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For Debate

Interleukin-6 and insulin sensitivity: friend or foe?

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Introduction

With the discovery of a number of immunomodulatory cytokines 15 to 20 years ago [1, 2] came the observation that so-called "inflammatory cytokines" such as TNFα, IL-6, IL-1α and IL-1β were capable of exerting effects on the body's major metabolic tissues: skeletal muscle, adipose tissue and the liver [3]. These cytokines normally circulate in low concentrations and are expressed in a wide variety of tissues, including those mentioned above. Since these cytokines often appear to be elevated in various disease states, such as cachexia [4], Type 1 [5] and Type 2 [6] diabetes, the hypothesis that these cytokines may be involved in the aetiology of these diseases has been the subject of numerous investigations. Much work has focused on the role of TNFα in the induction of Type 2 diabetes and insulin resistance, and although somewhat controversial, it is generally accepted that TNFα has a role to play in the aetiology of insulin resistance [7]. However, because IL-6 is often, but somewhat incorrectly [8], grouped together with TNFα as the so-called "pro-inflammatory" cytokines, IL-6 is seen as having a similar role to TNFα in terms of metabolism. It is true that many studies suggest that both IL-6 and TNFα have similar functions, but there are also quite a number that make observations to the contrary (for review see [9]). While some studies have shown a link between IL-6 and impaired insulin action, there is a strong body of literature suggesting that IL-6 does not cause insulin resistance, and in some instances may have beneficial effects on this disease. This review attempts to summarise the roles of IL-6 in insulin action, and show that IL-6 may induce insulin resistance in certain instances, but not in others.

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Abbreviations: Akt, acute transforming retrovirus thymoma · IL-6r, interleukin-6 receptor · Jak, Janus-activated protein kinase · mTOR, mammalian target of rapamycin · PI3-K, phosphatidylinositol-3-kinase · rh, recombinant human · sIL-6r, soluble interleukin-6 receptor · SOCS, suppressor of cytokine signalling · STAT, signal transducer and activator of transcription

IL-6 signal transduction

Although the protein was originally sequenced almost 25 years ago [10], IL-6 was first cloned and structurally characterised by Hirano et al. [11, 12]. Its nomenclature was changed soon after to IL-6 (for review see [13]). IL-6 is expressed in carcinoma cells [12], T and B cells [14], endothelial cells [15], and in recent years has been found to be expressed in, and released from, adipocytes [16, 17] and skeletal muscle [18, 19, 20]. Although a multitude of immunomodulatory functions have been ascribed to IL-6 (for review see [13]), it has also been shown to act on a number of tissues unrelated to inflammation or immunity (for review see [9]).

IL-6 transduces cellular signals through binding with either its membrane-bound receptor (IL-6r) [21]

or a cleaved soluble version of the membrane-bound receptor (sIL-6r) [22, 23]; the bound IL-6/IL-6r can then associate with the membrane-bound glycoprotein gp130. Heterodimers of IL-6, IL-6r and gp130 then form, and the intracellular signals are initiated. Since heterodimers of sIL-6r bound to IL-6 are able to bind to and activate the gp130 receptor system, cells that have either extremely low or no expression of IL-6r, but that express gp130, such as skeletal muscle [24, 25, 26], are often still responsive to cytokine stimulation given the presence of soluble receptors (for reviews see [27, 28]).

The cellular effects of IL-6 are known to be induced through signalling through the Jak-STAT pathway, whereby the IL-6r–gp130 heterodimer activates members of the Janus-activated protein kinases (Jak), Jak1, Jak2 and Tyk2. These proteins then phosphorylate and activate the signal transducer and activator of transcription (STAT)-3 in a multitude of cell types [29]. As a result of STAT-3 signalling, cytokines induce transcription of a family of proteins termed the suppressors of cytokine signalling (SOCS) [30]. The importance of interaction between these proteins and other cell signalling pathways will be discussed later in the article.

