs from the cytokine cascade induced by infections. Typically, IL-6 is the first cytokine released into the circulation during exercise. The level of circulating IL-6 increases in an exponential fashion (up to 100-fold) in response to exercise, and declines in the post-exercise period [34–37] (Figure 3).

The circulating levels of well-known anti-inflammatory cytokines and cytokine inhibitors such as IL-1ra and sTNF-R also increase after exercise.

Taken together, exercise provokes an increase primarily in IL-6, followed by an increase in IL-1ra and IL-10. The appearance of IL-6 in the circulation is by far the most marked and its appearance precedes that of the other cytokines. The IL-6 response to exercise has recently been reviewed [10,23,34–36]. A marked increase in circulating levels of IL-6 after exercise without muscle damage has been a remarkably consistent finding. Plasma IL-6 increases in an exponential fashion with exercise and is related to exercise intensity, duration, the mass of muscle recruited and one's endurance capacity [10,34–36]. In 2000, Steensberg et al. [38] published the first article demonstrating that most of the IL-6 seen in the circulation was likely to be derived from the contracting limb. Using a single-legged kicking model and measuring arteriovenous difference and blood flow across the contracting and non-contracting limb, it was clear that net release from the contracting limb was marked. This study has been followed by many others that confirmed the net limb release of IL-6 is marked and that the IL-6 mRNA levels in biopsy samples taken from the contracting limb rapidly increases above baseline values. However, it was only recently confirmed that the myocytes themselves produce IL-6. A qualitative elevation in IL-6 protein measured in muscle cells within human muscle biopsy sections using immunohistochemistry has been reported. In a follow-up study, however, definitive evidence was found that myocytes themselves are a major source of contraction-induced IL-6. In addition to immunohistochemistry techniques, *in situ* hybridization assays were performed on muscle cross-sections before and after exercise. Consistent with the immunohistochemical data, IL-6 mRNA was almost absent in cross-sections before exercise, but prominent after contraction.

The cytokine IL-6 exerts it actions via the IL-6R (IL-6 receptor) in conjunction with the ubiquitously expressed gp130 receptor. IL-6 is regulated in an autocrine fashion [39]. In accordance, acute exercise induces IL-6R expression in the post-exercise period after exercise suggesting a post-exercise-sensitizing mechanism to IL-6. We further demonstrated, that after a ten week training

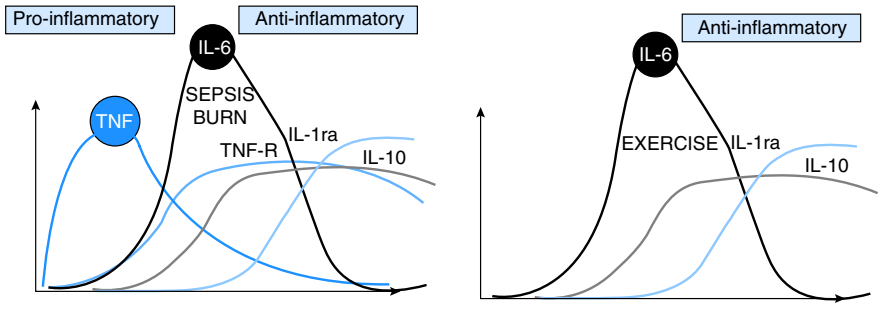

Figure 3. In sepsis, the cytokine cascade within the first few hours consists of TNF-α, IL-6, IL-1ra, sTNF-R and IL-10

The cytokine response to exercise does not include TNF-α but does show a marked increase in IL-6, which is followed by IL-1ra, sTNF-R and IL-10. Increased CRP levels do not appear until 8–12 h later.

period, IL-6R mRNA production was increased in skeletal muscle, suggesting a sensitization of skeletal muscle to IL-6 at rest.

Studies have reported that carbohydrate ingestion attenuates elevations in plasma IL-6 during both running and cycling. Low muscle glycogen concentration further enhances IL-6 mRNA and the transcription rate for IL-6. Therefore, pre-exercise intramuscular glycogen content appears to be an important stimulus for IL-6 gene transcription. It appears that muscle-derived IL-6 acts as an energy sensor.

Most studies of muscle-derived IL-6 have been performed in healthy young volunteers, exercised at high intensities. However, the clinical relevance of muscle-derived IL-6 is supported by the findings that even moderate exercise has major effects on muscle-derived IL-6. Young healthy individuals performed 3 h of dynamic two-legged knee-extensor exercise at 50% of their individual maximal power output. This exercise induced an only moderate increase in heart rate (from 113 to 122 beats·min^{-1}), but induced a 16-fold increase in IL-6 mRNA, a 20-fold increase in plasma IL-6 and a marked IL-6 release from working muscle. When the same model was applied in elderly healthy untrained subjects, even higher amounts of IL-6 were released from working muscle during exercise at the same relative intensity.

