Muscle-derived interleukin-6: mechanisms for activation and possible biological roles

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ABSTRACT **It has recently been demonstrated that the marked increase in the systemic concentration of cytokine interleukin-6 (IL-6) seen with exercise originates from the contracting limb and that skeletal muscle cells per se are the likely source of the production. This review summarizes the possible mechanisms for activation and biological consequences of muscle-derived IL-6. It appears that intramuscular IL-6 is stimulated by complex signaling cascades initiated by both calcium (Ca^{2+}) -dependent and -independent stimuli. It also seems likely that skeletal muscle produces IL-6 to aid in maintaining metabolic homeostasis during periods of altered metabolic demand such as muscular exercise or insulin stimulation. It may do so via local and/or systemic effects. This review also explores the efficacy that IL-6 may be used as a therapeutic drug in treating metabolic disorders such as obesity, type 2 diabetes, and atherosclerosis.—Febbraio, M. A., Pedersen, B. K. Muscle-derived interleukin 6: mechanisms for activation and possible biological roles. *FASEB J.* 16, 1335–1347 (2002)**

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IT HAS BEEN well demonstrated that the plasma concentration of interleukin-6 (IL-6) increases up to > 100-fold during muscular exercise (1, 2). This increase is followed by the appearance of cytokine inhibitors IL-1 receptor antagonist (IL-1ra) and tumor necrosis factor receptors (TNF-R) and the anti-inflammatory cytokine IL-10 (3–5). Concentrations of the chemokines, IL-8, macrophage inflammatory protein α (MIP-1α), and MIP-1β are elevated after strenuous exercise (6). Strenuous, prolonged exercise such as marathon running results in a small increase in the plasma concentration of TNF-α (7–10). Even though there is 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 and that its appearance precedes that of the other cytokines (**Fig. 1**).

IL-6 is a member of a family of cytokines that consists of leukemia inhibitory factor, IL-11, ciliary neurotropic factor, oncostatin M, and cardiotrophin 1 (11). Their membership is based on similarities in helical protein structure and a shared receptor subunit (the transmembrane glycoprotein 130) (12, 13). IL-6 is a variably glycosylated protein with a molecular mass of 22–27 kDa depending on the cellular source and amount of post-translational modification. It is synthesized as a precursor protein of 212 amino acids (aa), with a 28 aa signal sequence and a 184 aa mature segment (13). IL-6 is produced by many different cells, but the main sources in vivo are stimulated monocytes/macrophages, fibroblasts, and vascular endothelial cells (14), indicative of its role in the modulation of the immune system. Other cells known to express IL-6 include keratinocytes, osteoblasts, T cells, B cells, neutrophils, eosinophils, mast cells, smooth muscle cells (14), and skeletal muscle cells (15). Typical stimuli for IL-6 production are IL-1, TNF-α, and bacterial endotoxin (14). Hypoxia induces IL-6 in cultured endothelial cells (16) and hypoxia in vivo elevates plasma IL-6 (17, 18). Therefore, like many cytokines, IL-6 is a ubiquitous protein, stimulated by many physiological and pathological stressors (for review, see ref 2). Recently, however, the observation that 10–35% of the body's basal circulating IL-6 is derived from adipose tissue (19) has stimulated interest in this cytokine as a possible mediator of metabolic processes.

THE IL-6 RESPONSE TO EXERCISE

Factors affecting the IL-6 response

The finding of increased levels of IL-6 after exercise is remarkably consistent (3–6, 8, 10, 21–36). However, the appearance of IL-6 into the circulation depends on several factors including exercise intensity, duration, and mode. Surprisingly, few studies have examined the effect of intense exercise on plasma IL-6. However, Nielsen et al. (34) observed a twofold increase in plasma IL-6 after only 6 min of maximal rowing exercise. In contrast, during prolonged endurance activity, IL-6 does not appear until later during exercise. Data from the Copenhagen Marathon race (1996, 1997, and 1998, n=56) suggest a correlation between intensity of

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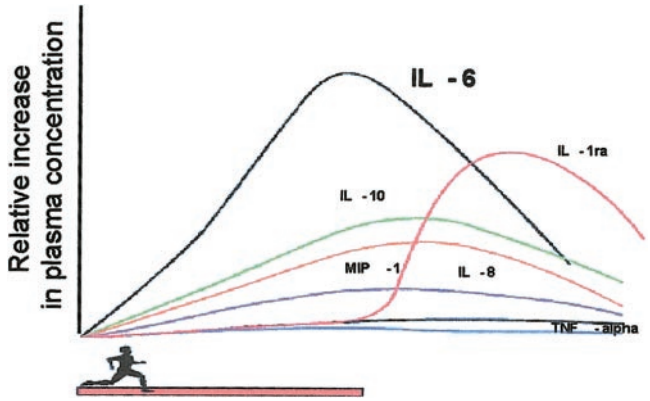

Figure 1. The plasma cytokine response to strenuous exercise.

exercise and the increase in plasma IL-6 (6). Although these studies suggest that exercise intensity plays a role in the IL-6 response, these results may be related to the mode of exercise in that the mass of skeletal muscle recruitment may play a role. During the study by Nielsen et al. (34) rowing was chosen as the mode of exercise, a type of exercise that results in the recruitment of large muscle groups from the upper and lower limbs. In support of the notion that the mass of muscle recruited is important in the appearance of IL-6 in the plasma, two studies (29, 36) have observed higher systemic concentrations of IL-6 during running compared with cycling exercise. In the study by Starkie et al. (29), exercise intensity was normalized to each individual's onset of blood lactate accumulation. Therefore, the 'metabolic load' when comparing the two forms of exercise was matched. The fact that the IL-6 response was higher during running, which involves the recruitment of more muscle groups than cycling, provides sound evidence that the mass of muscle recruited has a major effect on the systemic concentration of IL-6. Indeed, during studies using either the one- (30) or two- (31, 37) legged concentric knee extensor exercise model, which recruits only muscles from the upper legs, the appearance of IL-6 in the plasma is observed later and is less pronounced compared with exercise that results in the recruitment of more motor units.

The type of muscle contraction appears to have a great effect on the time course of the systemic appearance of IL-6. During prolonged (1 h) (38) or intermittent (27) eccentric, one-legged knee extensor exercise or two-legged eccentric knee extensor exercise lasting 30 min (39), the IL-6 level does not peak until well after the cessation of exercise. In contrast, during running, cycling, or concentric knee extensor exercise the IL-6 level peaks at the cessation of exercise before progressively declining into recovery (4, 8, 24, 31). It is clear therefore that the kinetics of IL-6 differ between that induced by concentric muscle contractions and that induced by eccentric exercise associated with muscle damage. In fact, Bruunsgaard et al. (39), using an eccentric exercise model, observed that peak IL-6 was associated not with exercise intensity or duration but with creatine kinase (CK) levels, a traditional marker of muscle damage. Because these observations, it was commonly thought that the IL-6 response to exercise represented a reaction to exercise-induced muscle injury in that the exercise-induced increase in IL-6 was a result of an immune response due to local damage in the working muscles (36). Although an earlier study provided some evidence that the increase in plasma IL-6 was a consequence of an immune response due to local damage in the working muscles (39), more recent studies from our group (4, 5) and others (40) did not show an association between peak IL-6 and peak CK levels. We recently examined plasma IL-6, CK, and myoglobin (another indicator of muscle membrane damage) during and for 5 days after eccentric exercise in healthy young and elderly subjects. Despite marked increases in CK and myoglobin, the plasma IL-6 peaked into recovery at ~5 $pg \cdot mL^{-1}$ in both groups (41). These findings suggest that the large increase in plasma levels of IL-6 in exercise models, where the CK level does not change or is enhanced only a few fold, is related to mechanisms other than muscle damage. It is most likely that the marked and immediate increase in plasma IL-6 in response to exercise of long duration is independent of muscle damage whereas muscle damage per se is followed by repair mechanisms including invasion of macrophages into the muscle leading to IL-6 production. The IL-6 production in relation to muscle damage occurs later and is of smaller magnitude than IL-6 production related to muscle contractions.

Apart from exercise, intensity duration, and mode, it has been suggested that the exercise-induced increase in plasma IL-6 is related to the sympathoadrenal response (24, 36, 43). A study performed in animals suggested that the increase in epinephrine during stress was responsible for the increase in IL-6 (44). However, recent data from our group showed that when epinephrine was infused to volunteers to closely mimic the increase in plasma epinephrine during 2.5 h of running exercise, plasma IL-6 increased only 4-fold during the infusion but 30-fold during the exercise (32). When we blunted the epinephrine response by carbohydrate ingestion (45) or increased it by the addition of heat stress (R. L. Starkie et al., unpublished results), the IL-6 response was unaffected. Thus, it seems that epinephrine plays only a minor role in the exercise-induced increase in plasma IL-6. It was previously demonstrated that peak plasma IL-6 during exercise correlated with plasma lactate (4). However, a recent study (46) examined patients with mitochondrial myopathy, characterized by high plasma lactate levels. These patients were treated with dichloroacetate (DCA) for 15 days, an agent that increases the activity of the pyruvate-dehydrogenase complex. The same exercise test was repeated on days with and without treatment. DCA lowered the plasma lactate levels and increased plasma IL-6 at rest. IL-6 increased in response to exercise only during DCA treatment. Thus, IL-6 production was not a direct result of high lactate levels.

In summary, it appears that the exercise-induced

increase in IL-6 is related to exercise intensity, duration, the mass of muscle recruited, and one's endurance capacity. The time course for the increase and peak in IL-6 appears to be vastly different when comparing concentric with eccentric exercise. Neither the plasma epinephrine response nor lactate concentration, however, appear to influence plasma IL-6 during exercise, as previously thought.

Are monocytes the cellular origin of the exercise-induced increase in plasma IL-6?

Until recently, the cellular origin of the exercise-induced increase in plasma IL-6 has largely been ignored. However, since it was commonly thought that the exercise-induced increase in IL-6 was a consequence of an immune response due to local damage in the working muscles (36), it was hypothesized that the immune cells were responsible for this increase (24). An earlier study by our research group (22) and a recent study by others (47) demonstrated, however, that IL-6 mRNA in monocytes, the blood mononuclear cells responsible for the increase in plasma IL-6 during sepsis (1), did not increase as a result of exercise. More recent work from our group has demonstrated clearly that monocytes are not the source of the exercise-induced increase in plasma IL-6. Using flow cytometric techniques, we have demonstrated that the number, percentage, and mean fluorescence intensity of monocytes staining positive for IL-6 either does not change during cycling exercise (45) or in fact decreases during prolonged running (8). Therefore, the previously held assumption that the IL-6 response to exercise may involve immune cells does not appear to be correct.

