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REVIEW

The role of gp130 receptor cytokines in the regulation of metabolic homeostasis

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ABSTRACT

It is well known that obesity is responsible, at least in part, for the increased incidence of chronic diseases such as type 2 diabetes, cardiovascular disease and certain types of cancer. Despite public education programs emphasizing lifestyle modifications to arrest this global pandemic, it is now estimated that 10–15% of the world's population are overweight or obese. As a result, new therapeutic options for the treatment of obesity-related disorders are clearly warranted. Much of the benefit of physical activity has been attributed to several mechanisms including reduced adiposity, increased cardiorespiratory fitness, reduced circulating lipids and the maintenance of muscle mass. However, the observation that the gp130 receptor cytokine interleukin-6 (IL-6) was released from skeletal muscle during exercise to improve metabolic homeostasis altered our understanding of the health benefits of exercise and opened avenues for research into potential novel therapeutics to treat metabolic disease. One gp130 receptor cytokine in particular, ciliary neurotrophic factor (CNTF), a pluripotent neurocytokine, showed efficacy as a potential anti-obesogenic therapy. This review examines the potential of gp130 receptor ligands, with a focus on IL-6 and CNTF as therapeutic strategies to treat obesity-related disorders.

KEY WORDS: Obesity, Skeletal muscle, Type 2 diabetes

Introduction

Historically, our hunter–gatherer ancestors became well adapted to the environment in which they lived, experiencing food shortages whilst still needing to be very physically active. The 'thrifty gene' theory states a strong evolutionary pressure to build energy reserves in the form of fat and to prioritize glucose consumption in the brain to enable survival in times of food scarcity (Zimmet and Thomas, 2003). This adaptation, which likely ensured survival for our ancestors, when combined with the modern transition to a more sedentary lifestyle with unrestricted calorically dense food, may be responsible for the increasing incidence of obesity and diabetes in modern society (Zimmet and Thomas, 2003; Lazar, 2005).

The prevalence of obesity (i.e. BMI $\geq$30 kg m^{-2}) has almost doubled from 1980 to 2014. It is estimated that 23% of the European adult population are now obese. Obesity is associated with an increased risk of other diseases such as atherosclerosis, myocardial infarction, stroke, metabolic syndrome, hypertension, infertility, type 2 diabetes mellitus (T2D) as well as certain types of cancer (Boughton and Murphy, 2013).

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In line with the obesity pandemic, diabetes is highly prevalent as well, with an estimated 347 million sufferers worldwide in 2008. Current projections predict the global prevalence will double by 2030 (Danaei et al., 2011; Wild et al., 2004) and that more than a third of young adults will develop diabetes within their lifetime (Magliano et al., 2008). Diabetes has profound effects for the individual. The associated mortality rate is high, with 3.8 million deaths globally attributed to diabetes in 2007 (Ford et al., 2008). While cardiovascular disease is the leading cause of morbidity and mortality for those with diabetes, diabetic nephropathy is now the most common cause of end-stage renal failure in the Western world (Gilbertson et al., 2005). The WHO reports that the direct cost of the disease and its complications accounts for up to 15% of healthcare budgets (WHO, 2006).

In view of the growing incidence of T2D and the fact that available treatments become ineffective over time (Turner et al., 1999), new therapeutic interventions are needed to help treat people with T2D.

Current therapies for T2D

The frontline therapies for T2D are still biguanide metformin and the sulfonylureas, which were introduced in the 1950s. No new treatment has been shown to be more effective. The efficacy of monotherapy with these agents declines with time as the treatments fail to halt the underlying pathology of the disease. In addition, recent studies indicate that sulfonylureas could contribute to pancreatic β-cell exhaustion. Despite the presence of a number of well-established drug classes for the treatment of T2D, there is still a significant unmet need for an effective, safe drug therapy with the ability to halt, or reverse, disease progression. Newer classes of incretin mimetics – the injectable glucagon-like peptide 1 (GLP-1) agonists and the orally available dipeptidyl peptidase-4 (DPP-4) inhibitors, have the potential to improve time to secondary failure, through pancreatic β-cell salvage. In addition, sodium glucose co-transporter 2 (SGLT2) inhibitors have recently entered clinic. However, the American Food and Drug Administration (FDA) recently warned that T2D patients who take SGLT2 inhibitors such as Farxiga, Invokana and Jardiance may experience ketoacidosis.

Despite the high prevalence of obesity, currently only six therapeutics are approved by the FDA to treat it (Yanovski and Yanovski, 2014). Xenical (Orlistat) is the only approved drug to act at the intestine. This drug inhibits intestinal lipases and hence intestinal fat absorption by 30%. There are four drugs that activate the noradrenergic system via action on the central nervous system (CNS). These drugs are called Phentermine, Diethylpropion, Phendimetrazine and Benzphetamine, and decrease appetite. The last drug, Lorcaserin, is a selective serotonin 2C (5HT2c) receptor agonist (Yanovski and Yanovski, 2014). Lorcaserin stimulates pro-opiomelanocortin (POMC) release in the hypothalamus and thereby decreases appetite (Zhang et al., 2014).

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Taken together, the need for novel diabetes and obesity therapeutics is likely to remain, given the complex and progressive nature of the disease and the side-effects of newer therapies.

gp130 receptor cytokines

Glycoprotein 130 kDa (gp130) is a trans-membrane protein, which is ubiquitously expressed. It serves as the signal transduction unit of a family of cytokines: the IL-6 family. The members of this family are IL-6, IL-11, IL-27, IL-30, IL-31, oncostatin M (OSM), leukemia inhibitory factor (LIF), cardiotrophin-1 (CT-1), cardiotrophin-like cytokine (CLC) and ciliary neurotrophic factor (CNTF) (Fig. 1) (Wagener et al., 2014). Despite varying degrees of homology, all IL-6 family cytokines share a very similar 4-helix bundle structure with an up–up–down–down topology (Kallen et al., 1999; Heinrich et al., 2003). Each IL-6 cytokine has different epitopes, called sites, which bind to the respective cytokine receptor. IL-6, IL-11, CNTF and CLC first bind to their respective α-receptor via interaction on site I (Garbers et al., 2012). The α-receptor recruits two β-receptors, but does not signal itself (Heinrich et al., 2003). Once bound to the α-receptor, the complex binds to two β-receptors via interactions on sites II and III and causes them to form a dimer (Heinrich et al., 2003). Contrary to this, other IL-6 family cytokines do not bind to an α-receptor and bind their respective β-receptor directly via interactions on sites II and III (Heinrich et al., 2003). All IL-6 family cytokines use gp130 as a β-receptor. The only exception to this rule is IL-31, which binds to the OSM receptor and the gp130-like protein (Garbers et al., 2012).

