Cytokine regulation of AMPK signalling

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1. ABSTRACT

The dynamic control of energy metabolism is dependent on balancing energy demand with energy supply. In mammals this balance is maintained through the integration of many different cytokine signals that communicate the nutrient status of the organism to the hypothalamus, liver and skeletal muscle. Adipose tissue and resident macrophages secrete many of these cytokine factors including leptin, tumour necrosis factor α, adiponectin, interleukin-6 and resistin. Other secreted factors including ciliary neurotrophic factor and ghrelin have also been shown to regulate energy metabolism. The AMP-activated protein kinase (AMPK) has emerged as an important integrator of these cytokine signals regulating both central and peripheral pathways controlling food intake, energy expenditure and substrate utilization and as such is the focus of this review.

2. INTRODUCTION

The epidemics of obesity, insulin resistance and type 2 diabetes has led to a dire need for a greater understanding of mechanisms regulating energy intake, energy expenditure and insulin sensitivity. The findings over the past several decades that cytokine signals play an essential role in regulating body mass and insulin sensitivity has been critical for understanding the causes and consequences of over nutrition. At the forefront of discoveries in this area are recent findings that the AMP-activated protein kinase (AMPK), is a key node integrating cytokine signals to control appetite, energy expenditure and substrate utilization. AMPK regulates whole-body energy metabolism through direct effects on gene transcription and key metabolic enzymes in both peripheral and central pathways In this review we will discuss the role of adipocytes and macrophage secreted proteins (leptin,

TNFα, adiponectin, interleukin-6 and resistin) as well as other factors including ciliary neurotrophic factor (CNTF) and ghrelin and their effect on AMPK signalling in the hypothalamus, liver, skeletal muscle and adipose tissue.

3. REGULATION OF AMPK ACTIVITY BY UPSTREAM KINASES AND PROTEIN PHOSPHATASES

AMPK activity is dependent on the phosphorylation of Thr172 in the activation loop of the α subunit by upstream kinases. In the past several years three upstream kinases have been identified as LKB1 (1-3), Ca2+/calmodulin-dependent protein kinase (CaMKK) (4-6) and transforming growth factor beta activated kinase (TAK1) (7) In skeletal muscle the activity of AMPK appears to be primarily dependent on LKB1 as genetic deletion of LKB1 in muscle results in a marked decrease in AMPK Thr172 phosphorylation (8, 9), reduced exercise capacity (10) and an inability of both AICAR and contraction to stimulate glucose uptake (8) Genetic deletion of LKB1 in liver also results in a dramatic loss of AMPK and ACC phosphorylation suggesting that like skeletal muscle LKB1 is the principal upstream kinase regulating AMPK activity both basally and in response to metformin in the liver (11) In the brain however, CAMKK is highly expressed suggesting that the central regulation of AMPK may be very sensitive to regulation by calcium (4) The tissue specific roles that individual upstream kinases may play in the complex regulation of AMPK by phosphorylation remain to be fully elucidated.

Binding of AMP to AMPK γ subunits has been suggested to result in a conformational change promoting phosphorylation of the α subunit on Thr172, presumably because the conformational change makes the phosphorylation site more accessible to upstream kinases (12) Recent crystal structures do not necessarily support this hypothesis but it should be noted that these structures have lacked the full enzyme complex (13-15) AMPK can also be readily de-phosphorylated *in vitro* by protein phosphatise 2C (PP2C), however, this effect is blocked by increasing AMP concentrations suggesting this may also be critical for the regulation of AMPK phosphorylation/activity (16, 17)

4. ROLE OF AMPK IN REGULATING SKELETAL MUSCLE METABOLISM

In obesity, defects in both glucose and fatty acid metabolism and mitochondrial density/function contribute to the development of skeletal muscle insulin resistance (18) AMPK plays a critical role in regulating skeletal muscle metabolism (for review see (19, 20)) AMPK activation results in increasing rates of skeletal muscle fatty acid oxidation through a mechanism involving phosphorylation of acetyl-CoA carboxylase (ACC) (21) and potentially malonyl-CoA decarboxylase (MCD) (22), leading to reduced content of malonyl-CoA and increased long-chain fatty acyl CoA flux into the mitochondria via carnitine palmitoyl transferase-1. AMPK activation also inhibits triglyceride lipase activity (23) and triglyceride

synthesis (24) through phosphorylation of hormone sensitive lipase (HSL) (25) and sn-glycerophosphate acyl transferase (GPAT) (24), respectively. Skeletal muscle glucose uptake is regulated by AMPK through a mechanism involving the phosphorylation of Akt substrate of 160 kDa (AS160) (26-28) and may improve skeletal muscle insulin sensitivity through phosphorylation of insulin receptor substrate (IRS) 1 (29) Lastly, AMPK is important for mitochondrial biogenesis (30-34) which may be mediated through direct phosphorylation of peroxisome-proliferator activated receptor γ coactivator 1α (PGC1α) (35) Given the central role of AMPK in regulating muscle metabolism, it is somewhat surprising then that the genetic disruption of AMPK signalling in mice with muscle specific over expression of an AMPKα2 dominant negative (36) or AMPKα2 (37) and γ3 (38) null deletion does not directly result in muscle insulin resistance, under low-fat dietary conditions, suggesting that either AMPK is not essential for substrate metabolism under basal conditions or that alternative signalling pathways may have developed to regulate glucose and fatty acid metabolism in these mice models.

5. HEPATIC GLUCOSE PRODUCTION AND AMPK

The regulation of hepatic glucose production is central for the maintenance of glucose homeostasis during fasting and refeeding. Fasting markedly upregulated AMPK activity in the liver and is associated with significant suppression of ACC activity (39), effects that were also observed in response to changes in insulin (40) Subsequent studies in rat hepatoma cells have showed that AMPK activation can repress the transcription of two key gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) (41) The repression of key gluconeogenic enzymes by AMPK is dependent on AMPK phosphorylation of Ser171 of the transducer of CREB activity 2 (TORC2), which blocks TORC2 nuclear translocation and thereby prevents CREB dependent transcription of PEPCK and G6Pase (42) In support of this role for AMPK, AMPK α2 knockout mice display fasting hyperglycemia, glucose intolerance and increased hepatic glucose output although this appears to be largely due to increased α-adrenergic signalling (37, 43) Similarly, LKB1 null mice are also hyperglycaemic and exhibit increased mRNA expression of gluconeogenic enzymes although since this phenotype appears to be more severe then the AMPKα1α2 liver specific null mice it is currently not clear whether this effect is due to non specific effects of LKB1 independent of AMPK (11) Taken together though these data to support and important for liver AMPK in the regulation of hepatic glucose production.

6. CENTRAL REGULATION OF APPETITE BY AMPK

While most research to date has focused on mechanisms mediating AMPK activity in peripheral tissues such as skeletal muscle and liver, more recently a critical role of AMPK as a regulator of food intake has become apparent. Hypothalamic AMPK is tightly regulated under

physiological conditions; fasting increases AMPK activity and refeeding inhibits AMPK activity. In elegant experiments where adenovirus encoding dominant negative (DN) and constitutively active (CA) mutations of AMPK were injected into the ventral medial hypothalamus, Minikoshi et al. (44) showed that a CA-AMPK increased body weight and food intake while a DN-AMPK had the opposite effect. Under fed conditions a CA-AMPK increased expression of arcuate neuropeptide Y (NPY) and agouti related peptide (AgRP) leading to hyperphagia while a DN-AMPK prevented feeding and the expression of NPY and AgRP during fasting. Lee et al. (45) provided a possible mechanism for these findings in a neuroblastoma cell line by showing that the modulation of cellular ATP and therefore AMPK by glucose, 2-deoxyglucose, pyruvate or ATP synthesis inhibitors also altered the expression of AgRP. Similarly, the fatty acid synthase inhibitor C75 was also found to suppress AMPK activity and NPY expression in the arcuate nucleus an effect that was reversed in the presence of the AMPK activator AICAR (46) Additional anorexigenic signals namely, insulin, glucose and re-feeding also suppress AMPK activity in the brain, whilst the appetite-stimulating agouti related protein activates hypothalamic AMPK (44) It is currently unclear whether AMPK effects on neuropeptide expression are mediated directly or whether this is an indirect effect since alterations in hypothalamic fatty acid oxidation mediated via AMPK would be anticipated to alter both malonyl-CoA and hypothalamic fatty acyl-CoA contents (47) Alternatively, since fatty acyl-CoA allosterically activates AMPK increasing fatty acid oxidation (48) it remains possible that the infusion of fatty acids into the brain may stimulate appetite in an AMPK dependent manner. Future studies are required to determine whether the effects of AMPK signaling on orexigenic peptide expression are direct or act through changes in fatty acyl-CoA content.

7. ADIPOKINES AND AMPK: HISTORICAL BACKGROUND AND INTRODUCTION

Nearly 55 years ago Kennedy hypothesized about the presence of a circulating, lipostatic, negative feedback signal which acted centrally to alter energy expenditure and food intake (49) Parabiosis experiments the following decade confirmed the presence of such a circulating factor and identified mouse mutations that lacked the lipostatic signal (*ob/ob* mice) or caused insensitivity to the signal (*db/db* mice) (50) It would take another forty years before this lipostatic factor was identified as being the protein product of the *ob* gene named leptin (from *leptos*, for thin) (51) It was subsequently discovered this protein rapidly reduced body mass and restored euglycemia in *ob/ob* mice (52-54) Around the same time another group was also laying the foundation for a new paradigm that in obesity, adipocytes could also secrete the inflammatory factor tumour necrosis factor α (TNFα) which would cause systemic insulin resistance (55, 56) These seminal discoveries, established the adipocyte as an endocrine organ which communicates changes in adipose mass and energy status to other organs such as the brain, skeletal muscle and liver to ultimately regulate whole body energy metabolism. The remainder of the review will be focused

on how regulation of AMPK by the adipokines such as leptin, adiponectin, IL-6, TNFα and resistin and other factors such as CNTF and ghrelin control substrate utilization, appetite and energy expenditure in both central and peripheral pathways.

