Regulation of metabolism and inflammation by two protein kinases – AMPK and STK25

Emmelie Cansby

Department of Molecular and Clinical Medicine
Institute of Medicine
Sahlgrenska Academy at University of Gothenburg

UNIVERSITY OF GOTHENBURG
Gothenburg 2014

Cover illustration: STK25 expression in mouse liver by Manoj Amrutkar

Regulation of metabolism and inflammation by two protein kinases – AMPK and STK25
© Emmelie Cansby 2014
emmelie.cansby@gu.se

ISBN 978-91-628-9067-4

Printed in Gothenburg, Sweden 2014
Ale Tryckteam AB, Bohus

“Fascinating is a word I use for the unexpected.”

- Spock, Star Trek: The Original Series

Regulation of metabolism and inflammation by two protein kinases – AMPK and STK25

Emmelie Cansby

Department of Molecular and Clinical Medicine, Institute of Medicine
Sahlgrenska Academy at University of Gothenburg
Göteborg, Sweden

ABSTRACT

Type 2 diabetes mellitus (T2DM) is a widespread metabolic disorder that has reached epidemic proportions globally, and is now considered to be one of the main threats to human health. The currently available treatment options in T2DM suffer from inadequate efficacy and durability, as well as liabilities, including hypoglycemia, weight gain, edema and gastrointestinal intolerance. Since the efficacy and patient compliance of existing treatments are in many cases unsatisfactory, there is a pronounced need for novel targets, which could complement current treatment.

Evidence accumulated during the last two decades indicates that excessive caloric intake and obesity lead to a chronic low-grade inflammation in metabolic tissues, and increased inflammation may be directly involved in the pathogenesis of T2DM. Therefore, novel pharmacological treatments targeting both the metabolic and inflammatory disruptions seen in T2DM are warranted.

AMP-activated protein kinase (AMPK) is a central master switch important for regulating energy homeostasis at cellular as well as whole body level. Recently, evidence for a role of AMPK in regulation of inflammatory balance has emerged. By using AMPK agonists AICAR and metformin in liver cell lines, primary hepatocytes and in mouse model system *in vivo*, we demonstrate that AMPK activation in liver leads to decreased inflammatory response to the proinflammatory cytokine IL-6. We further show that the anti-inflammatory action of AMPK was mediated via decreased phosphorylation of several downstream components of the canonical IL-6 receptor signalling pathway. Inhibition of the IL-6 signalling cascade in liver by AMPK supports a role of this kinase as a crucial point of convergence of metabolic and inflammatory pathways in hepatocytes.

Serine/threonine protein kinase 25 (STK25) is broadly expressed in mouse, rat and human tissues. When activated, this kinase is part of several cell processes, such as development, migration and apoptosis. We, for the first time, show that STK25 also has metabolic effects. By using small interfering RNA for *Stk25* in rodent muscle cell line L6, we demonstrate that STK25 is involved in regulation of glucose uptake and lipid oxidation. Furthermore, mice overexpressing STK25, when challenged with a high-fat diet, develop reduced glucose tolerance and insulin sensitivity compared to wild-type siblings. Increased triglyceride deposition in liver and skeletal muscle, and adipocyte hypertrophy, as observed in *Stk25* transgenic mice, suggest that the underlying cause of insulin resistance in conditions of excess dietary fuels is a shift in the metabolic balance from lipid oxidation toward lipid storage in peripheral tissues. Furthermore, *Stk25* transgenic mice show increased infiltration of inflammatory cells in liver.

Taken together, both AMPK and STK25 emerge as interesting targets for future treatment of T2DM, enabling to target the dysregulation of both metabolism and inflammation seen in connection with this disease.

Keywords: AMPK, STK25, Metabolism, Type 2 diabetes, Inflammation

ISBN: 978-91-628-9067-4

SAMMANFATTNING PÅ SVENSKA

Typ 2 diabetes är en sjukdom som påverkar ämnesomsättningen och som kännetecknas av förhöjda glukos- och insulinhalter i blodet. På senare år har antalet fall av denna förödande sjukdom ökat dramatiskt och diabetes har blivit ett av de stora globala hoten mot människors hälsa. Det har även framkommit att inflammation i vävnader där ämnesomsättning sker är en viktig del av diabetes. Eftersom den behandling av diabetes som finns tillgänglig idag uppvisar begränsad effekt samt har oönskade biverkningar, finns det ett stort behov av nya mediciner som kan ersätta eller komplettera dagens läkemedel. För att hitta nya vägar att påverka de negativa effekterna man ser på ämnesomsättningen vid diabetes och som samtidigt påverkar den inflammation som uppstår, har vi studerat två proteiner med potentiella effekter på både metabolism och inflammation.

