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The IL-6 Paradox: Context Dependent Interplay of SOCS3 and AMPK

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Abstract

Insulin resistance is the principle step towards the progression of type 2 diabetes, and has been linked to increased circulating levels of cytokines, leading to chronic low-grade inflammation. Specifically, in chronic disease states increased IL-6 is thought to play a critical role in the regulation of insulin resistance in the peripheral tissues, and has been used as a marker of insulin resistance. There is also an endogenous up-regulation of IL-6 in response to exercise, which has been linked to improved insulin sensitivity. This leads to the question "how can elevated IL-6 lead to the development of insulin resistance, and yet also lead to increased insulin sensitivity?" Resolving the dual role of IL-6 in regulating insulin resistance/sensitivity is critical to the development of potential therapeutic interventions. This review summarizes the literature on the seemingly paradoxical role of elevated IL-6 on insulin signalling, including the activation of AMPK and the involvement of leptin and SOCS3.

Keywords: Type 2 diabetes; Insulin resistance; Insulin sensitivity; Chronic low-grade inflammation; SOCS3; AMPK; Leptin signalling

Abbreviations: T2D: Type 2 Diabetes; IL-6: Interleukin-6; HFD: High Fat Diet; IR: Insulin Receptor; IRS-1 and IRS-2: Insulin Receptor Substrate 1 and 2; PI3 kinase: Phosphoinositide 3 Kinase; GLUT-4: Glucose Transporter 4; STAT3: Signal Transducer and Activator of Transcription 3; SOCS3: Suppressor of Cytokine Signalling 3; AMPK: AMP Activated Protein Kinase

Introduction

It is estimated that 347 million people worldwide have diabetes, with approximately 90% of those cases being Type 2 Diabetes (T2D) (World Health Organization). There are many grave pathophysiological outcomes of T2D, leading to increased morbidity and mortality. T2D leads to a greater incidence of tissue damage leading to complications in the cardiovascular system, kidneys, retina, and peripheral nervous system. Furthermore, the World Health Organization projects that deaths attributed to diabetes will double between 2005 and 2030. Altered function of insulin at peripheral tissues leads to insulin resistance in skeletal muscle, liver, and adipose tissue, which is critical to the development and progression of T2D.

Insulin is an anabolic hormone that is released by the β-cells in the pancreas to maintain glucose homeostasis within the body. The insulin signaling cascade begins when insulin binds to the Insulin Receptor (IR) on the cell membrane. Insulin binding results in autophosphorylation and activation of the IR beta subunit. Once activated, IR phosphorylates and activates several molecules, including the Insulin Receptor Substrate (IRS) proteins 1 and 2. IR binds to IRS-1 and IRS-2 through the Pleckstrin Homology (PH), and Phosphotyrosine Binding (PTB) domains [1]. IRS-1 functions primarily in skeletal muscle and adipose tissues, whereas IRS-2 functions primarily in the liver [2]. The tyrosine phosphorylation of IRS proteins activates binding sites for Src Homology 2 (SH2) domain proteins including Phosphoinositide 3-kinase (PI3 kinase) [3]. IRS phosphorylates PI3 kinase by binding to the regulatory subunit p85, and generates membrane phosphatidyl-inositol-3,4,5-Trisphosphate (PIP3). In turn, PIP3 recruits and activates phosphoinositide 3-dependent kinase 1 and 2, protein kinase C, and Akt leading to subsequent phosphorylation of downstream targets such as mammalian Target of Rapamycin (mTOR), and glycogen synthase kinase-3β. Insulin is released in response to increased levels of circulating glucose, causing a coordinated response in peripheral tissues with the overall goal to take up and store glucose. Insulin stimulates glucose uptake in skeletal muscle and adipose tissue and causes translocation of Glucose Transporter 4 (GLUT-4) vesicles to the plasma membrane to facilitate glucose transport into the cells (Figure 1). In the liver, insulin signaling regulates gluconeogenesis by inhibiting key enzymes, resulting in reduced hepatic glucose output. Although skeletal muscle accounts for approximately 75% of whole body insulin-stimulated glucose uptake, glycogen synthesis is also stimulated to store large amounts of glucose in the liver [4]. The effects of insulin on glucose metabolism are complex and highly regulated. Adding to this complexity, these signaling pathways can be altered or influenced by various pathophysiological conditions, such as inflammation, infection, and obesity.

The current literature provides evidence that elevated IL-6 plays an important role both in the development of insulin resistance, and as a mediator of increased exercise induced insulin sensitivity. However, there are still many gaps in knowledge related to these context dependent physiological outcomes of elevated IL-6. This review summarizes the literature on the paradoxical role of elevated IL-6 on insulin signaling, and highlights novel questions concerning the relationship between IL-6, SOCS3, and AMPK.