IL-6 cytokine family signaling

Depending on the cytokine, it first binds to its α-receptor or directly to its β-receptors (see above). In both cases, dimerization of the β-receptors occurs and brings janus kinases (JAK1 and JAK2) as well as tyrosine kinase (Tyk2) into close contact and activates them (Jones et al., 2011; Mihara et al., 2012). The activated kinases phosphorylate tyrosine residues on the cytoplasmic domain of the signaling receptor, e.g. gp130 in the case of IL-6 and gp130/LIF receptor (LIFR) in the case of CNTF. This step leads to the activation of two different pathways. The first signaling pathway recruits signal transducer and activator of transcription 3 (STAT3) and STAT1 to the phosphorylated YXXQ/YXPQ motifs on gp130.

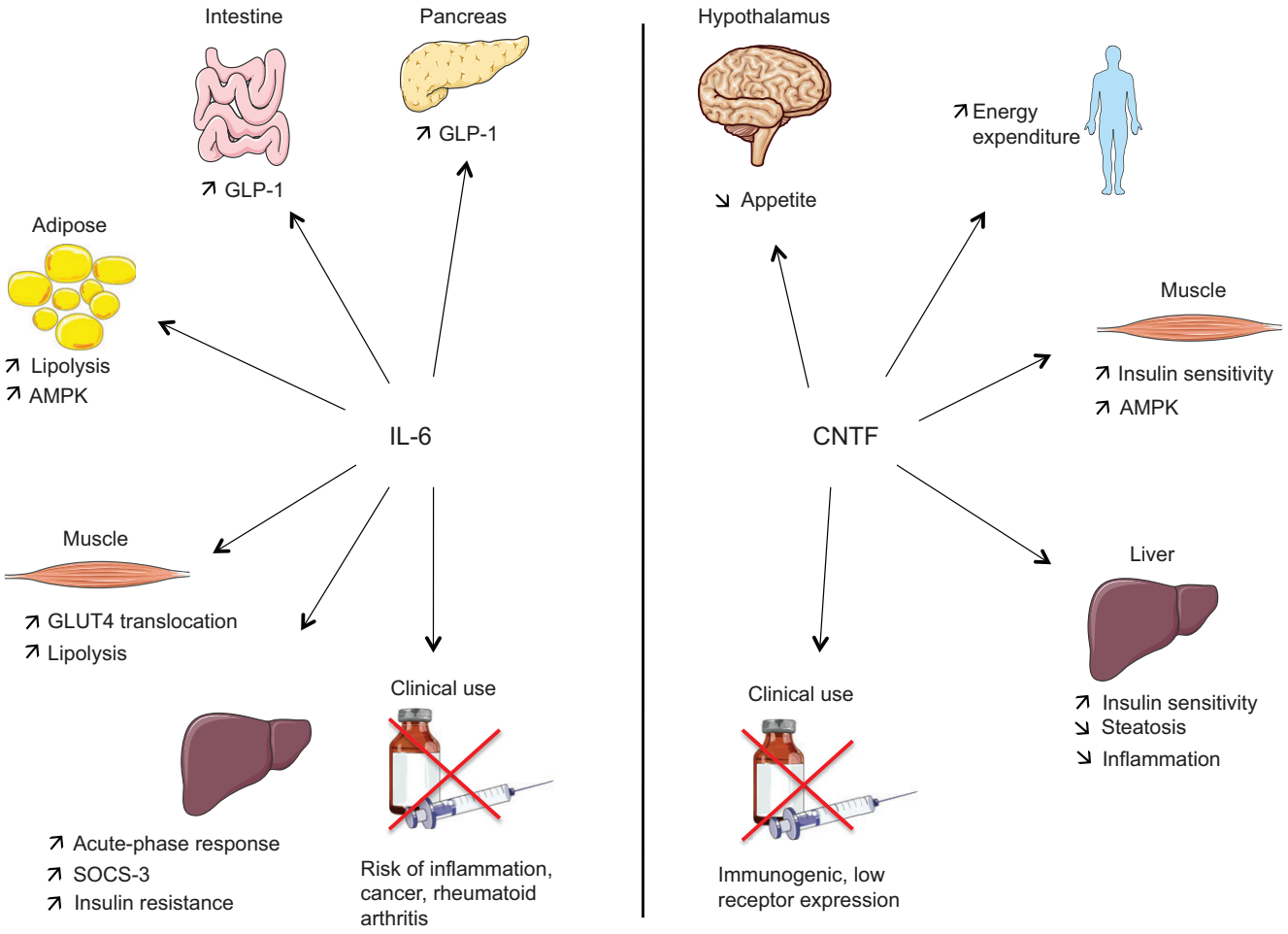

Fig. 1. Metabolic actions of the gp130 ligands IL-6 and CNTF. IL-6 (interleukin-6) can be released by the muscle in response to exercise or by the adipose tissue, especially in the case of obesity. IL-6 causes GLUT4 translocation in the muscle and activates lipolysis in the adipose tissue. It also acts on the intestine and the pancreas to induce production of the incretin GLP-1. IL-6 signaling in the liver leads to elevated levels of SOCS-3, which is linked to insulin resistance. Its association with different types of cancers, rheumatoid arthritis and a generally pro-inflammatory state make IL-6 unsuitable for clinical use. CNTF (ciliary neurotrophic factor) acts centrally on the hypothalamus, where it causes neurogenesis in orexigenic and anorexigenic nuclei, resulting in a decrease in appetite. Peripherally, CNTF increases overall energy expenditure and improves insulin sensitivity in the muscle via AMPK signaling. In the liver, CNTF increases insulin sensitivity and decreases inflammation and steatosis. In clinical trials, many patients have developed neutralizing antibodies to CNTF. Besides, the low receptor (CNTFR) expression makes CNTF an unpractical therapeutic option for obesity and T2D.

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STAT1/3 is phosphorylated by JAK and forms heterodimers or homodimers. Dimerized STATs enter the nucleus and promote transcription of different genes, including SOCS-3 and genes of the acute phase response (Rezende et al., 2012). SOCS-3 inhibits JAK activity and is thus a negative feedback to the signaling cascade. It has been shown that SOCS-3 inhibits insulin signaling and is increased in diet-induced obesity (Febbraio, 2007). The second signaling pathway is mediated by phosphorylation of Tyr759 on gp130 and results in the recruitment of SHP-2 (Src homology domain-containing protein tyrosine phosphatase-2). SHP-2 is phosphorylated by JAK and interacts with Grb2 (growth-factor receptor bound protein 2), which triggers the activation of the mitogen-activated protein kinase (MAPK) pathway. Ras activates Raf, which activates ERK1/2 (extracellular signal regulated kinase) (Hassan et al., 2014). ERK1/2 activates different transcription factors, including NF-IL-6 (nuclear factor for IL-6 expression), which consequently amplifies the signal by producing more IL-6. Binding of the cytokines to their respective receptors causes rapid internalization of the complex (Heinrich et al., 2003).