Aktivering av signalproteinet AMPK ökar glukosupptaget i muskel samt minskar frisättningen av glukos i lever, och därmed motverkas de metabola störningar som man ser vid diabetes. Resultat från våra studier tyder på att AMPK även motverkar inflammation orsakad av inflammationsframkallande molekyler i lever, vilket indikerar att AMPK kan fungera som en länk mellan regleringen av inflammatoriska och metabola processer.

Vi har nyligen identifierat ett nytt enzym, STK25, som fungerar som en katalysator för utvecklingen av insulinresistens i kroppen, som i sin tur kan leda till diabetes. Vår senaste forskning utförd på cellnivå samt i genmodifierade möss visar att om man hämmar STK25 så förbättras glukosregleringen markant, medan aktivering av STK25 har motsatt effekt, det vill säga glukosintolerans och insulinresistens. Genom att påverka aktiviteten av STK25 skulle vi potentiellt kunna förbättra energibalansen i celler och därmed motverka de kanske viktigaste orsakerna till att diabetes uppstår.

Sammanfattningsvis så har vi visat att både AMPK och STK25 är intressanta mål för framtida behandling av diabetes, då de har effekter på de metabola och inflammatoriska komplikationer man ser vid denna sjukdom.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Nerstedt A, Johansson A, Andersson CX, Cansby E, Smith U, Mahlapuu M. **AMP-activated protein kinase inhibits IL-6-stimulated inflammatory response in human liver cells by suppressing phosphorylation of signal transducer and activator of transcription 3 (STAT3).** *Diabetologia. 2010 Nov;53(11):2406-16.*

II. Nerstedt A, Cansby E, Amrutkar M, Smith U, Mahlapuu M. **Pharmacological activation of AMPK suppresses inflammatory response evoked by IL-6 signalling in mouse liver and in human hepatocytes.** *Mol Cell Endocrinol. 2013 Aug 15;375(1-2):68-78.*

III. Cansby E, Nerstedt A, Amrutkar M, Nuñez Durán E, Smith U, Mahlapuu M. **Partial hepatic resistance to IL-6-induced inflammation develops in type 2 diabetic mice, while the anti-inflammatory effect of AMPK is maintained.** *Manuscript.*

IV. Nerstedt A, Cansby E, Andersson CX, Laakso M, Stancakova A, Bluher M, et al. **Serine/threonine protein kinase 25 (STK25): a novel negative regulator of lipid and glucose metabolism in rodent and human skeletal muscle.** *Diabetologia. 2012 Jun;55(6):1797-807.*

V. Cansby E, Amrutkar M, Manneras Holm L, Nerstedt A, Reyahi A, Stenfeldt E, et al. **Increased expression of STK25 leads to impaired glucose utilization and insulin sensitivity in mice challenged with a high-fat diet.** *Faseb J. 2013 Sep;27(9):3660-71.*

CONTENT

ABBREVIATIONS III
1 INTRODUCTION 1
1.1 Type 2 diabetes mellitus and insulin resistance 1
1.2 Inflammation as part of metabolic disease 2
1.3 Protein kinases in regulation of metabolism and inflammation 3
1.3.1 AMP-activated protein kinase 3
1.3.2 Serine/threonine protein kinase 25 9
2 AIM 13
3 METHODS 14
3.1 Ethical statement 14
3.2 Experiments in human subjects 14
3.3 Animal experiments 14
3.4 Cell culture experiments 17
3.5 Techniques for quantitative assessment of mRNA and protein level . 19
3.6 Enzyme-linked immunosorbent assay 20
4 RESULTS 21
5 DISCUSSION 26
ACKNOWLEDGEMENT 30
REFERENCES 32