The cellular origin of muscle-derived IL-6

Since it appears that the exercise-induced increase in IL-6 is related to exercise intensity, duration, and the mass of muscle recruited, we hypothesized that the contracting muscle may be responsible for the increase in plasma IL-6. Our initial study was performed to test the hypothesis that IL-6 was produced in skeletal muscle in response to intense exercise of long duration (3). Muscle biopsies were collected before and after a marathon race. A comparative polymerase chain reaction (PCR) technique was established to detect mRNA for IL-6 in skeletal muscle RNA extracted from the biopsies. Before exercise, mRNA for IL-6 could not be detected in muscle but we did detect IL-6 in the postexercise samples. The observation that intramuscular (i.m.) IL-6 gene expression increases in skeletal muscle in response to exercise was confirmed in a rat exercise model using the quantitative competitive reverse transcription (RT) -PCR method (48). Rats were subjected to electrically stimulated eccentric or concentric contractions of the one hind leg while the other leg remained at rest. The eccentric and concentric contractions both resulted in elevated levels of IL-6 mRNA in the exercised muscle whereas the level in the resting leg was not elevated. It appears therefore that the local IL-6 production is connected with contracting muscle and is not due to a systemic effect, because IL-6 mRNA was elevated only in the muscle from the exercising leg and not in the other resting leg. As discussed, in these previous studies we were unable to detect IL-6 mRNA in resting skeletal muscle. In the previous human study IL-6 mRNA could be detected in only 5 of 8 muscle biopsies after 197 ± 7 min of exercise (6). In contrast, we have recently demonstrated that IL-6 mRNA is expressed in resting skeletal muscle (29, 31). In the study by Starkie et al. (29), subjects exercised for only 60 min on four separate occasions (twice running and twice cycling) and gene expression was markedly elevated and similar in all postexercise samples. The finding of similar levels of IL-6 mRNA in concentric and eccentric exercised muscle (29, 48) supports the idea that the cytokine production cannot be as closely related to muscle damage as first thought. The disparity in gene expression between our recent (29, 31) and previous (3) studies may be due to differences in the methodologies used. We recently used the real-time PCR technique, which measures the PCR product when cDNA amplification is first detected by fluorescence, not after a fixed number of PCR cycles. Hence, this method appears more sensitive when compared with conventional or comparative PCR, as that performed by Ostrowski et al. (3) because PCR precision is highest at early cycles.

Although the earlier studies demonstrate that IL-6 mRNA is increased in skeletal muscle biopsy samples, they do not prove that skeletal muscle is the source of the increase in the contraction-induced increase in IL-6. Recently, however, we demonstrated that the net IL-6 release from the contracting limb contributes to the exercise-induced increase in arterial plasma concentrations (30). By obtaining arterial-femoral venous differences over an exercising leg, we found that exercising limbs released IL-6. During the last 2 h of exercise the release per unit time was ~17-fold higher than the amount accumulating in the plasma. We have recently confirmed that IL-6 is released from a contracting limb during knee extensor (31) and bicycle (M. A. Febbraio et al, unpublished results) exercise. Although IL-6 appears to be produced in the contracting skeletal muscle, it is still not fully clear which cell type within the muscle is responsible for the production. Whereas myoblasts have been shown to be capable of producing IL-6 (49, 50), endothelial cells (16), fibroblasts (50), and smooth muscle cells (51) have been shown to produce IL-6 under certain circumstances. Langberg et al. (52) have recently demonstrated that IL-6 is produced by the peritendinous tissue of active muscle during exercise. 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. Using RT-PCR, it was demonstrated that the nuclear transcription rate for IL-6 increased rapidly and markedly after the onset of exercise (37). This suggested that a factor associated with contraction increase IL-6 transcriptional rate, probably in the nu-

clei from myocytes, given the observation that IL-6 protein is expressed within muscle fibers (53). It appears that most, if not all, of the IL-6 produced during exercise originates from the contracting limbs and that skeletal muscle cells per se are the likely source.

Several studies have reported that carbohydrate ingestion attenuates elevations in plasma IL-6 during running and cycling (24, 36). In contrast, we (45) have reported that plasma IL-6 was unaffected by carbohydrate ingestion during cycling. However, in this study the subjects were highly endurance trained and plasma IL-6 increased to only ~2 pg·mL^{-1}, even without carbohydrate ingestion. This increase is markedly less than that previously observed in moderately trained subjects (36). We recently we reported that carbohydrate ingestion did attenuate the increase in plasma IL-6 in response to cycling and running (29). In the latter experiment, we used subjects of similar aerobic fitness to those previously reported (36). We found that the IL-6 gene expression in the contracting muscles was not affected by carbohydrate ingestion. IL-6 protein release from contracting muscle was not measured, and it is possible that carbohydrate ingestion did affect the release of IL-6 without altering the gene expression. However, carbohydrate ingestion may have increased the clearance of IL-6 and/or the production of IL-6 by other sources. Administration of IL-6 into rats results in an increase in blood glucose and a decrease in hepatic glycogen content (54). IL-6 has been demonstrated to act directly on hepatocytes to increase hepatic glucose release (55), demonstrating that IL-6 does not mediate changes in blood glucose concentration simply through its effect on glucoregulatory hormones. Taken collectively, these studies indicate that IL-6 plays roles in liver function other than stimulating production of acute-phase proteins. The liver has been identified as a potential source of IL-6 in rats (56, 57). It is well known that either CHO ingestion (58, 59) or infusion (60) during prolonged exercise suppresses hepatic glucose production. These previous studies lead to the suggestion that if blood glucose levels fall, as happens during prolonged exercise without carbohydrate ingestion, the liver will produce IL-6 to stimulate its own glucose output. Conversely, if glucose is supplied exogenously via oral carbohydrate feedings, hepatic IL-6 production will be down-regulated as the demand for endogenous glucose is decreased. Stouthard et al. (61) have demonstrated that the infusion of recombinant human (rh) IL-6 into resting subjects increases endogenous glucose production, and it well known that the liver accounts for most of the glucose produced in the body. Alternatively, differences in clearance of IL-6 may be responsible for the attenuated plasma IL-6 during carbohydrate ingestion.

INTRACELLULAR SIGNALING FOR IL-6 PRODUCTION IN SKELETAL MUSCLE

Role of calcium (Ca^{2+})

As discussed, we have demonstrated that the nuclear transcriptional rate of the IL-6 gene is remarkably rapid after the onset of exercise, with a 10 to -20-fold increase when comparing 30 min of exercise with rest (**Fig. 2**). We therefore hypothesized that this rapid increase in nuclear transcriptional rate was related to a glycogen-independent mechanism, possibly the cytosolic Ca^{2+} levels, since mechanical load is a potent stimulus for liberating Ca^{2+} from the lateral sacs of the sarcoplasmic reticulum (62). To test this hypothesis, muscle cells isolated from human biopsies were harvested and grown in a culture medium until they fused into myotubes (63). The cell cultures were then stimulated with the Ca^{2+} ionophore ionomycin and IL-6 mRNA was measured by real-time PCR over the next 48 h. IL-6 mRNA increased progressively over 48 h compared with preincubation levels. It is clear from an examination of the literature, there is a signaling cascade in other cell types that indeed implicates intracellular Ca^{2+} ion concentration ($[Ca^{2+}]_i$) as a potent signaling factor for IL-6 transcription. It is well known that $[Ca^{2+}]_i$ controls a diverse range of cellular functions, including gene expression and proliferation (64, 65). In B lymphocytes, the amplitude and duration of the $[Ca^{2+}]_i$ controls the differential activation of the proinflammatory transcriptional regulators nuclear factor κβ (NF-κB), c-Jun amino-terminal kinase (JNK), and nuclear factor of activated T cells (NFAT) (66). These authors observed that NF-κB and JNK are selectively activated by a large $[Ca^{2+}]_i$ rise, whereas activation of NFAT was induced by a low sustained $[Ca^{2+}]_i$. We propose therefore that during prolonged contractile activity that results in an increase in IL-6 mRNA in skeletal muscle (3, 29, 31, 37), initial IL-6 transcription occurs via a Ca^{2+}/NFAT-dependent pathway. It is tempting to sug-

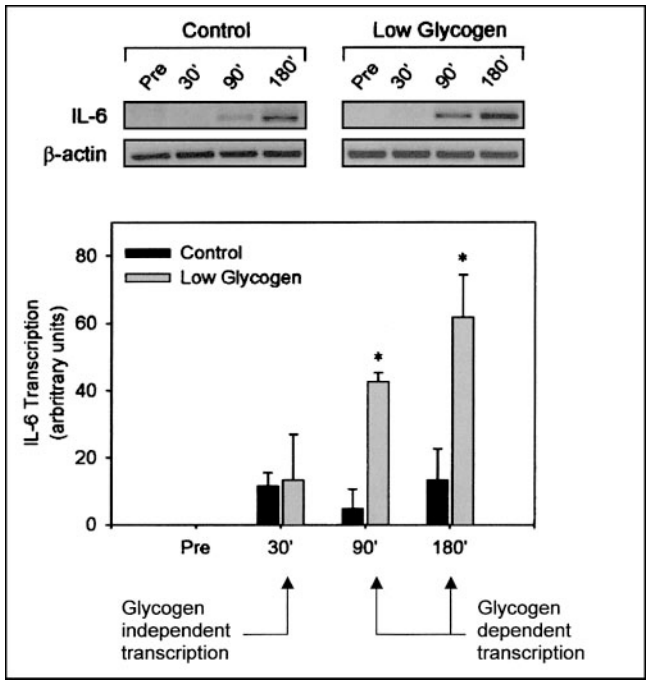

Figure 2. Biphasic transcription of IL-6 in isolated nuclei from muscle biopsies obtained before and during exercise. Adapted from ref 37.

gest such a hypothesis because of several factors. First, NFAT is activated in many cells including skeletal myocytes via the upstream activation of calcineurin. Calcineurin, also called protein phosphatase 2B, is a serine-threonine protein phosphatase cytosolically located (67, 68). Although it was first shown to be activated by Ca^{2+} in T cells, it is present in ~10-fold higher concentrations in neuronal and muscle cells than other cell types (62). Second, when activated, calcineurin binds to and dephosphorylates NFAT, allowing it to translocate to the nucleus where it associates with other transcription factors (62). Although NFAT in itself can lead to cytokine gene transcription, it can bind to the transcription factor AP-1, which can lead to cytokine gene transcription (67, 68). Although this pathway is likely to lead to IL-6 gene transcription during sustained muscular contractions, it is possible that large Ca^{2+} transients as seen with maximal contraction can activate IL-6 via NF-κB and JNK. It is known that skeletal muscle expresses JNK and muscle contraction markedly increases JNK activation (69). Even though the effect of JNK activation on IL-6 gene transcription in skeletal muscle is unknown, Tuyt et al. (70) have demonstrated that JNK regulates IL-6 gene expression in monocytes via activation of NF-κB. Although the degree to which IL-6 is activated in skeletal muscle by these signaling pathways is not known, it is possible that during more intense muscular activity serial activation of these various pathways gives rise to the more pronounced IL-6 response (see **Fig. 3**).