The IL-6 receptor exists both as a membrane-bound form (mIL-6R) on the cell surface and as a soluble form (sIL-6R) in the circulation. sIL-6R results either from shedding of the mIL-6R from the plasma membrane by a disintegrin and metalloproteinase (ADAM) 10 and 17 (Kraakman et al., 2013) or in 10% of cases from alternative splicing of the IL-6R (Wolf et al., 2014). The cells that shed sIL-6R are mainly activated neutrophils before apoptosis as well as immune cells upon exposure to increased levels of C-reactive protein (CRP), NO and reactive oxygen species (Rabe et al., 2008). During classic signaling, IL-6 binds to its membrane-bound receptor. During trans-signaling, however, IL-6 forms a complex with sIL-6R in the circulation. This IL-6/sIL-6R complex only requires gp130 to signal, meaning that it can signal in any cell of the body (Jones et al., 2011). Normal concentrations of sIL-6R are about 30–50 ng ml^{-1} (Garbers et al., 2012). This number can increase up to 3-fold during inflammation (Garbers et al., 2011), which increases the potential to trans-signal. In addition to the sIL-6R that allows trans-signaling, there is also a soluble form of gp130 (sgp130). sgp130 can bind to the IL-6/sIL-6R complex and inhibit binding of the complex to the membrane-bound gp130 (mgp130). In physiological conditions, the concentration of sgp130 is around 400 ng ml^{-1} (Rose-John, 2012) and thus higher than the concentration of the IL-6/sIL-6R complex and also has a higher affinity for the IL-6/sIL-6R complex than the affinity of IL-6 for the sIL-6R. Similarly to IL-6, there is also a soluble version of the CNTF receptor, called sCNTFR (Mihara et al., 2012). At high concentrations, CNTF has been shown to signal by binding to the IL-6R in addition to the CNTFR.

Metabolic actions of gp130 ligands

As the criteria for belonging to the gp130 cytokine family are based on structure homology rather than sequence homology, the members of this group have very diverse and usually pleiotropic functions. These functions may be as different as inhibiting the growth of tumor cell lines (LIF and OSM), providing protection from acute myocardial infarction (CT-1) (López-Yoldi et al., 2015) and supporting the myelination of neurons (CT-1) or platelet formation (LIF). Several gp130 ligands have both pro- and anti-inflammatory effects and induce the production of acute phase proteins in the liver. However, these cytokines also play a role in metabolism. For example, LIF has been shown to provoke cancer cachexia and to inhibit lipoprotein lipase (Seto et al., 2015), whereas OSM is thought to play a role in hepatic insulin resistance, hepatic steatosis and liver inflammation (Elks and Stephens, 2015). CT-1 has been shown to regenerate cirrhotic livers and to improve steatosis in non-alcoholic fatty liver disease (NAFLD) (López-Yoldi et al., 2015). At least the three cytokines neuropoietin (NP), IL-11 and OSM have been shown to inhibit adipocyte differentiation (White and Stephens, 2011).

At a molecular level, it is important to acknowledge the fact that gp130 resembles very much the leptin receptor (LRb). The two receptors have a very similar sequence and also signal via both the JAK/STAT and ERK pathways (Febbraio, 2007). Whereas obese people become resistant to leptin, gp130 ligands do not cause resistance, making gp130 an attractive target for developing new obesity therapeutics.

Of all the gp130 ligands, the two that have been studied most are IL-6 and CNTF. These two cytokines will be discussed in more depth in this review.

The role of IL-6 in metabolic processes

IL-6 is a cytokine produced by many cells including T-cells, B-cells, monocytes, fibroblasts, keratinocytes, vascular endothelial cells, mesangial cells, adipocytes and some tumor cells (Mihara et al., 2012). IL-6Rs are predominantly found on hemopoietic cells (Mihara et al., 2012) and hepatocytes (Wolf et al., 2014), but can also be found in muscle, adipose tissue, heart and brain (Hassan et al., 2014; Zvonic et al., 2003). Circulating levels of IL-6 are elevated in a broad array of pathological settings. Whereas basal IL-6 concentrations range from 1 to 10 pg ml^{-1}, levels increase as much as 1000-fold during septic shock (Waage et al., 1989). Triggers of IL-6 transcription include viruses, bacterial lipopolysaccharides (LPS), IL-1 or tumor necrosis factor α (TNFα) (Wolf et al., 2014). IL-6 is involved in various functions, including triggering the acute-phase response in the liver, angiogenesis in an inflammatory context, attraction of neutrophils, differentiation of B-cells, bone, cartilage and lipid metabolism (Mihara et al., 2012). It can act as both a pro- and an anti-inflammatory cytokine (Wolf et al., 2014).

IL-6 is associated with promoting tumor growth in several types of cancer (Weidle et al., 2010). IL-6 is also highly upregulated in Castleman's disease, systemic juvenile idiopathic arthritis and rheumatoid arthritis. In these diseases, IL-6 is the main inflammatory mediator and causes heavy collateral damage in the affected tissues. In light of this, a drug blocking the IL-6R called Tocilizumab (Actemra) was approved in 2010 by the FDA for the treatment of rheumatoid arthritis (Ogata et al., 2015). Recently, a novel role for IL-6 signaling in the etiology of inflammation and immunity was uncovered. Mauer and co-workers conditionally inactivated the IL-6R gene in myeloid cells of mice. These animals showed an increased expression of M1-macrophage-associated genes and a reduction of markers for M2-macrophages, indicating a shift in macrophage polarization (Mauer et al., 2014). Furthermore, in bone marrow-derived macrophages of control but not of myeloid-specific IL6R-deficient mice, IL-6 treatment induced cAMP response element-binding protein (CREB)-dependent IL-4 receptor expression and augmented the IL-4-mediated transcriptional program (Mauer et al., 2014). Therefore, intact IL-6 signaling in hematopoietic cells is absolutely required for IL-4-mediated alternative macrophage polarization.