ABBREVIATIONS

2-DG	2-deoxy-D-glucose
ACC	Acetyl-CoA carboxylase
AICAR	5-aminoimidazole-4-carboxamide ribonucleotide
AMPK	AMP-activated protein kinase
AS160	Akt substrate of 160 kDa
CaMKKβ	Calcium/calmodulin-dependent protein kinase kinase β
CBS	Cystathionine-β synthase
CCM	Cerebral cavernous malformation
cDNA	Complementary DNA
CNTF	Ciliary neurotrophic factor
CRP	C-reactive protein
EHC	Euglycemic-hyperinsulinemic clamp
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescence-activated cell sorting
FAS	Fatty acid synthase
FFA	Free fatty acid
G6Pase	Glucose-6-phosphatase
GCK	Germinal centre kinases
GP130	Glycoprotein 130
GPAT	Glycerol-3-phosphate acyltransferase
GTT	Glucose tolerance test
HDL	High-density lipoprotein
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
HP	Haptoglobin
HSL	Hormone sensitive lipase
IKK	IkappaB kinase
IL	Interleukin
IL6Rα	IL-6 receptor subunit α
IP	Intraperitoneal
ITT	Insulin tolerance test
JAK1	Janus kinase 1
LKB1	Liver Kinase B1
LM04	LIM domain only 4
MAPK	Mitogen-activated protein kinase
MBP	Myelin basic protein
MIF	Macrophage migration inhibitory factor
MST	Mammalian sterile 20-like
NF-κB	Nuclear factor-kappaB
PAK	p21-activated kinases
PCR	Polymerase chain reaction

PEPCK	Phosphoenolpyruvate carboxykinase
PGC1α	PPAR gamma coactivator 1-alpha
PPAR	Peroxisome proliferator-activated receptor
PTP	Protein tyrosine phosphatase
qRT-PCR	Quantitative real-time PCR
ROS	Reactive oxygen species
SAA	Serum amyloid A
SCD-1	Stearoyl CoA desaturase 1
SHP	Small heterodimer partner
SHP2	SH2-domain containing protein tyrosine phosphatase 2
shRNA	Short hairpin RNA
siRNA	Small interfering RNA
SIRT1	Sirtuin 1
SOCS3	Suppressor of cytokine signalling 3
SREBP-1c	Sterol regulatory element binding protein 1
STAT3	Signal transducer and activator of transcription 3
Ste20	Sterile 20
T2DM	Type 2 diabetes
TAK1	Transforming growth factor-beta-activated kinase 1
TNF-α	Tumour necrosis factor α
TZD	Thiazolidinedione
UCP	Uncoupling protein
WAT	White adipose tissue

1 INTRODUCTION

1.1 Type 2 diabetes mellitus and insulin resistance

Type 2 diabetes mellitus (T2DM) is a widespread metabolic disorder, characterized by chronic hyperglycaemia due to a malfunction of the interrelationship between insulin secretion and insulin action. This disease has reached epidemic proportions globally, with approximately 366 million people worldwide living with T2DM today, and numbers are expected to rise to 552 million by 2030 (1). Alarmingly, T2DM has gone from being a disease typically seen later in life to now also affecting children and adolescents (2). Despite the fact that inherited features predispose to T2DM, environmental and lifestyle factors are held mainly responsible for the increasing prevalence of the disease over the past decades. Interestingly, there is now strong evidence from controlled clinical trials that T2DM can be prevented by interventions that deliver relatively modest lifestyle changes (3). T2DM is associated with a reduced life expectancy owing to a greater risk of heart disease, stroke, renal disease, blindness and amputation (4).

Obesity is generally considered to be correlated with the development of T2DM, with a relative risk for an obese subject to develop T2DM being approximately 10-fold higher, compared to lean controls (5). Furthermore, insulin resistance is also correlated with T2DM, and considered to be a predictor of T2DM, independent of obesity (6, 7). Insulin resistance is a state of reduced insulin sensitivity, i.e. an inability of insulin to lower plasma glucose levels through effects on insulin-responsive tissues, such as liver, skeletal muscle and white adipose tissue (WAT). In the liver, insulin stimulates the storage of glucose in the form of glycogen by activating several enzymes, such as glucokinase and glycogen synthase. Furthermore, insulin inhibits gluconeogenesis and glucogenolysis in liver, overall leading to suppressed hepatic glucose output (8). In skeletal muscle, insulin facilitates entry of glucose via increased translocation of the glucose uptake transporter GLUT4 to the plasma membrane, resulting in lower plasma glucose levels (9). In WAT, insulin represses lipolysis by inhibition of hormone sensitive lipase (HSL), which ultimately leads to decreased circulating levels of free fatty acids (FFAs). Increased plasma levels of FFAs have been shown to

decrease glucose uptake in liver and skeletal muscle (10). Overall, insulin resistance in these tissues results in an increase in plasma glucose levels, and to compensate for this, the β-cells in pancreas initially secrete more insulin, which ultimately results in β-cell dysfunction, hyperglycaemia and progression towards T2DM (11).