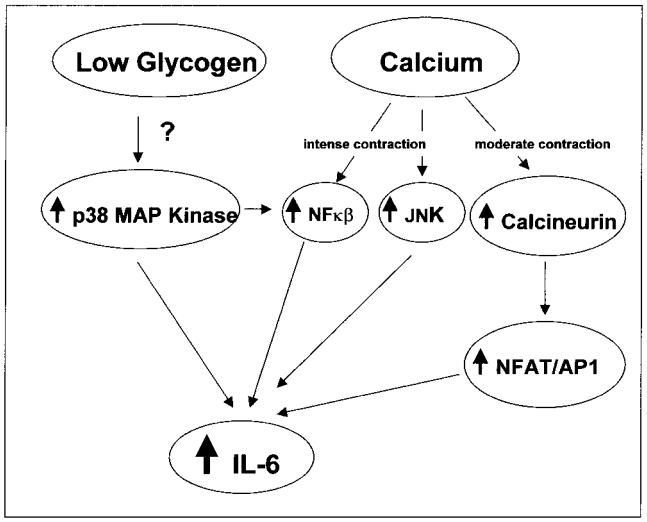

Figure 3. Proposed signaling cascade for the transcription of IL-6 in skeletal muscle. At the onset of muscle contraction, IL-6 gene transcriptional rate is activated by cytosolic Ca^{2+} levels via activation of upstream signaling molecules. During prolonged exercise when cytosolic Ca^{2+} levels plateau, IL-6 is activated via the calcineurin-NFAT/AP1 pathway. During more intense contraction, when the amplitude of the influx of Ca^{2+} into the cytosol is marked, IL-6 is activated via the upstream activation of NF-κB and JNK. During prolonged exercise, a secondary increase in the transcription of IL-6 occurs due to reduced glycogen levels. This likely occurs via the activation of p38 MAP kinase directly acting on IL-6 and/or via the activation of NF-κB.

Role of glycogen

In the first study where we observed IL-6 protein release from the contracting limb during prolonged exercise, we did not observe such a phenomenon until 120 min of single-legged knee extensor exercise (30). Thereafter, it rose steadily and increased markedly after 240 min of exercise. It is well known that exercise of this duration results in glycogen depletion and possibly hypoglycemia (for review, see ref 71). This led us to the hypothesis that IL-6 gene transcription and ultimately protein translation and release was linked to glycogen depletion. In a recent study, an elevated plasma IL-6 response was observed when subjects exercised in a glycogen-depleted state (72). To test the hypothesis that IL-6 gene transcription and protein release is related to glycogen content, we conducted a study where subjects completed 1 h of single-legged bicycle exercise, followed by 1 h or double-arm cranking 16 h before performing 4–5 h of exhaustive two-legged knee extensor exercise at 40% of their maximal knee extensor power output. In the intervening 16 h, subjects consumed a low-carbohydrate diet. This protocol was designed to deplete glycogen content in one leg and it allowed us to test the hypothesis that pre-exercise glycogen availability affected IL-6 production. The experimental model had the advantage that delivery of substrates and hormones to each limb was the same. Subjects commenced exercise with a 40% lower glycogen content in the low- vs. high-glycogen leg (31). We found that in the postexercise samples, those with the lowest glycogen content expressed the highest levels of IL-6 mRNA. The release of IL-6 from the low-glycogen exercising leg occurred after only 60 min of exercise whereas it occurred after 120 min in the other limb (31). Thus, we concluded that muscle glycogen content is a determining factor for production of IL-6 across contracting limbs. One potential concern from this previous study was that one leg performed exercise the day before and the other did not. Since mechanical load can activate the calcineurin/NFAT signaling cascade, we could not rule out the possibility that the exercise the day before was the stimulus for the IL-6 transcription, even though resting IL-6 on the day of the experiment was similar when comparing legs. To rule out this possibility, we had subjects perform exercise on two different occasions, once with a normal and once with a low pre-exercise muscle glycogen content (37). We demonstrated that prolonged exercise activated transcription of the IL-6 gene in skeletal muscle of humans, a response that was dramatically enhanced under conditions in which muscle glycogen concentrations were low (Fig. 2). Therefore, pre-exercise i.m. glycogen content appears to be an important determinant of IL-6 gene transcription and ultimate release.

The signaling cascade that would result in IL-6 gene transcription due to altered glycogen availability is not well understood. It is possible that a low glycogen content within the muscle may simply result in an

impaired resequestering of Ca^{2+} by the sarcoplasmic reticulum, thus activating IL-6 by the previously discussed Ca^{2+}-dependent pathway. It has been demonstrated that during exercise, impaired calcium uptake and release is associated with low glycogen content in both animals (73–75) and humans (76). However, it is possible that low glycogen may activate IL-6 via a Ca^{2+}-independent pathway. Apart from NFAT, JNK, and NF-κB, it is well known that IL-6 is activated by the mitogen-activated protein (MAP) kinase, p38. p38 MAP kinase induces IL-6 in neonatal rat cardiomyocytes via the activation of NF-κB (77). Pharmacological blockade of p38 MAP kinase with a specific inhibitor decreases IL-6 expression in MC3T3E-1 osteoblasts (78). It is well known that p38 MAP kinase increases markedly in contracting skeletal muscle (69). No studies have determined whether glycogen availability influences p38 MAP kinase expression in contracting skeletal muscle. However, since p38 MAP kinase is a stress-activated protein kinase and low glycogen decreases the energy availability in contracting muscle, this scenario appears possible. Low glycogen increases Akt phosphorylation and activity in rat skeletal muscle during insulin stimulation (79). Akt is a signaling serine-threonine molecule downstream of phosphoinositide 3-kinase, a molecule essential for stimulation of insulin mediated glucose transport (80). Therefore, there is some evidence that glycogen availability influences at least one key signaling molecule in skeletal muscle during altered homeostasis. Although further research is required to determine the precise signaling cascade that would lead to IL-6 gene transcription in muscle as a result of low glycogen, we suggest that this process may involve the activation of p38 MAP kinase (see Fig. 3).

BIOLOGICAL ROLES OF MUSCLE-DERIVED IL-6

Regulation of glucose homeostasis

Since recent data suggest that the transcription and ultimate release of IL-6 during prolonged exercise is related to i.m. glycogen content (31, 37), a hypothesis has been developed that suggests that IL-6 may be involved in the regulation of glucose homeostasis either by affecting hepatic glucose production and/or muscle glucose uptake (**Fig. 4**, from ref 81)

At the onset of exercise, the increase in hepatic glucose production (HGP) is marked and the magnitude of increase depends on the intensity of muscular work (82). However, despite the large body of research focused on the regulation of HGP during exercise, this phenomenon is still not fully elucidated (83). Although the exercise-induced changes in insulin and/or glucagon (84), cortisol (85), epinephrine (86), or adrenergic neural stimulation (87, 88) have been proposed to be the major neurohumoral mediators of HGP during exercise, they cannot account for the rapid increase. Indeed, we have concluded that the possibility exists that an as yet unidentified factor, released from contracting muscle cells, may contribute to the increase in hepatic glucose production (84).

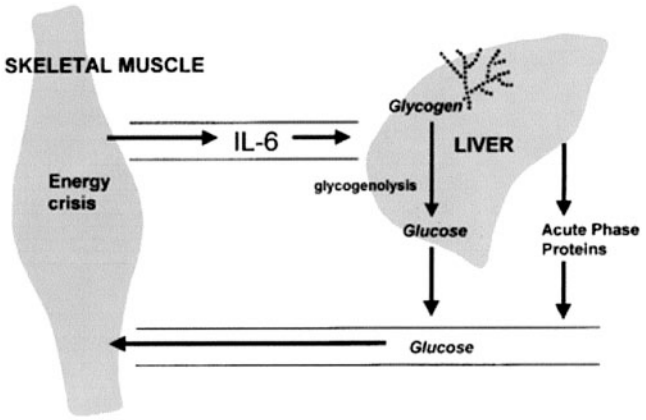

Figure 4. Scheme outlining the possible role of interleukin-6 in the maintenance of glucose homeostasis during muscle contraction. From ref 81.

Some evidence suggests that IL-6 may have a marked influence on hepatic glucose metabolism. IL-6 has been shown to inhibit glycogen synthase activity and accelerate glycogen phosphorylase activity (89). It has been demonstrated that injection of rhIL-6 into humans increases HGP (90) and fasting blood glucose concentration in a dose-dependent manner (90). These data raise the possibility that the IL-6 produced by contracting skeletal muscle may contribute to mediating the hepatic glucose output necessary to maintain blood glucose homeostasis when the uptake of blood glucose by skeletal muscles is increased by prolonged exercise. In addition to the potential effect of IL-6 on HGP, there is some evidence that IL-6 may be involved in processes involved in glucose uptake by insulin-stimulated tissue and/or contracting skeletal muscle. Glucose uptake into cells is facilitated by a specific family of proteins referred to as the glucose transporter proteins (GLUT) (91). In insulin-responsive cells such as muscle, GLUT4 is expressed in high levels in intracellular pools of specific vesicles. During insulin stimulation, the signaling cascade that results in GLUT4 translocation from the intracellular pools to the plasma membrane and transverse tubule is well categorized. This process involves a complex signaling cascade initiated by insulin binding to the α-subunit of the insulin receptor resulting in autophosphorylation of tyrosine residues in the receptor β-subunit and activation of a tyrosine kinase intrinsic to the β-subunit (92). The receptor kinase then tyrosine phosphorylates insulin receptor substrate 1 (IRS-1) (93). IRS-1 can activate the enzyme phosphatidylinositol 3-kinase (PI 3-kinase) (94), which is thought to be an important component of intracellular signaling events that leads to the biological actions of insulin and, ultimately, trafficking of GLUT4 from its intracellular pool to the plasma membrane where it can facilitate the uptake of glucose molecules.