Several studies have shown that obese people and/or patients with T2D have increased serum levels of IL-6 (Pradhan et al., 2001). Conversely, IL-6 levels decrease when obese people lose weight (Nicklas et al., 2005). In the last 20 years it has become clear that obesity is associated with low grade chronic inflammation

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(Hotamisligil, 2006). Given that inflammation results in the secretion of a wide array of pro-inflammatory cytokines including IL-6 from macrophages and/or adipocytes (Weisberg et al., 2003) along with the observation of elevated circulating IL-6 levels in obesity and patients with T2D (Bastard et al., 2000; Carey et al., 2004; Vozarova et al., 2001), the prevailing dogma has been that IL-6 causes insulin resistance (Lazar, 2005). Indeed, when IL-6 concentrations are chronically elevated in rodents, hepatic insulin resistance ensues, probably via activation of SOCS-3 (Klover et al., 2003). In humans, increased levels of IL-6 and CRP correlate with the risk of developing T2D (Pradhan et al., 2001). One theory suggests that elevated IL-6 might be a mere consequence of high TNFα levels (Pedersen and Febbraio, 2008), which seems to be caused by both aging and obesity. Importantly, in a carefully conducted human study, Carey and co-workers found that IL-6 was highly correlated with fat mass, but not correlated at all with glucose infusion rate during a hyperinsulinemic euglycemic clamp (Carey et al., 2004). These data suggested that circulating IL-6 is a marker of adiposity, rather than a cause of insulin resistance. It has been proposed that the negative effects of IL-6 may be caused by trans-signaling of IL-6, whereas the positive effects of IL-6 would be due to classical IL-6 signaling (Kraakman et al., 2013). Recently, Kraakman and co-workers have shown that by inhibiting IL-6 trans-signaling by overexpressing sgp130, fewer macrophages are recruited into the adipose tissue. Interestingly, this did not improve insulin sensitivity (Kraakman et al., 2015).

In contrast with the prevailing dogma that IL-6 has a negative effect on metabolic processes, there are several lines of evidence suggesting that IL-6 can indeed be viewed as promoting insulin action. The harbinger of this theory came from observations at the turn of the millennium that demonstrated that IL-6 is produced (Hiscock et al., 2004) and subsequently released (Steensberg et al., 2001; Steensberg et al., 2000) from contracting skeletal muscle cells. Physical exercise training is known to increase insulin sensitivity (Carey et al., 2004), while, in the immediate post-exercise period, insulin action is enhanced (Wojtaszewski and Richter, 2006). IL-6 can increase as much as 120-fold after running a marathon (Starkie et al., 2001). In the context of exercise, IL-6 release seems to be higher when muscle glycogen stores are lower (Steensberg et al., 2001) and it has been shown to increase skeletal muscle glucose uptake (Carey et al., 2006). Thus, the discovery that IL-6 was the proteotypical ‘myokine’ which triggered favourable metabolic actions opened new avenues for therapeutic targets. Muscle secretory factors may result in a new class of biologics to treat diseases such as T2D. To explore this avenue, several studies using recombinant human IL-6 (rhIL-6) were conducted. rhIL-6 was shown to activate lipolysis and fat oxidation in humans (van Hall et al., 2003), while increasing insulin-stimulated glucose uptake via 5′ AMP-activated protein kinase (AMPK) activation (Carey et al., 2006). In contrast, patients treated for rheumatoid arthritis with the IL-6R antibody Tocilizumab experienced an increase in serum total cholesterol (Smolen et al., 2008), as well as an increase in body weight and serum triglycerides (Nishimoto et al., 2005). These effects point towards a possible beneficial role of IL-6 signaling for glucose and lipid metabolism. In mice, IL-6 signaling protects against hepatic inflammation and has positive effects on insulin sensitivity (Wunderlich et al., 2010; Matthews et al., 2010) and improves leptin action (Sadagurski et al., 2010). In a particularly elegant study, Ellingsgaard and co-workers demonstrated that IL-6 stimulated GLP-1 secretion, which in turn activated insulin release by the pancreas (Ellingsgaard et al., 2011). *In vitro*, IL-6 causes the translocation of the GLUT4 transporter to the plasma membrane in skeletal myotubes (Carey et al., 2006), a finding consistent with the increased IL-6 levels during exercise.

It seems to make a crucial difference whether IL-6 is elevated chronically or acutely and how high the experimental IL-6 concentrations are. The tissue in which IL-6 is produced might also make a difference. During exercise, IL-6 is almost exclusively released by the muscle (Steensberg et al., 2000), whereas in obesity, up to 35% of the circulating IL-6 is produced by the adipose tissue (Pedersen and Febbraio, 2008). It is also important to note that rodent models do not necessarily react to IL-6 in the same way as humans (Pedersen and Febbraio, 2008). Taken together, many different factors seem to determine whether IL-6 exerts positive or negative metabolic effects. Furthermore, it is possible that an as yet unknown circulating factor regulates the signaling response to IL-6. It is clear, however, that by activating AMPK, increasing GLUT4 translocation in skeletal muscle (Carey et al., 2006), stimulating GLP-1 (Ellingsgaard et al., 2011) and enhancing leptin action (Sadagurski et al., 2010), there are many aspects of IL-6 signaling that would favor enhanced rather than impaired metabolic homeostasis in obesity. Notwithstanding this, IL-6 could never be used as a treatment for metabolic disease because, as discussed, there are negative, pro-inflammatory effects of IL-6 caused by trans-signaling of IL-6 (Kraakman et al., 2013).

The role of CNTF in metabolic processes

CNTF can be found primarily in the cerebrospinal fluid but may also be detected at very low concentrations in the blood. It is produced by Schwann cells in the periphery and by astrocytes in the CNS (Sleeman et al., 2000). It is also a survival and differentiation factor for neurons and glial cells. CNTF lacks a signal peptide necessary for secretion and is, therefore, thought to be released after cell damage in glial cells (Schuster et al., 2003). CNTF is also upregulated following brain damage (Schuster et al., 2003). Its receptor is mainly expressed in the nervous system and in skeletal muscle, but it can also be found in the kidney, liver, testis, lungs, bone marrow and adrenal gland (Allen et al., 2011). CNTF has also been shown to signal in adipocytes and is upregulated during obesity (Matthews and Febbraio, 2008). The CNTFR is attached to the membrane via a glycosyl–phosphatidyl–inositol link and can be cleaved by phospholipase C, which results in the release of soluble CNTFR (sCNTFR) (Allen et al., 2011). As with IL-6, sCNTFR circulates in the blood and the complex of CNTF/sCNTFR is able to elicit a response in cells expressing gp130 and LIFR. At high concentrations, CNTF is able to bind to the IL-6R instead of the CNTFR to induce signaling (Lambert et al., 2001). However, the affinity of CNTF to the IL-6R is about 50 times lower than that of IL-6 to the IL-6R (Schuster et al., 2003).