8. LEPTIN

Leptin signalling in the hypothalamus is essential for the regulation of body mass and neuroendocrine homeostasis (57) These effects are attributed to the binding of leptin to the long form of the leptin receptor (LRb) and the phosphorylation of tyrosine 1138 (58) within hypothalamic nuclei, which regulate food intake and energy expenditure. Contrary to the situation in muscle, leptin administration results in suppression of AMPK activity in the paraventricular and arcuate sections of the hypothalamus (44) The effects of leptin on AMPK signalling in the brain appear to be downstream of the MC receptor as icv administration of the MC4 receptor agonist, MT-II, inhibits AMPK activity in the paraventricular nucleus, while both refeeding and leptin fail to inhibit hypothalamic AMPK activity in MC4 receptor knockout mice (44)

Leptin's effects on metabolism are not limited to the hypothalamus as almost all tissues examined express leptin receptors (59) Leptin acts directly in isolated skeletal muscle to increase fatty acid oxidation (60) in an AMPK-dependent manner (61), and this process appears to involve an increase in the AMP:ATP ratio. The acute activation of AMPK is limited to α2 containing heterotrimers (62) in oxidative muscle fibres (61) In addition to the direct activation of leptin in skeletal muscle, there is also a delayed action of leptin that requires inputs from the central nervous system (CNS) The CNS-mediated activation of AMPK involves the stimulation of α-adrenergic signalling (61) and is dependent on the melanocortin (MC) system since intracerebroventricular (icv) delivery of an MC4 receptor antagonist inhibits leptin activation of AMPK in skeletal muscle while a melanocortin agonist directly increases AMPK signalling in muscle (63) Since α-adrenergic receptors couple to G proteins and activate CAMKK signalling, it is possible that the chronic effects of AMPK activation in skeletal muscle are mediated through CAMKK signalling. Conversely, the acute effects of leptin alters the AMP:ATP ratio and may be dependent on reducing PP2C dephosphorylation of Thr172. Future studies are required to delineate the upstream pathways mediating the acute and chronic effects of leptin on AMPK signalling and to understand. A second important question is how leptin mediates opposite effects on AMPK, activating in muscle but suppressing in hypothalamus an effect which may be related to different upstream AMPK kinase expression, which in skeletal muscle primarily is dependent on LKB1, while in the hypothalamus more likely involves CAMKKβ.

Chronic leptin treatment is believed to help reverse insulin resistance independent of caloric restriction by reducing lipid storage in skeletal muscle and liver (64) We have shown that chronic leptin administration increases

skeletal muscle AMPK α and β expression (65) and fatty acid oxidation (66) There is also evidence in cultured myotubes that AMPK activation by leptin increases AMPKα2 nuclear translocation and results in PPARα transcription (62) In the liver while leptin dramatically reduces lipid storage this effect does not appear to be dependent on the activation of AMPK (67) Chronic adenovirus-induced hyperleptinemia rapidly reduces adipocyte size and mass, induces mitochondrial biogenesis and concomitantly increases AMPK Thr-172 phosphorylation (68, 69) Whether AMPK is required for leptin induced mitochondrial biogenesis and transforming adipocytes into fat-burning cells is currently unknown. In summary, while it is commonly believed that these insulin sensitizing effects of leptin are mediated through AMPK effects, direct evidence in genetic models of AMPK deficiency are required to validate this hypothesis.

Despite the pronounced effects of leptin in models of leptin deficiency (53) or lipodystrophy (70), rodents fed a high-fat diet are resistant to the effects of leptin (71) and recombinant leptin infusion has minimal effects on body mass and food intake under these conditions (72) Skeletal muscle leptin resistance develops following high-fat feeding in rodents (73) and is also prevalent in obese humans (74, 75) The development of leptin resistance in obese skeletal muscle is characterized by suppressed rates of leptin stimulated AMPK signalling (75-78) Similarly, high fat feeding inhibits the ability of leptin to suppress hypothalamic AMPK signalling (78, 79) Two important mediators of leptin resistance are the suppressor of cytokine signalling 3 (SOCS3) (80) and the protein tyrosine phosphatase 1B (PTP1B) (81, 82) SOCS3 is a member of a family of proteins (SOCS1-SOCS7, and CIS) in which their central SH2 domains bind to phosphotyrosine residues in cytokine receptors (83) Initial studies by Bjorbaek et al. (80) demonstrated that inhibition of leptin signalling via the signal transducer and activator of transcription (STAT)-3 in hypothalamic nuclei was mediated by SOCS3 binding of Tyr985 of the leptin receptor (84, 85) Both SOCS3 hypothalamic specific null mice (86) and SOCS3 mice with haploinsufficiency (87) have enhanced leptin sensitivity and are resistant to diet-induced obesity. SOCS3 is also up-regulated in skeletal muscle with high-fat feeding (88) and is associated with leptin resistance (89) Moreover, the over-expression of SOCS3 via adenovirus-mediated infection in skeletal muscle cells, to a similar degree as observed in skeletal muscle of mice fed a high-fat diet (88), or in obese humans (89), inhibited leptin but not AICAR activation of AMPK α2 activity (89) These data demonstrate that SOCS3 inhibits leptin activation of AMPK and suggest that the down-regulation of leptin signalling in skeletal muscle may contribute to the aberrant regulation of fatty acid metabolism. This paradigm requires experimental support *in vivo*. It also appears as though blunted leptin signalling to AMPK in the hypothalamus may contribute to hyperphagia as elevated hypothalamic AMPKα2 activity in diabetic rats was associated with elevated NPY and suppressed POMC mRNA, whereas these effects were reversed with pharmacological AMPK inhibition (90).

9. ADIPONECTIN

Adiponectin is secreted exclusively from adipose tissue and is an abundant plasma protein that is reduced with obesity (91) Structurally, adiponectin is related to the complement 1q family and contains a carboxyl-terminal globular domain and an amino-terminal collagenous domain (92) Adiponectin circulates in serum as a range of multimers from low molecular weight trimers to high molecular weight (HMW) dodecamers (93) Adiponectin has been shown to improve whole-body insulin sensitivity in models of genetic and diet-induced obesity (94-99) In muscle cells *in vitro* improvements in insulin sensitivity by adiponectin are dependent on the activation of AMPK and a subsequent reduction in mTOR/S6 kinase activity which in turn results in a reduction of insulin receptor substrate 1 inhibitory serine phosphorylation (100) Adiponectin stimulates fatty acid oxidation and glucose uptake in skeletal muscle (101, 102) and adipose tissue (103) effects which are dependent on AMPK signaling. Purification and characterisation of adiponectin multimers from human plasma suggests that high molecular weight adiponectin is the most potent in stimulating AMPK activity, at least in C2C12 cells (104) Future studies are required to determine whether improvements in insulin sensitivity following adiponectin treatment *in vivo* are dependent on AMPK signalling in skeletal muscle.

The activation of AMPK signalling is dependent on signalling through the adiponectin receptor 1 (AdipoR1) while the adiponectin receptor 2 (AdipoR2) appears to be essential for regulating PPARα gene expression (105) Two reports (106, 107) in human skeletal muscle and a recent study in primary myotubes (108) suggest that skeletal muscle contains abundant levels of both AdipoR1 and AdipoR2 but that liver primarily expresses AdipoR2. Adiponectin receptor signalling appears to be dependent on the adaptor protein containing pleckstrin homology domain, phosphotyrosine binding domain and leucine zipper motif (APPL1) however it is still unknown if AAPL1 regulates AMPK signalling and if so what mechanisms may be involved (109) Adiponectin activation of AMPK signalling is blunted in obesity (110, 111), despite similar AdipoR1 and AdipoR2 expression, suggesting that defects in AAPL1, or other distal signaling events, may be important in this process.

An important role for adiponectin is the suppression of hepatic glucose output through activation of AMPK (112) Adenovirus expressing a dominant negative AMPK (101) or the knockout of the AMPK α2 subunit (43) results in increased hepatic glucose output and glucose intolerance, which can be suppressed by insulin, but no longer by adiponectin. In agreement with these findings, the deletion of the AdipoR1 receptor in liver using siRNA results in reduced AMPK and increased gluconeogenesis and glucose intolerance (113)

Kubota and colleagues (114) have recently added an important new role of adiponectin in regulating appetite and energy expenditure by showing that adiponectin activates AMPK in the hypothalamus . They show that in

the hypothalamus, AdipoR1 and AdipoR2 colocalize with the leptin receptor Ob-R and that cerebrospinal fluid (CSF) contains low levels of adiponectin that comes from the blood because intravenous injection of adiponectin raises CSF levels in adiponectin deficient (adipo-/-) mice. The form of adiponectin in the CSF is restricted to the trimer and hexamer forms and not the HMW form present in blood and this may be an important point of difference between a previous study which infused HMW recombinant adiponectin into the brain and found it induced weight loss and increased energy expenditure (115) Importantly, the effects of adiponectin to stimulate appetite and reduce energy expenditure were eliminated following the ablation of AdipoR1 (AdipoR1 siRNA) or AMPK signaling (AMPK DN) (114) Another critical finding was that leptin sensitivity is markedly increased adiponectin -/- mice leading to the proposal that the central actions of leptin and adiponectin function to provide a homeostatic mechanism to maintain fat levels/energy stores through the suppression or stimulation of appetite and energy expenditure. Future challenges in the field will be to determine how and why leptin and adiponectin signalling which act very similarly in the periphery are such polar opposites centrally and to determine whether CNS adiponectin levels are reduced in human obesity or in response to fasting or with obesity (116)

10. TUMOUR NECROSIS FACTOR α (TNFα)

TNFα has been most strongly implicated in the pathogenesis of insulin resistance because its expression is correlated with reduced insulin-stimulated glucose disposal (117-120) Direct evidence supporting the role of TNFα in mediating insulin resistance comes from studies in humans demonstrating that acute infusion of TNFα inhibits insulin stimulated glucose disposal (121) while the genetic ablation of TNFα or TNFα signalling restores skeletal muscle insulin sensitivity in obese rodents (122) Although an early study reported that TNFα neutralization in a small cohort did not reverse insulin resistance in patients with established Type 2 diabetes (123) more extensive trials for the treatment of rheumatoid arthritis have demonstrated the efficacy of this treatment in improving insulin sensitivity (124) Since suppressed rates of fatty acid oxidation in skeletal muscle of obese humans (125) and rodents (126, 127) has in some, but not all (75), cases been associated with reduced AMPK activity we tested the hypothesis that this effect may be mediated via TNFα-induced inhibition of AMPK signalling.

Chronic TNFα treatment in muscle cells downregulated fatty acid oxidation and suppressed AMPK signalling via the induction of PP2C (128) *In vivo* reductions in AMPK Thr172 phosphorylation were associated with elevated PP2C expression, reduced ACC phosphorylation and suppressed rates of fatty acid oxidation in wild type mice treated with TNFα for 24 h; however, these effects were not observed in TNF receptor -/- mice. The suppressed AMPK signalling in wildtype mice treated with TNFα was also associated with intramuscular diacylglycerol accumulation, protein kinase C ε and θ activation and the development of skeletal muscle insulin resistance. Interestingly, when TNFα was neutralized in *ob/ob* mice, or when TNFα signalling was genetically ablated (*ob/ob* TNF receptor -/- mice), the reductions in AMPK activation were reversed. Thus, elevated levels of TNFα directly inhibit AMPK signalling through PP2C activation and this is an important contributing factor to the suppressed rates of fatty acid oxidation and the development of lipid induced insulin resistance. Confirmation of these effects in humans is required.

