Proceedings of the Nutrition Society (2004), **63**, 263–267
DOI:10.1079/PNS2004338

The 12th Conference of the International Research Group on the Biochemistry of Exercise was held at Maastricht University, Maastricht, The Netherlands on 13–16 July 2003

Plenary Lecture

The metabolic role of IL-6 produced during exercise: is IL-6 an exercise factor?

B. K. Pedersen[1,2]*, A. Steensberg[1,2], C. Fischer[1,2], C. Keller[1,2], P. Keller[1,2], P. Plomgaard[1,2], E. Wolsk-Petersen[1,2] and M. Febbraio[1]

[1]*The Copenhagen Muscle Research Centre and* [2]*Department of Infectious Diseases, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, DK-2100 Copenhagen, Denmark*

For most of the last century, researchers have searched for a muscle contraction-induced factor that mediates some of the exercise effects in other tissues such as the liver and the adipose tissue. It has been called the 'work stimulus', the 'work factor' or the 'exercise factor'. In the search for such a factor, a cytokine, IL-6, was found to be produced by contracting muscles and released into the blood. It has been demonstrated that IL-6 has many biological roles such as: (1) induction of lipolysis; (2) suppression of TNF production; (3) stimulation of cortisol production. The *IL-6* gene is rapidly activated during exercise, and the activation of this gene is further enhanced when muscle glycogen content is low. In addition, carbohydrate supplementation during exercise has been shown to inhibit the release of IL-6 from contracting muscle. Thus, it is suggested that muscle-derived IL-6 fulfils the criteria of an exercise factor and that such classes of cytokines could be termed 'myokines'.

IL-6: Cytokines: Muscle: Exercise: Training

For most of the last century researchers searched for a muscle contraction-induced factor that could mediate some of the exercise-induced changes in other organs such as the liver and the adipose tissue. Erling Asmussen discussed this factor in his introductory talk in a symposium held in Dallas in January 1966 and published in *Circulation* (see Winocour *et al*. 1992): 'For every state of physical exercise, there is a carefully controlled level of pulmonary function, ventilation of cardiac output, and of deep body temperature. These levels are maintained at least as precisely as the resting level, and the controlling feedback systems are the same in exercise as during rest; only the set-point has been changed. For years the search for the stimulus that initiates and maintains this change of excitability or sensibility of the regulating centers in exercise has been going on. For lack of more precise knowledge, it has been called the 'work stimulus' or 'the work factor''.

In the present paper 'exercise factor' is used as the preferred term to cover the effects of muscle contractions as such. It is clear that the signalling pathways from contracting muscles to other organs are not associated with the nervous system, as electrical stimulation of paralysed muscles in patients with spinal cord injuries induces in essence the same physiological changes as those in intact subjects (Kjaer *et al*. 1996).

Recently, it was demonstrated that exercise induces *IL-6* gene transcription locally in contracting skeletal muscle (Pedersen *et al*. 2001; Febbraio & Pedersen, 2002). In addition, an exercising limb releases high amounts of IL-6 into the blood (Pedersen *et al*. 2001; Febbraio & Pedersen, 2002). The present review will discuss whether IL-6 may represent a link between skeletal muscle and peripheral organs such as adipose tissue.

Plasma concentrations of IL-6 during exercise

Plasma IL-6 levels increase dramatically (≤100-fold) in response to exercise (Pedersen & Hoffman-Goetz, 2000;

Abbreviation: rh, recombinant human.

***Corresponding author:** Professor Bente Klarlund Pedersen, fax +45 35 45 76 44, email bkp@rh.dk

Pedersen *et al*. 2001; Febbraio & Pedersen, 2002). The finding of increased levels of IL-6 after exercise is a remarkably consistent finding (for review, see Pedersen *et al.* 2003).

Recent studies clearly demonstrate that muscle contractions without any muscle damage induce a marked elevation in plasma IL-6 (Pedersen *et al*. 2001, 2003; Febbraio & Pedersen, 2002).

In addition to the effects of exercise intensity, duration and mode, it has also been suggested that the exercise-induced increase in plasma IL-6 is related to the sympatho-adrenal response to exercise. However, when volunteers are infused with adrenaline in order to closely mimic the increase in plasma adrenaline during 2·5 h of running exercise, plasma IL-6 increases only 4-fold during the infusion but increases 30-fold during the exercise (Steensberg *et al.* 2001*b*). Thus, it seems that adrenaline only plays a minor role in the exercise-induced increase in plasma IL-6.