CNTF was initially discovered as a factor promoting survival of chick ciliary ganglion neurons (Adler et al., 1979) and was later found to promote the survival of peripheral motor neurons (Forger et al., 1993). These findings prompted a clinical study aiming to improve amyotrophic lateral sclerosis (ALS) with recombinant human (rh) CNTF injections. This study found no treatment effect for the ALS but, instead, patients had reduced appetite and lost weight (ACTS, 1996). These findings opened a new field in the research of obesity. Upon the findings of the weight-lowering effects of CNTF, Regeneron Pharmaceuticals created a recombinant version of human CNTF and coined it Axokine®. Compared with CNTF, Axokine® has a deletion of 15 amino acids at the C-terminus. Also, the cysteine at position 17 was changed for an alanine and the glutamine at position 63 was changed to an arginine, which corresponds to the rat sequence (Panayotatos et al., 1993;

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Preti, 2003). In comparison with human CNTF, Axokine® has a 3- to 5-fold increased potency *in vitro* and *in vivo* (Preti, 2003). This new anti-obesity drug showed very promising results in early clinical trials. In a phase IIB clinical trial, patients treated with Axokine (1 or 2 µg kg^{-1}) for 84 days lost 3–4 kg, while placebo-treated patients did not lose any weight. However, up to 87% of the participants who received the treatment produced neutralizing antibodies against Axokine (Ettinger et al., 2003), which led the patients to become refractory to the drug's effects (Preti, 2003).

CNTF was first reported to be a possible treatment for obesity over 15 years ago. Gloaguen and co-workers demonstrated that the CNTF and leptin receptors co-localized in the hypothalamus (Gloaguen et al., 1997). Moreover, systemic administration of both CNTF and leptin activated genes in the arcuate nuclei, suggesting that both cytokines activated anorexogenic neuronal signaling. Unlike leptin, CNTF also reduced obesity-related phenotypes in *db*/*db* mice, and in mice fed a high fat diet, which are partially leptin resistant. These authors concluded that CNTF represented a cytokine-mediated anti-obesity mechanism that acts independently of leptin signaling, offering alternative treatment strategies for obesity-associated leptin resistance. This initial study was confirmed by others, who showed that Axokine® markedly reduced weight in leptin-resistant rodent models in the absence of side effects such as fever and other metabolic abnormalities associated with cytokine treatment (Kalra et al., 1998; Lambert et al., 2001). While these initial studies demonstrated that CNTF exerted its effects via activation of neurons in the arcuate nuclei of the hypothalamus, it was the work of Brüning and co-workers that delineated the precise central signaling mechanism of CNTF (Janoschek et al., 2006). These authors created a mouse with selective deletion of the gp130R in POMC expressing neurons. When fed a high fat diet, these mice displayed a normal phenotype, suggesting that endogenous CNTF plays no role in energy balance. However, in contrast with littermates expressing gp130 normally, when CNTF was directly administered into the hypothalamus of POMC/gp130R deficient mice it had no effect. This identified the precise neuronal circuit by which CNTF signals in the hypothalamus (Janoschek et al., 2006). It has also been shown that CNTF decreases the activation of AMPK in the hypothalamus, markedly reducing food intake and body weight in animals fed both a chow and high fat diet (Steinberg et al., 2006). Clearly, therefore, CNTF or CNTF derivatives are effective mediators of energy intake via multiple pathways in the hypothalamus. They activate POMC neurons in the arcuate nuclei and decrease AMPK activity.

It should be noted, however, that not all of the positive effects of CNTF are due to central effects. In one of the early studies examining the metabolic effects of CNTF, weight loss was observed when compared with pair-fed animals, suggesting that at least some of the metabolic actions of CNTF involve increased energy expenditure (Lambert et al., 2001). CNTF stimulates STAT3, MAPK, Akt and p70S6K in brown adipocytes (Ott et al., 2002) and in 3T3-L1 adipocytes (Zvonic et al., 2003). Moreover, *in vivo*, CNTF increases energy expenditure and upregulates the uncoupling mechanism in the brown adipose tissue (Crowe et al., 2008), as well as inducing mitochondrial biogenesis (Liu et al., 2007). In addition, in line with work conducted with IL-6, CNTF increases fat oxidation in skeletal muscle via the activation of AMPK (Watt et al., 2006). Mice treated with CNTF for 7 days lost more weight compared with pair-fed vehicle-treated animals fed a high fat diet (Watt et al., 2006). These studies using CNTF and/or CNTF analogs have heightened interest in the possibility of using CNTF or its analogs as a treatment for metabolic disease (Febbraio, 2007).

Are gp130 receptor ligands still viable treatment strategies for metabolic disease?

Taken together, it is clear that neither IL-6, because of its capacity to produce pro-inflammatory reactions, nor CNTF, because it causes immunogenicity, could be used as a viable treatment strategy. However, this does not necessarily mean that gp130 receptor ligands offer no therapeutic utility to treat metabolic disease. Both IL-6 and CNTF have the capacity to reduce body weight and improve insulin action in both rodents and humans. Therefore, if treatment with peptides that activate the gp130 receptor can maintain their signaling properties, but overcome their negative effects, they could represent a breakthrough in the treatment of obesity and T2D. It should be noted that while the cytokines IL-6 and CNTF only share 6% sequence identity and 24% sequence similarity (Bazan, 1991), their structure is strikingly similar. Many years ago, Kallen and co-workers showed that the receptor-binding epitopes of these two cytokines can be swapped and the resulting recombinant proteins bind to a new combination of receptors (Kallen et al., 1999). In this process, these researchers produced seven recombinant human cytokines by removing one of the gp130 binding epitopes of IL-6 on site III and replacing it with the LIFR epitope from CNTF on site III (Kallen et al., 1999). The resulting designer cytokines were named IC1–7. These cytokines signal, therefore, by completely novel tripartite receptors: IL-6R, LIFR and gp130R. The rationale for testing IC1 to IC7 therapeutically is to avoid the three major problems that were encountered with the administration of IL-6 and CNTF. Firstly, it is hypothesized that IC1–7 would not be pro-inflammatory like IL-6 because they signal through a LIFR/gp130 heterodimer and not a gp130/gp130 homodimer. Secondly, IC1–7 should not be immunogenic like Axokine® because the majority of the molecule is from human IL-6 (as opposed to human CNTF). Thirdly, the peripheral expression of IL-6R is much higher than the expression of CNTFR, which would mean that the efficacious dose of IC1–7 would be lower than that of Axokine®. Whether one or any members of these new class of designer gp130 receptor ligands have therapeutic utility to treat metabolic disease remains to be tested.

Acknowledgements

We would like to thank all of our collaborators over the years, especially Bente Klarlund Pedersen, University of Copenhagen, Denmark, and Stefan Rose-John, University of Kiel, Germany. We also thank all past and present members of the Cellular & Molecular Metabolism Laboratory.