11. INTERLEUKIN (IL-6)

The gene encoding the interleukin-6 (IL-6) protein was originally sequenced more then 25 years ago and is the founding member of a "family" of cytokines whose membership is based not on sequence homology, but of a shared four helical bundle structure (129) and a shared subunit in their receptor complex, that being the transmembrane signal transduction protein gp130 (130) The IL-6 family also known as "gp130 cytokines" consists of IL-6, IL-11, leukemia inhibitory factor (LIF), oncostatin M (OsM), cardiotrophin 1 (CT-1), ciliary neurotrophic factor (CNTF) and cardiotrophin-like cytokine (CLC) (131) Although there is a degree of cross talk among the IL-6 family cytokines (132), the complex signal transduction cascade is not common to all gp130 cytokines. CNTF, CT-1 and CLC first bind to their specific α receptors which are not involved in signal transduction per se, but binding of the ligand to the specific receptor induces a heterodimer of the signal transducing β-receptors gp130 and LIF receptor (LIFR) to allow signal transduction. LIF and OSM directly induce a gp130/LIFR heterodimer, while IL-6 and IL-11 induce a gp130 homodimer after binding their specific α receptors IL-6Rα and IL-11Rα (132) The intricacies of these complicated ligand-receptor interactions are not within the scope of this review, although a pertinent observation is that like signaling through the long form leptin receptor, signaling through the gp130 receptor activates the Jak/Stat pathway.

Interleukin-6 is elevated in patients with obesity and metabolic syndrome and, as such, IL-6 is thought to play a negative role in metabolic processes (133) However, the discovery that IL-6 can be produced and released from skeletal muscle during exercise (134), led to renewed interest into the role of IL-6 in the aetiology of insulin resistance because insulin action is enhanced in the immediate post-exercise period (135) Presently, the role of IL-6 in insulin resistance is both unclear and the subject of intense debate. However, several studies (89, 136-138) have reported that IL-6 can increase skeletal muscle fatty acid oxidation, an effect that is associated with the activation of AMPK (139) Supporting these observations, IL-6 infusion in humans enhances the glucose rate of disappearance during a hyperinsulinemic-euglycaemic clamp, indicating that IL-6 enhances muscle glucose uptake (89) Recent studies in myotubes (89, 140) have found that the activation of AMPK by IL-6 is essential for increases in both fatty acid oxidation and glucose uptake. The mechanisms by which IL-6 increases AMPK and enhances insulin stimulated glucose uptake are presently not understood but will be critical for our understanding of IL-6 signalling in skeletal muscle.

12. CILIARY NEUROTROPHIC FACTOR (CNTF)

CNTF is a neurotrophic factor that was originally used as a therapeutic strategy to treat severe neurodegenerative disorders such as amyotrophic lateral sclerosis. Although not successful in slowing the progression of amyotrophic lateral sclerosis, CNTF was found to induce severe anorexia and weight loss in these clinical trials (141) While CNTF-induced weight loss was initially attributed to a cachectic response, subsequent studies in diet-induced (142) and genetic (*ob/ob*, MC4R$^{-/-}$) obesity (143, 144) demonstrated that low doses of CNTF and the CNTF homologue, Axokine® (CNTF$_{AX15}$), induced weight loss without causing the typical deleterious effects of other related cytokines. Indeed, recent clinical trials demonstrated the efficacy and safety of CNTF$_{AX15}$, as prolonged treatment in obese human subjects induced a modest decrease in body weight with minor adverse side effects (145) The weight loss effects of CNTF were initially attributed to reductions in food intake, mediated by hypothalamic STAT3 phosphorylation and hypothalamic neurogenesis (146, 147), but since some effect of CNTF on weight loss is still present in calorically matched mice, we hypothesized that CNTF may have a potentially important role in skeletal muscle. We have recently demonstrated that CNTF increases AMPK activity in skeletal muscle by acutely decreasing the ATP:AMP ratio, resulting in increased fatty acid oxidation (77) The administration of CNTF, at doses demonstrated to induce weight loss, stimulated the expression of mitochondrial oxidative genes in skeletal muscle, which supports the concept that AMPK contributes to mitochondrial biogenesis. In addition, the stimulatory effect of CNTF on AMPK activation and fatty acid oxidation was associated with a reversal of insulin resistance induced by acute exposure to high fatty acid levels in rodents *in vivo (*148) and *in vitro* (77) Critically, these effects were not maintained when cells were infected with a dominant negative AMPK, demonstrating that AMPK signalling is essential for CNTF effects on skeletal muscle insulin sensitivity and fatty acid metabolism (77).

CNTF and leptin display similar expression patterns and signalling homology within hypothalamic regions involved in food intake (142, 149) The activation of the signal transducer and activator of transcription 3 (STAT3) within the arcuate nucleus by CNTF and leptin is associated with the suppression of orexigenic peptides such as NPY and AgRP that in turn leads to suppression of food intake through a diverse and multifaceted signaling cascade (For review see (150)) As discussed above, despite the pronounced effects of leptin on reducing food intake in a model of leptin deficiency (*ob/ob* mice), these effects are abrogated in diet-induced mice fed a high-fat diet (151) In contrast, the effect of CNTF on food intake persists in diet-induced obesity and in *db/db* mice, which lack a functional leptin receptor (143) and appear to be due to CNTF's capacity to reduce hypothalamic AMPK signaling (79) This effect was transient and is consistent with the short half-life (~45 min) of CNTF in circulation (152) These data demonstrate that

an impairment of AMPK signalling by leptin contributes to diet induced leptin resistance and that CNTF bypasses diet-induced leptin resistance to reduce hypothalamic AMPK activity and food intake. The capacity of CNTF to bypass leptin resistance to reduce food intake via AMPK signalling highlights its potential role in the therapeutic treatment of human obesity.

13. RESISTIN

Resistin (or FIZZ3) is an adipocyte-derived secretory factor which was first identified as a novel transcript produced exclusively by adipocytes (153) Despite the significant interest generated by the discovery of resistin in 2001, very little is known about the intracellular signalling pathways by which resistin induces its metabolic effects. A consistent finding *in vivo* is that resistin suppresses liver and muscle AMPK signalling (154-156) an effect also observed in L6 muscle cells (157) The mechanisms mediating this inhibition of AMPK signalling are still unclear and it is unknown whether the effects observed in vivo are due to direct effects on AMPK signalling or may be mediated through indirect pathways One possibility may be that resistin induced increases in SOCS3 (158) may inhibit cytokine signalling through to AMPK but future studies are required to establish direct evidence identifying the mechanisms by which resistin suppresses AMPK signalling.

14. GHRELIN

The gut derived, appetite-stimulating peptide ghrelin, promotes food intake (159) and has been shown to increase AMPK activity in the hypothalamus (160) Ghrelin has differential effects on AMPK activity depending on the tissue studied with reports of ghrelin treatment suppressing AMPK activity in liver (161, 162) whilst activating AMPK activity in heart (161) Ghrelin appears to have no effect on skeletal muscle AMPK which may be related to the fact that ghrelin signalling is dependent on the G protein-coupled receptor (163) and therefore most likely CAMKK signalling, which as discussed previously may not be important for regulating AMPK activity in skeletal muscle.

15. SUMMARY AND PERSPECTIVE

Collectively, these studies demonstrate that cytokine regulation of whole body energy expenditure and food intake is dependent on the differential regulation of AMPK signalling in multiple tissues throughout the body (Table 1) These studies suggest that in obesity the combined effect of reduced adiponectin production (91) and sensitivity (110, 111) when compounded with leptin resistance (76) and increased circulating TNFα (128) and resistin (155) contribute to reduced AMPK signalling and fatty acid oxidation in obesity. Several important questions remain. One important question is while AMPK is indeed activated or inhibited by adipokines and that these adipokines in turn regulate insulin sensitivity it remains to be determined in genetic models of AMPK deficiency whether AMPK signalling is an absolute requirement for this effect. Other questions are related to the mechanism

Table 1. Summary of regulators of AMPK, the mechanisms of activation or inhibition, and downstream effects shown to be dependent on AMPK

Cytokine	Effect on AMPK and Tissue Involved	Mechanism of Activation or Inhibition	Downstream Effects Shown to be Dependent on AMPK
Leptin	↑ Muscle (61, 65) ↑Adipose (69) ↔ Liver (67) ↓ Hypothalamus (44)	Muscle 0-30 min: ↑ AMP (61) >2 h: CNS (melanocortin)→α Adernergic (61, 63) Chronic: ↑ protein expression (65) Hypothalamus: MC4 (44)	Muscle ↑ACC-P → ↑ FAOx (61) Hypothalamus ↓ NPY → ↓ Food Intake (44)
Adiponectin	↑ Muscle (101) ↑ Liver (101) ↑ Adipose (103) ↑ Hypothalamus (114)	Muscle ↑ AMP (101)	Muscle ↑ACC-P → ↑ FAOx (101) Muscle and Adipose ↑ Glucose Uptake (101) (103) Liver ↓ PEPCK → ↓ Gluconeogenesis (43, 101) Hypothalamus ↑NPY → ↑ Food Intake (114)
Resistin	↓ Muscle (156, 157) ↓ Liver (156, 164) ↓ Adipose (156)	??	??
TNFα	Acute (2-5 min) ↑ (7) Chronic ↓ Muscle (128)	Acute ↑ TAK1 activity (7) Chronic ↑ PP2C expression (128)	Chronic ↓ACC-P → ↓ FAOx (128)
CNTF	↑ Muscle (77) ↓ Hypothalamus (79)	Muscle ↑ AMP (77)	Muscle ↑ACC-P → ↑ FAOx (77)
IL-6	↑ Muscle (89, 139, 140, 165) ↑ Adipose (139) ↑ Liver (139)	??	Muscle ↑ACC-P → ↑ FAOx (89, 140) ↑GLUT4 translocation → ↑ Glucose uptake (89)
Ghrelin	↓ Liver (161) ↓ Adipose (161) ↑ Heart (161) ↑ Hypothalamus (160, 161) ↔ Muscle (161)	??	??

by which AMPK is regulated because while the up regulation of PP2C is important for long-term TNFα-induced down-regulation of AMPK activity (76), changes in the cellular energy charge appear to underpin AMPK activation in several instances (61, 77, 101); however, the signalling events controlling these responses are not yet understood. Lastly, while our understanding of adipokine signalling to AMPK has been enhanced over the last decade, only a small number of adipokines have been studied with respect to AMPK regulation. In this regard, recent studies have identified a multitude of adipokines central to metabolic regulation, such as retinol binding protein-4, visfatin, adipsin and CC chemokine ligand 2 (monocyte chemoattractant protein-1); however, their role in AMPK regulation remains unresolved.