Sources of circulating IL-6

Concentrations of circulating IL-6 are low in healthy humans, generally at levels of <4 pg/ml [31, 32, 33, 34, 35]. Since a large number of cells are able to produce IL-6, it is difficult to isolate the sources of the IL-6 found in circulation, but it is to be expected that immune cells contribute to some extent in the basal state, although probably not during heavy muscular work [35, 36]. In addition, because IL-6 is rapidly cleared when large doses are infused [37, 38], or because plasma levels rise during exercise [39], isolating the cellular/tissue sources in vivo becomes more difficult. Rodent studies have shown that hepatocytes produce IL-6 in response to corticosterone [40], epinephrine [41], partial hepactectomy [42], thermal injury [43], and endotoxin [44]. In addition, the protein "proteolysis-inducing factor" increases the production of IL-6 in cultured human hepatocytes [45]. To our knowledge, only one study has measured IL-6 flux across the hepatosplanchnic viscera. In healthy resting humans, there is a small (~1.0 ng/min) net uptake of IL-6 by hepatosplanchnic tissue, which increases markedly during heavy exercise [46]. Whether these tissues in disease states release IL-6 is not known.

It has been shown that IL-6 is expressed in and released from subcutaneous adipocytes in humans in vivo [17]. Furthermore, adipose tissue might contribute as much as 35% to the basal circulating levels [17].

It is now well known that skeletal muscle produces a significant amount of IL-6 during exercise (for review see [9]), although the contribution from skeletal muscle at rest is unclear. Despite this, skeletal muscle cells do express *IL-6* mRNA [19] and protein [18, 19] in the basal state. Muscle tissue beds release small amounts of IL-6 into the circulation in older subjects [47], although this may not be the case in the young [20]. Furthermore, it has recently been shown that IL-6 stimulates its own expression in skeletal muscle [48] and adipocytes [49].

Elevated IL-6 in insulin resistance: correlative or causative?

In a series of pioneering investigations, Hotamisligil et al. demonstrated that TNFα is elevated in adipocytes and plasma of insulin-resistant rodents, and that neutralisation of circulating TNFα improved this condition [50]. Their in vitro work demonstrated that TNFα inhibited activation of various insulin-signalling proteins [51]. Since IL-6 is a cytokine that is not only often elevated in Type 2 diabetes, but its production is also stimulated by TNFα [52], it was hypothesised that IL-6 might contribute to the induction of Type 2 diabetes [6, 53]. Although plasma IL-6 levels are often significantly elevated in Type 2 diabetes [6, 31, 32, 33, 34, 54], they are rarely >5 pg/ml. In recent studies, we have matched patients with Type 2 diabetes with control subjects, for age, weight, sex and BMI. In three separate cohorts we found no evidence that circulating IL-6 is elevated in the patient population [47] (and unpublished observations). The possible reason for these apparent anomalous results will be discussed later in the article.

As discussed, *IL-6* mRNA expression in human subcutaneous adipose tissue has been shown previously [17]. In more recent studies, adipose tissue *IL-6* mRNA has been shown to be elevated in insulin-resistant humans [33, 55, 56], which correlated with reduced rates of insulin-stimulated glucose disposal [57]. These data provide some evidence that IL-6 is related to insulin resistance. Critically, however, they do not provide any evidence that elevated IL-6 actually causes insulin resistance through a specific pathway. Given that much of the IL-6 is produced by adipose tissue at rest, it is possible that the elevated IL-6 seen in patients with Type 2 diabetes is the result of elevated adiposity in these patients, rather than IL-6 exerting a negative effect on insulin action. Indeed, Vozarova et al. [32] demonstrated that in an ethnic population susceptible to insulin resistance, IL-6 was negatively correlated to insulin action and positively correlated to adiposity. However, after adjustment for adiposity, there was no correlation between IL-6 and insulin action. We have recently conducted a study in which we measured BMI and IL-6 in patients with Type 2 diabetes and in healthy matched control subjects. We performed hyperinsulinaemic–euglycaemic

clamps on these populations and measured circulating IL-6. We found no relationship between IL-6 and insulin sensitivity as measured by glucose infusion rate during a clamp, but we did find a strong relationship (r=0.85; p<0.00001) between IL-6 and BMI [47]. On balance, therefore, we would suggest that IL-6 is indeed associated with insulin resistance, but that this association is more likely to be due to the elevated fat mass characteristic of patients with Type 2 diabetes.