Competing interests

The authors declare no competing or financial interests.

Funding

This work was funded by a project grant (APP1042465) and a Research Fellowship to M.A.F. (APP1021168) from the National Health & Medical Research Council of Australia (NHMRC).

References

ACTS (1996). A double-blind placebo-controlled clinical trial of subcutaneous recombinant human ciliary neurotrophic factor (rHCNTF) in amyotrophic lateral sclerosis. ALS CNTF Treatment Study Group. *Neurology* **46**, 1244-1249.

Adler, R., Landa, K. B., Manthorpe, M. and Varon, S. (1979). Cholinergic neuronotrophic factors: intraocular distribution of trophic activity for ciliary neurons. *Science* **204**, 1434-1436.

Allen, T. L., Matthews, V. B. and Febbraio, M. A. (2011). Overcoming insulin resistance with ciliary neurotrophic factor. *Handb. Exp. Pharmacol.* **203**, 179-199.

Bastard, J. P., Jardel, C., Bruckert, E., Blondy, P., Capeau, J., Laville, M., Vidal, H. and Hainque, B. (2000). Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J. Clin. Endocrinol. Metab.* **85**, 3338-3342.

Bazan, J. F. (1991). Neuropoietic cytokines in the hematopoietic fold. *Neuron* **7**, 197-208.