16. ACKNOWLEDGEMENTS

17. REFERENCES

1. Hawley, S., J. Boudeau, J. Reid, K. Mustard, L. Udd, T. Makela, D. Alessi & D. G. Hardie: Complexes between the LKB1 tumor suppressor, STRADalpha/beta and MO25alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. *Journal of Biology*, 2, 28 (2003)

2. Woods, A., S. R. Johnstone, K. Dickerson, F. C. Leiper, L. G. Fryer, D. Neumann, U. Schlattner, T. Wallimann, M. Carlson & D. Carling: LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Curr Biol*, 13, 2004-2008 (2003)

3. Shaw, R. J., M. Kosmatka, N. Bardeesy, R. L. Hurley, L. A. Witters, R. A. DePinho & L. C. Cantley: Inaugural Article: The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. *PNAS*, 101, 3329-3335 (2004)

4. Hurley, R. L., K. A. Anderson, J. M. Franzone, B. E. Kemp, A. R. Means & L. A. Witters: The Ca2+/calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. *J Biol Chem*, 280, 29060-6 (2005)

5. Woods, A., K. Dickerson, R. Heath, S. P. Hong, M. Momcilovic, S. R. Johnstone, M. Carlson & D. Carling: Ca2+/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells. *Cell Metab*, 2, 21-33 (2005)

6. Hawley, S. A., D. A. Pan, K. J. Mustard, L. Ross, J. Bain, A. M. Edelman, B. G. Frenguelli & D. G. Hardie: Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. *Cell Metab*, 2, 9-19 (2005)

7. Momcilovic, M., S. P. Hong & M. Carlson: Mammalian TAK1 activates Snf1 protein kinase in yeast and phosphorylates AMP-activated protein kinase in vitro. *J Biol Chem*, 281, 25336-43 (2006)

8. Sakamoto, K., A. McCarthy, D. Smith, K. A. Green, D. Grahame Hardie, A. Ashworth & D. R. Alessi: Deficiency

of LKB1 in skeletal muscle prevents AMPK activation and glucose uptake during contraction. *Embo J*, 24, 1810-20 (2005)

9. Koh, H. J., D. E. Arnolds, N. Fujii, T. T. Tran, M. J. Rogers, N. Jessen, Y. Li, C. W. Liew, R. C. Ho, M. F. Hirshman, R. N. Kulkarni, C. R. Kahn & L. J. Goodyear: Skeletal muscle-selective knockout of LKB1 increases insulin sensitivity, improves glucose homeostasis, and decreases TRB3. *Mol Cell Biol*, 26, 8217-27 (2006)

10. Thomson, D. M., B. B. Porter, J. H. Tall, H. J. Kim, J. R. Barrow & W. W. Winder: Skeletal muscle and heart LKB1 deficiency causes decreased voluntary running and reduced muscle mitochondrial marker enzyme expression in mice. *Am J Physiol Endocrinol Metab*, 292, E196-202 (2007)

11. Shaw, R. J., K. A. Lamia, D. Vasquez, S. H. Koo, N. Bardeesy, R. A. Depinho, M. Montminy & L. C. Cantley: The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. *Science*, 310, 1642-6 (2005)

12. Hardie, D. G., D. Carling & M. Carlson: The AMP-activated/SNF1 protein kinase subfamily: Metabolic sensors of the Eukaryotic cell? *Annu Rev Biochem*, 67, 821-855 (1998)

13. Amodeo, G. A., M. J. Rudolph & L. Tong: Crystal structure of the heterotrimer core of Saccharomyces cerevisiae AMPK homologue SNF1. *Nature*, 449, 492-5 (2007)

14. Xiao, B., R. Heath, P. Saiu, F. C. Leiper, P. Leone, C. Jing, P. A. Walker, L. Haire, J. F. Eccleston, C. T. Davis, S. R. Martin, D. Carling & S. J. Gamblin: Structural basis for AMP binding to mammalian AMP-activated protein kinase. *Nature*, 449, 496-500 (2007)

15. Townley, R. & L. Shapiro: Crystal structures of the adenylate sensor from fission yeast AMP-activated protein kinase. *Science*, 315, 1726-9 (2007)

16. Davies, S. P., N. R. Helps, P. T. Cohen & D. G. Hardie: 5'-AMP inhibits dephosphorylation, as well as promoting phosphorylation, of the AMP-activated protein kinase. Studies using bacterially expressed human protein phosphatase-2C alpha and native bovine protein phosphatase-2AC. *FEBS Lett.*, 377, 421-425 (1995)

17. Sanders, M. J., P. O. Grondin, B. D. Hegarty, M. A. Snowden & D. Carling: Investigating the mechanism for AMP activation of the AMP-activated protein kinase cascade. *Biochem J* (2006)

18. Savage, D. B., K. F. Petersen & G. I. Shulman: Disordered lipid metabolism and the pathogenesis of insulin resistance. *Physiol Rev*, 87, 507-20 (2007)

19. Kahn, B. B., T. Alquier, D. Carling & D. G. Hardie: AMP-activated protein kinase: Ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metabolism*, 1, 15-25 (2005)

20. Steinberg, G. R. & S. B. Jorgensen: The AMP-activated protein kinase: role in regulation of skeletal muscle metabolism and insulin sensitivity. *Mini Rev Med Chem*, 7, 519-26 (2007)

21. Carling, D., V. A. Zammit & D. G. Hardie: A common bicyclic protein kinase cascade inactivates the regulatory enzymes of fatty acid and cholesterol biosynthesis. *FEBS Lett*, 223, 217-22 (1987)

22. Saha, A. K., A. J. Schwarsin, R. Roduit, F. Masse, V. Kaushik, K. Tornheim, M. Prentki & N. B. Ruderman: Activation of malonyl-CoA decarboxylase in rat skeletal muscle by contraction and the AMP-activated protein kinase activator 5- aminoimidazole-4-carboxamide-1-beta -D-ribofuranoside. *J Biol Chem*, 275, 24279-24283 (2000)

23. Watt, M. J., G. R. Steinberg, S. Chan, A. Garnham, B. E. Kemp & M. A. Febbraio: β-adrenergic stimulation of skeletal muscle HSL can be overridden by AMPK signaling. *FASEB J.*, 18, 1445-1446 (2004)

24. Muoio, D. M., K. Seefeld, L. A. Witters & R. A. Coleman: AMP-activated kinase reciprocally regulates triacylglycerol synthesis and fatty acid oxidation in liver and muscle: evidence that sn-glycerol- 3-phosphate acyltransferase is a novel target. *Biochem. J.*, 338, 783-91 (1999)

25. Garton, A. J., D. G. Campbell, D. Carling, D. G. Hardie, R. J. Colbran & S. J. Yeaman: Phosphorylation of bovine hormone-sensitive lipase by the AMP-activated protein kinase. A possible antilipolytic mechanism. *Eur J Biochem*, 179, 249-254 (1989)

26. Kramer, H. F., C. A. Witczak, N. Fujii, N. Jessen, E. B. Taylor, D. E. Arnolds, K. Sakamoto, M. F. Hirshman & L. J. Goodyear: Distinct Signals Regulate AS160 Phosphorylation in Response to Insulin, AICAR, and Contraction in Mouse Skeletal Muscle. *Diabetes*, 55, 2067-2076 (2006)

27. Thong, F. S., P. J. Bilan & A. Klip: The Rab GTPase-activating protein AS160 integrates Akt, protein kinase C, and AMP-activated protein kinase signals regulating GLUT4 traffic. *Diabetes*, 56, 414-23 (2007)

28. Treebak, J. T., S. Glund, A. Deshmukh, D. K. Klein, Y. C. Long, T. E. Jensen, S. B. Jorgensen, B. Viollet, L. Andersson, D. Neumann, T. Wallimann, E. A. Richter, A. V. Chibalin, J. R. Zierath & J. F. P. Wojtaszewski: AMPK-Mediated AS160 Phosphorylation in Skeletal Muscle Is Dependent on AMPK Catalytic and Regulatory Subunits. *Diabetes*, 55, 2051-2058 (2006)

29. Jakobsen, S. N., D. G. Hardie, N. Morrice & H. E. Tornqvist: 5'-AMP-activated   Protein   Kinase Phosphorylates IRS-1 on Ser-789 in Mouse C2C12 Myotubes   in   Response   to   5-Aminoimidazole-4-

carboxamide Riboside. *J Biol Chem*, 276, 46912-46916 (2001)

30. Winder, W. W., B. F. Holmes, D. S. Rubink, E. B. Jensen, M. Chen & J. O. Holloszy: Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle. *J.Appl.Physiol.*, 88, 2219-2226 (2000)

31. Bergeron, R., J. M. Ren, K. S. Cadman, I. K. Moore, P. Perret, M. Pypaert, L. H. Young, C. F. Semenkovich & G. I. Shulman: Chronic activation of AMP kinase results in NRF-1 activation and mitochondrial biogenesis. *Am J Physiol*, 281, E1340-E1158 (2001)

32. Zong, H., J. M. Ren, L. H. Young, M. Pypaert, J. Mu, M. J. Birnbaum & G. I. Shulman: AMP kinase is required for mitochondrial biogenesis in skeletal muscle in response to chronic energy deprivation. *Proc Natl Acad Sci*, 99, 15983-15987 (2002)

33. Jorgensen, S. B., J. F. Wojtaszewski, B. Viollet, F. Andreelli, J. B. Birk, Y. Hellsten, P. Schjerling, S. Vaulont, P. D. Neufer, E. A. Richter & H. Pilegaard: Effects of alpha-AMPK knockout on exercise-induced gene activation in mouse skeletal muscle. *Faseb J*, 19, 1146-1148 (2005)

34. Jorgensen, S. B., J. T. Treebak, B. Viollet, P. Schjerling, S. Vaulont, J. F. Wojtaszewski & E. A. Richter: Role of {alpha}2-AMPK in basal, training- and AICAR-induced GLUT4, hexokinase II and mitochondrial protein expression in mouse muscle. *Am J Physiol Endocrinol Metab*, 292, E331-E339 (2006)