Another finding in relation to exercise is increased circulating levels of other anti-inflammatory cytokines and cytokine inhibitors, such as IL-1 receptor antagonist and TNF-α receptors and the anti-inflammatory cytokine IL-10 (Pedersen *et al*. 2001, 2003; Febbraio & Pedersen, 2002).

Most studies have reported that exercise does not induce an increase in plasma levels of TNF-α (Pedersen *et al.* 2001, 2003; Febbraio & Pedersen, 2002) and it seems that exercise induces a very strong anti-inflammatory cytokine response, with the appearance of IL-6 in the circulation being by far the most marked and its appearance preceding that of other cytokines.

Muscle-derived IL-6: source of origin?

Based on the common belief that the exercise-induced increase in IL-6 is a consequence of an immune response it has been hypothesized that the immune cells are responsible for this increase (Nehlsen-Canarella *et al*. 1997). However, IL-6 mRNA in monocytes does not increase with exercise (Ullum *et al*. 1994; Moldoveanu *et al*. 2000). Furthermore, monocytes staining positive for IL-6 either do not change (Starkie *et al*. 2000) or decrease during exercise (Starkie *et al*. 2001*b*). Recently, the hypothesis that the liver releases IL-6 during exercise was tested in human subjects by measuring IL-6 across the hepato-splanchnic viscera. It was observed that rather than releasing IL-6, the liver actually eliminates this cytokine during exercise (Febbraio *et al*. 2003*a*).

A number of studies have demonstrated that working muscle produces IL-6. Thus, muscle biopsies obtained before and after exercise in human subjects (Ostrowski *et al*. 1998; Starkie *et al*. 2001*a*; Steensberg *et al*. 2001*a*) and rats (Jonsdottir *et al*. 2000) demonstrate very little IL-6 mRNA in resting muscle but a ≤100-fold increase in exercising skeletal muscle. In one study (Jonsdottir *et al.* 2000) rats were subjected to electrically-stimulated eccentric or concentric contractions of the one hind leg, while the other leg remained at rest. Both the eccentric and concentric contractions resulted in elevated levels of IL-6 mRNA locally in the exercised muscle, whereas the level in resting muscle was not elevated. It appears, therefore, that IL-6 production is associated with contracting muscle, and is not a systemic effect.

By measuring femoral arterial–venous differences across an exercising and a resting leg it has been found that only the exercising limbs release IL-6 (Steensberg *et al*. 2000). Moreover, it has been reported that IL-6 is released from an exercising limb during both knee extensor (Steensberg *et al*. 2001*a*) and bicycle (Febbraio *et al*. 2002) exercise. Keller *et al*. (2001) isolated nuclei from muscle biopsies obtained before and during exercise and demonstrated that the transcription rate for IL-6 increased rapidly and markedly after the onset of exercise. The finding that human muscle cell lines can be stimulated to produce IL-6 further supports the possibility that myocytes could be the origin of IL-6 (C Keller, unpublished results). Recently, the expression of IL-6 was studied by immunohistochemical analysis of biopsies from human muscle tissue undergoing concentric bicycle exercise. IL-6 expression was clearly increased after exercise and remained high even after 24 h relative to pre-exercise or resting individuals (Penkowa *et al*. 2003).

Muscle-derived IL-6: effect of glycogen

Carbohydrate ingestion attenuates elevations in plasma IL-6 during exercise (Nehlsen-Canarella *et al*. 1997). It has been demonstrated that carbohydrate ingestion during moderate exercise has no effect on the exercise-induced increase in IL-6 mRNA levels in the working muscle, but instead attenuates the release of IL-6 from working muscle (Febbraio *et al*. 2003*b*).