Chronic Low-grade Inflammation vs. Acute Inflammation

Interleukin-6 (IL-6) is a pleiotropic cytokine that is secreted by

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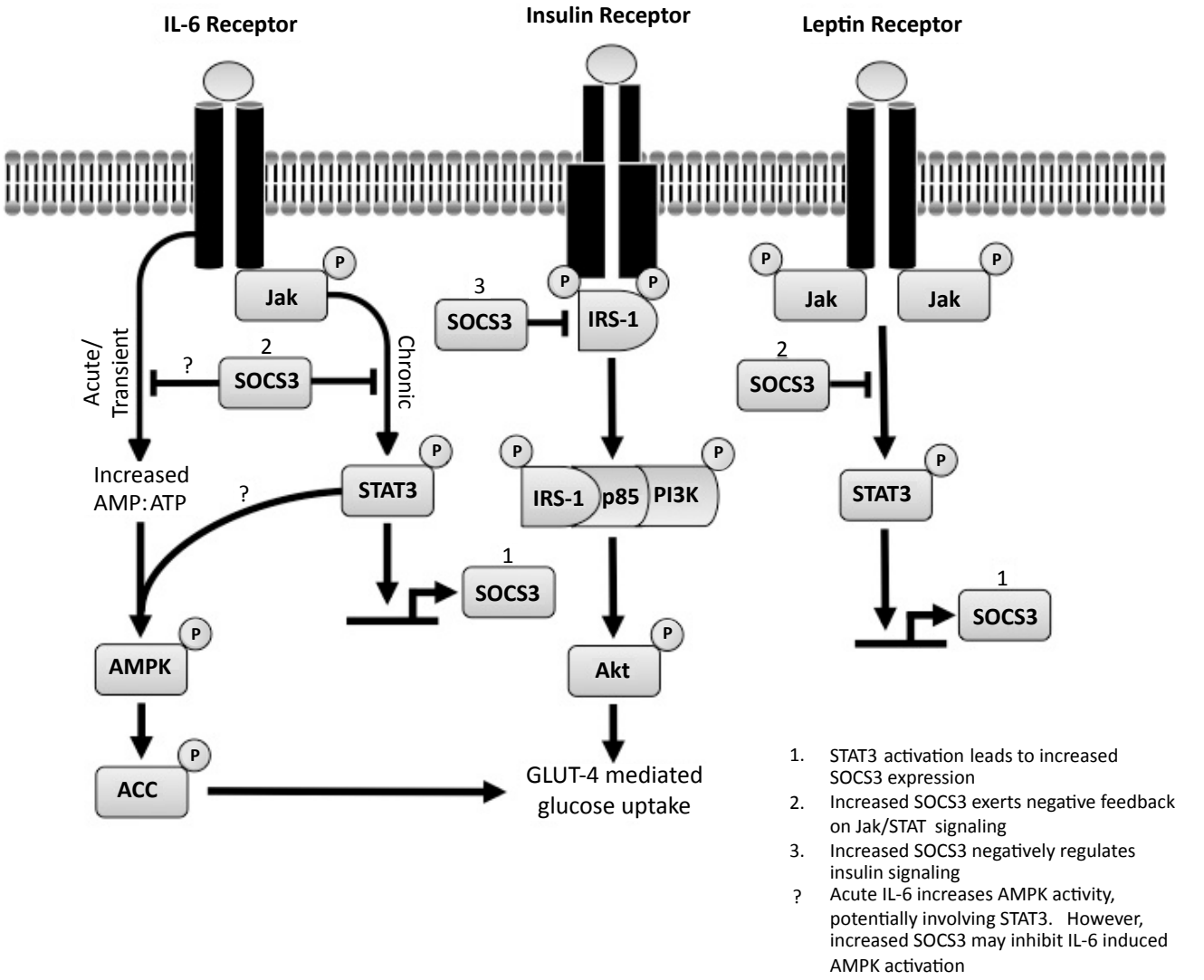