35. Jager, S., C. Handschin, J. St-Pierre & B. M. Spiegelman: AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. *Proc Natl Acad Sci U S A*, 104, 12017-22 (2007)

36. Mu, J., J. T. Brozinick, Jr., O. Valladares, M. Bucan & M. J. Birnbaum: A role for AMP-activated protein kinase in contraction- and hypoxia- regulated glucose transport in skeletal muscle. *Mol Cell*, 7, 1085-94. (2001)

37. Viollet, B., F. Andreelli, S. B. Jorgensen, C. Perrin, A. Geloen, D. Flamez, J. Mu, C. Lenzner, O. Baud, M. Bennoun, E. Gomas, G. Nicolas, J. F. Wojtaszewski, A. Kahn, D. Carling, F. C. Schuit, M. J. Birnbaum, E. A. Richter, R. Burcelin & S. Vaulont: The AMP-activated protein kinase alpha2 catalytic subunit controls whole-body insulin sensitivity. *J Clin Invest*, 111, 91-8 (2003)

38. Barnes, B. R., S. Marklund, T. L. Steiler, M. Walter, G. Hjalm, V. Amarger, M. Mahlapuu, Y. Leng, C. Johansson, D. Galuska, K. Lindgren, M. Abrink, D. Stapleton, J. R. Zierath & L. Andersson: The AMPK-gamma 3 isoform has a key role for carbohydrate and lipid metabolism in glycolytic skeletal muscle. *J. Biol. Chem.*, 279, 38441-38447 (2004)

39. Witters, L. A., G. Gao, B. E. Kemp & B. Quistorff: Hepatic 5'-AMP-activated protein kinase: zonal distribution and relationship to acetyl-CoA carboxylase activity in varying nutritional states. *Arch Biochem Biophys*, 308, 413-9 (1994)

40. Witters, L. A. & B. E. Kemp: Insulin activation of acetyl-CoA carboxylase accompanied by inhibition of the 5'-AMP-activated protein kinase. *J Biol Chem*, 267, 2864-7 (1992)

41. Lochhead, P. A., I. P. Salt, K. S. Walker, D. G. Hardie & C. Sutherland: 5-aminoimidazole-4-carboxamide riboside mimics the effects of insulin on the expression of the 2 key gluconeogenic genes PEPCK and glucose-6-phosphatase. *Diabetes*, 49, 896-903 (2000)

42. Koo, S. H., L. Flechner, L. Qi, X. Zhang, R. A. Screaton, S. Jeffries, S. Hedrick, W. Xu, F. Boussouar, P. Brindle, H. Takemori & M. Montminy: The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. *Nature*, 437, 1109-11 (2005)

43. Andreelli, F., M. Foretz, C. Knauf, P. D. Cani, C. Perrin, M. A. Iglesias, B. Pillot, A. Bado, F. Tronche, G. Mithieux, S. Vaulont, R. Burcelin & B. Viollet: Liver adenosine monophosphate-activated kinase-alpha2 catalytic subunit is a key target for the control of hepatic glucose production by adiponectin and leptin but not insulin. *Endocrinology*, 147, 2432-41 (2006)

44. Minokoshi, Y., T. Alquier, N. Furukawa, Y. B. Kim, A. Lee, B. Xue, J. Mu, F. Foufelle, P. Ferre, M. J. Birnbaum, B. J. Stuck & B. B. Kahn: AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature*, 428, 569-572 (2004)

45. Lee, K., B. Li, X. Xi, Y. Suh & R. J. Martin: Role of neuronal energy status in the regulation of adenosine 5'-monophosphate-activated protein kinase, orexigenic neuropeptides expression, and feeding behavior. *Endocrinology*, 146, 3-10 (2005)

46. Kim, E.-K., I. Miller, S. Aja, L. E. Landree, M. Pinn, J. McFadden, F. P. Kuhajda, T. H. Moran & G. V. Ronnett: C75, a Fatty Acid Synthase Inhibitor, Reduces Food Intake via Hypothalamic AMP-activated Protein Kinase. *J. Biol. Chem.*, 279, 19970-19976 (2004)

47. Pocai, A., E. D. Muse & L. Rossetti: Did a muscle fuel gauge conquer the brain? *Nat Med*, 12, 50-1; discussion 51 (2006)

48. Watt, M. J., G. R. Steinberg, Z. P. Chen, B. E. Kemp & M. A. Febbraio: Fatty acids stimulate AMP-activated protein kinase and enhance fatty acid oxidation in L6 myotubes. *J Physiol*, 574, 139-47 (2006)

49. Kennedy, G. C.: The role of depot fat in the hypothalmic control of food intake in the rat. *Proc. R. Soc.*, 140, 578-592 (1953)

50. Coleman, D. L. & K. P. Hummel: Effects of parabiosis of normal with genetically diabetic mice. *Am J Physiol*, 217, 1298-1304 (1969)

51. Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold & J. M. Friedman: Positional cloning of the mouse obese gene and its human homologue. *Nature*, 372, 425-32. (1994)

52. Campfield, L. A., F. J. Smith, Y. Guisez, R. Devos & P. Burn: Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science*, 269, 546-549 (1995)

53. Halaas, J. L., K. S. Gajiwala, M. Maffei, S. L. Cohen, B. T. Chait, D. Rabinowitz, R. L. Lallone, S. K. Burley & J. M. Friedman: Weight reducing effects of the plasma protein encoded by the obese gene. *Science*, 269, 543-546 (1995)

54. Pelleymounter, M. A., M. J. Cullen, B. M. Baker, R. Hecht, D. Winter, T. Boone & F. Collins: Effects of the obese gene product on body weight regulation in *ob/ob* mice. *Science*, 269, 540-543 (1995)

55. Hotamisligil, G., N. Shargill & B. M. Spiegelman: Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science*, 259, 87-91 (1993)

56. Hotamisligil, G., D. Murray, L. Choy & B. Spiegelman: Tumor Necrosis Factor {alpha} Inhibits Signaling From the Insulin Receptor. *Proc Natl Acad Sci*, 91, 4854-4858 (1994)

57. Luca, C., T. J. Kowalski, Y. Zhang, J. K. Elmquist, C. Lee, M. W. Kilmann, T. Ludwig, S. M. Liu & S. C. Chua, Jr.: Complete rescue of obesity, diabetes, and infertility in db/db mice by neuron-specific LEPR-B transgenes. *J Clin Invest* (2005)

58. Bates, S. H., W. H. Stearns, T. A. Dundon, M. Schubert, A. W. Tso, Y. Wang, A. S. Banks, H. J. Lavery, A. K. Haq, E. Maratos-Flier, B. G. Neel, M. W. Schwartz & M. G. Myers, Jr.: STAT3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature*, 421, 856-859 (2003)

59. Tartaglia, L., M. Dembski, X. Weng, N. Deng, J. Culpepper, R. Devos, G. J. Richards, L. A. Campfield, F. T. Clark, J. Deeds, C. Muir, S. Sanker, A. Moriarty, K. J. Moore, J. S. Smutko, G. G. Mays, E. A. Woolf, C. A. Monroe & R. I. Tepper: Identification and expression cloning of a leptin receptor, OB-R. *Cell*, 83, 1263-1271 (1995)

60. Muoio, D. M., G. L. Dohm, F. T. J. Fiedorek, E. B. Tapscott & R. A. Coleman: Leptin directly alters lipid partitioning in skeletal muscle. *Diabetes*, 46, 1360-1363 (1997)

61. Minokoshi, Y., Y. B. Kim, O. D. Peroni, L. G. Fryer, C. Muller, D. Carling & B. B. Kahn: Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature*, 415, 339-343 (2002)

62. Suzuki, A., S. Okamoto, S. Lee, K. Saito, T. Shiuchi & Y. Minokoshi: Leptin stimulates fatty acid oxidation and peroxisome proliferator-activated receptor alpha gene expression in mouse C2C12 myoblasts by changing the subcellular localization of the alpha2 form of AMP-activated protein kinase. *Mol Cell Biol*, 27, 4317-27 (2007)

63. Tanaka, T., H. Masuzaki, S. Yasue, K. Ebihara, T. Shiuchi, T. Ishii, N. Arai, M. Hirata, H. Yamamoto, T. Hayashi, K. Hosoda, Y. Minokoshi & K. Nakao: Central melanocortin signaling restores skeletal muscle AMP-activated protein kinase phosphorylation in mice fed a high-fat diet. *Cell Metab*, 5, 395-402 (2007)

64. Shimabukuro, M., K. Koyama, G. Chen, M.-Y. Wang, F. Trieu, Y. Lee, C. B. Newgard & R. H. Unger: Direct antidiabetic effect of leptin through triglyceride depletion of tissues. *Proc Natl Acad Sci*, 94, 4637-4641 (1997)

65. Steinberg, G. R., J. W. E. Rush & D. J. Dyck: AMPK expression and phosphorylation are increased in rodent muscle after chronic leptin treatment. *Am J Physiol Endocrinol Metab*, 284, E648-654 (2003)

66. Steinberg, G. R., A. Bonen & D. J. Dyck: Fatty acid oxidation and triacylglycerol hydrolysis are enhanced following chronic leptin treatment in rats. *Am J Physiol*, 282, E593-E600 (2002)

67. Lee, Y., X. Yu, F. Gonzales, D. J. Mangelsdorf, M.-Y. Wang, C. Richardson, L. A. Witters & R. H. Unger: PPARalpha is necessary for the lipopenic action of hyperleptinemia on white adipose and liver tissue. *Proc Natl Acad Sci*, 99, 11848-11853 (2002)

68. Wang, M. Y., L. Orci, M. Ravazzola & R. H. Unger: Fat storage in adipocytes requires inactivation of leptin's paracrine activity: implications for treatment of human obesity. *Proc Natl Acad Sci U S A*, 102, 18011-6 (2005)

69. Orci, L., W. S. Cook, M. Ravazzola, M. Y. Wang, B. H. Park, R. Montesano & R. H. Unger: Rapid transformation of white adipocytes into fat-oxidizing machines. *Proc Natl Acad Sci U S A*, 101, 2058-63 (2004)

70. Asilmaz, E., P. Cohen, M. Miyazaki, P. Dobrzyn, K. Ueki, G. Fayzikhodjaeva, A. A. Soukas, C. R. Kahn, J. M. Ntambi, N. D. Socci & J. M. Friedman: Site and mechanism of leptin action in a rodent form of congenital lipodystrophy. *J. Clin. Invest.*, 113, 414-424 (2004)