Following exercise a low muscle glycogen level is associated with high levels of IL-6 mRNA (Steensberg *et al*. 2001*a*), even when there are no changes in blood glucose levels. In addition, IL-6 is released from the low-glycogen exercising leg after only 60 min of exercise, but after 120 min from the other limb (Steensberg *et al*. 2001*a*). Thus, it was concluded that muscle glycogen content is a determining factor for the production of IL-6 across contracting limbs. However, exercise increases the transcription rate of the *IL-6* gene in skeletal muscle of human subjects (Keller *et al*. 2001), a response that is dramatically enhanced under conditions in which muscle glycogen concentrations are low. Thus, pre-exercise intramuscular glycogen content appears to be an important stimulus for the transcription of the *IL-6* gene. It is important to note that the recent observation that carbohydrate ingestion during moderate exercise has no effect on exercise-induced increase in IL-6 mRNA (Nehlsen-Canarella *et al*. 1997) is consistent with the theory that the transcription of the *IL-6* gene is mediated by glycogen content, since carbohydrate ingestion does not attenuate the rate of muscle glycogenolysis.

Furthermore, it has been demonstrated that the release of IL-6 from working skeletal muscle is positively related to work intensity, glucose uptake and plasma adrenaline concentration (Helge *et al*. 2003). Thus, there is evidence that suggests that IL-6 release may be linked to the regulation of glucose homeostasis during exercise and/ or that IL-6 may work as a sensor of carbohydrate availability.

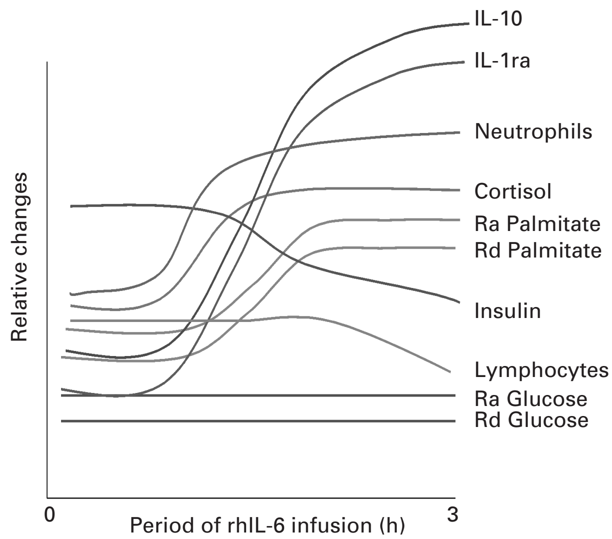

Fig. 1. Schematic representation of the cytokine response to exercise. The diagram illustrates the relative changes in a number of metabolic and immune variables in response to 3 h of infusion with recombinant human (rh) IL-6 eliciting plasma concentrations of IL-6 comparable with that obtained during exercise. The following variables are shown: rate of appearance (Ra) of glucose; rate of disappearance (Rd) of glucose; Ra of palmitate; Rd of palmitate; cortisol concentration, insulin concentration (reflecting data in patients with diabetes); the plasma concentrations of the anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist (IL-1ra); numbers of circulating neutrophils and lymphocytes.

IL-6: effect on glucose metabolism

As discussed earlier, it has been shown that the increase in IL-6 mRNA (Keller *et al*. 2001; Steensberg *et al*. 2001*a*), the transcription rate (Keller *et al*. 2001) and protein release from skeletal muscle (Steensberg *et al*. 2001*a*) is augmented when muscle glycogen availability is reduced. Furthermore, in a previous study (Steensberg *et al*. 2001*a*) the increased expression of IL-6 was shown to be associated with increased glucose uptake during exercise. This finding suggests that IL-6 may be involved, at least in part, in mediating glucose uptake during exercise. However, when recombinant human (rh) IL-6 is infused into healthy volunteers at rest at physiological concentrations corresponding to the plasma IL-6 levels during prolonged exercise, IL-6 has no influence on glucose metabolism (Fig. 1; Steensberg *et al*. 2003*b*). Thus, IL-6 alone at physiological concentrations does not induce hepatic glucose output or increase glucose disposal. It should not be excluded, however, that IL-6 together with another stimulus from contracting muscles is involved in glucose homeostasis during exercise.