Figure 1: IL-6, insulin, and leptin signaling in skeletal muscle. Chronic low-grade inflammation is associated with increased levels of IL-6, which leads to increased phosphorylation of STAT3, and subsequent increased SOCS3 expression. Increased SOCS3 mediates the inhibitory effects of IL-6 on insulin signaling and glucose uptake. SOCS3 directly disrupts insulin signaling by binding to IR and preventing interaction with IRS-1 (primarily in skeletal muscle and adipose tissue) / IRS-2 (primarily in the liver), and may target IRS-1/IRS-2 for degradation. Disrupting the early signaling events also has downstream effects on other proteins in the insulin pathway including PI3K and Akt, ultimately leading to insulin resistance and attenuated GLUT-4 mediated glucose uptake. Chronically elevated leptin levels also leads to increased SOCS3 expression, which can also directly inhibit insulin mediated glucose uptake. In addition, SOCS3 can have indirect effects on glucose uptake; increased SOCS3 leading to leptin resistance, and impaired leptin mediated AMPK activation. In contrast, acute/transient IL-6 elevations in response to exercise do not cause sustained increased SOCS3 expression, but cause increased insulin sensitivity in peripheral tissues by increased AMPK activity and subsequent increased glucose uptake. IL-6 mediated activation of AMPK in response to exercise is blunted in chronic inflammatory states. Since IL-6 is required for exercise-induced AMPK activation and obesity results in a blunted exercise-induced activation of AMPK, it is possible that increased SOCS3 may cause IL-6 resistance, which results in diminished AMPK activity and increased insulin resistance. IL-6: Interleukin-6; STAT3: Signal Transducer and Activator of Transcription 3; SOCS3: Suppressor of Cytokine Signaling 3; IR: Insulin Receptor; IRS: Insulin Receptor Substrate; PI3K: Phosphoinositide 3 Kinase; GLUT-4: Glucose Transporter 4; AMPK: AMP activated Protein Kinase

and acts on a wide variety of tissues and cells. IL-6 mediates several steps in the activation of inflammatory responses, by regulating the synthesis of pro-inflammatory cytokines [5]. However, IL-6 also promotes the synthesis of anti-inflammatory cytokines such as, IL-1 receptor antagonist and IL-10 [5,6]. Therefore, IL-6 exhibits both pro- and anti-inflammatory properties, and there seems to be context dependent effects. The plasma levels of IL-6 in healthy humans are typically less than 5 pg/ml [7] (Table 1). Although many different cell types are capable of producing IL-6, the release differs under varying physiological conditions within the body. In healthy humans, adipose tissue releases 10-35% of IL-6 in basal circulating levels [8]. Immune cells, specifically macrophages that are present within adipose tissue are responsible for releasing the majority of IL-6 from this tissue [9]. In obesity, increased numbers of macrophages begin to infiltrate the white adipose tissue, and the macrophage content correlates positively with adiposity and adipocyte size [10]. This increased number of

Inflammatory Marker	Normal	Obesity	Sepsis	Exercise
IL-6	<5 pg/ml [7,12,97,103-109]	1.5-38 pg/ml [105,106,110-113]	3.5-16000 pg/ml [114- 16]	~100 fold elevation in resting concentration [6,16-18,97]
TNF-α	<3.5 pg/ml [12,16,103,106,116]	1.8-88 pg/ml [106,110,113]	Undetectable-1000 pg/ml [117]	~2 fold elevation in resting concentration [6,16]
IL-10	<3.0 pg/ml [103,109,118]	0.35-10 pg/ml [108,111,112,118]	Undetectable-1700 pg/ml [119]	~8-27 fold elevation in resting concentration [6,16]
CRP	<10 mg/l [107]	1.3-8.5 mg/l [105,106,108,110,112]	>10 mg/l [120]	Up to 100 fold elevation in resting concentration [6,16,121]

IL-6: Interleukin-6; TNF-α: Tumor Necrosis Factor alpha; IL-10: Interleukin-10; CRP: C Reactive Protein

Table 1: Plasma levels of inflammatory markers in humans under varying physiological and pathophysiological conditions.

macrophages leads to increased production of C-reactive protein, and inflammatory cytokines, including TNFα, IL-1β and IL-6 [11]. Additionally, it was found that TNFα and IL-6 were more highly expressed in macrophages compared to adipocyte cells in adipose tissue obtained from an obese mouse model (*ob/ob*). Therefore, obesity has been characterized as a state of chronic low-grade inflammation, due to the increased secretion and subsequent ~2-3-fold elevation in systemic inflammatory markers from macrophages in the adipose tissue [10,12] (Table 1).

In contrast to chronically elevated IL-6, there can also be elevated IL-6 in response to acute infection, sepsis or exercise. An acute phase response results in the release of similar inflammatory markers that are seen in chronic low-grade inflammation, however, the acute circulating levels of these markers are much higher than levels associated with systemic inflammation. An acute transient increase in inflammatory markers also occurs during physical activity [11]. While it is true that eccentric contractions cause damage to skeletal muscle and can elicit an inflammatory response, there is also an acute transient increase in cytokine levels in response to physical activity in undamaged muscle [13]. However, unlike sepsis and infection, pro-inflammatory cytokines, TNF-α and IL-1β, do not typically increase [14,15]. It has been reported that plasma IL-6 concentrations increased approximately 100 fold during exercise, and the magnitude of increase in IL-6 depends on the duration and intensity of the exercise [16-18] (Table 1). Increases in IL-6 mRNA and protein were found in skeletal muscle during exercise, and the skeletal muscle cells produced enough IL-6 to account for the large increase in plasma IL-6 levels [19-21]. In contrast to chronic low-grade inflammation, infection and sepsis, it was shown that this acute increase in IL-6 levels was not due to activation of macrophages [22].