71. Maffei, M., J. Halaas, E. Ravussin, R. E. Pratley, G. H. Lee, Y. Zhang, H. Fei, S. Kim, R. Lallone, S. Ranganathan, P. A. Kern & J. M. Friedman: Leptin levels in human and rodent: measurement of plasma

leptin and ob RNA in obese and weight-reduced subjects. *Nat Med*, 1, 1155-1161 (1995)

72. Heymsfield, S. B., A. S. Greenberg, K. Fujioka, R. M. Dixon, R. Kuschner, T. Hunt, J. A. Lubina, J. Patane, B. Self, P. Hunt & M. McCamish: Recombinant leptin for weight loss in obese and lean adults: A randomized, controlled, dose-escallation trial. *JAMA*, 282, 1568-1575 (1999)

73. Steinberg, G. R. & D. J. Dyck: Development of leptin resistance in rat soleus muscle in response to high-fat diets. *Am J Physiol*, 279, E1374-E1382 (2000)

74. Steinberg, G. R., M. L. Parolin, G. J. Heigenhauser & D. J. Dyck: Leptin increases FA oxidation in lean but not obese human skeletal muscle: evidence of peripheral leptin resistance. *Am J Physiol Endocrinol Metab*, 283, E187-92. (2002)

75. Steinberg, G. R., A. C. Smith, B. J. W. van Denderen, Z. Chen, S. Murthy, D. J. Campbell, G. J. F. Heigenhauser, D. J. Dyck & B. E. Kemp: AMP-Activated Protein Kinase Is Not Down-Regulated in Human Skeletal Muscle of Obese Females. *J Clin Endocrinol Metab*, 89, 4575-4580 (2004)

76. Steinberg, G. R., A. J. McAinch, M. B. Chen, P. E. O'Brien, J. B. Dixon, D. Cameron-Smith & B. E. Kemp: The suppressor of cytokine signaling 3 inhibits leptin activation of AMP-kinase in cultured skeletal muscle of obese humans. *J Clin Endocrinol Metab*, 91, 3592-7 (2006)

77. Watt, M. J., N. Dzamko, W. G. Thomas, S. Rose-John, M. Ernst, D. Carling, B. E. Kemp, M. A. Febbraio & G. R. Steinberg: CNTF reverses obesity-induced insulin resistance by activating skeletal muscle AMPK. *Nat Med*, 12, 541-8 (2006)

78. Martin, T. L., T. Alquier, K. Asakura, N. Furukawa, F. Preitner & B. B. Kahn: Diet-induced obesity alters AMP kinase activity in hypothalamus and skeletal muscle. *J Biol Chem*, 281, 18933-41 (2006)

79. Steinberg, G. R., M. J. Watt, B. C. Fam, J. Proietto, S. Andrikopoulos, A. M. Allen, M. A. Febbraio & B. E. Kemp: Ciliary neurotrophic factor suppresses hypothalamic AMP-kinase signaling in leptin-resistant obese mice. *Endocrinology*, 147, 3906-14 (2006)

80. Bjorbaek, C., J. K. Elmquist, J. D. Frantz, S. E. Shoelson & J. S. Flier: Identification of SOCS-3 as a potential mediator of central leptin resistance. *Mol Cell*, 1, 619-25 (1998)

81. Cheng, A., N. Uetani, P. D. Simoncic, V. P. Chaubey, A. Lee-Loy, C. J. McGlade, B. P. Kennedy & M. L. Tremblay: Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. *Dev Cell*, 2, 497-503 (2002)

82. Zabolotny, J. M., K. K. Bence-Hanulec, A. Stricker-Krongrad, F. Haj, Y. Wang, Y. Minokoshi, Y. B. Kim, J. K. Elmquist, L. A. Tartaglia, B. B. Kahn & B. G. Neel: PTP1B regulates leptin signal transduction in vivo. *Dev Cell*, 2, 489-95 (2002)

83. Wormald, S. & D. J. Hilton: Inhibitors of Cytokine Signal Transduction. *J. Biol. Chem.*, 279, 821-824 (2004)

84. Bjorbaek, C., H. J. Lavery, S. H. Bates, R. K. Olson, S. M. Davis, J. S. Flier & M. G. Myers, Jr.: SOCS3 Mediates Feedback Inhibition of the Leptin Receptor via Tyr985. *J Biol Chem*, 275, 40649-40657 (2000)

85. Bjorbaek, C., K. El-Haschimi, J. D. Frantz & J. S. Flier: The role of SOCS-3 in leptin signaling and leptin resistance. *J Biol Chem*, 274, 30059-65 (1999)

86. Mori, H., R. Hanada, T. Hanada, D. Aki, R. Mashima, H. Nishinakamura, T. Torisu, K. R. Chien, H. Yasukawa & A. Yoshimura: Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity, 10, 739-743 (2004)

87. Howard, J. K., B. J. Cave, L. J. Oksanen, I. Tzameli, C. Bjorbaek & J. S. Flier: Enhanced leptin sensitivity and attenuation of diet-induced obesity in mice with haploinsufficiency of Socs3, 10, 734-738 (2004)

88. Steinberg, G. R., A. C. Smith, S. Wormald, P. Malenfant, C. Collier & D. J. Dyck: Endurance training partially reverses dietary-induced leptin resistance in rodent skeletal muscle. *Am J Physiol Endocrinol Metab*, 286, E57-63 (2004)

89. Carey, A. L., G. R. Steinberg, S. L. Macaulay, W. G. Thomas, A. G. Holmes, G. Ramm, O. Prelovsek, C. Hohnen-Behrens, M. J. Watt, D. E. James, B. E. Kemp, B. K. Pedersen & M. A. Febbraio: 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, (2006)

90. Namkoong, C., M.-S. Kim, P.-G. Jang, S. M. Han, J.-Y. Park, E. H. Koh, I.-K. Lee, J. Y. Kim, I.-S. Park & K.-U. Lee: Enhanced hypothalamic AMP-activated protein kinase activity contributes to hyperphagia in diabetic rats. *Diabetes*, 54, 63-68 (2005)

91. Hu, E., P. Liang & B. M. Spiegelman: AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem*, 271, 10697-703 (1996)

92. Scherer, P. E., S. Williams, M. Fogliano, G. Baldini & H. F. Lodish: A Novel Serum Protein Similar to C1q, Produced Exclusively in Adipocytes. *J. Biol. Chem.*, 270, 26746-26749 (1995)

93. Barre, L., C. Richardson, M. F. Hirshman, J. T. Brozinick, S. Fiering, B. E. Kemp, L. J. Goodyear & L. A. Witters: A Genetic Model for the Chronic Activation of Skeletal Muscle AMP-activated Protein Kinase Leads to Glycogen Accumulation. *Am J Physiol Endocrinol Metab* 00369.2.006 (2006)

94. Yamauchi, T., J. Kamon, H. Waki, Y. Terauchi, N. Kubota, K. Hara, Y. Mori, T. Ide, K. Murakami, N. Tsuboyama-Kasaoka, O. Ezaki, Y. Akanuma, O. Gavrilova, C. Vinson, M.L. Reitman, H. Kagechika, K. Shudo, M. Yoda, Y. Nakano, K. Tobe, R. Nagai, S. Kimura, M. Tomita, P. Froguel & T. Kadowaki: The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med*, 7, 941-946 (2001)

95. Yamauchi, T., J. Kamon, H. Waki, Y. Imai, N. Shimozawa, K. Hioki, S. Uchida, Y. Ito, K. Takakuwa, J. Matsui, M. Takata, K. Eto, Y. Terauchi, K. Komeda, M. Tsunoda, K. Murakami, Y. Ohnishi, T. Naitoh, K. Yamamura, Y. Ueyama, P. Froguel, S. Kimura, R. Nagai & T. Kadowaki: Globular Adiponectin Protected ob/ob Mice from Diabetes and ApoE-deficient Mice from Atherosclerosis. *J. Biol. Chem.*, 278, 2461-2468 (2003)

96. Ouchi, N., S. Kihara, Y. Arita, M. Nishida, A. Matsuyama, Y. Okamoto, M. Ishigami, H. Kuriyama, K. Kishida, H. Nishizawa, K. Hotta, M. Muraguchi, Y. Ohmoto, S. Yamashita, T. Funahashi & Y. Matsuzawa: Adipocyte-Derived Plasma Protein, Adiponectin, Suppresses Lipid Accumulation and Class A Scavenger Receptor Expression in Human Monocyte-Derived Macrophages. *Circulation*, 103, 1057-1063 (2001)

97. Masaki, T., S. Chiba, T. Yasuda, T. Tsubone, T. Kakuma, I. Shimomura, T. Funahashi, Y. Matsuzawa & H. Yoshimatsu: Peripheral, But Not Central, Administration of Adiponectin Reduces Visceral Adiposity and Upregulates the Expression of Uncoupling Protein in Agouti Yellow (Ay/a) Obese Mice. *Diabetes*, 52, 2266-2273 (2003)

98. Fruebis, J., T.-S. Tsao, S. Javorshi, D. Ebbets-Reed, M. R. Erickson, F. T. Yen, B. E. Bihain & H. F. Lodish: Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci*, 98, 2004-2010 (2001)

99. Lin, H. V., J. Y. Kim, A. Pocai, L. Rossetti, L. Shapiro, P. E. Scherer & D. Accili: Adiponectin resistance exacerbates insulin resistance in insulin receptor transgenic/knockout mice. *Diabetes*, 56, 1969-76 (2007)

100. Wang, C., X. Mao, L. Wang, M. Liu, M. D. Wetzel, K. L. Guan, L. Q. Dong & F. Liu: Adiponectin sensitizes insulin signaling by reducing p70 S6 kinase-mediated serine phosphorylation of IRS-1. *J Biol Chem*, 282, 7991-6 (2007)

101. Yamauchi, T., J. Kamon, Y. Minokoshi, Y. Ito, H. Waki, S. Uchida, S. Yamashita, M. Noda, S. Kita, K. Ueki, K. Eto, Y. Akanuma, P. Froguel, F. Foufelle, P. Ferre, D. Carling, S. Kimura, R. Nagai, B. B. Kahn & T. Kadowaki: Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med*, 8, 1288-1295 (2002)

102. Tomas, E., T.-S. Tsao, A. K. Saha, H. E. Murrey, C. c. Zhang, S. I. Itani, H. F. Lodish & N. B. Ruderman: Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: Acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. *Proc Natl Acad Sci U S A.*, 99, 16309-16313 (2002)

103. Wu, X., H. Motoshima, K. Mahadev, T. J. Stalker, R. Scalia & B. J. Goldstein: Involvement of AMP-Activated Protein Kinase in Glucose Uptake Stimulated by the Globular Domain of Adiponectin in Primary Rat Adipocytes. *Diabetes*, 52, 1355-1363 (2003)