Of particular importance is the fact that skeletal muscle is the most important organ in relation to insulin sensitivity, since it contributes >90% of insulin-stimulated glucose disposal in healthy patients [58]. It is somewhat surprising, therefore, that there are few studies that have measured *IL-6* expression in insulin-resistant skeletal muscle. We have previously shown that the basal expression of *IL-6* mRNA is not different in an insulin-resistant rodent from in its littermate control [59]. We have also shown that IL-6 protein release at rest and following 45 min of contraction does not differ between patients with Type 2 diabetes and healthy matched subjects [60]. Finally, we have recently demonstrated that *IL-6* mRNA does not differ in the skeletal muscle between patients with Type 2 diabetes and control subjects either before or after insulin stimulation [47]. Further research examining the IL-6 protein content in skeletal muscle from patients with Type 2 diabetes is warranted.

Interaction between IL-6 and glucose transport in vitro

The observations that insulin-sensitive tissues such as adipose tissue [17] and skeletal muscle [20] are sources of IL-6 have prompted many recent investigations into the effect of IL-6 on insulin signalling and glucose transport [37, 55, 57, 61, 62, 63, 64]. However, the first study to examine the effect of cytokines on cellular glucose transport in vitro was published over 15 years ago [65]. These authors obtained an extract from stimulated macrophages, termed a "macrophage monokine preparation" and subjected L6 myotubes to this extract. They found it to increase glucose transport. TNFα and IL-1α were immunoprecipitated from the preparation media, and also used to treat the cells. IL-1α did not affect glucose transport but, somewhat surprisingly, TNFα increased it. In addition, the remaining preparation also enhanced glucose uptake, implicating other molecule/s within the solution, such as IL-6, as stimulators of glucose transport. Stouthard et al. [66] later confirmed that IL-6 is likely to have been a contributor to this effect. These authors demonstrated that treating 3T3-L1 adipocytes with recombinant murine IL-6 for 5 h increased basal glucose transport by ~20%. An increase in IL-6-stimulated basal glucose transport has also been observed in both smooth muscle [67] and chondrocytes [68], although this effect is not always seen, as two studies have found no effect of IL-6 on basal glucose transport in 3T3 L1 adipocytes [55, 69].

Insulin-stimulated glucose transport rates are equally inconclusive, as data suggest that IL-6 either enhances [66] or suppresses [55, 63] insulin-stimulated glucose transport in 3T3-L1 adipocytes. Why are the data so inconclusive? An obvious reason is the use of various cell types, as the mechanisms for glucose uptake may differ. For example, unlike 3T3-L1 adipocytes, chondrocytes do not express GLUT4. Nonetheless, even in differentiated 3T3-L1 adipocytes that are highly responsive to insulin and that express quantifiable amounts of GLUT4, the data are equivocal [55, 63, 66]. One study that observed an increase in glucose uptake [66] and another that observed a decrease in this measure [55] treated the cells for a similar period of ~24 h. Hence, differences in incubation time cannot account for the differences in results. More studies are required to determine the effect of IL-6 on glucose transport in adipocytes to resolve the conflict within the literature.

What are the mechanisms by which IL-6 may impair or increase insulin sensitivity? The reduction in insulin sensitivity in 3T3-L1 adipocytes as a result of IL-6 treatment could be due to the fact that IL-6 reduces the expression of the adipocyte insulin-sensitising adipokine, *adiponectin* [70]. In addition, IL-6 may reduce *GLUT4* and *IRS-1* mRNA, IRS-1 protein and tyrosine phosphorylation [55], although short-term IL-6 treatment, unlike TNFα, does not serine phosphorylate IRS-1 [55]. Data from tumour cells might provide evidence for the improved IL-6-stimulated glucose transport seen in some studies, as it activates the phosphatidylinositol-3-kinase (PI3-K)/acute transforming retrovirus thymoma (Akt) signalling pathway [71, 72, 73], although this remains to be investigated in normal mammalian cell lines.