Although both chronic low-grade inflammation and exercise result in increased plasma IL-6 levels, there are important differences between the two conditions. Chronic low-grade inflammation is characterized by a slight yet significant systemic increase in IL-6 levels, whereas exercise results in an acute and transient increase in IL-6 levels. During chronic low-grade inflammation the increased IL-6 is released primarily from macrophages in adipose tissue [10,12], while during exercise the IL-6 is released from skeletal muscle [19,23]. IL-6 released from muscle during exercise allows accumulation within the skeletal muscle compartment and potentially increases specificity of IL-6 signaling at skeletal muscle, acting in an autocrine/paracrine fashion. This may also account for differences observed in the effects of IL-6 in response to exercise (acute/transient) versus chronic low-grade inflammation. Although IL-6 released from skeletal muscle will enter the circulation, and acts in an endocrine manner during exercise, the elevated IL-6 is only transient, and therefore, does not have the negative effects on tissues seen with chronically elevated IL-6 levels. The differences between these chronic and acute IL-6 elevations may be important with respect to the effects that the increased IL-6 has on target tissues, and more specifically, the effects on insulin signaling within these tissues.

IL-6 Increases SOCS3 Expression

IL-6 initiates cell signaling by binding to the IL-6 Receptor (IL-6R), which is also known as a type I cytokine receptor. The IL-6R exists as membrane bound and soluble receptors and IL-6 regulates the inflammatory state by coordinated signaling through both forms. The interaction between IL-6 and IL-6R forms a heterodimer with a non-ligand binding membrane glycoprotein, gp130 [24]. The complex formed between IL-6/IL-6R and gp130 activates the Janus kinase-signal transducer and activator of transcription (Jak/STAT) signal transduction pathway in IL-6 target cells, leading to the phosphorylation of the cytoplasmic portion of gp130 [25]. The phospho-tyrosine residues on gp130 are docking sites for STAT (signal transducer and activator of transcription) proteins, which are able to bind to these docking sites via SH2 domains. Several studies, both *in vitro* and *in vivo*, have shown that IL-6 stimulation increases the phosphorylation of STAT3 proteins. Activated STAT3 is translocated to the nucleus, where it is able to regulate the transcription of IL-6 target genes. IL-6 mediated Jak/STAT signaling can be induced rapidly, and results in increased phosphorylation of STAT3 under acute inflammatory conditions [26-29] (Figure 1).

As a negative feedback control, activated STAT proteins induce the expression of Suppressor of Cytokine Signaling (SOCS) proteins, which inhibit signaling events in response to various cytokines, including IL-6, IL-10, and interferon gamma. SOCS3 is able to down regulate IL-6 signaling by exerting negative feedback control on the Jak/STAT pathway through various mechanisms [30-32]. Both *in vivo* and *in vitro* studies provide evidence that elevated circulating levels of IL-6 result in increased expression of SOCS3 proteins in skeletal muscle [26,33-35], liver [36], and adipose tissue [26,37]. Consequently, this increase in SOCS3 expression has various important downstream effects on the insulin signaling pathway in these tissues.

SOCS3 and Insulin Resistance

Chronically elevated IL-6 leads to increased expression of SOCS3 proteins in skeletal muscle, liver and adipose tissue. This increased expression of SOCS3 mediates the inhibitory effects of IL-6 on insulin signaling and glucose metabolism [36,38,39]. It has been shown that insulin resistance increases following SOCS3 adenoviral [36] and transgene [38] overexpression in liver and adipose tissue. SOCS3 adenoviral overexpression in the liver of *db/db* obese mice leads to increased plasma insulin concentrations, glucose intolerance, and insulin resistance. Furthermore, when these mice were given antisense treatment for SOCS3 protein, insulin sensitivity improved [39]. Similarly, after a muscle specific deletion of SOCS3, whole body glucose tolerance and insulin sensitivity increased. This was due to enhanced glucose uptake into skeletal muscle [40]. Additionally, it

was found that insulin sensitivity increased following an adipose tissue specific SOCS3 deletion, suggesting that SOCS3 negatively regulates insulin signaling [37,41]. Although it has been demonstrated that increased SOCS3 expression causes insulin resistance in multiple tissues, the mechanisms by which SOCS3 exerts these effects are less clear. There is a large body of evidence supporting that SOCS3 disrupts insulin signaling by binding to specific sites on IR, and IRS-1/IRS-2, as well as targeting IRS-1/IRS-2 for degradation [36]. Disrupting these early signaling events also has downstream effects on other important proteins in the insulin pathway, including PI3 kinase and Akt.