Studies have demonstrated that monocytes are not major contributors to the IL-6 response to exercise. However, small amounts of IL-6 are also produced and released from adipose tissue, and studies indicate that also the brain and peritendon tissue may release IL-6 in response to exercise. Although we have yet to determine the precise biological action of muscle-derived IL-6, accumulating data support the hypothesis that the role of IL-6 released from contracting muscle during exercise is to act in a hormone-like manner to mobilize extracellular substrates and/or augment substrate delivery during exercise. In addition, IL-6 has important anti-inflammatory effects.

The anti-inflammatory effects of IL-6

A couple of studies suggest that IL-6 may exert inhibitory effects on TNF-α [8]. IL-6 inhibits lipopolysaccharide-induced TNF-α production both in cultured human monocytes and in the human monocytic line U937. Furthermore, levels of TNF-α are markedly elevated in anti-IL-6-treated mice and in IL-6 deficient knockout mice, indicating that circulating IL-6 is involved in the regulation of TNF-α levels. In addition, rhIL-6 infusion inhibits the endotoxin-induced increase in circulating levels of TNF-α in healthy humans. Lastly, IL-6 stimulates the release of soluble TNF-α receptors, but not IL-1β and TNF-α, and appears to be the primary inducer of the hepatocyte derived acute-phase proteins, many of which have anti-inflammatory properties.

The anti-inflammatory effects of IL-6 are also demonstrated by the fact that IL-6 stimulates the production of IL-1ra and IL-10. The appearance of IL-10 and IL-1ra in the circulation following exercise also contributes to mediating the anti-inflammatory effects of exercise. IL-10 inhibits the production of IL-1α, IL-1β and TNF-α as well as the production of chemokines, including IL-8 and macrophage inflammatory protein-α from lipopolysaccharide-activated human monocytes. These cytokines and chemokines play a critical role in the activation of granulocytes, monocytes/macrophages and lymphocytes and in their recruitment to the sites of inflammation. Whereas IL-10 influences multiple cytokines, the biological role of IL-1ra is to inhibit signal transduction through the IL-1 receptor complex.

The anti-inflammatory effects of acute exercise and regular training

An association between physical inactivity and low-grade systemic inflammation has been demonstrated in cross-sectional studies including healthy younger individuals, elderly people, as well as in patients with intermittent claudication [40]. These data, however, do not provide any information with regard to a possible causal relationship. Longitudinal studies show that regular training induces a reduction in CRP levels and suggest that physical activity may suppress systemic low-grade inflammation. To study whether acute exercise induces a true anti-inflammatory response, a model of 'low grade inflammation' was established in which we injected a low dose of *Escherichia coli* endotoxin to healthy volunteers, who had been randomized to either rest or exercise prior to endotoxin administration. In resting subjects, endotoxin induced a 2- to 3-fold increase in circulating levels of TNF-α. In contrast, when the subjects performed 3 h of ergometer cycling and received the endotoxin bolus at 2.5 h, the TNF-α response was totally blunted.

Following exercise, the high circulating levels of IL-6 are followed by an increase in IL-1ra and IL-10, and the latter two anti-inflammatory cytokines can be induced by IL-6. Therefore, IL-6 induces an anti-inflammatory environment by inducing the production of IL-1ra and IL-10, but it also inhibits

TNF-α production as suggested by *in vitro* and animal studies. In addition, rhIL-6 infusion inhibited the endotoxin-induced increase in plasma TNF-α in humans. The possibility exists that with regular exercise, the anti-inflammatory effects of an acute bout of exercise will protect against chronic systemic low-grade inflammation, but such a link between the acute effects of exercise and the long-term benefits has not yet been proven. Given that the atherosclerotic process is characterized by inflammation, one alternative explanation would be that regular exercise, which offers protection against atherosclerosis, indirectly offers protection against vascular inflammation and hence systemic low-grade inflammation.

Conclusion

The long-term effect of exercise on the progression of disease may be ascribed to the anti-inflammatory response elicited by an acute bout of exercise, which in part is mediated by muscle-derived IL-6. These anti-inflammatory effects of exercise may offer protection against TNF-induced insulin resistance. It is suggested that muscle contraction-induced factors, so-called myokines, may be involved in mediating the health benefits of exercise and play important roles in the protection against diseases associated with low-grade inflammation such as cardiovascular diseases and type 2 diabetes.