During muscle contraction, the process of GLUT4 translocation is less well understood. Several studies in the literature provide evidence that the pathways re-

sponsible for GLUT4 translocation and glucose uptake when comparing insulin- and contraction-mediated glucose transport are independent of one another. First, the effect of contraction is a potent and additive stimulus for glucose disposal in skeletal muscle. The addition of exercise during a euglycemic, hyperinsulinemic clamp increases glucose disposal by a further 70% in humans (95). Second, although IRS-1 phosphorylation and PI 3-kinase activity are essential components of insulin-stimulated glucose uptake (96–98), they are not increased by muscle contraction (99). Indeed, when PI-3 kinase is selectively inhibited by wortmannin, insulin-mediated glucose transport is inhibited but contraction-mediated glucose transport is not impaired (96). Recently, Bergeron et al. (100) demonstrated that the compound 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR) activated the AMP-activated kinase (AMPK) pathway and increased muscle glucose uptake in conscious rats. In that study, the increase in glucose uptake in the presence of AICAR was observed independent of insulin or the PI 3-kinase inhibitor wortmannin. In this respect, AICAR appeared to mimic the action of contraction-induced GLUT4 translocation and glucose transport. This study suggests that AMPK may have a strong role as the kinase responsible for translocating GLUT4 to the plasma membrane during muscle contraction, ultimately leading to an increase in glucose uptake (For review, see ref 101). However, Mu et al. (102) recently demonstrated that although contraction-induced glucose uptake was reduced in an AMPK null transgenic mouse it was only partially reduced. This suggests that although AMPK is responsible for a portion of the contraction mediated increase in glucose transport, other AMPK-independent pathways contribute to the response.

In our recent study (31), although glucose uptake was increased during contraction in a leg depleted of glycogen and one with normal glycogen levels, the glucose uptake was markedly higher in the depleted leg after 60 min of two-legged contraction. IL-6 was produced by the glycogen depleted leg, but not the leg with normal glycogen levels, at this corresponding time point. Although these results demonstrate only a temporal relationship between IL-6 production and glucose uptake by skeletal muscle, they raise the possibility that one biological role of IL-6 may be to signal molecules to ultimately result in GLUT4 translocation and enhanced glucose uptake. Work from Stouthard and co-workers has shown a more direct relationship between IL-6 and glucose transport. In the first of these studies, Stouthard et al. (61) demonstrated that infusion of rhIL-6 into human subjects increased whole body glucose disposal and subsequent oxidation compared with a control trial. Even though endogenous glucose production was increased with rhIL-6 infusion, the metabolic clearance rate of glucose was higher in this trial, suggesting that relative hyperglycemia was not responsible for the augmented glucose disposal. In a followup study, Stouthard et al. (103) demonstrated that IL-6 increased basal and insulin-stimulated glucose uptake in cultured 3T3-L1 adipocytes. These authors concluded that IL-6 acted by increasing glucose transporter intrinsic activity. Recent evidence from others provides a mechanism as to how IL-6 may act to increase glucose uptake. Increased glucose transport was found in jejunal tissue incubated with IL-6 compared with controls (104). Moreover, IL-6 seems to be able to increase the absorption of glucose in the gut, thereby increasing the plasma glucose levels (104). The effect of IL-6 on glucose uptake in skeletal muscle cells has not been investigated. However, Bedard et al. (105) have implicated other cytokines, namely, TNF-α and interferon γ (IFN-γ) in the process of glucose uptake in skeletal muscle via an increase in nitric oxide synthase (NOS) expression. Bradley et al. (106) have demonstrated that leg glucose uptake during muscle contraction in humans is reduced with administration of L-NMMA, a pharmacological NOS inhibitor; when inducible NOS is pharmacologically inhibited in an osteoblast cell line, IL-6 expression is concomitantly decreased (78). Although speculative, these studies suggest the possibility that muscle-derived IL-6 may contribute to contraction-mediated glucose uptake.

Regulation of fat metabolism

Besides the glucoregulatory effect of IL-6, emerging evidence suggests that this cytokine may be involved in other metabolic pathways. Stouthard et al. (103) found an increase in circulating free fatty acids (FFA) with rhIL-6 infusion concomitant with the increase in liver glucose output during IL-6 infusion. In this previous study, however, epinephrine was elevated and therefore the authors could not determine whether IL-6 acted directly on adipocytes, as epinephrine is a powerful lipolytic hormone. Infusion of IL-6 into rats increased serum triglyceride and FFA levels in a dose-dependent manner (107). The hypertriglyceridemia was due to increased secretion by the liver and not decreased clearance. In an important study, Wallenius et al. (108) have demonstrated that an IL-6-deficient mouse developed mature-onset obesity compared with wild-type control mice. When the mice were treated with IL-6 for 18 days, there was a significant decrease in body weight in transgenic but not the wild-type mice. These data suggest that IL-6 is important in lipolytic processes. IL-6 is secreted by adipose tissue (19, 109, 110), suggesting it may have paracrine effects there. However, a recent study has demonstrated that IL-6 production by adipose tissue is suppressed during exercise but elevated after exercise (111). Taken together, these data suggest that IL-6 is a powerful lipolytic factor and may indicate that during exercise the increase in arterial FFA concentration is mediated at least in part by IL-6 released from the muscle. Hence, we propose that muscle-derived IL-6 acts in a neuroendocrine hormone-like manner.

IMPLICATIONS OF MUSCLE-DERIVED IL-6 IN HEALTH AND DISEASE

Given the many beneficial effects of physical exercise on health and the marked increase in IL-6 gene transcription within skeletal muscle and protein release, it is hard to believe that muscle-derived IL-6 is detrimental to health. However, several studies have suggested that high circulating IL-6 may exert pathogenic effects in age-related diseases such as obesity, atherosclerosis, and type 2 diabetes (112–117). In several population-based studies, plasma concentrations of IL-6 have been shown to predict total and cardiovascular mortality (118, 119). Aging is associated with increased levels of IL-6 (120). It has been proposed that IL-6 is the mediator that links the acute-phase response to visceral obesity, insulin resistance, and atherosclerosis (117; Munford Grand Rounds of July 6, 2000). These studies demonstrate only a putative association between IL-6 and disease. To our knowledge, only one study has directly manipulated IL-6 and examined altered metabolic homeostasis. Wallenius (108) demonstrated that an IL-6-deficient mouse developed mature-onset obesity vs. a wild-type control mouse. When mice were treated with IL-6 for 18 days, there was a pronounced decrease in body weight in the transgenic but not wild-type mice. Contrary to the popular theory, this study suggests a positive metabolic role for IL-6 in health and in the treatment of disease. There are several reasons why IL-6 may be considered detrimental to health. First, it is important to distinguish between acute increases in IL-6 and IL-6 hyperproduction. IL-6 hyperproduction has been implicated in glucocorticoid receptor dysfunction that may negatively affect metabolic homeostasis (121). When circulating IL-6 is acutely elevated by exercise (31) or rhIL-6 infusion (A. Steensberg, unpublished results), however, the decline in systemic IL-6 upon withdrawal of the stimulus is rapid, with values returning to baseline within hours. Therefore, the distinction between acute and chronic elevations in IL-6 may be important when determining the health implications of IL-6. IL-6 may be elevated in disease states as a consequence rather than a cause of the perturbation in order to down-regulate other metabolic dysfunctions. For example, high levels of IL-6 in patients with so-called 'metabolic syndrome' may be explained by the fact that IL-6 is produced in adipose tissue, and these subjects have a higher body mass index than the general population (19, 122).

Anti-inflammatory effects of muscle-derived IL-6

Increased levels of TNF-α and IL-6 have been observed in obese individuals, smokers, and patients with non-insulin-dependent diabetes mellitus (type 2 diabetes) (112, 123). Therefore, IL-6 and TNF-α have both been implicated in disease. The evidence that insulin resistance in skeletal muscle is linked to TNF-α is well established. TNF-α is expressed in human skeletal muscle and its expression is augmented in the skeletal muscle of patients with type 2 diabetes (124). TNF-α decreases insulin-stimulated rates of glucose storage in cultured human muscle cells (125). TNF-α administration impairs insulin-mediated capillary recruitment and glucose uptake in anesthetized rats (126) whereas TNF-α null mice are protected from insulin resistance (127). TNF-α down-regulates GLUT4 and inhibits insulin receptor activity (128). In contrast, the association between IL-6 and insulin resistance and/or type 2 diabetes is putative rather than causal. Although type 2 diabetes is associated with IL-6 gene polymorphism (129), higher plasma concentrations of IL-6 (130), and IL-6 release from adipose tissue (113), there is no direct evidence for an association between IL-6 expression and insulin resistance, particularly in skeletal muscle.

Although many studies suggest that IL-6 and TNF-α have similar functions, numerous studies demonstrate this is not the case. In elderly people, serum levels of leptin and TNF-α are correlated even when adjusting for the effect of gender and body mass index (131). TNF-α is elevated in elderly patients with atherosclerosis compared with age-matched subjects without this diagnosis (120, 132). However, although TNF-α correlates with IL-6, the latter cytokine was not associated with atherosclerosis (120). Infusion of IL-6 into humans will result in fever but does not cause shock or capillary leakage-like syndrome as observed with the prototypical proinflammatory cytokines IL-1 and TNF-α (132). IL-6 administration in humans induces the induction of IL-1ra and soluble TNF receptors, but not IL-1β and TNF-α (132). IL-6 induces the production of C reactive protein, which has a role in the induction of anti-inflammatory cytokines in circulating monocytes and in suppression of the synthesis of proinflammatory cytokines in tissue macrophages (133). IL-6 is involved in the regulation of hematopoiesis, and can inhibit myeloid leukemic cell lines and their differentiation into macrophages (14). Hence, muscle-derived IL-6 can have positive immunological suppressive effects (for review, see ref 132).