It has been demonstrated that the β subunit of IR and SOCS3 co-immunoprecipitated in muscle lysates, and adenoviral mediated overexpression of SOCS3 resulted in decreased insulin stimulated phosphorylation of both IRS-1 and IRS-2 [36]. Since the decreased phosphorylation of IRS-1 and IRS-2 occurred without a decrease in IR phosphorylation, SOCS3 may bind to residues on IR that are involved in IR/IRS binding. Tyrosine 960 of IR is an important residue in IR/IRS binding [42], and when mutated to phenylalanine, SOCS3 was unable to bind to the β subunit of IR [36]. This finding suggests that when SOCS3 binds to the IR, it inhibits IR/IRS1 interaction, and consequently, disrupts insulin signaling. Co-immunoprecipitation of SOCS3 with both the β subunit of IR and IRS-1 was increased in skeletal muscle from rats fed High Fat Diets (HFD), and obese Zucker rats compared to the control rats [43,44]. HFDs cause elevated IL-6, which leads to increased SOCS3 expression, and acts as barrier between IR and IRS-1 binding. Consequently, insulin stimulated tyrosine phosphorylation of IRS-1 was decreased in both the HFD rats and obese Zucker rats compared to the control rats [43,44]. The decreased IRS-1 phosphorylation caused decreased activity and phosphorylation of PI3 kinase, and increased insulin resistance [44]. Following a skeletal muscle specific SOCS3 deletion, there was no difference in IR, IRS-1, and Akt protein expression. However, IRS-1 association with the p85 subunit of PI3 kinase and Akt phosphorylation increased in SOCS3 deficient mice after insulin stimulation [40]. These studies suggest that muscle specific SOCS3 deletion improves insulin sensitivity in mice fed a HFD.

Along with skeletal muscle, increased SOCS3 expression can also induce insulin resistance in the liver. When the HepG2 cells (human liver cell line) and primary hepatocytes were treated first with IL-6 and then insulin, SOCS3 expression increased, and tyrosine phosphorylation of IRS-1 and IRS-2 were decreased, but the phosphorylation of IR did not differ [45,46]. Similar to skeletal muscle, it was found that SOCS3 co-immunoprecipitated with the β subunit of IR in liver lysates, and these findings suggest that SOCS3 attenuates insulin signaling by inhibiting IR/IRS binding in the liver [36]. Increased SOCS3 suppressed IRS-1 association with the p85 subunit of PI3 kinase, and attenuated Akt phosphorylation [39,45,46]. When treated with antisense oligonucleotides against SOCS3, the phosphorylation of both IRS-1 and IRS-2 was restored, and the activity of PI3 kinase and Akt was improved [39]. In a Lipopolysaccharide (LPS) model for sepsis, SOCS3 protein expression was increased in the liver, which resulted in a drastic decrease in IRS-1 and IRS-2 phosphorylation in response to insulin. Furthermore, insulin stimulated PI3 kinase and Akt activity were both significantly decreased [36]. However, in contrast to other studies, IR phosphorylation was decreased in the mice that were treated with LPS. It should be noted that LPS injection simulates endotoxemia in the liver tissue, and this is a severe model of systemic inflammation compared to chronic low-grade inflammation. This may account for discrepancies in results between studies using these different models of inflammation.

In addition to decreased tyrosine phosphorylation of IRS-1 and IRS-2, SOCS has also been shown to mediate the degradation of these proteins. Adenoviral mediated expression of SOCS1 in liver lysates resulted in reduced levels of IRS-1 and IRS-2 proteins, and these levels returned to normal when SOCS1 was no longer detected [47]. SOCS proteins contain a highly conserved binding domain known as the SOCS box [30-32]. The SOCS box has an Elongin C binding motif that can form a complex with Elongin B [48]. This Elongin BC complex assembles an E3 ubiquitin ligase complex, which can degrade IRS-1 and IRS-2 proteins [49]. The deletion of SOCS box residues resulted in no reduction of IRS-1 and IRS-2 proteins levels when SOCS1 was expressed [47]. Although these results were only shown with SOCS1, the Elongin C binding motif is present in the SOCS box of both SOCS1 and SOCS3. Therefore, under certain circumstances degradation via the E3 ubiquitin ligase complex is a plausible explanation for the reduced IRS-1 and IRS-2 protein levels following SOCS3 expression [36].