IL-6: effect on fat metabolism

Wallenius *et al*. (2002) have demonstrated that IL-6-deficient mice develop mature-onset obesity. In addition, when the mice are treated with IL-6 for 18 d, there is a marked decrease in body weight in the IL-6-knock-out mice, but not in the wild-type mice. A study conducted recently to determine whether physiological concentrations of IL-6 affect lipid metabolism in human subjects (van Hall *et al*. 2003) has shown that rhIL-6 is associated with an increase in the plasma fatty acid concentration and the rate of appearance of endogenous fatty acids from 90 min after the start of the infusion. The elevated levels reached at the end of rhIL-6 infusion persist for at least 3 h post infusion (Fig. 1). Triacylglycerol concentrations are unchanged during, and reduced after, rhIL-6 infusion, while whole-body fat oxidation together with fatty acid re-esterification increases after the second hour of rhIL-6 infusion. These data identify IL-6 as a potent modulator of fat metabolism in man, increasing lipolysis and fat oxidation without causing hypertriacylglycerolaemia. Importantly, the increase in lipolysis and the rate of appearance of fatty acids do not result in transient impaired glucose disposal (Steensberg *et al*. 2003*b*), and this effect is likely to be a result of the concomitant occurrence of fat oxidation. Recent data (E Wolsk-Petersen, BK Pedersen, A Steensberg, C Fischer, C Keller, P Keller, P Plomgaard and M Febbraio, unpublished results) demonstrate that acute rhIL-6 administration increases lipolysis and fatty acid oxidation, with a concomitant decrease in the insulin level to normal values in patients with type 2 diabetes. Thus, IL-6 induces lipolysis without enhancing endogenous glucose production.

The anti-inflammatory effects of exercise

Regular exercise protects against insulin resistance and CVD and low-grade inflammation, including elevated levels of plasma TNF-α, and has been suggested as an important mechanism in these disorders (for review, see Pederson *et al*. 2003). Recently, it was hypothesized that exercise could inhibit the endotoxin-stimulated increase in circulating levels of TNF-α (Starkie *et al*. 2003). To test the hypothesis that IL-6 as well as physical exercise inhibits TNF-α production, eight healthy males participated in three experiments in which they either rested, performed bicycling for 3 h or were infused with rhIL-6 for 3 h while they rested. After 2·5 h, the volunteers received a bolus of *Escherichia coli* lipopolysaccharide endotoxin (0·06 ng/kg) intravenously to induce low-grade inflammation. In the control study plasma TNF-α increased markedly in response to endotoxin. In contrast during exercise, which resulted in elevated IL-6, and rhIL-6 infusion the endotoxin-induced increase in TNF-α was completely abolished. The study demonstrated that physical exercise inhibits the production of TNF-α elicited by low-grade endotoxaemia in human subjects, and suggested that exercise-induced IL-6 production may be involved in mediating the effect of exercise on endotoxin-induced TNF-α production. However, other mediators such as adrenaline may contribute to the anti-inflammatory effects of exercise. In general, the findings relating to the effect of exercise on plasma cytokines suggest that exercise induces a strong anti-inflammatory effect (Febbraio & Pedersen, 2002). Thus, following exercise classical anti-inflammatory cytokines such as IL-1 receptor antagonist and IL-10 are present in the circulation. When rhIL-6 is infused into healthy volunteers IL-6 appears to induce both IL-1 receptor antagonist and IL-10 (Fig. 1; Steensberg *et al*. 2003*a*).

In addition, rhIL-6 at physiological concentrations induces elevated levels of cortisol (Fig. 1), which closely correspond with changes in the kinetics and concentrations of cortisol in response to exercise (Steensberg *et al.* 2003*a*). This finding together with the finding that exercise and rhIL-6 inhibit TNF production may provide a mechanism to explain why physical exercise either reduces the susceptibility to, or improves the symptoms of, diseases associated with low-grade inflammation such as type 2 diabetes and atherosclerosis.

In the study (Wallenius *et al*. 2002) with IL-6-knock-out mice the mice developed maturity-onset obesity and insulin resistance, which was reversed by administration of IL-6. These results clearly show that lack of IL-6 causes insulin resistance. Given that TNF-α may induce insulin resistance (Hotamisligil, 2000), the present findings suggest that exercise may also enhance insulin sensitivity through suppression of TNF-α production. In this connection it is interesting that it has been demonstrated that while IL-6 is released from exercising muscles, TNF is not (Steensberg *et al*. 2002). Taken together, these findings clearly show that muscle-derived IL-6 is a strong mediator of the anti-inflammatory effects of exercise.

Conclusion

Muscle-derived IL-6 possesses some of the characteristics of a true 'exercise factor'. Thus, IL-6 may be one of several 'myokines', a new term for cytokines produced and released by skeletal muscle that exert their effect in other parts of the body.