Boughton, C. K. and Murphy, K. G. (2013). Can neuropeptides treat obesity? A review of neuropeptides and their potential role in the treatment of obesity. *Br. J. Pharmacol.* **170**, 1333-1348.
Carey, A. L., Bruce, C. R., Sacchetti, M., Anderson, M. J., Olsen, D. B., Saltin, B., Hawley, J. A. and Febbraio, M. A. (2004). Interleukin-6 and tumor necrosis factor-alpha are not increased in patients with Type 2 diabetes: evidence that plasma interleukin-6 is related to fat mass and not insulin responsiveness. *Diabetologia* **47**, 1029-1037.
Carey, A. L., Steinberg, G. R., Macaulay, S. L., Thomas, W. G., Holmes, A. G., Ramm, G., Prelovsek, O., Hohnen-Behrens, C., Watt, M. J., James, D. E. et al. (2006). Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. *Diabetes* **55**, 2688-2697.
Crowe, S., Turpin, S. M., Ke, F., Kemp, B. E. and Watt, M. J. (2008). Metabolic remodeling in adipocytes promotes ciliary neurotrophic factor-mediated fat loss in obesity. *Endocrinology* **149**, 2546-2556.
Danaei, G., Finucane, M. M., Lu, Y., Singh, G. M., Cowan, M. J., Paciorek, C. J., Lin, J. K., Farzadfar, F., Khang, Y.-H., Stevens, G. A. et al. (2011). National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants. *Lancet* **378**, 31-40.
Ellingsgaard, H., Hauselmann, I., Schuler, B., Habib, A. M., Baggio, L. L., Meier, D. T., Eppler, E., Bouzakri, K., Wueest, S., Muller, Y. D. et al. (2011). Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. *Nat. Med.* **17**, 1481-1489.
Elks, C. M. and Stephens, J. M. (2015). Oncostatin m modulation of lipid storage. *Biology* **4**, 151-160.
Ettinger, M. P., Littlejohn, T. W., Schwartz, S. L., Weiss, S. R., McIlwain, H. H., Heymsfield, S. B., Bray, G. A., Roberts, W. G., Heyman, E. R., Stambler, N. et al. (2003). Recombinant variant of ciliary neurotrophic factor for weight loss in obese adults: a randomized, dose-ranging study. *JAMA* **289**, 1826-1832.
Febbraio, M. A. (2007). gp130 receptor ligands as potential therapeutic targets for obesity. *J. Clin. Invest.* **117**, 841-849.
Ford, E. S., Li, C., Little, R. R. and Mokdad, A. H. (2008). Trends in A1C concentrations among U.S. adults with diagnosed diabetes from 1999 to 2004. *Diabetes Care* **31**, 102-104.
Forger, N. G., Roberts, S. L., Wong, V. and Breedlove, S. M. (1993). Ciliary neurotrophic factor maintains motoneurons and their target muscles in developing rats. *J. Neurosci.* **13**, 4720-4726.
Garbers, C., Thaiss, W., Jones, G. W., Waetzig, G. H., Lorenzen, I., Guilhot, F., Lissilaa, R., Ferlin, W. G., Grotzinger, J., Jones, S. A. et al. (2011). Inhibition of classic signaling is a novel function of soluble glycoprotein 130 (sgp130), which is controlled by the ratio of interleukin 6 and soluble interleukin 6 receptor. *J. Biol. Chem.* **286**, 42959-42970.
Garbers, C., Hermanns, H. M., Schaper, F., Müller-Newen, G., Grötzinger, J., Rose-John, S. and Scheller, J. (2012). Plasticity and cross-talk of interleukin 6-type cytokines. *Cytokine Growth Factor Rev.* **23**, 85-97.
Gilbertson, D. T., Liu, J., Xue, J. L., Louis, T. A., Solid, C. A., Ebben, J. P. and Collins, A. J. (2005). Projecting the number of patients with end-stage renal disease in the United States to the year 2015. *J. Am. Soc. Nephrol.* **16**, 3736-3741.
Gloaguen, I., Costa, P., Demartis, A., Lazzaro, D., Di Marco, A., Graziani, R., Paonessa, G., Chen, F., Rosenblum, C. I., Van Der Ploeg, L. H. T. et al. (1997). Ciliary neurotrophic factor corrects obesity and diabetes associated with leptin deficiency and resistance. *Proc. Natl. Acad. Sci. USA* **94**, 6456-6461.
Hassan, W., Ding, L., Gao, R.-Y., Liu, J. and Shang, J. (2014). Interleukin-6 signal transduction and its role in hepatic lipid metabolic disorders. *Cytokine* **66**, 133-142.
Heinrich, P. C., Behrmann, I., Haan, S., Hermanns, H. M., Müller-Newen, G. and Schaper, F. (2003). Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *Biochem. J.* **374**, 1-20.
Hiscock, N., Chan, M. H., Bisucci, T., Darby, I. A. and Febbraio, M. A. (2004). Skeletal myocytes are a source of interleukin-6 mRNA expression and protein release during contraction: evidence of fiber type specificity. *FASEB J.* **18**, 992-994.
Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature* **444**, 860-867.
Janoschek, R., Plum, L., Koch, L., Munzberg, H., Diano, S., Shanabrough, M., Muller, W., Horvath, T. L. and Bruning, J. C. (2006). gp130 signaling in proopiomelanocortin neurons mediates the acute anorectic response to centrally applied ciliary neurotrophic factor. *Proc. Natl. Acad. Sci. USA* **103**, 10707-10712.
Jones, S. A., Scheller, J. and Rose-John, S. (2011). Therapeutic strategies for the clinical blockade of IL-6/gp130 signaling. *J. Clin. Invest.* **121**, 3375-3383.
Kallen, K.-J., Grotzinger, J., Lelievre, E., Vollmer, P., Aasland, D., Renne, C., Mullberg, J., Zum Buschenfelde, K.-H. M., Gascan, H. and Rose-John, S. (1999). Receptor recognition sites of cytokines are organized as exchangeable modules: transfer of the leukemia inhibitory factor receptor-binding site from ciliary neurotrophic factor to interleukin-6. *J. Biol. Chem.* **274**, 11859-11867.
Kalra, S. P., Xu, B., Dube, M. G., Moldawer, L. L., Martin, D. and Kalra, P. S. (1998). Leptin and ciliary neurotropic factor (CNTF) inhibit fasting-induced suppression of luteinizing hormone release in rats: role of neuropeptide Y. *Neurosci. Lett.* **240**, 45-49.
Klover, P. J., Zimmers, T. A., Koniaris, L. G. and Mooney, R. A. (2003). Chronic exposure to interleukin-6 causes hepatic insulin resistance in mice. *Diabetes* **52**, 2784-2789.
Kraakman, M. J., Allen, T. L., Whitham, M., Iliades, P., Kammoun, H. L., Estevez, E., Lancaster, G. I. and Febbraio, M. A. (2013). Targeting gp130 to prevent inflammation and promote insulin action. *Diabetes Obes. Metab.* **15** Suppl. 3, 170-175.
Kraakman, M. J., Kammoun, H. L., Allen, T. L., Deswaerte, V., Henstridge, D. C., Estevez, E., Matthews, V. B., Neill, B., White, D. A., Murphy, A. J. et al. (2015). Blocking IL-6 trans-signaling prevents high-fat diet-induced adipose tissue macrophage recruitment but does not improve insulin resistance. *Cell Metab.* **21**, 403-416.
Lambert, P. D., Anderson, K. D., Sleeman, M. W., Wong, V., Tan, J., Hijarunguru, A., Corcoran, T. L., Murray, J. D., Thabet, K. E., Yancopoulos, G. D. et al. (2001). Ciliary neurotrophic factor activates leptin-like pathways and reduces body fat, without cachexia or rebound weight gain, even in leptin-resistant obesity. *Proc. Natl. Acad. Sci. USA* **98**, 4652-4657.
Lazar, M. A. (2005). How obesity causes diabetes: not a tall tale. *Science* **307**, 373-375.
Liu, Q.-S., Wang, Q.-J., Du, G.-H., Zhu, S.-Y., Gao, M., Zhang, L., Zhu, J.-M. and Cao, J.-F. (2007). Recombinant human ciliary neurotrophic factor reduces weight partly by regulating nuclear respiratory factor 1 and mitochondrial transcription factor A. *Eur. J. Pharmacol.* **563**, 77-82.
López-Yoldi, M., Moreno-Aliaga, M. J., Bustos, M. (2015). Cardiotrophin-1: a multifaceted cytokine. *Cytokine Growth Factor Rev.* **26**, 523-532.
Magliano, D. J., Shaw, J. E., Shortreed, S. M., Nusselder, W. J., Liew, D., Barr, E. L. M., Zimmet, P. Z. and Peeters, A. (2008). Lifetime risk and projected population prevalence of diabetes. *Diabetologia* **51**, 2179-2186.
Matthews, V. B. and Febbraio, M. A. (2008). CNTF: a target therapeutic for obesity-related metabolic disease? *J. Mol. Med.* **86**, 353-361.
Matthews, V. B., Allen, T. L., Risis, S., Chan, M. H. S., Henstridge, D. C., Watson, N., Zaffino, L. A., Babb, J. R., Boon, J., Meikle, P. J. et al. (2010). Interleukin-6-deficient mice develop hepatic inflammation and systemic insulin resistance. *Diabetologia* **53**, 2431-2441.
Mauer, J., Chaurasia, B., Goldau, J., Vogt, M. C., Ruud, J., Nguyen, K. D., Theurich, S., Hausen, A. C., Schmitz, J., Brönneke, H. S. et al. (2014). Signaling by IL-6 promotes alternative activation of macrophages to limit endotoxemia and obesity-associated resistance to insulin. *Nat. Immunol.* **15**, 423-430.
Mihara, M., Hashizume, M., Yoshida, H., Suzuki, M. and Shiina, M. (2012). IL-6/IL-6 receptor system and its role in physiological and pathological conditions. *Clin. Sci.* **122**, 143-159.
Nicklas, B. J., You, T. and Pahor, M. (2005). Behavioural treatments for chronic systemic inflammation: effects of dietary weight loss and exercise training. *CMAJ* **172**, 1199-1209.
Nishimoto, N., Kanakura, Y., Aozasa, K., Johkoh, T., Nakamura, M., Nakano, S., Nakano, N., Ikeda, Y., Sasaki, T., Nishioka, K. et al. (2005). Humanized anti-interleukin-6 receptor antibody treatment of multicentric Castleman disease. *Blood* **106**, 2627-2632.
Ogata, A., Morita, T., Yoshida, Y. and Tanaka, T. (2015). Subcutaneous formulation of Tocilizumab for treatment of rheumatoid arthritis. *Ther. Deliv.* **6**, 283-295.
Ott, V., Fasshauer, M., Dalski, A., Klein, H. H. and Klein, J. (2002). Direct effects of ciliary neurotrophic factor on brown adipocytes: evidence for a role in peripheral regulation of energy homeostasis. *J. Endocrinol.* **173**, R1-R8.
Panayotatos, N., Radziejewska, E., Acheson, A., Pearsall, D., Thadani, A. and Wong, V. (1993). Exchange of a single amino acid interconverts the specific activity and gel mobility of human and rat ciliary neurotrophic factors. *J. Biol. Chem.* **268**, 19000-19003.
Pedersen, B. K. and Febbraio, M. A. (2008). Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol. Rev.* **88**, 1379-1406.
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. *JAMA* **286**, 327-334.
Preti, A. (2003). Axokine (Regeneron). *IDrugs* **6**, 696-701.
Rabe, B., Chalaris, A., May, U., Waetzig, G. H., Seegert, D., Williams, A. S., Jones, S. A., Rose-John, S. and Scheller, J. (2008). Transgenic blockade of interleukin 6 transsignaling abrogates inflammation. *Blood* **111**, 1021-1028.
Rezende, L. F., Santos, G. J., Carneiro, E. M. and Boschero, A. C. (2012). Ciliary neurotrophic factor protects mice against streptozotocin-induced type 1 diabetes through SOCS3: the role of STAT1/STAT3 ratio in beta-cell death. *J. Biol. Chem.* **287**, 41628-41639.
Rose-John, S. (2012). IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. *Int. J. Biol. Sci.* **8**, 1237-1247.
Sadagurski, M., Norquay, L., Farhang, J., D'aquino, K., Copps, K. and White, M. F. (2010). Human IL6 enhances leptin action in mice. *Diabetologia* **53**, 525-535.