Along with skeletal muscle and liver, SOCS3 is also a negative regulator of insulin signaling in adipose tissue. When SOCS3 was overexpressed in primary adipocytes or adipose tissue, insulin stimulated IRS-1 tyrosine phosphorylation, PI3 kinase activation of p85 subunit, and Akt phosphorylation decreased resulting in increased insulin resistance [38,50]. There was also a significant reduction in IRS-1 protein levels, which suggests that SOCS3 may be capable of degrading IRS-1 via ubiquitin ligase in adipose tissue [38,50]. SOCS3 deficient mouse embryonic fibroblasts, differentiated into adipocytes, showed increased IRS-1 and IRS-2 tyrosine phosphorylation compared to wild type adipocytes when stimulated with insulin [37]. Additionally, in the SOCS3 deficient adipocytes, p85 subunit binding to IRS-1, PI3 kinase activity, and glucose uptake were all increased. During chronic insulin treatment, which simulates conditions that lead to increased SOCS3 expression, IRS-1 protein levels were decreased in wild type adipocytes, which were not seen in the SOCS3 deficient adipocytes [37]. HFD mice with an adipose tissue specific deletion SOCS3 deletion (AKO) had increased glucose infusion rate with hyperinsulinaemic-euglyceamic clamp tests compared to the control mice [41]. Following a bolus of insulin, there was decreased IRS-1 tyrosine phosphorylation and IRS-1 protein levels in the HFD control mice compared to the HFD AKO mice. These findings demonstrate that the deletion of SOCS3 can protect against HFD induced insulin resistance in adipose tissue.

Collectively, these studies provide evidence that SOCS3 is a negative regulator of insulin signaling in skeletal muscle, liver, and adipose tissue. The ability of SOCS3 to inhibit insulin signaling suggests that these proteins influence energy balance and glucose homeostasis within the body. In support of this, SOCS3 is also known to have a role in the development of leptin resistance.

SOCS3 and Leptin Resistance

Leptin is a hormone secreted by adipocytes that regulates energy balance and caloric intake in the body. The leptin receptor (LRb) is a class I cytokine receptor, and is membrane bound as a homodimer [51]. In the hypothalamus, leptin binds to its receptor on the plasma membrane, and this leads to tyrosine phosphorylation of Jak2, and subsequent tyrosine phosphorylation of LRb [52,53]. Phosphorylated LRb binds to, and activates STAT3 proteins. STAT3 activation increases Pro-opiomelanocortin (POMC) expression, as well as inhibits Neuropeptide Y (NPY) and agouti-related peptide (AgRP) activity, all resulting in appetite suppression and increased energy expenditure [54-56]. The activation of STAT3 by leptin also mediates the transcription of SOCS3 protein [57]. Similar to IL-6

signal transduction, leptin induces SOCS3 expression, and SOCS3 then inhibits leptin signaling (Figure 1). SOCS3 inhibits leptin signaling by binding to Tyr985 on LRb, and blocking further signal transduction through STAT3, and also by inhibiting Jak2 phosphorylation [58,59]. Mice with haploinsufficiency of SOCS3 had lower plasma leptin levels, and had prolonged activation of STAT3 proteins compared to wild type mice when administered the same does of leptin [60]. When leptin was infused into neuron specific SOCS3 deficient mice, these mice had greater weight loss compared to the wild type mice. Furthermore, when these mice were fed a HFD, the wild type gained significantly more weight than the SOCS3 deficient mice [61]. SOCS3 mediated inhibition of leptin signaling prevents leptin from effectively modulating energy intake and suppressing appetite, and exacerbates obesity.

Under normal conditions, leptin stimulation results in increased activity of AMP activated Protein Kinase (AMPK), and downstream target acetyl-CoA carboxylase (ACC) in peripheral tissues [62]. AMPK is a regulator of cellular energy balance, and once activated switches on energy producing pathways. AMPK phosphorylates target proteins leading to increased fatty acid oxidation, glucose transport, and lipolysis in skeletal muscle, liver, and adipose tissue [63]. However, leptin failed to increase AMPK or ACC phosphorylation in rat soleus muscle following a HFD, indicating that the tissue had become leptin resistant [63,64]. Leptin stimulation resulted in decreased AMPK mediated Jak2, IRS-1, and Akt phosphorylation in the liver of rats fed a HFD compared to control rats [65]. Additionally, AMPK expression was decreased in the HFD liver. Leptin resistance has also been connected to increased SOCS3 mRNA and protein expression in skeletal muscle cells [66]. When SOCS3 was overexpressed in skeletal muscle, there was decreased α2AMPK activity, and decreased ACC phosphorylation [67]. These results imply that increased SOCS3 can lead to the development of leptin resistance in the muscle cells.