Summary

- *Low-grade chronic systemic inflammation accompanies chronic diseases such as cardiovascular disease and type 2 diabetes.*
- *Regular exercise induces an anti-inflammatory response.*
- *During exercise, skeletal muscle releases IL-6.*
- *IL-6 has anti-inflammatory actions and modulates glucose and lipid metabolism.*
- *Muscle-derived cytokines, termed myokines, are likely to mediate the health benefits against chronic diseases.*

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References

1. Murray, C.J. & Lopez. A.D. (1997) Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. *Lancet* **349**, 1436–1442
2. Blair, S.N., Cheng, Y. & Holder, J.S. (2001) Is physical activity or physical fitness more important in defining health benefits? *Med. Sci. Sports Exercise* **33**, S379–S399
3. Pedersen, B.K. & Saltin, B. (2006) Evidence for prescribing exercise as therapy in chronic disease. *Scand. J. Med. Sci. Sports* **16** (Suppl 1), 3–63
4. Libby, P. (2002) Inflammation in atherosclerosis. *Nature* **420**, 868–874
5. Dandona, P., Aljada, A. & Bandyopadhyay, A. (2004) Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol.* **25**, 4–7
6. Ross, R. (1999) Atherosclerosis: an inflammatory disease. *N. Engl. J. Med.* **340**, 115–126
7. Festa, A., D'Agostino, Jr, R., Tracy, R.P. & 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
8. Petersen, A.M. & Pedersen, B.K. (2005) The anti-inflammatory effect of exercise. *J. Appl. Physiol.* **98**, 1154–1162
9. Pedersen, B.K. & Febbraio, M. (2005) Muscle-derived interleukin-6: A possible link between skeletal muscle, adipose tissue, liver, and brain. *Brain Behav. Immun.* **19**, 371–376
10. Pedersen, B.K., Steensberg, A., Fischer, C., Keller, C., Keller, P., Plomgaard, P., Febbraio, M. & Saltin, B. (2003) Searching for the exercise factor: is IL-6 a candidate. *J. Muscle Res. Cell Motil.* **24**, 113–119
11. Pedersen, B.K., Steensberg, A., Fischer, C., Keller, C., Keller, P., Plomgaard, P., Wolsk-Petersen, E. & Febbraio, M. (2004) The metabolic role of IL-6 produced during exercise: is IL-6 an exercise factor? *Proc. Nutr. Soc.* **63**, 263–267
12. Taylor, R.S., Brown, A., Ebrahim, S., Jolliffe, J., Noorani, H., Rees, K., Skidmore, B., Stone, J.A., Thompson, D.R. & Oldridge, N. (2004) Exercise-based rehabilitation for patients with coronary heart disease: systematic review and meta-analysis of randomized controlled trials. *Am. J. Med.* **116**, 682–692
13. 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
14. Knowler, W.C., Barrett-Connor, E., Fowler, S.E., Hamman, R.F., Lachin, J.M., Walker, E.A. & Nathan, D.M. (2002) Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N. Engl. J. Med.* **346**, 393–403
15. Boule, N.G., Haddad, E., Kenny, G.P., Wells, G.A. & Sigal, R.J. (2001) Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. *J. Am. Med. Assoc.* **286**, 1218–1227
16. UK Prospective Diabetes Study (UKPDS) Group. (1998) Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet* **352**, 854–865
17. Coutinho, M., Gerstein, H.C., Wang, Y. & Yusuf, S. (1999) The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95783 individuals followed for 12.4 years. *Diabetes Care* **22**, 233–240
18. Bruunsgaard, H. (2005) Physical activity and modulation of systemic low-level inflammation. *J. Leukocyte Biol.* **78**, 819–835
19. Bruunsgaard, H., Andersen-Ranberg, K., Jeune, B., Pedersen, A.N., Skinhoj, P. & Pedersen, B.K. (1999) A high plasma concentration of TNF-α is associated with dementia in centenarians. *J. Gerontol. Ser. A* **54**, M357–M364
20. Plomgaard, P., Bouzakri, K., Krogh-Madsen, R., Mittendorfer, B., Zierath, J.R. & Pedersen, B.K. (2005) Tumor necrosis factor-α induces skeletal muscle insulin resistance in healthy human subjects via inhibition of Akt substrate 160 phosphorylation. *Diabetes* **54**, 2939–2945