1.2 Inflammation as part of metabolic disease

Traditionally, inflammation is described as a complex biological defence response of the body to injuries or infections, with several different inflammatory cells present at various stages of the inflammatory process (12). Evidence during the last two decades indicates that excessive caloric intake and obesity lead to a chronic low-grade inflammation in metabolic tissues, with elevations in markers and mediators of inflammation and the acute phase response.

The first link between obesity, T2DM and chronic inflammation was the finding that tumour necrosis factor α (TNF-α), a proinflammatory cytokine, is overexpressed in the adipose tissue of obese mice (13). TNF-α is also overexpressed in the adipose and muscle tissues of obese humans. Moreover, TNF-α administration leads to insulin resistance, which suggests that increased inflammation may be a causative factor for metabolic dysregulation (14-16).

Of all the proinflammatory cytokines which are increased during chronic inflammation, circulating levels of interleukin (IL)-6 show the strongest correlation with insulin resistance (17, 18). Importantly, recent evidence emphasizes that metabolic effects of IL-6 differ in a tissue-specific manner. In obese subjects with or without T2DM, adipose tissue IL-6 content strongly correlates with decreased whole body insulin-mediated glucose uptake and glucose tolerance (17, 19). At the same time, physical exercise increases secretion of IL-6 from skeletal muscle (20), while improving insulin sensitivity. A large body of evidence demonstrates that IL-6 impairs insulin action in rodent and human liver cells (21, 22) as well as induces hepatic inflammation, lipid accumulation and insulin resistance in liver *in vivo* in mice (23-26).

Taken together, inflammation in metabolic tissues, with increased production of proinflammatory cytokines, may be directly involved in the

pathogenesis of T2DM. Therefore, novel pharmacological treatments targeting both the metabolic and inflammatory disruptions seen in T2DM are warranted.

1.3 Protein kinases in regulation of metabolism and inflammation

Protein phosphorylation is a post-translational modification, carried out by kinases (27). Phosphorylation by protein kinases regulates signalling pathways and cellular processes that mediate metabolism, transcription, differentiation, cytoskeleton arrangement, apoptosis, and immunological functions (28). Protein kinases have long been the focus of development of drugs for therapy of a wide range of diseases, and protein kinases represent as much as 30% of all protein targets under investigation by pharmaceutical companies today. Currently, most of the approved drugs that target protein kinases are in cancer therapy. A few drugs targeting kinases are approved for the treatment of inflammatory conditions, such as fostamatinib and tofacitinib, while no kinase agonist or antagonist is to date approved for the treatment of T2DM or other metabolic complications (29). Kinases displaying both metabolic and inflammatory effects are of great interest as potential drug targets, and combining conventional therapies with novel kinase activators or inhibitors could prove to be a successful strategy for treating metabolic and inflammatory diseases in the future. The focus of my work has been to characterize the effect of two protein kinases in regulation of metabolism and inflammation

1.3.1 AMP-activated protein kinase

AMP-activated protein kinase (AMPK) was first described by Prof. D. Carling and Prof. G. Hardie in 1987 (30), although the general interest in this kinase first started growing during the last decade. Today, the number of published papers with AMPK in their title alone has risen to more than 4000, linking AMPK to a wide range of key physiological functions such as regulation of glucose and lipid metabolism, lipid oxidation and inflammatory signalling.

The primary function of AMPK is to monitor changes in the intracellular level of ATP and to couple this to phosphorylation of downstream substrates

leading to an increase in the rate of ATP-producing pathways and/or a decrease in the rate of ATP-utilizing pathways (31), making AMPK a central master switch important for regulating energy homeostasis. In addition to AMPK exerting its effects by acute phosphorylation of metabolic enzymes, this kinase also acts in the longer term by regulating gene and protein expression.

1.3.1.1 Structure and regulation

AMPK is a heterotrimeric serine/threonine protein kinase complex, orthologues of which are expressed in virtually all eukaryotes. AMPK is composed of three subunits, α, β and γ, which all are required for its activity (Figure 1)(32). In mammalian cells there are two isoforms of the α subunit, two isoforms of the β subunit and three isoforms of the γ subunit. Although AMPK is ubiquitously expressed in mammalian cells, the expression of certain subunit isoforms is tissue-specific. The α1 and β1 subunits are ubiquitously expressed, whereas expression of α2 and β2 is relatively higher in cardiac and skeletal muscle (33, 34). Of the γ isoforms, γ1 and γ2 are expressed uniformly throughout different tissues, whereas γ3 has only been detected in skeletal muscle (35).