Chronic low-grade inflammation is associated with both increased circulating IL-6 and leptin levels, and consequently increased SOCS3 expression. Therefore, SOCS3 expression has both direct and indirect effects on the pathway under these conditions. As previously discussed, SOCS3 causes insulin resistance by directly inhibiting IR and IRS-1/IRS-2, which consequently causes decreased activity of downstream components in the insulin signaling pathway. SOCS3 can also negatively regulate leptin signaling leading to impaired leptin induced glucose uptake via AMPK, and cause subsequent interactions between the leptin and insulin signaling pathways (Figure 1). These interactions allow SOCS3 to mediate further indirect effects on insulin signaling.

Exercise and Insulin Signaling

It has been well documented that regular physical activity can alleviate or protect against T2D by enhancing insulin sensitivity in peripheral tissues [68-76]. Metformin is an antidiabetic drug that lowers fasting plasma insulin and glucose levels, and improves glucose tolerance by suppressing hepatic glucose production and increasing glucose uptake in skeletal muscle [77,78]. Due to the effectiveness of metformin on lowering plasma glucose concentrations, and the inexpensive cost, metformin is the most commonly prescribed drug for T2D patients [79]. Studies comparing the effectiveness of exercise, metformin and the combination treatment on insulin sensitivity have yielded interesting findings. Insulin resistant individuals were either treated with metformin, underwent a single bout of exercise, or both combined, and insulin sensitivity was measured 4 hours post exercise. Euglycemic hyperinsulinemic clamp tests found that insulin sensitivity increased by 54% in the individuals that exercised, and these changes were not seen in the metformin or combination treatments [79]. When prediabetic individuals participated in exercise training with or without metformin for 12 weeks, insulin sensitivity was increased in all treatments. However, the increased in insulin sensitivity was 25-30% higher in the exercise without metformin treatments compared to the others [80]. Additionally, prediabetic individuals prescribed either metformin or 150 minutes of physical activity per week for approximately 3 years resulted in the incidence of diabetes being reduced by 31% and 58% in the metformin and physical activity groups respectively [81]. Life style changes associated with regular exercise and treatment with metformin both enhance insulin sensitivity, and reduced the incidence of diabetes in high risk candidates. Pharmacological agents are more often chosen as the prescribed treatment in T2D, however it has been shown that the endogenous response to exercise is more effective. It is important to understand the mechanisms behind the increase in insulin sensitivity in response to regular exercise, as well as, why these mechanisms are more effective than current pharmacological treatments.

As previously stated, IL-6 is elevated during exercise, and plasma levels increase up to 100 fold (Table 1). IL-6 infusion during exercise caused increased glucose disposal [82], and stimulated the production of anti inflammatory cytokines IL-1 receptor antagonist and IL-10. The production of IL-10 is important because it inhibits the production of pro inflammatory cytokines IL-1, TNF alpha, and IL-8 [5,16,83]. Furthermore, glucose uptake rate was lower in IL-6 knockout mice compared to wild type, and the knockout mice did not benefit from exercise [84]. These studies suggest that acute elevations in IL-6 increase insulin sensitivity, whereas the lack of IL-6 prevents the exercise induced increases in insulin sensitivity.

The mechanism by which elevated IL-6 improves insulin sensitivity following physical activity may involve the regulation of AMPK activity. AMPK is an evolutionary conserved αβγ heterotrimer that consists of an α catalytic subunit, and βγ regulatory subunits [85,86]. Mice that overexpressed a skeletal muscle specific kinase dead form of AMPKα2 had reduced exercise tolerance during a single bout of exercise compared to wild type mice [87,88]. Additionally, AMPKβ2 knockout mice had reduced maximal exercise capacity and AMPK activity during treadmill running compared to wild type mice [89], and muscle specific AMPKβ1β2 knockout mice showed decreased AMPKα1α2 activity and AMPK phosphorylation following exercise compared to wild type mice [90]. Following muscle contraction, glucose uptake rates did not increase until the last five minutes in hind limb muscles from AMPKα2 dominant negative mice, whereas glucose uptake rates increased rapidly and remained elevated throughout the contraction period in wild type mice [91]. A similar study on AMPKα2 dominant negative mice found that contraction induced glucose uptake was reduced by 50% in extensor digitorum longus muscle compared to the wild type mice, suggesting that AMPK activity has important role in exercise induced glucose uptake [92]. However, another study found that contraction stimulated glucose uptake into tibialis anterior, extensor digitorum longus, and gastrocnemius muscles were similar in muscle specific transgenic mice with inactive AMPKα2 catalytic subunits when compared to wild type mice [93]. In order to visualize GLUT-4 translocation and localization, mice quadriceps muscle fibers were transfected with GLUT4-Enhanced Green Fluorescent Protein (EGFP). Following ablation of AMPKα2 activity in transgenic mice, GLUT4-EGFP basal localization, and contraction stimulated GLUT4-EGFP translocation was similar compared to wild type mice [94]. In contrast to above, these studies suggest that AMPKα2 activation may not be required for exercise induced glucose uptake. Therefore, AMPK activity may be necessary for full activation of exercise induced glucose