As discussed previously, there is a paucity of research examining the expression of *IL-6* in the skeletal muscles of patients with metabolic dysregulation. Likewise, few studies have examined the effect of IL-6 on metabolism in myocytes or muscle tissue in vitro. In fact, to our knowledge, only one study has examined the effect of IL-6 on insulin sensitivity in myocytes [64]. In this study, muscle glucose uptake was not measured, but the authors found no suppression of insulin receptor signal transduction in the skeletal muscles of rats exposed to chronic IL-6 treatment, even though such an effect was prevalent in hepatic tissue.

In contrast with adipocytes and myocytes, it is generally accepted that IL-6 causes hepatic insulin resistance. Hepatocytes become insulin resistant when insulin is unable to blunt gluconeogenesis and increase glycogen synthesis. Cultured primary rat hepatocytes treated with IL-6 demonstrate reduced insulin-stimulated glycogen synthase activity, a blunted suppression of glycogen phosphorylase, and a decrease in

glycogen synthesis and increased breakdown [74]. Furthermore, treatment of Hep2G cells and primary rat hepatocytes with IL-6 inhibits insulin receptor autophosphorylation, tyrosine phosphorylation of IRS-1 and subsequent activation of downstream targets PI3-K and Akt [61, 64]. The IL-6-induced reduction in insulin signalling appears to be due to stimulation of SOCS-3, which can directly inhibit the insulin receptor [61]. While the studies on hepatocytes, which are glucose-producing cells, appear conclusive, the same cannot be said for studies on adipocytes and myocytes, which are the primary sink for glucose disposal. We suggest that further work is required in these cell types before definitive conclusions can be drawn. In addition, results obtained from isolated cell systems should be treated with some caution, because they are not representative of multi-organ systems. Indeed, even when different cell types, for example adipocytes and myocytes, are co-cultured and subjected to a pharmacological intervention, the results obtained are different from those obtained through culturing the cell types in isolation and subjecting them to the identical intervention [75].

Interaction between IL-6 and glucose transport in vivo

As discussed, IL-6 protein content in adipose tissue has been negatively correlated to insulin-stimulated glucose disposal [57], and a single polymorphism in the *IL-6* gene promoter has repeatedly been linked to reduced insulin sensitivity and Type 2 diabetes [76, 77, 78]. However, these studies cannot prove that IL-6 is the cause of insulin resistance. More information can be obtained from recombinant human (rh) IL-6 infusion studies.

There have been several studies that have infused IL-6 into humans and measured the glucose and/or insulin response. The first study [79] to use such a protocol saw investigators infuse rhIL-6 into patients with metastatic renal cancer, and observed large increases in glucose rates of appearance and disappearance using isotopic tracer dilution methods. These data support those obtained in hepatocytes in vitro, in that glucose production was increased. Critically, however, the increase in production was matched by an increase in whole-body glucose disposal, suggesting that in insulin-sensitive tissue, glucose uptake is potentiated by IL-6. However, due to the health of the patients, and the extremely high dose of IL-6 administered, resulting in dramatically elevated hormone levels, these results must be interpreted with caution. In contrast, others [80] demonstrated that an extremely high dose (10 μg/kg) of IL-6, resulting in a circulating plasma IL-6 concentration of >1000 pg/ml, caused modest hyperglycaemia. Somewhat interesting was the observation that at low doses (0.1–0.3 μg/kg), blood glucose levels declined while insulin levels were unchanged. These data may suggest, therefore, that at these low doses, IL-6 is actually insulin sensitising. These data must also be treated with caution since a control trial was not performed and the glucose response at low doses of IL-6 may have been entirely due to fasting. In humans, infusion of IL-6 in the high physiological range (~150 pg/ml) increases fatty acid rate of appearance and oxidation [38] but does not alter glucose uptake or oxidation in the same cohort of subjects [37]. These data [37] argue against a negative effect of IL-6 on glucose transport mechanisms in the basal state. In this study, IL-6 was infused directly into a femoral artery, and yet leg glucose uptake measured by arterio–venous balance in the limb infused with IL-6 was not impaired. In addition, whole-body glucose uptake measured via the isotopic tracer dilution method indicated that endogenous glucose production (glucose rate of appearance) was not increased and whole-body glucose disposal (glucose rate of disappearance) was not impaired. In addition, in a separate study, rhIL-6 infusion resulted in a significantly lower plasma glucose concentration than that following a saline infusion after 1.5 h [81]. Of note, at this corresponding time point, subcutaneous adipose tissue glucose uptake was 1.5±0.5 vs 0.3±0.2 μmol·100 g^{-1}·min^{-1} for IL-6 and the control respectively, supporting earlier in vitro work [66] showing that IL-6 is capable of increasing basal glucose uptake in adipocytes. To our knowledge, there are no studies that have infused IL-6 during a hyperinsulinaemic–euglycaemic clamp. However, a recent investigation [77] demonstrated that subjects who possessed the −174 C/C IL-6 promoter genotype had lower insulin sensitivity than subjects who had the 174 G/G polymorphism, which is comparatively a stronger inducer of the IL-6 gene. Taken together, studies in humans suggest that IL-6 has no effect or indeed enhances insulin sensitivity when physiological doses are administered.