104. Hada, Y., T. Yamauchi, H. Waki, A. Tsuchida, K. Hara, H. Yago, O. Miyazaki, H. Ebinuma & T. Kadowaki: Selective purification and characterization of adiponectin multimer species from human plasma. *Biochem Biophys Res Commun*, 356, 487-93 (2007)

105. Yamauchi, T., J. Kamon, Y. Ito, A. Tsuchida, T. Yokomizo, S. Kita, T. Sugiyama, M. Miyagishi, K. Hara, M. Tsunoda, K. Murakami, T. Ohteki, S. Uchida, S. Takekawa, H. Waki, N. H. Tsuno, Y. Shibata, Y. Terauchi, P. Froguel, K. Tobe, S. Koyasu, K. Taira, T. Kitamura, T. Shimizu, R. Nagai & T. Kadowaki: Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature*, 423, 762-769 (2003)

106. Debard, C., M. Laville, V. Berbe, E. Loizon, G. C, B. Morio-Liondore, Y. Boirie & H. Vidal: Expression of key genes of fatty acid oxidation, including adiponectin receptors, in skeletal muscle of Type 2 diabetic patients. *Diabetaologia*, 47, 816-820 (2004)

107. Civitarese, A. E., C. P. Jenkinson, D. . Richardson, M. Bajaj, K. Cusi, S. Kashyap, R. Berria, R. Belfort, R. A. DeFronzo, L. J. Mandarino & E. Ravussin: Adiponectin receptors gene expression and insulin sensitivity in non-diabetic Mexican Americans with or without a family history of Type 2 diabetes. *Diabetologia*, 47, 816-820 (2004)

108. Staiger, H., S. Kaltenbach, K. Staiger, N. Stefan, A. Fritsche, A. Guirguis, C. Peterfi, M. Weisser, F. Machicao, M. Stumvoll & H.-U. Haring: Expression of Adiponectin Receptor mRNA in Human Skeletal Muscle Cells Is Related to In Vivo Parameters of Glucose and Lipid Metabolism. *Diabetes*, 53, 2195-2201 (2004)

109. Mao, X., C. K. Kikani, R. A. Riojas, P. Langlais, L. Wang, F. J. Ramos, Q. Fang, C. Y. Christ-Roberts, J. Y. Hong, R. Y. Kim, F. Liu & L. Q. Dong: APPL1 binds to adiponectin receptors and mediates adiponectin signalling and function. *Nat Cell Biol*, 8, 516-23 (2006)

110. Chen, M. B., A. J. McAinch, S. L. Macaulay, L. A. Castelli, E. O'Brien P, J. B. Dixon, D. Cameron-Smith, B. E. Kemp & G. R. Steinberg: Impaired Activation of AMP-Kinase and Fatty Acid Oxidation by Globular Adiponectin in Cultured Human Skeletal Muscle of Obese Type 2 Diabetics. *J Clin Endocrinol Metab*, 90, 3665-3672 (2005)

111. Bruce, C. R., V. A. Mertz, G. J. Heigenhauser & D. J. Dyck: The stimulatory effect of globular adiponectin on insulin-stimulated glucose uptake and fatty acid oxidation is impaired in skeletal muscle from obese subjects. *Diabetes*, 54, 3154-60 (2005)

112. Combs, T. P., A. H. Berg, S. Obici, P. E. Scherer & L. Rossetti: Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. *J Clin Invest*, 108, 1875-81 (2001)

113. Yamauchi, T., Y. Nio, T. Maki, M. Kobayashi, T. Takazawa, M. Iwabu, M. Okada-Iwabu, S. Kawamoto, N. Kubota, T. Kubota, Y. Ito, J. Kamon, A. Tsuchida, K. Kumagai, H. Kozono, Y. Hada, H. Ogata, K. Tokuyama, M. Tsunoda, T. Ide, K. Murakami, M. Awazawa, I. Takamoto, P. Froguel, K. Hara, K. Tobe, R. Nagai, K. Ueki & T. Kadowaki: Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nat Med*, 13, 332-9 (2007)

114. Kubota, N., W. Yano, T. Kubota, T. Yamauchi, S. Itoh, H. Kumagai, H. Kozono, I. Takamoto, S. Okamoto, T. Shiuchi, R. Suzuki, H. Satoh, A. Tsuchida, M. Moroi, K. Sugi, T. Noda, H. Ebinuma, Y. Ueta, T. Kondo, E. Araki, O. Ezaki, R. Nagai, K. Tobe, Y. Terauchi, K. Ueki, Y. Minokoshi & T. Kadowaki: Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. *Cell Metab*, 6, 55-68 (2007)

115. Qi, Y., N. Takahashi, S. M. Hileman, H. R. Patel, A. H. Berg, U. B. Pajvani, P. E. Scherer & R. S. Ahima: Adiponectin acts in the brain to decrease body weight. *Nat Med*, 10, 524-9 (2004)

116. Steinberg, G. R. & B. E. Kemp: Adiponectin: starving for attention. *Cell Metab*, 6, 3-4 (2007)

117. Katsuki, A., Y. Sumida, S. Murashima, K. Murata, Y. Takarada, K. Ito, M. Fujii, K. Tsuchihashi, H. Goto, K. Nakatani & Y. Yano: Serum levels of tumor necrosis factor-alpha are increased in obese patients with noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab*, 83, 859-62. (1998)

118. Zinman, B., A. J. G. Hanley, S. B. Harris, J. Kwan & I. G. Fantus: Circulating tumor necrosis factor-alpha concentrations in a native Canadian population with high rates of Type 2 diabetes mellitus. *J Clin Endocrinol Metab*, 84, 272-278 (1999)

119. Saghizadeh, M., J. M. Ong, W. T. Garvey, R. R. Henry & P. A. Kern: The Expression of TNFalpha by Human Muscle . Relationship to Insulin Resistance. *J. Clin. Invest.*, 97, 1111-1116 (1996)

120. Kern, P. A., S. Ranganathan, C. Li, L. Wood & G. Ranganathan: Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol*, 280, E745-E751 (2001)

121. Plomgaard, P., K. Bouzakri, R. Krogh-Madsen, B. Mittendorfer, J. R. Zierath & B. K. Pedersen: Tumor Necrosis Factor-{alpha} Induces Skeletal Muscle Insulin Resistance in Healthy Human Subjects via Inhibition of Akt Substrate 160 Phosphorylation. *Diabetes*, 54, 2939-45 (2005)

122. Uysal, K. T., S. M. Wiesbrock, M. W. Marino & G. S. Hotamisligil: Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature*, 389, 610-4 (1997)

123. Ofei, F., S. Hurel, J. Newkirk, M. Sopwith & R. Taylor: Effects of an engineered human anti-TNF-alpha antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. *Diabetes*, 45, 881-885 (1996)

124. Gonzalez-Gay, M. A., J. M. De Matias, C. Gonzalez-Juanatey, C. Garcia-Porrua, A. Sanchez-Andrade, J. Martin & J. Llorca: Anti-tumor necrosis factor-alpha blockade improves insulin resistance in patients with rheumatoid arthritis. *Clin Exp Rheumatol*, 24, 83-6 (2006)

125. Bandyopadhyay, G. K., J. G. Yu, J. Ofrecio & J. M. Olefsky: Increased Malonyl-CoA Levels in Muscle From Obese and Type 2 Diabetic Subjects Lead to Decreased Fatty Acid Oxidation and Increased Lipogenesis; Thiazolidinedione Treatment Reverses These Defects. *Diabetes*, 55, 2277-85 (2006)

126. Yu, X., S. McCorkle, M. Wang, Y. Lee, J. Li, A. K. Saha, R. H. Unger & N. B. Ruderman: Leptinomimetic effects of the AMP kinase activator AICAR in leptin-resistant rats: prevention of diabetes and ectopic lipid deposition. *Diabetaologia*, 47, 2012-2021 (2004)

127. Barnes, B. R., J. W. Ryder, T. L. Steiler, L. G. Fryer, D. Carling & J. R. Zierath: Isoform specific regulation of 5' AMP-activated protein kinase in skeletal muscle from obese Zucker (fa/fa) rats in response to contraction. *Diabetes*, 51, 2703-2708 (2002)

128. Steinberg, G. R., B. J. Michell, B. J. van Denderen, M. J. Watt, A. L. Carey, B. C. Fam, S. Andrikopoulos, J. Proietto, C. Z. Gorgun, D. Carling, G. S. Hotamisligil, M. A. Febbraio, T. W. Kay & B. E. Kemp: Tumor necrosis factor alpha-induced skeletal muscle insulin resistance involves suppression of AMP-kinase signaling. *Cell Metab*, 4, 465-74 (2006)

129. Gadient, R. A. & P. H. Patterson: Leukemia inhibitory factor, Interleukin 6, and other cytokines using the GP130 transducing receptor: roles in inflammation and injury. *Stem Cells*, 17, 127-37 (1999)

130. Taga, T., M. Hibi, Y. Hirata, K. Yamasaki, K. Yasukawa, T. Matsuda, T. Hirano & T. Kishimoto: Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. *Cell*, 58, 573-81 (1989)

131. Boulay, J. L., J. J. O'Shea & W. E. Paul: Molecular phylogeny within type I cytokines and their cognate receptors. *Immunity*, 19, 159-63 (2003)

132. Ernst, M. & B. J. Jenkins: Acquiring singalling specificity from the cytokine receptor gp130. *Trends in Genetics*, 20, 23-32 (2004)

133. Lazar, M. A.: How obesity causes diabetes: not a tall tale. *Science*, 307, 373-375 (2005)

134. Febbraio, M. A. & B. K. Pedersen: Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. *FASEB J.*, 16, 1335-1347 (2002)

135. Richter, E. A., L. P. Garetto, M. N. Goodman & N. B. Ruderman: Muscle glucose metabolism following exercise in the rat: increased sensitivity to insulin. *J Clin Invest*, 69, 785-93 (1982)

136. Petersen, E. W., A. L. Carey, M. Sacchetti, G. R. Steinberg, S. L. Macaulay, M. A. Febbraio & B. K. Pedersen: Acute IL-6 treatment increases fatty acid turnover in elderly humans in vivo and in tissue culture in vitro. *Am J Physiol*, 288, E155-E162 (2005)

137. van Hall, G., A. Steensberg, M. Sacchetti, C. Fischer, C. Keller, P. Schjerling, N. Hiscock, K. Moller, B. Saltin, M. A. Febbraio & B. K. Pedersen: Interleukin-6 stimulates lipolysis and fat oxidation in humans. *J Clin Endocrinol Metab*, 88, 3005-10 (2003)