transport, but it appears there are also AMPK independent mechanisms involved in this process [92].

Several studies have shown that IL-6 is an important factor involved in exercise-mediated activation of AMPK. AMPK is activated by decreases in the energy state of the cell, or increases in the AMP: ATP ratio, and it was shown that incubation of skeletal muscle cells with IL-6 resulted in increased concentrations of AMP [95]. Incubating extensor digitorum longus muscle and cultured F442a adipocytes with IL-6 resulted in increased AMPK and ACC phosphorylation in the cells [96,97]. Additionally, exercise caused increased AMPK and ACC phosphorylation in skeletal muscle, liver, and adipose tissue of control mice, and these effects were diminished in IL-6 knockout mice [96,97]. IL-6 infusion into humans at a plasma concentration that mimics levels reached during strenuous exercise increased glucose disposal rate. Furthermore, L6 myotubes, when treated with IL-6, resulted in increased insulin stimulated translocation of GLUT-4 to the plasma membrane, and was accompanied by increased AMPK activity [26]. Following adenoviral mediated infection of myotubes with a dominant negative AMPK α subunit, the effect of IL-6 on insulin stimulated GLUT-4 translocation was diminished. AMPK phosphorylation was reduced in obese rats compared to lean rats, and contraction failed to increase AMPK activity in the obese rats following exercise [98,99]. Similarly, HFD fed mice had reduced exercise tolerance and attenuated AMPKα2 activity during a single bout of exercise compared to chow fed mice [100]. IL-6 has been shown to be required for exercise-mediated increases in AMPK activity [96,97], but it remains unclear why IL-6 is inhibited from activating AMPK in response to exercise in chronic inflammatory states. In contrast, it was found that T2D subjects had similar exercise induced AMPKα2 activity compared to non-diabetic subjects [101]. However, none of these subjects were obese, and may not be suffering from chronic low-grade inflammation in conjunction with T2D. This interpretation was supported by another study that compared AMPK activity between obese non-diabetic, non-obese T2D, and obese T2D subjects. The obese non-diabetic and obese T2D subjects showed diminished exercise induced increases in AMPK phosphorylation, AMPKα2 activity, and total AMPK activity compared to non-obese T2D subjects [102]. Furthermore, obese non-diabetic and obese T2D subjects had attenuated increases in ACC phosphorylation compared to non-obese T2D subjects. These results indicate that the inhibition of exercise induced increases in AMPK activity occurs under obese or chronic low-grade inflammatory conditions, and that IL-6 resistance may be related to the reduced AMPK response.

Conclusion

The role of elevated IL-6 in insulin resistance and insulin sensitivity is an active area of investigation. The current literature provides evidence that IL-6 induces insulin resistance, and that it can also improve insulin sensitivity. These studies suggest that the effects of IL-6 on insulin signaling are context dependent, and that this is a critical factor in this paradox that cannot be overlooked. Several studies have provided evidence that leptin resistance results in decreased AMPK activity, and more specifically that this resistance is mediated by increased SOCS3 expression. There are parallels between IL-6 and leptin signaling pathways associated with insulin resistance, including coincident activation of STAT3 and SOCS3 as a common inhibitory molecule. Since it has been demonstrated that SOCS3 can cause leptin resistance, then it may be true that IL-6 resistance can also occur. Under normal physiological conditions significant IL-6 elevations occur following exercise, and then return back to basal circulating levels shortly after. These acute transient IL-6 elevations do not cause sustained increases in SOCS3 expression but increase insulin sensitivity, and have positive effects on insulin signaling in peripheral tissues. In contrast, IL-6 is chronically elevated in pathophysiological conditions such as obesity and T2D leading to sustained increases in SOCS3. It is feasible that similar to leptin signaling increased SOCS3 may lead to IL-6 resistance by negative feedback, resulting in diminished AMPK activity (Figure 1). Understanding and resolving the intricate relationship between IL-6 and insulin action is important for the development of treatment strategies and regimes for inflammatory diseases.

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