Perhaps the most convincing evidence implicating a positive effect of IL-6 on insulin resistance comes from work using a mouse knockout model. In the first of these studies [82], an IL-6 knockout mouse developed mature onset obesity and glucose intolerance, while treating these mice with IL-6 partially arrested the pathophysiology. In the subsequent study [83] treatment of rats with intracerebroventricular IL-6 decreased body fat. In the latter study, however, insulin sensitivity was not measured.

Although IL-6 administration affects neither the regulation of gluconeogenesis nor the expression of hepatic *PEPCK*, it is capable of inhibiting glucagon-induced expression and activation of PEPCK [84]. This may suggest that IL-6 can actually improve hepatic insulin resistance in patients with Type 2 diabetes. In contrast, IL-6 also inhibits insulin activation of glucokinase [85] while it has been shown to induce

hepatic triglyceride secretion in rats [86]. As discussed, chronic treatment with IL-6 induces hepatic insulin resistance in rats, due to marked reductions in insulin receptor autophosphorylation and IRS-1 tyrosine phosphorylation [64]. The authors suggested that this effect was exerted via induction of SOCS-3, since researchers from this group had previously demonstrated that SOCS-3 was a potential mediator of IL-6-dependent insulin resistance in hepatocytes [61]. The above-mentioned studies imply that the negative action of IL-6 on the liver is not to activate gluconeogenesis, but to increase glycogen breakdown, which is consistent with previous in vivo [87] and in vitro [74] work.

Like the in vitro studies, studies conducted in vivo suggest that IL-6 induces hepatic insulin resistance, but if anything, augments peripheral glucose disposal. The reasons why IL-6 would promote insulin resistance in hepatic but not in other insulin-sensitive organs have not been elucidated, although one might suggest that the low expression of the IL-6/gp130 receptor system in muscle explains a lack of induction of IL-6-induced inhibitory proteins such as SOCS proteins. As discussed, physical exercise results in skeletal muscle production and release [19, 20] but hepatic uptake [46] of IL-6. This may indicate that during periods of accelerated metabolic stress, IL-6 acts in an 'endocrine-like' manner, signalling to the liver to produce IL-6 during periods of reduced glucose availability. We recently tested this hypothesis, and showed that IL-6 infused during low-intensity exercise increases hepatic glucose production, but also increases glucose disposal, such that euglycaemia is maintained [88]. Of note, in this study, there were no differences in any metabolic hormones between an IL-6 infusion trial and a control trial, implying a direct effect of IL-6. In addition, the metabolic clearance rate of glucose, a measure of glucose disappearance independent of glycaemia, was higher when IL-6 was infused, implying that IL-6 was enhancing local factors within the tissues responsible for glucose clearance.