It is clear that TNF-α can adversely affect metabolic function. We therefore suggest that one function of muscle-derived IL-6 is to down-regulate TNF-α, giving rise to the observation that in some circumstances both cytokines may be elevated. Although TNF-α has been measured in human skeletal muscle (125), we have recently measured i.m. IL-6 and TNF-α mRNA and protein release across contracting and nonconcontracting limbs in healthy subjects and patients with type 2 diabetes (A. Steensberg et al. and M. A. Febbraio, unpublished results). In the first of these studies (M. A. Febbraio, unpublished results), we demonstrated that TNF-α was not released either at rest or after 25 min of semirecumbent cycling in either patients with type 2 diabetes or healthy aged and BMI matched controls. In contrast, 25 min of

exercise was sufficient to result in a marked increase in IL-6 release, which appeared to be augmented in the patient group. In the latter study (A. Steensberg et al., unpublished results), despite 3 h of continual contractile activity, TNF-α mRNA was not significantly increased compared with rest. However, there was a small (~4-fold) and insignificant increase in TNF-α mRNA after 30 min of exercise, after which time it decreased. In contrast, the ~100-fold increase in IL-6 mRNA peaked after 180 min of exercise. We have been able to demonstrate in preliminary experiments, that TNF-α gene expression is inducible in human skeletal muscle cells. We have shown that when stimulated with the Ca^{2+} ionophore ionomycin, cultured primary human muscle cells significantly increase TNF-α gene expression after 6 h of incubation (C. Keller, Y. Hellsten, H. Pilegaard, M. A., Febbraio, and B. K. Pedersen, unpublished observations). In contrast, IL-6 peaked in the same stimulated culture preparation after 24 h (63). Given these preliminary results and those from the present experiment, we propose that one function of the marked increase in IL-6 gene expression in skeletal muscle during muscle contraction may be to inhibit any increase in TNF-α production. During contraction, muscle glucose uptake is markedly increased compared with rest and therefore our hypothesis is consistent with the observation that TNF-α impairs glucose disposal in skeletal muscle. Indeed, some data demonstrate that IL-6 can attenuate increases in TNF-α. Tanaka et al. (134) have recently demonstrated that during viral myocarditis, the serum TNF-α concentration is markedly reduced in transgenic mice, which overexpress IL-6 compared with wild-type mice. IL-6 inhibits LPS-induced TNF-α production in cultured human monocytes and in the human monocytic line U937 (135). The suppressive effect occurs at the level of transcription in human peripheral blood mononuclear cells (136). In in vivo endotoxin models, levels of TNF-α are elevated in anti-IL-6-treated mice (137) and in IL-6-deficient knockout mice compared with control mice (137), suggesting that circulating IL-6 regulates TNF-α. Whereas exercise decreases the percentage of type 1 T cells, IL-6 may stimulate type 2 T cells, thereby maintaining a relatively unaltered percentage of these cells in the circulation compared with total circulating lymphocyte number (138). Thus, the current view is that IL-6 has primarily anti-inflammatory effects.

IL-6, glucose uptake, and insulin resistance: implications for type 2 diabetes

Although the precise cause/s of peripheral insulin resistance associated with type 2 diabetes is/are not fully clear, recent research suggests that type 2 diabetics have defects in insulin signaling and GLUT4 trafficking (139). As discussed, Stouthard et al. (103) have demonstrated that basal and insulin-stimulated glucose uptake in cultured 3T3-L1 adipocytes was augmented when the cells were incubated with IL-6. If, as we suspect, IL-6 expression may increase glucose transport by up-regulating the processes involved in the trafficking of GLUT4 from the intracellular pools to the plasma membrane, it is possible that IL-6 expression may be up-regulated in insulin resistant skeletal muscle in an attempt to overcome the impaired glucose uptake.

A current theory is that IL-6 causes insulin resistance because type 2 diabetes is associated with IL-6 gene polymorphism (129), higher plasma concentrations of IL-6 (130), and IL-6 release from adipose tissue (113). However, there is no direct evidence that IL-6 expression causes insulin resistance in a manner similar to that of TNF-α. On the contrary, there is evidence suggesting that IL-6 may enhance insulin sensitivity rather than cause insulin resistance. For example, transgenic non-obese diabetic (NOD) mice that overexpress human IL-6 have delayed onset of diabetes and prolonged survival compared with NOD mice in the absence of the overexpressed IL-6 gene (140). Wallenius et al. (108) have demonstrated that IL-6-deficient mice have higher basal glucose levels and markedly impaired glucose disposal during an intravenous glucose tolerance test. We have recently shown that a rodent rendered insulin resistant in skeletal muscle due to an overexpression of the gluconeogenic regulatory enzyme phosphoenolpyruvate carboxykinase in the kidney and liver markedly increases IL-6 gene expression in skeletal muscle during a hyperinsulinemic, euglycemic clamp (141). We hypothesized that the higher IL-6 mRNA in insulin-resistant, insulin-stimulated tissue was an attempt by the organ to overcome the defect. This hypothesis was based on the consistent observation that during contraction, when glucose uptake is markedly elevated above resting levels, skeletal muscle IL-6 gene transcription is remarkably high. These studies could suggest that IL-6 production and subsequent release by skeletal muscle may play a role in the regulation of glucose homeostasis in insulin-sensitive tissue and that IL-6 may be up-regulated in insulin resistant tissue in an attempt to overcome such a metabolic dysfunction.

CONCLUSION

In conclusion, the literature demonstrates that exercise results in a marked increase in circulating IL-6 and that much is derived from the contracting limb. It appears that the skeletal muscle cells per se produce IL-6, stimulated by complex signaling cascades that are initiated by Ca^{2+}-dependent and -independent stimuli. We propose that the muscle produces IL-6 to aid in maintaining metabolic homeostasis during periods of altered metabolic demand such as muscular exercise or insulin stimulation. It may do so via local effects and/or via systemic effects, acting in a neurohumoral manner (2, see **Fig. 5**). Given recent findings, we think there is a scope for IL-6 to be used as a therapeutic drug in

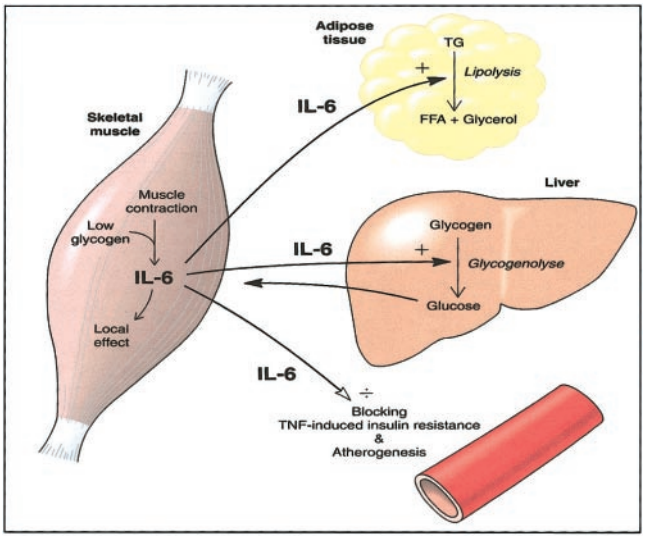

Figure 5. Schematic presentation of the biological effect of muscle-derived IL-6 (adapted from ref 2).

treating metabolic disorders such as obesity, type 2 diabetes and atherosclerosis. FJ