138. Bruce, C. R. & D. J. Dyck: Cytokine regulation of skeletal muscle fatty acid metabolism: effect of interleukin-6 and tumor necrosis factor-alpha. *Am J Physiol Endocrinol Metab*, 287, E616-21 (2004)

139. Kelly, M., C. Keller, P. R. Aviluea, P. Keller, Z. Luo, X. Ziang, M. Giralt, J. Hidalgo, A. K. Saha, B. K. Pedersen & N. B. Ruderman: AMPK activity is diminished in tissues of IL-6 knockout mice: the effect of exercise. *Biochem Biophys Res Commun*, 320, 449-454 (2004)

140. Al-Khalili, L., K. Bouzakri, S. Glund, F. Lonnqvist, H. A. Koistinen & A. Krook: Signaling specificity of interleukin-6 action on glucose and lipid metabolism in skeletal muscle. *Mol Endocrinol*, 20, 3364-75 (2006)

141. Miller, R. G., J. H. Petajan & W. W. Bryan: A placebo-controlled trial of recombinant human ciliary neurotrophic factor (rhCNTF) in amyotrophic lateral sclerosis. rhCNTF ALS Study Group. *Ann Neurol*, 1996, 256-260 (1996)

142. Lambert, P. D., K. D. Anderson, M. W. Sleeman, V. Wong, J. Tan, A. Hijarunguru, T. L. Corcoran, J. D. Murray, K. E. Thabet, G. D. Yancopoulos & S. J. Wiegand: From the Cover: 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*, 98, 4652-4657 (2001)

143. Gloaguen, I., P. Costa, A. Demartis, D. Lazzaro, A. Di Marco, R. Graziani, G. Paonessa, F. Chen, C. I. Rosenblum, L. H. T. Van der Ploeg, R. Cortese, G. Ciliberto & R. Laufer: Ciliary neurotrophic factor corrects obesity and diabetes associated with leptin deficiency and resistance. *PNAS*, 94, 6456-6461 (1997)

144. Sleeman, M. W., K. Garcia, R. Liu, J. D. Murray, L. Malinova, M. Moncrieffe, G. D. Yancopoulos & S. J. Wiegand: Ciliary neurotrophic factor improves diabetic parameters and hepatic steatosis and increases basal metabolic rate in db/db mice. *PNAS*, 100, 14297-14302 (2003)

145. Ettinger, M., T. Littlejohn, S. Schwartz, S. Weiss, H. McIlwain, S. Heymsfield, G. Bray, W. Roberts, E. Heyman, N. Stambler, S. Heshka, C. Vicary & H. Guler: Recombinant variant of ciliary neurotrophic factor for weight loss in obese adults: a randomized, dose-ranging study. *JAMA*, 289, 1826-1832 (2003)

146. Kelly, J. F., C. F. Elias, C. E. Lee, R. S. Ahima, R. J. Seeley, C. Bjorbaek, T. Oka, C. B. Saper, J. S. Flier & J. K. Elmquist: Ciliary Neurotrophic Factor and Leptin Induce Distinct Patterns of Immediate Early Gene Expression in the Brain. *Diabetes*, 53, 911-920 (2004)

147. Kokoeva, M. V., H. Yin & J. S. Flier: Neurogenesis in the hypothalamus of adult mice: potential role in energy balance. *Science*, 310, 679-83 (2005)

148. Watt, M. J., A. Hevener, G. I. Lancaster & M. A. Febbraio: Ciliary neurotrophic factor prevents acute lipid-induced insulin resistance by attenuating ceramide accumulation and phosphorylation of JNK in peripheral tissues. *Endocrinology*, 147, 2077-2085 (2006)

149. Anderson, K. D., P. D. Lambert, T. L. Corcoran, J. D. Murray, K. E. Thabet, G. D. Yancopoulos & S. J. Wiegand: Activation of the Hypothalamic Arcuate Nucleus Predicts the Anorectic Actions of Ciliary Neurotrophic Factor and Leptin in Intact and Gold Thioglucose-Lesioned Mice. *J Neuroendocrinol*, 15, 649-660 (2003)

150. Schwartz, M. W. & D. Porte: Diabetes, Obesity and the Brain. *Science*, 307, 375-379 (2005)

151. Frederich, R. C., A. Hamann, S. Anderson, B. Lollmann, B. B. Lowell & J. S. Flier: Leptin levels reflect body lipid content in mice: Evidence for diet-induced resistance to leptin action. *Nat Med*, 1, 1311-1314 (1995)

152. Bluher, S., S. Moschos, J. J. Bullen, E. Kokkotou, E. Maratos-Flier, S. Wiegand, M. Sleeman & C. Mantzoros: Ciliary neurotrophic factorAx15 alters energy homeostasis, decreases body weight, and improves metabolic control in diet-induced obese and UCP1-DTA mice. *Diabetes*, 53, 2787-2796 (2004)

153. Steppan, C. M., S. T. Bailey, S. Bhat, E. J. Brown, R. R. Banerjee, C. M. Wright, H. R. Patel, R. S. Ahima & M.

A. Lazar: The hormone resistin links obesity to diabetes. *Nature*, 409, 307-12 (2001)

154. Qi, Y., Z. Nie, Y. S. Lee, N. S. Singhal, P. E. Scherer, M. A. Lazar & R. S. Ahima: Loss of resistin improves glucose homeostasis in leptin deficiency. *Diabetes*, 55, 3083-90 (2006)

155. Banerjee, R. R., S. M. Rangwala, J. S. Shapiro, A. S. Rich, B. Rhoades, Y. Qi, J. Wang, M. W. Rajala, A. Pocai, P. E. Scherer, C. M. Steppan, R. S. Ahima, S. Obici, L. Rossetti & M. A. Lazar: Regulation of Fasted Blood Glucose by Resistin. *Science*, 303, 1195-1198 (2004)

156. Satoh, H., M. T. A. Nguyen, P. D. G. Miles, T. Imamura, I. Usui & J. M. Olefsky: Adenovirus-mediated chronic "hyper-resistinemia" leads to in vivo insulin resistance in normal rats. *J. Clin. Invest.*, 114, 224-231 (2004)

157. Palanivel, R. & G. Sweeney: Regulation of fatty acid uptake and metabolism in L6 skeletal muscle cells by resistin. *FEBS Lett*, 579, 5049-54 (2005)

158. Steppan, C. M., J. Wang, E. L. Whiteman, M. J. Birnbaum & M. A. Lazar: Activation of SOCS-3 by resistin. *Mol Cell Biol*, 25, 1569-75 (2005)

159. Nakazato, M., N. Murakami, Y. Date, M. Kojima, H. Matsuo, K. Kangawa & S. Matsukura: A role for ghrelin in the central regulation of feeding. *Nature*, 409, 194-198 (2001)

160. Andersson, U., K. Filipsson, C. R. Abbott, A. Woods, K. Smith, S. R. Bloom, D. Carling & C. J. Small: AMP-activated Protein Kinase Plays a Role in the Control of Food Intake. *J. Biol. Chem.*, 279, 12005-12008 (2004)

161. Kola, B., E. Hubina, S. A. Tucci, T. C. Kirkham, E. A. Garcia, S. E. Mitchell, L. M. Williams, S. A. Hawley, D. G. Hardie, A. B. Grossman & M. Korbonits: Cannabinoids and ghrelin have both central and peripheral metabolic and cardiac effects via AMP-activated protein kinase. *J Biol Chem*, 280, 25196-201 (2005)

162. Barazzoni, R., A. Bosutti, M. Stebel, M. R. Cattin, E. Roder, L. Visintin, L. Cattin, G. Biolo, M. Zanetti & G. Guarnieri: Ghrelin regulates mitochondrial-lipid metabolism gene expression and tissue fat distribution in liver and skeletal muscle. *Am J Physiol Endocrinol Metab*, 288, E228-35 (2005)

163. Howard, A. D., S. D. Feighner, D. F. Cully, J. P. Arena, P. A. Liberator, C. I. Rosenblum, M. Hamelin, D. L. Hreniuk, O. C. Palyha, J. Anderson, P. S. Paress, C. Diaz, M. Chou, K. K. Liu, K. K. McKee, S. S. Pong, L. Y. Chaung, A. Elbrecht, M. Dashkevicz, R. Heavens, M. Rigby, D. J. Sirinathsinghji, D. C. Dean, D. G. Melillo, A. A. Patchett, R. Nargund, P. R. Griffin, J. A. DeMartino, S. K. Gupta, J. M. Schaeffer, R. G. Smith & L. H. Van der Ploeg: A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science*, 273, 974-7 (1996)

164. Rangwala, S. M., A. S. Rich, B. Rhoades, J. S. Shapiro, S. Obici, L. Rossetti & M. A. Lazar: Abnormal Glucose Homeostasis due to Chronic Hyperresistinemia. *Diabetes* 53, 1937-41 (2004)

165. Glund, S., A. Deshmukh, Y. C. Long, T. Moller, H. A. Koistinen, K. Caidahl, J. R. Zierath & A. Krook: Interleukin-6 directly increases glucose metabolism in resting human skeletal muscle. *Diabetes*, 56, 1630-7 (2007)

Abbreviations: AMPK: AMP-activated protein kianse; ACC: acetyl-CoA carboxylase; PP2C: protein phosphatase 2C; CNS: central nervous system; NPY: neuropeptide Y; TAK1: TGFβ activated kinase; MCR4: Melanocortin receptor 4; PEPCK: phosphoenolpyruvate carboxykinase; CaMKK: Ca2+/calmodulin-dependent protein kinase; G6Pase: glucose-6-phosphatase; MCD: malonyl-CoA decarboxylase; ciliary neurotrophic factor: CNTF; HSL: hormone sensitive lipase; GPAT; sn-glycerophosphate acyl transferase; AS160: Akt substrate of 160 kDa; IRS: insulin receptor substrate 1; PGC1α: peroxisome-proliferator activated receptor γ coactivator 1α ; DN: dominant negative; CA: constitutively active; NPY: neuropeptide Y; AgRP: agouti related peptide; TORC2: transducer of CREB activity 2; SOCS3: suppressor of cytokine signalling 3; CSF: cerebrospinal fluid; AdipoR1: adiponectin receptor 1; AdiporR2: adiponectin receptor 2; APPL1: phosphotyrosine binding domain and leucine zipper motif; IL-6: interleukin-6; CNTF: ciliary neurotrophic factor; STAT3: signal transducer and activator of transcription 3

Key Words: Leptin, Adiponectin, TNFα, IL-6, CNTF, Resistin, Ghrelin, Nutrient Signalling, Cytokines, Insulin Sensitivity, Obesity

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