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Schuster, B., Kovaleva, M., Sun, Y., Regenhard, P., Matthews, V., Grötzinger, J., Rose-John, S. and Kallen, K.-J. (2003). Signaling of human ciliary neurotrophic factor (CNTF) revisited. The interleukin-6 receptor can serve as an alpha-receptor for CTNF. *J. Biol. Chem.* **278**, 9528-9535.

Seto, D. N., Kandarian, S. C. and Jackman, R. W. (2015). A key role for leukemia inhibitory factor in C26 cancer cachexia. *J. Biol. Chem.* **290**, 19976-19986.

Sleeman, M. W., Anderson, K. D., Lambert, P. D., Yancopoulos, G. D. and Wiegand, S. J. (2000). The ciliary neurotrophic factor and its receptor, CNTFR alpha. *Pharm. Acta Helv.* **74**, 265-272.

Smolen, J. S., Beaulieu, A., Rubbert-Roth, A., Ramos-Remus, C., Rovensky, J., Alecock, E., Woodworth, T. and Alten, R. (2008). Effect of interleukin-6 receptor inhibition with Tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial. *Lancet* **371**, 987-997.

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. Cell Physiol.* **280**, C769-C774.

Steensberg, A., van Hall, G., Osada, T., Sacchetti, M., Saltin, B. and Pedersen, B. K. (2000). Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J. Physiol.* **529**, 237-242.

Steensberg, A., Febbraio, M. A., Osada, T., Schjerling, P., van Hall, G., 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.* **537**, 633-639.

Steinberg, G. R., Watt, M. J., Fam, B. C., Proietto, J., Andrikopoulos, S., Allen, A. M., Febbraio, M. A. and Kemp, B. E. (2006). Ciliary neurotrophic factor suppresses hypothalamic AMP-kinase signaling in leptin-resistant obese mice. *Endocrinology* **147**, 3906-3914.

Turner, R. C., Cull, C. A., Frighi, V., Holman, R. R. and **for theUK Prospective Diabetes Study (UKPDS) Group** (1999). Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). *JAMA* **281**, 2005-2012.

van Hall, G., Steensberg, A., Sacchetti, M., Fischer, C., Keller, C., Schjerling, P., Hiscock, N., Møller, K., Saltin, B., Febbraio, M. A. et al. (2003). Interleukin-6 stimulates lipolysis and fat oxidation in humans. *J. Clin. Endocrinol. Metab.* **88**, 3005-3010.

Vozarova, B., Weyer, C., Hanson, K., Tataranni, P. A., Bogardus, C. and Pratley, R. E. (2001). Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obes. Res.* **9**, 414-417.

Waage, A., Brandtzaeg, P., Halstensen, A., Kierulf, P. and Espevik, T. (1989). The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin 6, interleukin 1, and fatal outcome. *J. Exp. Med.* **169**, 333-338.

Wagener, E.-M., Aurich, M., Aparicio-Siegmund, S., Floss, D. M., Garbers, C., Breusing, K., Rabe, B., Schwanbeck, R., Grotzinger, J., Rose-John, S. et al. (2014). The amino acid exchange R28E in ciliary neurotrophic factor (CNTF) abrogates interleukin-6 receptor-dependent but retains CNTF receptor-dependent signaling via glycoprotein 130 (gp130)/leukemia inhibitory factor receptor (LIFR). *J. Biol. Chem.* **289**, 18442-18450.

Watt, M. J., Dzamko, N., Thomas, W. G., Rose-John, S., Ernst, M., Carling, D., Kemp, B. E., Febbraio, M. A. and Steinberg, G. R. (2006). CNTF reverses obesity-induced insulin resistance by activating skeletal muscle AMPK. *Nat. Med.* **12**, 541-548.

Weidle, U. H., Klostermann, S., Eggle, D. and Kruger, A. (2010). Interleukin 6/interleukin 6 receptor interaction and its role as a therapeutic target for treatment of cachexia and cancer. *Cancer Genomics Proteomics* **7**, 287-302.

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L. and Ferrante, A. W., Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* **112**, 1796-1808.

White, U. A. and Stephens, J. M. (2011). The gp130 receptor cytokine family: regulators of adipocyte development and function. *Curr. Pharm. Des.* **17**, 340-346.

WHO (2006). http://www.who.int/mediacentre/factsheets/fs311/en/index.html. World Health Organization.

Wild, S., Roglic, G., Green, A., Sicree, R. and King, H. (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* **27**, 1047-1053.

Wojtaszewski, J. F. P. and Richter, E. A. (2006). Effects of acute exercise and training on insulin action and sensitivity: focus on molecular mechanisms in muscle. *Essays Biochem.* **42**, 31-46.

Wolf, J., Rose-John, S. and Garbers, C. (2014). Interleukin-6 and its receptors: a highly regulated and dynamic system. *Cytokine* **70**, 11-20.

Wunderlich, F. T., Ströhle, P., Könner, A. C., Gruber, S., Tovar, S., Brönneke, H. S., Juntti-Berggren, L., Li, L.-S., van Rooijen, N., Libert, C. et al. (2010). Interleukin-6 signaling in liver-parenchymal cells suppresses hepatic inflammation and improves systemic insulin action. *Cell Metab.* **12**, 237-249.

Zhang, Y., Liu, J., Yao, J., Ji, G., Qian, L., Wang, J., Zhang, W., Tian, J., Nie, Y., Zhang, Y. E. et al. (2014). Obesity: pathophysiology and intervention. *Nutrients* **6**, 5153-5183.

Zimmet, P. and Thomas, C. R. (2003). Genotype, obesity and cardiovascular disease: has technical and social advancement outstripped evolution?. *J. Intern. Med.* **254**, 114-125.

Zvonic, S., Cornelius, P., Stewart, W. C., Mynatt, R. L. and Stephens, J. M. (2003). The regulation and activation of ciliary neurotrophic factor signaling proteins in adipocytes. *J. Biol. Chem.* **278**, 2228-2235.