The *IL-6* gene is not activated in resting muscles, but is rapidly activated by contractions. The IL-6 acts as an energy sensor, being dependent on the glycogen content in the muscle. IL-6 is released from contracting muscles in high amounts and IL-6 exerts its effect on adipose tissue, inducing lipolysis and gene transcription in abdominal subcutaneous fat. Furthermore, IL-6 induces strong anti-inflammatory effects. By its ability to inhibit low-grade TNF-α production, IL-6 may inhibit TNF-α-induced insulin resistance and thereby have an important role in mediating the beneficial health effects of exercise in inactivity and obesity-related disorders such as diabetes and CVD.

Acknowledgements

The work on IL-6 has been supported by The Danish National Research Foundation (no. 504-14), the Novo Nordisk Foundation, the Lundbeck Foundation, Rigshospital, H:S Copenhagen Hospital Corporation, the AIDS Foundation, the Farmacy Foundation of 1991, the Foundation of Augustinus, the Foundation of Engineer Frode V. Nyegaard and Wife, Danfoss (the Foundation of Manufacturer Mads Clausen), the Memorial Legacy of Manufacturer Vilhelm Pedersen and Wife, Foundation of 17.12.1981, the Foundation for Advancement of Medical Science, the Foundation of Gerda and Aage Haensch, the Memorial Legacy of Jens Peter Nielsen, the Foundation of A.P. Møller and the Jubilee Foundation of Rigshospital.

References

Febbraio MA, Ott P, Nielsen HB, Steensberg A, Keller C, Krustrup P, Secher NH & Pedersen BK (2003*a*) Hepatosplanchnic clearance of interleukin-6 in humans during exercise. *American Journal of Physiology* **285**, E397–E402.

Febbraio MA & Pedersen BK (2002) Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. *FASEB Journal* **16**, 1335–1347.

Febbraio MA, Steensberg A, Keller C, Starkie RL, Nielsen HB, Krustrup P, Ott P, Secher NH & Pedersen BK (2003*b*) Glucose ingestion attenuates interleukin-6 release from contracting skeletal muscle in humans. *Journal of Physiology (London)* **549**, 607–612.

Febbraio MA, Steensberg A, Starkie RL, McConell GK & Kingwell BA (2002) Skeletal muscle interleukin-6 and tumor necrosis factor-alpha release in healthy subjects and patients with type 2 diabetes at rest and during exercise. *Metabolism* **52**, 939–944.

Helge JW, Stallknecht B, Pedersen BK, Galbo H, Kiens B & Richter EA (2003) The effect of graded exercise on IL-6 release and glucose uptake in skeletal muscle. *Journal of Physiology (London)* **546**, 299–305.

Hotamisligil GS (2000) Molecular mechanisms of insulin resistance and the role of the adipocyte. *International Journal of Obesity and Related Metabolic Disorders* **24**, Suppl. 4, S23–S27.

Jonsdottir I, Schjerling P, Ostrowski K, Asp S, Richter EA & Pedersen BK (2000) Muscle contractions induces interleukin-6 mRNA production in rat skeletal muscles. *Journal of Physiology (London)* **528**, 157–163.

Keller C, Steensberg A, Pilegaard H, Osada T, Saltin B, Pedersen BK & Neufer PD (2001) Transcriptional activation of the IL-6 gene in human contracting skeletal muscle: influence of muscle glycogen content. *FASEB Journal* **15**, 2748–2750.

Kjaer M, Secher NH, Bangsbo J, Perko G, Horn A, Mohr T & Galbo H (1996) Hormonal and metabolic responses to electrically induced cycling during epidural anesthesia in humans. *Journal of Applied Physiology* **80**, 2156–2162.

Moldoveanu AI, Shephard RJ & Shek PN (2000) Exercise elevates plasma levels but not gene expression of IL-1beta IL-6, and TNF-alpha in blood mononuclear cells. *Journal of Applied Physiology* **89**, 1499–1504.

Nehlsen-Canarella SL, Fagoaga OR & Nieman DC (1997) Carbohydrate and the cytokine response to 2·5 hours of running. *Journal of Applied Physiology* **82**, 1662–1667.

Ostrowski K, Rohde T, Zacho M, Asp S & Pedersen BK (1998) Evidence that IL-6 is produced in skeletal muscle during intense long-term muscle activity. *Journal of Physiology (London)* **508**, 949–953.

Pedersen BK & Hoffman-Goetz L (2000) Exercise and the immune system: Regulation integration and adaption. *Physiological Reviews* **80**, 1055–1081.