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REFERENCES

1. Pedersen, B. K., and Hoffman-Goetz, L. (2000) Exercise and the immune system: regulation, integration and adaption. *Physiol. Rev.* **80,** 1055–1081
2. Pedersen, B. K., Steensberg, A., and Schjerling, P. (2001) Muscle-derived interleukin-6: possible biological effects. *J. Physiol. (London)* **536,** 329–337
3. Ostrowski, K., Rohde, T., Zacho, M., Asp, S., and Pedersen, B. K. (1998) Evidence that IL-6 is produced in skeletal muscle during intense long-term muscle activity. *J. Physiol. (London)* **508,** 949–953
4. Ostrowski, K., Hermann, C., Bangash, A., Schjerling, P., Nielsen, J. N., and Pedersen, B. K. (1998) A trauma-like elevation in plasma cytokines in humans in response to treadmill running. *J. Physiol. (London)* **508,** 949–953
5. Ostrowski, K., Rohde, T., Asp, S., Schjerling, P., and Pedersen, B. K. (1999) The cytokine balance and strenuous exercise: TNF-alpha, IL-2β, IL-6, IL-1ra, sTNF-r1, sTNF-r2, and IL-10. *J. Physiol. (London)* **515,** 287–291
6. Ostrowski, K., Rohde, T., Asp, S., Schjerling, P., and Pedersen, B. K. (2001) Chemokines are elevated in plasma after strenuous exercise. *Eur. J. Cell. Physiol.* **84,** 244–245
7. Pedersen, B. K., Ostrowski, K., Rohde, T., and Bruunsgaard, H. (1998) The cytokine response to strenuous exercise. *Can. J. Physiol. Pharmacol.* **76,** 505–511
8. Starkie, R. L., Rolland, J., Angus, D. J., Anderson, M. J., and Febbraio, M. A. (2001) Circulating monocytes are not the source of elevations in plasma IL-6 and TNF-alpha levels after prolonged running. *Am. J. Physiol.* **280,** C769–C774
9. Toft, A. D., Ostrowski, K., Asp, S., Møller, K., Iversen, S., Hermann, C., Søndergaard, S. R., and Pedersen, B. K. (2000). The effects of n-3 PUFA on the cytokine response to strenuous exercise. *J. Cell. Physiol.* **89,** 2401–2405
10. Suzuki, K., Yamada, M., Kurakake, S., Okamura, N., Yamaya, K., Liu, Q., Kudoh, S., Kowatari, K., Nakaji, S., and Sugawara, K. (2000) Circulating cytokines and hormones with immunosuppressive but neutrophil-priming potentials rise after endurance exercise in humans. *Eur. J. Cell. Physiol.* **81,** 281–287
11. Gadient, R. A., and Patterson, P. H. (1999) Leukemia inhibitory factor. *Interleukin* **6,** and other cytokines using the GP130 transducing receptor: roles in inflammation and injury. *Stem Cells* **17,** 127–137
12. Hibi, M., Nakajima, K., and Hirano, T. (1996) IL-6 cytokine family and signal transduction: a model of the cytokine system. *J. Mol. Med.* **74,** 1–12
13. Hirano, T., Matsuda, T., and Nakajima, K. (1994) Signal transduction through gp130 that is shared among the receptors for the interleukin 6 related cytokine subfamily. *Stem Cells* **12,** 262–277
14. Akira, S., Taga, T., and Kishimoto, T. (1993) Interleukin-6 in biology and medicine. *Adv. Immunol.* **54,** 1–78
15. Nagaraju, K., Raben, N., Merritt, G., Loeffler, L., Kirk, K., and Plotz, P. (1998) A variety of cytokines and immunologically relevant surface molecules are expressed by normal human skeletal muscle cells under proinflammatory stimuli. *Clin. Exp. Immunol.* **113,** 407–414
16. Yan, S. F., Tritto, I., Pinsky, D., Liao, H., Huang, J., Fuller, G., Brett, J., May, L., and Stern, D. (1995) Induction of interleukin 6 (IL-6) by hypoxia in vascular cells. Central role of the binding site for nuclear factor-IL-6. *J. Biol. Chem.* **270,** 11463–11471
17. Klausen, T., Richalet, J. P., Olsen, N. V., and Pedersen, B. K. (1997) Hypoxemia increases serum interleukin-6 in humans. *Eur. J. Cell. Physiol.* **76,** 480–482
18. Mazzeo, R. S., Donovan, D., Fleshner, M., Butterfield, G. E., Zamudio, S., Wolfel, E. E., and Moore, L. G. (2001) Interleukin-6 response to exercise and high altitude exposure: influence of alpha-adrenergic blockade *J. Cell. Physiol.* **91,** 2143–2149
19. Mohamed-Ali, V., Goodrick, S., Rawesh, A., Katz, D. R., Miles, J. M., Yudkin, J. S., and Klein, S., Coppack, C. (1997) Subcutaneous adipose tissue releases interleukin-6, but not tumour necrosis factor-alpha, in vivo. *J. Clin. Endocrinol. Metab.* **82,** 4196–4200
20. Northoff, H., and Berg, A. (1991) Immunologic mediators as parameters of the reaction to strenuous exercise. *Int. J. Sports Med.* **12,** S9–S15
21. Sprenger, H., Jacobs, C., Nain, M., Gressner, A. M., Prinz, H., Wesemann, W., and Gemsa, D. (1992) Enhanced release of cytokines, interleukin-2 receptors, and neopterin after long-distance running. *Clin. Immunol. Immunopathol.* **63,** 188–95
22. Ullum, H., Haahr, P. M., Diamant, M., Palmo, J., Halkjaer Kristensen, J., Pedersen, B. K. (1994) Bicycle exercise enhances plasma IL-6 but does not change IL-1alpha. IL-1beta:IL-6, or TNF-alpha pre-mRNA in BMNC. *J. Cell. Physiol.* **77,** 93–97
23. Drenth, J. P., van Uum, S. H., van Deuren, M., Pesman, G. J., van der ven Jongekrug, J., and van der Meer, J. W. (1995) Endurance run increases circulating IL-6 and IL-1ra but downregulates ex vivo TNF-alpha and IL-1beta production. *J. Cell. Physiol.* **79,** 1497–1503
24. Nehlsen-Canarella, S. L., Fagoaga, O. R., and Nieman, D. C. (1997) Carbohydrate and the cytokine response to 2.5 hours of running. *J. Cell. Physiol.* **82,** 1662–1667
25. Castell, L. M., Poortmans, J. R., Leclercq, R., Brasseur, M., Duchateau, J., and Newsholme, E. A. (1997) Some aspects of the acute phase response after a marathon race, and the effects of glutamine supplementation. *Eur. J. Cell. Physiol.* **75,** 47–53
26. Rohde, T., MacLean, D. A., Richter, E. A., Kiens, B., and Pedersen, B. K. (1997) Prolonged submaximal eccentric exercise is associated with increased levels of plasma IL-6. *Am. J. Physiol.* **273,** E85–E91
27. Hellsten, Y., Frandsen, U., Orthenblad, N., Sjodin, N., and Richter, E. A. (1997) Xanthine oxidase in human skeletal muscle following eccentric exercise: a role of inflammation. *J. Physiol. (London)* **498,** 239–248
28. Nieman, D. C., Nehlsen-Canarella, S. L., Fagoaga, O. R., Henson, D. A., Utter, A., Davis, J. M., Williams, F., and Butterworth, D. E. (1998) Effects of mode and carbohydrate on the granulocyte and monocyte response to intensive prolonged exercise. *J. Cell. Physiol.* **84,** 1252–1259
29. Starkie, R. L., Arkinstall, M. J., Koukoulas, I., Hawley, J. A., and Febbraio, M. A. (2001) Carbohydrate ingestion attenuates the increase in plasma interleukin 6, but not skeletal muscle

interleukin 6 mRNA, during exercise in humans. *J. Physiol. (London)* **533,** 585–591
30. Steensberg, A., van Hall, G., Osada, T., Sacchetti, M., Saltin, B., and Pedersen, B. K. (2000) Production of IL-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma IL-6. *J. Physiol. (London)* **529,** 237–242
31. Steensberg, A., Febbario, M., van Hall, G., Osada, T., Sacchetti, M., 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. (London)* **537,** 633–639
32. Steensberg, A., Toft, A. D., S. P., Halkjaer-Kristensen, J., and Pedersen, B. K. (2001) Plasma interleukin-6 during strenuous exercise—role of adrenaline. *Am. J. Physiol.* **281,** C1001–C1004
33. Toft, A. D., Ostrowski, K., Asp, S., Møller, K., Iversen, S., Hermann, C., Søndergaard, S. R., and Pedersen, B. K. (2000). The effects of n-3 PUFA on the cytokine response to strenuous exercise. *J. Cell. Physiol.* **89,** 2401–2405
34. Nielsen, H. B., Secher, N., and Perdersen, B. K. (1996) Lymphocytes and NK cell activity during repeated bouts of maximal exercise. *Am. J. Physiol.* **271,** R222–R227
35. Ostrowski, K., Schjerling, P., and Pedersen, B. K. (2000) Physical activity and plasma interleukin-6 in humans—effect of intensity of exercise. *Eur. J. Cell. Physiol.* **86,** 512–515
36. Nieman, D. C., Nehlsen-Canarella, S. L., Fagoaga, O. R., Henson, D. A., Utter, A., Davis, J. M., Williams, F., and Butterworth, D. E. (1998) Influence of mode and carbohydrate on the cytokine response to heavy exertion. *Med. Sci. Sports Exerc.* **30,** 671–678
37. 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
38. Pedersen, B. K., Bruunsgaard, H., Klokker, M., Kappel, M., MacLean, D. A., Nielsen, H. B., Rohde, T., Ullum, H., and Zacho, M. (1997) Exercise-induced immunomodulation—possible roles of neuroendocrine factors and metabolic factors. *Int. J. Sports Med.* **18,** S2–S7
39. Bruunsgaard, H., Galbo, H., Halkjaer-Kristensen, J., Johansen, T. L., MacLean, D. A., and Pedersen, B. K. (1997) Exercise-induced increase in interleukin-6 is related to muscle damage. *J. Physiol. (London)* **499,** 833–841
40. Croisier, J. L., Camus, G., Venneman, I., Deby-Dupont, G., Juchmes-Ferir, A., Lamy, M., Crielaard, J. M., Deby, C., and Duchateau, J. (1999) Effects of training on exercise-induced muscle damage and interleukin 6 production. *Muscle Nerve* **22,** 208–212
41. Toft, A. D., Jensen, L. B., Bruunsgaard, H., Ibfelt, T., Halkjær-Kristensen, J., Febbraio, M. A., and Pederse, B. K. (2002) The cytokine response to eccentric exercise in young and elderly humans. *Am. J. Physiol.* In press
42. Nehlsen Cannarella, S. L., Fagoaga, O. R., Nieman, D. C., Henson, D. A., Butterworth, D. E., Schmitt, R. L., Bailey, E. M., Warren, B. J., Utter, A., and Davis, J. M. (1997) Carbohydrate and the cytokine response to 2.5 h of running. *J. Cell. Physiol.* **82,** 1662–1667
43. Rhind, S. G., Castellani, J. W., Brenner, I. K., Shephard, R. J., Zamecnik, J., Montain, S. J., Young, A. J., and Shek, P. N. (2001) Intracellular monocyte and serum cytokine expression is modulated by exhausting exercise and cold exposure. *Am. J. Physiol.* **281,** R66–R75
44. DeRijk, R. H., Boelen, A., Tilders, F. J., and Berkenbosch, F. (1994) Induction of plasma interleukin-6 by circulating adrenaline in the rat. *Psychoneuroendocrinology* **19,** 155–163
45. Starkie, R. L., Angus, D. J., Rolland, J., Hargreaves, M., and Febbraio, M. (2000) Effect of prolonged submaximal exercise and carbohydrate ingestion on monocyte intracellular cytokine production in humans. *J. Physiol. (London)* **528,** 647–655
46. Steensberg, A., Vissing, J., and Pedersen, B. K. (2001) Lack of IL-6 production during exercise in patients with mitochondrial myopathy. *Eur. J. Cell. Physiol.* **84,** 155–157
47. Moldoveanu, A. I., Shephard, R. J., and Shek, P. N. (2000) Exercise elevates plasma levels but not gene expression of IL-1beta. IL-6, and TNF-alpha in blood mononuclear. *J. Appl. Physiol.* **89,** 1499–1504
48. Jonsdottir, I., Schjerling, P., Ostrowski, K., Asp, S., Richter, E. A., and Pedersen, B. K. (2000). Muscle contractions induces interleukin-6 mRNA production in rat skeletal muscles. *J. Physiol. (London)* **528,** 157–163
49. Bartoccioni, E., Michaelis, D., and Hohlfeld, R. (1994) Constitutive and cytokine-induced production of interleukin-6 by human myoblasts. *Immunol. Lett.* **42,** 135–138
50. De Rossi, M., Bernasconi, P., Baggi, F., de Waal, M. R., and Mantegazza, R. (2000) Cytokines and chemokines are both expressed by human myoblasts: possible relevance for the immune pathogenesis of muscle inflammation. *Int. Immunol.* **12,** 1329–1335
51. Klouche, M., Bhakdi, S., Hemmes, M., and Rose-John, S. (1999) Novel path to activation of vascular smooth muscle cells: up-regulation of gp130 creates an autocrine activation loop by IL-6 and its soluble receptor. *J. Immunol.* **163,** 4583–4589
52. Langberg, H., Olesen, J., Gemmer, C., and Kjaer, M. (2002) IL-6 production in various types of tissues as measured by microdialysis in response to exercise in humans. *J. Physiol. (London)* **539P,** abstr.
53. Malm, C., Nyberg, P., Engstrom, M., Sjodin, B., Lenkei, R., Ekblom, B., and Lundberg, I. (2000) Immunological changes in human skeletal muscle and blood after eccentric exercise and multiple biopsies. *J. Physiol. (London)* **529,** 243–262
54. Stith, R. D., and Luo, J. (1994) Endocrine and carbohydrate responses to interleukin-6 in vivo. *Circ. Shock.* **44,** 210–215
55. Ritchie, D. G. (1990) Interleukin 6 stimulates hepatic glucose release from prelabeled glycogen pools. *Am. J. Physiol.* **258,** E57–E64
56. Liao, J., Keiser, J. A., Scales, W. E., Kunkel, S. L., and Kluger, M. J. (1995) Role of epinephrine in TNF and IL-6 production from isolated perfused rat liver. *Am. J. Physiol.* **268,** R896–R901
57. Liao, J., Keiser, J. A., Scales, W. E., Kunkel, S. L., and Kluger, M. J. (1995) Role of epinephrine in TNF and IL-6 production from isolated perfused rat liver. *Am. J. Physiol.* **1995,** 268, R896–R901
58. McConell, G., Fabris, S., Proietto, J., and Hargreaves, M. (1994) Effect of carbohydrate ingestion on glucose kinetics during exercise. *J. Cell. Physiol.* **77,** 1537–1541
59. Jeukendrup, A. E., Raben, A., Gijsen, A., Stegen, J. H., Brouns, F., Saris, W. H., and Wagenmakers, A. J. (1999) Glucose kinetics during prolonged exercise in highly trained human subjects: effect of glucose ingestion. *J. Physiol. (London)* **515,** 579–589
60. Howlett, K., Angus, D., Proietto, J., and Hargreaves, M. (1998) Effect of increased blood glucose availability on glucose kinetics during exercise. *J. Cell. Physiol.* **1998,** 84, 1413–1417
61. Stouthard, J. M., Romijn, J. A., van der. P. T., Endert, E., Klein, S., Bakker, P. J., Veenhof, C. H., and Sauerwein, H. P. (1995) Endocrinologic and metabolic effects of interleukin-6 in humans. *Am. J. Physiol.* **268,** E813–E819
62. Olson, E. N., and Williams, R. S. (2000) Remodeling muscles with calcineurin. *Bioessays* **22,** 510–519
63. Keller, C., Hellsten, Y., Pilegaard, H., Febbraio, M. A., and Pedersen, B. K. (2002) Human muscle cells express IL-6 via a Ca^{2+} dependent pathway. *J. Physiol. (London)* **539P,** abstr.
64. Berridge, M. J. (1993) Inositol trisphosphate and calcium signalling. *Nature (London)* **361,** 315–325
65. Ghosh, A., and Greenberg, M. E. (1995) Calcium signaling in neurons: molecular mechanisms and cellular consequences. *Science* **268,** 239–247
66. Dolmetsch, R. E., Xu, K., and Lewis, R. S. (1998) Calcium oscillations increase the efficiency and specificity of gene expression. *Nature (London)* **392,** 933–936
67. Rao, A., Luo, C., and Hogan, P. G. (1997) Transcription factors of the NFAT family: regulation and function. *Annu. Rev. Immunol.* **15,** 707–747
68. Crabtree, G. R. (1999) Generic signals and specific outcomes: signaling through Ca^{2+}, calcineurin, and NF-AT. *Cell* **96,** 611–614
69. Boppart, M. D., Asp, S., Wojtaszewski, J. F., Fielding, R. A., Mohr, T., and Goodyear, L. J. (2000) Marathon running transiently increases c-Jun NH2-terminal kinase and p38 activities in human skeletal muscle. *J. Physiol. (London)* **526,** 663–669
70. Tuyt, L. M., Dokter, W. H., Birkenkamp, K., Koopmans, S. B., Lummen, C., Kruijer, W., and Vellenga, E. (1999) Extracellu-