21. Hotamisligil, G.S. (2003) Inflammatory pathways and insulin action. *Int. J. Obes. Relat. Metab. Disord.* **27** (Suppl 3), S53–S55
22. Plomgaard, P., Keller, P., Keller, C. & Pedersen, B.K. (2005) TNF-α, but not IL-6, stimulates plasminogen activator inhibitor-1 expression in human subcutaneous adipose tissue. *J. Appl. Physiol.* **98**, 2019–2023
23. Febbraio, M.A. & Pedersen, B.K. (2005) Contraction-induced myokine production and release: is skeletal muscle an endocrine organ? *Exerc. Sport Sci. Rev.* **33**, 114–119
24. Wallenius, V., Wallenius, K., Ahren, B., Rudling, M., Carlsten, H., Dickson, S.L., Ohlsson, C. & Jansson, J.O. (2002) Interleukin-6-deficient mice develop mature-onset obesity. *Nat. Med.* **8**, 75–79
25. Carling, D. (2004) The AMP-activated protein kinase cascade-a unifying system for energy control. *Trends Biochem. Sci.* **29**, 18–24
26. Kelly, M., Keller, C., Avilucea, P.R., Keller, P., Luo, Z., Xiang, X., Giralt, M., Hidalgo, J., Saha, A.K. & Pedersen, B.K. (2004) AMPK activity is diminished in tissues of the IL-6 knockout mice: the effect of exercise. *Biochem. Biophys. Res. Commun.* **320**, 449–454
27. Bruce, C.R. & Dyck, D.J. (2004) Cytokine regulation of skeletal muscle fatty acid metabolism: effect of interleukin-6 and tumor necrosis factor-α. *Am. J. Physiol. Endocrinol. Metab.* **287**, E616–E621
28. Nonogaki, K., Fuller, G.M., Fuentes, N.L., Moser, A.H., Staprans, I., Grunfeld, C. & Feingold, K.R. (1995) Interleukin-6 stimulates hepatic triglyceride secretion in rats. *Endocrinology* **136**, 2143-2149
29. Path, G., Bornstein, S.R., Gurniak, M., Chrousos, G.P., Scherbaum, W.A. & Hauner, H. (2001) Human breast adipocytes express interleukin-6 (IL-6) and its receptor system: increased IL-6 production by β-adrenergic activation and effects of IL-6 on adipocyte function. *J. Clin. Endocrinol. Metab.* **86**, 2281–2288
30. Petersen, E.W., Carey, A.L., Sacchetti, M., Steinberg, G.R., Macaulay, S.L., Febbraio, M.A. & Pedersen, B.K. (2005) 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
31. Stouthard, J.M., Romijn, J.A., van der P.T., Endert, E., Klein, S., Bakker, P.J., Veenhof, C.H. & Sauerwein, H.P. (1995) Endocrinologic and metabolic effects of interleukin-6 in humans. *Am. J. Physiol.* **268**, E813–E819
32. Van Hall, G., Steensberg, A., Sacchetti, M., Fischer, C., Keller, C., Schjerling, P., Hiscock, N., Moller, K., Saltin, B., Febbraio, M.A. & Pedersen, B.K. (2003) Interleukin-6 stimulates lipolysis and fat oxidation in humans. *J. Clin. Endocrinol. Metab.* **88**, 3005–3010
33. Akira, S., Taga, T. & Kishimoto, T. (1993) Interleukin-6 in biology and medicine. *Adv. Immunol.* **54**, 1–78
34. Febbraio, M.A. & Pedersen, B.K. (2002) Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. *FASEB J.* **16**, 1335–1347
35. Pedersen, B.K. & Hoffman-Goetz, L. (2000) Exercise and the immune system: regulation, integration and adaption. *Physiol. Rev.* **80**, 1055–1081
36. Pedersen, B.K., Steensberg, A. & Schjerling, P. (2001) Muscle-derived interleukin-6: possible biological effects. *J. Physiol. (London)* **536**, 329–337
37. Suzuki, K., Nakaji, S., Yamada, M., Totsuka, M., Sato, K. & Sugawara, K. (2002) Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. *Exercise Immun. Rev.* **8**, 6–48
38. Steensberg, A., Keller, C., Starkie, R.L., Osada, T., Febbraio, M.A. & Pedersen, B.K. (2002) IL-6 and TNF-α expression in, and release from, contracting human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* **283**, E1272–E1278
39. Keller, P., Keller, C., Carey, A.L., Jauffred, S., Fischer, C.P., Steensberg, A. & Pedersen, B.K. (2003) Interleukin-6 production by contracting human skeletal muscle: Autocrine regulation by IL-6. *Biochem. Biophys. Res. Commun.* **319**, 550–554
40. Tisi, P.V., Hulse, M., Chulakadabba, A., Gosling, P. & Shearman, C.P. (1997) Exercise training for intermittent claudication: does it adversely affect biochemical markers of the exercise-induced inflammatory response? *Eur. J. Vasc. Endovasc. Surg.* **14**, 344–350