Pedersen BK, 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? *Journal of Muscle Research and Cell Motility* **24**, 113–119.

Pedersen BK, Steensberg A & Schjerling P (2001) Muscle-derived interleukin-6: possible biological effects. *Journal of Physiology (London)* **536**, 329–337.

Penkowa M, Keller C, Keller P, Jauffred S & Pedersen BK (2003) Immunohistochemical detection of interleukin-6 in human skeletal muscle fibers following exercise. *FASEB Journal* **17**, 2166–2168.

Starkie RL, Angus DJ, Rolland J, Hargreaves M & Febbraio M (2000) Effect of prolonged submaximal exercise and carbohydrate ingestion on monocyte intracellular cytokine production in humans. *Journal of Physiology (London)* **528**, 647–655.

Starkie RL, Arkinstall MJ, Koukoulas I, Hawley JA & Febbraio MA (2001*a*) Carbohydrate ingestion attenuates the increase in plasma interleukin-6, but not skeletal muscle interleukin-6 mRNA, during exercise in humans. *Journal of Physiology (London)* **533**, 585–591.

Starkie RL, Ostrowski S, Jauffred S, Febbraio MA & Pedersen BK (2003) Exercise and IL-6 infusion inhibit endotoxin-induced TNF-α production in humans. *FASEB Journal* **17**, 884–886.

Starkie RL, Rolland J, Angus DJ, Anderson MJ & Febbraio MA (2001*b*) Circulating monocytes are not the source of elevations in plasma IL-6 and TNF-alpha levels after prolonged running. *American Journal of Physiology* **280**, C769–C774.

Steensberg A, Febbraio MA, Osada T, Schjerling P, van Hall G, Saltin B & Pedersen BK (2001*a*) Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. *Journal of Physiology (London)* **537**, 633–639.

Steensberg A, Fischer CP, Keller C, Moller K & Pedersen BK (2003*a*) IL-6 enhances plasma IL-1ra, IL-10 and cortisol in humans. *American Journal of Physiology* **285**, E443–E437.

Steensberg A, Fischer CP, Sacchetti M, Keller C, Osada T, Schjerling P, van Hall G, Febbraio MA & Pedersen BK (2003*b*) Acute interleukin-6 administration does not impair muscle glucose uptake or whole body glucose disposal in healthy humans. *Journal of Physiology (London)* **548**, 631–638.

Steensberg A, Keller C, Starkie RL, Osada T, Febbraio MA & Pedersen BK (2002) IL-6 and TNF-alpha expression in, and release from, contracting human skeletal muscle. *American Journal of Physiology* **283**, E1272–E1278.

Steensberg A, Toft AD, Schjerling P, Halkjaer-Kristensen J & Pedersen BK (2001*b*) Plasma interleukin-6 during strenuous exercise – role of adrenaline. *American Journal of Physiology* **281**, 1001–1004.

Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B & Pedersen BK (2000) Production of IL-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma IL-6. *Journal of Physiology (London)* **529**, 237–242.

Ullum H, Haahr PM, Diamant M, Palmo J, Halkjaer Kristensen J & Pedersen BK (1994) Bicycle exercise enhances plasma IL-6 but does not change IL-1alpha, IL-1beta, IL-6, or TNF-alpha pre-mRNA in BMNC. *Journal of Applied Physiology* **77**, 93–97.

van Hall G, Steensberg A, Sacchetti M, Fischer C, Keller C, Schjerling P, Hiscock N, Møller K, Saltin B, Febbraio MA & Pedersen BK (2003) Interleukin-6 stimulates lipolysis and fat oxidation in humans. *Journal of Clinical Endocrinology and Metabolism* **88**, 3005–3010.

Wallenius V, Wallenius K, Ahren B, Rudling M, Carlsten H, Dickson SL, Ohlsson C & Jansson JO (2002) Interleukin-6-deficient mice develop mature-onset obesity. *Nature Medicine* **8**, 75–79.

Winocour PH, Durrington PN, Bhatnagar D, Mbewu AD, Ishola M, Mackness M & Arrol S (1992) A cross-sectional evaluation of cardiovascular risk factors in coronary heart disease associated with type 1 (insulin-dependent) diabetes mellitus. *Diabetes Research and Clinical Practice* **18**, 173–184.