lar-regulated kinase 1/2. Jun N-terminal kinase, and c-Jun are involved in NF-kappa B-dependent IL-6 expression in human monocytes. *J. Immunol.* **162,** 4893–4902
71. Coggan, A. R., and Coyle, E. F. (1991) Carbohydrate ingestion during prolonged exercise: effects on metabolism and performance. *Exerc. Sport Sci. Rev.* **19,** 1–40
72. Gleeson, M., and Bishop, N. C. (2000) Special feature for the Olympics: effects of exercise on the immune system: modification of immune responses to exercise by carbohydrate, glutamine and anti-oxidant supplements. *Immunol. Cell Biol.* **78,** 554–561
73. Byrd, S. K., Bode, A. K., and Klug, G. A. (1989) Effects of exercise of varying duration on sarcoplasmic reticulum function. *J. Cell. Physiol.* **66,** 1383–1389
74. Chin, E. R., and Allen, D. G. (1996) The role of elevations in intracellular [Ca^{2+}] in the development of low frequency fatigue in mouse single muscle fibres. *J. Physiol. (London)* **491,** 813–824
75. Stephenson, D. G., and Nguyen, L. T., Stephenson, G. M. (1999) Glycogen content and excitation-contraction coupling in mechanically skinned muscle fibres of the cane toad. *J. Physiol. (London)* **519,** 177–187
76. Booth, J., McKenna, M. J., Ruell, P. A., Gwinn, T. H., Davis, G. M., Thompson, M. W., Harmer, A. R., Hunter, S. K., and Sutton, J. R. (1997) Impaired calcium pump function does not slow relaxation in human skeletal muscle after prolonged exercise. *J. Cell. Physiol.* **83,** 511–521
77. Craig, R., Larkin, A., Mingo, A. M., Thuerauf, D. J., Andrews, C., McDonough, P. M., and Glembotski, C. C. (2000) p38 MAPK and NF-kappa B collaborate to induce interleukin-6 gene expression and release. Evidence for a cytoprotective autocrine signaling pathway in a cardiac myocyte model system. *J. Biol. Chem.* **275,** 23814–23824
78. Chae, H. J., Kim, S. C., Chae, S. W., An, N. H., Kim, H. H., Lee, Z. H., and Kim, H. R. (2001) Blockade of the p38 mitogen-activated protein kinase pathway inhibits inducible nitric oxide synthase and interleukin-6 expression in MC3T3E-1 osteoblasts. *Pharmacol. Res.* **43,** 275–283
79. Derave, W., Hansen, B. F., Lund, S., Kristiansen, S., and Richter, E. A. (2000) Muscle glycogen content affects insulin-stimulated glucose transport and protein kinase B activity. *Am. J. Physiol.* **279,** E947–E955
80. Holman, G. D., and Kasuga, M. (1997) From receptor to transporter: insulin signalling to glucose transport. *Diabetologia* **40,** 991–1003
81. Gleeson, M. (2000) Interleukins and exercise. *J. Physiol. (London)* **529,** 1
82. Wahren, J., Felig, P., Ahlborg, G., and Jorfeldt, L. (1971) Glucose metabolism during leg exercise in man. *J. Clin. Invest.* **50,** 2715–2725
83. Kjaer, M. (1998) Hepatic glucose production during exercise. *Adv. Exp. Med. Biol.* **441,** 117–127
84. Coker, R. H., Simonsen, L., Bulow, J., Wasserman, D. H., and Kjaer, M. (2001) Stimulation of splanchnic glucose production during exercise in humans contains a glucagon-independent component. *Am. J. Physiol.* **280,** E918–E927
85. Cryer, P. E. (1993) Glucose counter regulation: prevention and correction of hypoglycemia in humans. *Am. J. Physiol.* **264,** E149–E155
86. Howlett, K., Febbraio, M., and Hargreaves, M. (1999) Glucose production during strenuous exercise in humans: role of epinephrine. *Am. J. Physiol.* **276,** E1130–E1135
87. Sigal, R. J., Fisher, S. J., Manzon, A., Morais, J. A., Halter, J. B., Vranic, M., and Marliss, E. B. (2000) Glucoregulation during and after intense exercise: effects of alpha-adrenergic blockade. *Metabolism* **49,** 386–394
88. Sigal, R. J., Purdon, C., Bilinski, D., Vranic, M., Halter, J. B., and Marliss, E. B. (1994) Glucoregulation during and after intense exercise: effects of beta-blockade. *J. Clin. Endocrinol. Metab.* **78,** 359–366
89. Kanemaki, T., Kitade, H., Kaibori, M., Sakitani, K., Hiramatsu, Y., Kamiyama, Y., Ito, S., and Okumura, T. (1998) Interleukin 1beta and interleukin 6, but not tumor necrosis factor alpha, inhibit insulin-stimulated glycogen synthesis in rat hepatocytes. *Hepatology* **27,** 1296–1303
90. Tsigos, C., Papanicolaou, D. A., Kyrou, I., Defensor, R., Mitsiadis, C. S., and Chrousos, G. P. (1997) Dose-dependent effects of recombinant human interleukin-6 on glucose regulation [see comments]. *J. Clin. Endocrinol. Metab.* **82,** 4167–4170
91. James, D. E., Strube, M., and Mueckler, M. (1980) Molecular cloning and characterization of an insulin-regulatable glucose transporter. *Nature (London)* **338,** 83–87
92. Kelly, K. L., Ruderman, N. B., and Chen, K. S. (1992) Phosphatidylinositol-3-kinase in isolated rat adipocytes. Activation by insulin and subcellular distribution. *J. Biol. Chem.* **267,** 3423–3428
93. Sun, X. J., Rothenberg, P., Kahn, C. R., Backer, J. M., Araki, E., Wilden, P. A., Cahill, D. A., Goldstein, B. J., and White, M. F. (1991) Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. *Nature (London)* **352,** 73–77
94. Backer, J. M., Schroeder, G. G., Kahn, C. R., Myers, M. G., Jr., Wilden, P. A., Cahill, D. A., and White, M. F. (1992) Insulin stimulation of phosphatidylinositol 3-kinase activity maps to insulin receptor regions required for endogenous substrate phosphorylation. *J. Biol. Chem.* **267,** 1367–1374
95. Bourey, R. E., Coggan, A. R., Kohrt, W. M., Kirwan, J. P., King, D. S., and Holloszy, J. O. (1990) Effect of exercise on glucose disposal: response to a maximal insulin stimulus. *J. Cell. Physiol.* **69,** 1689–1694
96. Lee, A. D., Gulve, E. A., Chen, M., Schluter, J., and Holloszy, J. O. (1995) Effects of Ca^{2+} ionophore ionomycin on insulin-stimulated and basal glucose transport in muscle. *Am. J. Physiol.* **268,** R997–R1002
97. Lund, S., Holman, G. D., Schmitz, O., and Pedersen, O. (1995) Contraction stimulates translocation of glucose transporter GLUT4 in skeletal muscle through a mechanism distinct from that of insulin. *Proc. Natl. Acad. Sci. USA* **92,** 5817–5821
98. Yeh, J. I., Gulve, E. A., Rameh, L., and Birnbaum, M. J. (1995) The effects of wortmannin on rat skeletal muscle. Dissociation of signaling pathways for insulin- and contraction-activated hexose transport. *J. Biol. Chem.* **270,** 2107–2111
99. Hayashi, T., Hirshman, M. F., Kurth, E. J., Winder, W. W., and Goodyear, L. J. (1998) Evidence for 5′ AMP-activated protein kinase mediation of the effect of muscle contraction on glucose transport. *Diabetes* **47,** 1369–1373
100. Bergeron, R., Russell, R. R., III, Young, L. H., Ren, J. M., Marcucci, M., Lee, A., and Shulman, G. I. (1999) Effect of AMPK activation on muscle glucose metabolism in conscious rats. *Am. J. Physiol.* **267,** E938–E944
101. Richter, E. A., Derave, W., and Wojtaszewski, J. F. (2001) Glucose, exercise and insulin: emerging concepts. *J. Physiol. (London)* **535,** 313–322
102. Mu, J., Brozinick, J. T., Jr., Valladares, O., Bucan, M., and Birnbaum, M. J. (2001) A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle. *Mol. Cell* **7,** 1085–1094
103. Stouthard, J. M., Oude Elferink, R. P., and Sauerwein, H. P. (1996) Interleukin-6 enhances glucose transport in 3T3–L1 adipocytes. *Biochem. Biophys. Res. Commun.* **220,** 241–245
104. Hardin, J., Kroeker, K., Chung, B., and Gall, D. G. (2000) Effect of proinflammatory interleukins on jejunal nutrient transport. *Gut* **47,** 184–191
105. Bedard, S., Marcotte, B., and Marette, A. (1997) Cytokines modulate glucose transport in skeletal muscle by inducing the expression of inducible nitric oxide synthase. *Biochem. J.* **325,** 487–493
106. Bradley, S. J., Kingwell, B. A., and McConell, G. K. (1999) Nitric oxide synthase inhibition reduces leg glucose uptake but not blood flow during dynamic exercise in humans. *Diabetes* **48,** 1815–1821
107. Nonogaki, K., Fuller, G. M., Fuentes, N. L., Moser, A. H., Staprans, I., Grunfeld, C., and Feingold, K. R. (1995) Interleukin-6 stimulates hepatic triglyceride secretion in rats. *Endocrinology* **136,** 2143–2149
108. 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
109. Mohamed-Ali, V., Goodrick, S., Bulmer, K., Holly, J. M., Yudkin, J. S., and Coppack, S. W. (1999) Production of soluble tumor necrosis factor receptors by human subcutaneous adipose tissue in vivo. *Am. J. Physiol.* **277,** E971–E9755