Conclusions and future directions

The role of IL-6 in the induction of insulin sensitivity appears inconclusive, with many findings suggesting a causative role in reduced glucose disposal, and others supporting the hypothesis that IL-6 might improve insulin sensitivity. We suggest that a critical factor, at least in vivo, which has been largely overlooked, is the mode of treatment. Generally, acute treatment with IL-6 results in positive effects with respect to insulin sensitivity. The rapid rate at which IL-6 is cleared after infusion [37, 38] or exercise [39] suggests that chronic elevation of IL-6 is not desirable. Indeed, patients with hepatic cirrhosis have compromised metabolic function [89] and it has been hypothesised that this may be due, in part, to elevated IL-6 [90]. Hence, although the elevated plasma concentrations seen in patients with Type 2 diabetes are relatively small, they are nonetheless chronically elevated, which may contribute to the pathogenesis of this disease, as previously suggested [91]. In support of this, Dovio et al. [92] have reported that chronic IL-6 hypersecretion can upregulate glucocorticoid receptor activity leading to hormonal dysregulation.

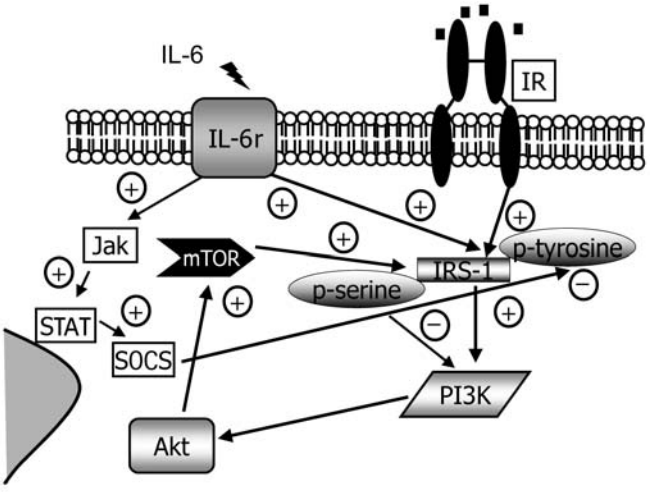

Fig. 1. Hypothesised role for IL-6 in the aetiology of insulin sensitivity within insulin responsive cells (IR). Like insulin, IL-6 may be capable of phosphorylating IRS-1 on tyrosine residues. However, when IL-6 is chronically elevated, Akt phosphorylation can lead to IRS-1 phosphorylation on serine residues via activation of the mammalian target of rapamycin (mTOR). This leads to impaired insulin signalling and cellular insulin resistance. In addition, IL-6 may activate suppressors of cytokine signalling through the Jak/Stat pathway to inhibit tyrosine phosphorylation of IRS-1. Akt, acute transforming retrovirus thymoma; Jak, Janus-activated protein kinase; p, phosphorylation; PI3-K, phosphatidylinositol-3-kinase; SOCS, suppressor of cytokine signalling; STAT, signal transducer and activator of transcription

After careful examination of the literature, we are testing the hypothesis that IL-6 may act upon IRS-1 in a fashion not dissimilar to insulin itself. In Type 2 diabetes, where insulin is chronically elevated, insulin may phosphorylate IRS-1 on serine residues to downregulate PI3-K via activation of the mammalian target of rapamycin (mTOR) which is activated by Akt [93, 94, 95]. Under normal circumstances, however, when insulin circulates at lower levels but is increased in a phasic manner after a meal, tyrosine phosphorylation of IRS-1 occurs, activating PI3-K and glucose uptake. However, Akt is not chronically elevated to stimulate mTOR (Fig. 1). Indeed, we have recently demonstrated that acute IL-6 stimulation phosphorylated Akt to 60% above basal levels in L6 myotubes (unpublished observations).

In summary, further research is required to determine the precise role of IL-6 in the aetiology of insulin sensitivity. However, this review has attempted to

demonstrate that the role of IL-6 in glucoregulatory processes is neither exclusively beneficial nor counter-regulatory.

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