110. Orban, Z., Remaley, A. T., Sampson, M., Trajanoski, Z., and Chrousos, G. P. (1999) The differential effect of food intake and beta-adrenergic stimulation on adipose-derived hormones and cytokines in man. *J. Clin. Endocrinol. Metab.* **84,** 2126–2133
111. Lyngsø, D., Simonsen, L., and Bülow, J. (2002) Interleukin-6 production in human subcutaneous tissue. The effect of exercise. *J. Physiol. (London)* In press
112. Ershler, W. B., and Keller, E. T. (2000) Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. *Annu. Rev. Med.* **51,** 245–270
113. Kern, P. A., Ranganathan, S., Li, C., Wood, L., and Ranganathan, G. (2001) Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am. J. Physiol.* **280,** E745–E751
114. Pradhan, A. D., Manson, J. E., Rifai, N., Buring, J. E., and Ridker, P. M. (2001) C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *J. Am. Med. Assoc.* **286,** 327–334
115. McCarty, M. F. (1999) Interleukin-6 as a central mediator of cardiovascular risk associated with chronic inflammation, smoking, diabetes, and visceral obesity: down-regulation with essential fatty acids, ethanol and pentoxifylline. *Med. Hypotheses* **52,** 465–477
116. Staels, B., Koenig, W., Habib, A., Merval, R., Lebret, M., Torra, I. P., Delerive, P., Fadel, A., Chinetti, G., Fruchart, J. C., Najib, J., Maclouf, J., and Tedgui, A. (1998) Activation of human aortic smooth-muscle cells is inhibited by PPARalpha but not by PPARgamma activators. *Nature (London)* **393,** 790–793
117. Yudkin, J. S., Kumari, M., Humphries, S. E., and Mohammed-Ali V (2000) Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis* **148,** 209–214
118. Ridker, P. M., Rifai N. Stampfer, M. J., and Hennekens, C. H. (2000) Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* **101,** 1767–1772
119. Ridker, P. M., Hennekens, C. H., Buring, J. E., and Rifai, N. (2000) C. reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N. Engl. J. Med.* **342,** 836–843
120. Bruunsgaard, H., Skinhoj, P., Qvist, J., and Pedersen, B. K. (1999) Elderly humans show prolonged in vivo inflammatory activity during pneumococcal infections. *J. Infect. Dis.* **180,** 551–554
121. Dovio, A., and Angeli, A. (2001) Cytokines and type 2 diabetes1mellitus. *J. Am. Med. Assoc.* **286,** 2233
122. Fried, S. K., Bunkin, D. A., and Greenberg, A. S. (1998) Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J. Clin. Endocrinol. Metab.* **83,** 847–850
123. Vgontzas, A. N., Papanicolaou, D. A., Bixler, E. O., Hopper, K., Lotsikas, A., and Lin, H. M. (2000) Sleep Apnea and daytime sleepiness and fatigue: relation to visceral obesity, insulin resistance, and hypercytokinemia. *J. Clin. Endocrinol. Metab.* **85,** 1151–1158
124. Saghizadeh, M., Ong, J. M., Garvey, W. T., Henry, R. R., and Kern, P. A. (1996) The expression of TNF alpha by human muscle. Relationship to insulin resistance. *J. Clin. Invest.* **97,** 1111–1116
125. Halse, R., Pearson, S. L., McCormack, J. G., Yeaman, S. J., and Taylor, R. (2001) Effects of tumor necrosis factor-beta on insulin action in cultured human muscle cells. *Diabetes* **50,** 1102–1109
126. Youd, J. M., Rattigan, S., and Clark, M. G. (2000) Acute impairment of insulin-mediated capillary recruitment and glucose uptake in rat skeletal muscle in vivo by TNF-alpha. *Diabetes* **49,** 1904–1909
127. Uysal, K. T., Wiesbrock, S. M., Marino, M. W., and Hotamisligil, G. S. (1997) Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature (London)* **389,** 610–614
128. Hotamisligil, G. S. (1999) The role of TNFalpha and TNF receptors in obesity and insulin resistance. *Intern. Med.* **245,** 621–625
129. Fernandez-Real, J. M., Broch, M., Vendrell, J., Gutierrez, C., Casamitjana, R., Pugeat, M., Richart, C., and Ricart, W. (2000) Interleukin-6 gene polymorphism and insulin sensitivity. *Diabetes* **49,** 517–520
130. Pickup, J. C., Mattock, M. B., Chusney, G. D., and Burt, D. (1997) NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. *Diabetologia* **40,** 1286–1292
131. Bruunsgaard, H., Skinhoj, P., Pedersen, A. N., Schroll, M., and Pedersen, B. K. (2000) Ageing, tumour necrosis factor-alpha (TNF-alpha) and atherosclerosis. *Clin. Exp. Immunol.* **121,** 255–260
132. Tilg, H., Dinarello, C. A., and Mier, J. W. (1997) IL-6 and APPs: anti-inflammatory and immunosuppressive mediators. *Immunol. Today* **18,** 428–432
133. Pue, C. A., Mortensen, R. F., Marsh, C. B., Pope, H. A., and Wewers, M. D. (1996) Acute phase levels of C-reactive protein enhance IL-1 beta and IL-1ra production by human blood monocytes but inhibit IL-1 beta and IL-1ra production by alveolar macrophages. *J. Immunol.* **156,** 1594–1600
134. Tanaka, T., Kanda, T., McManus, B. M., Kanai, H., Akiyama, H., Sekiguchi, K., Yokoyama, T., and Kurabayashi, M. (2001) Overexpression of interleukin-6 aggravates viral myocarditis: impaired increase in tumor necrosis factor-alpha. *J. Mol. Cell Cardiol.* **33,** 1627–1635
135. Schindler, R., Mancilla, J., Endres, S., Ghorbani, R., Clark, S. C., and Dinarello, C. A. (1990) Correlations and interactions in the production of interleukin-6 (IL-6). IL-1, and tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF. *Blood* **75,** 40–47
136. Matthys, P., Mitera, T., Heremans, H., van Damme, J., and Billiau, A. (1995) Anti-gamma interferon and anti-interleukin-6 antibodies affect staphylococcal enterotoxin B-induced weight loss, hypoglycemia and cytokine release in D-galactose-sensitized and unsensitized mice. *Infect. Immun.* **63,** 1158–1164
137. Mizuhara, H., O'Neill, E., Seki, N., Ogawa, T., Kusunoki, C., Otsuka, K., Satoh, S., Niwa, M., Senoh, H., and Fujiwara, H. (1994) T cell activation-associated hepatic injury: mediation by tumor necrosis factors and protection by interleukin 6. *J. Exp. Med.* **179,** 1529–1537
138. Steensberg, A., Toft, A. D., Bruunsgaard, H., Sandmand, M., Halkjær-Kristensen, J., and Pedersen, B. K. (2001) Strenuous exercise decreases the percentage of type 1 T cells in the circulation. *J. Cell. Physiol.* **91,** 1708–1712
139. Zierath, J. R., Krook, A., and Wallberg-Henriksson, H. (2000) Insulin action and insulin resistance in human skeletal muscle. *Diabetologia* **43,** 821–835
140. DiCosmo, B. F., Picarella, D., and Flavell, R. A. (1994) Local production of human IL-6 promotes insulitis but retards the onset of insulin-dependent diabetes mellitus in non-obese diabetic mice. *Int. Immunol.* **6,** 1829–1837
141. Febbraio, M. A., Carey, A. L., Lamont, B., Koukoulas, I., Andrikopoulos, S., and Proietto, J. (2002) Interleukin-6 expression in insulin-resistant rodent skeletal muscle *J. Physiol. (London)* **539P,** abstr.