The α subunit contains a serine/threonine protein kinase domain at the N-terminus, which becomes phosphorylated on the Thr^{172} residue by upstream kinases upon activation. Upstream kinases identified to date are the liver kinase B1 (LKB1) (36), the calcium/calmodulin-dependent protein kinase kinase β (CaMKKβ) (37), and the transforming growth factor-beta-activated kinase 1 (TAK1) (38), and phosphorylation by these kinases leads to >100-fold activation of AMPK (39). Furthermore, as its name suggests, AMPK is allosterically activated by binding of AMP to the cystathionine-β synthase (CBS) domains on the γ subunit (40). The degree of allosteric activation by AMP depends on the γ isoform present in the AMPK complex (41), although in comparison to activation through phosphorylation, allosteric activation causes a very modest effect (two- to five-fold increase in activity). Binding of AMP to the γ subunit causes a conformational change of the trimeric complex, protecting the kinase from dephosphorylation of the α subunit and subsequent inactivation, which makes this step important. Dephosphorylation of AMPK on Thr^{172} of the α subunit is *in vitro* achieved by members of the phosphoprotein phosphatase family and metal-dependent protein phosphatase family (42, 43). However, further investigation is required to determine the

physiologically relevant APMK phosphatases *in vivo.* Interestingly, a recent study reported that similarly to AMP, ADP can bind to the γ subunit and protect AMPK from dephosphorylation (44). The β subunit interacts with the α and γ subunits, acting as a scaffold for the interaction of the heterotrimeric complex (45).

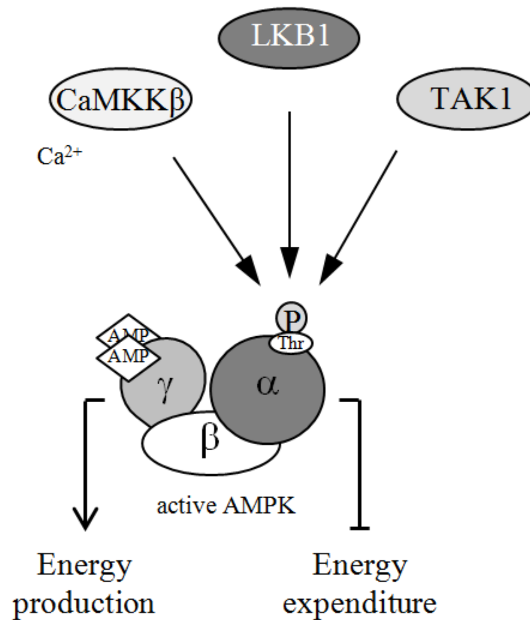

Figure 1. Structure and regulation of AMPK.

1.3.1.2 AMPK in regulation of metabolism

Activation of AMPK has several effects in main metabolic tissues, which are beneficial for whole body glucose homeostasis, insulin sensitivity and lipid metabolism.

Skeletal muscle is the major site of insulin-stimulated glucose disposal (46), and insulin resistance in this tissue is a contributing factor in the development of T2DM. Therefore, alternative pathways that stimulate glucose uptake in skeletal muscle could potentially improve glycemic control in this patient group. Activation of AMPK increases the glucose uptake in skeletal muscle either acutely by increased translocation of GLUT4, the main

glucose transporter in skeletal muscle, to the plasma membrane by AMPK phosphorylating the Akt substrate of 160 kDa (AS160), or chronically by increasing the expression of GLUT4 (47, 48). AMPK phosphorylates and inactivates acetyl-CoA carboxylase (ACC) 2 (49). Inhibition of this protein by AMPK promotes fatty acid uptake into mitochondria and subsequent β-oxidation (50). Furthermore, AMPK upregulates peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*Pgc1α*), which leads to increased mitochondrial biogenesis (51). Taken together, activation of AMPK in skeletal muscle leads to increased glucose uptake and lipid oxidation.

Elevated glucose production by the liver is a major cause of fasting hyperglycaemia in T2DM (52). Activation of AMPK suppresses the transcription of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), two key enzymes in gluconeogenesis (53). Additionally, AMPK has been implicated in regulation of liver lipid metabolism. AMPK phosphorylates the transcription factor sterol regulatory element binding protein 1 (SREBP-1c) (54), leading to decreased expression of enzymes in the fatty acid synthesis pathway, i.e. fatty acid synthase (FAS) (55). Furthermore, AMPK suppresses the expression of other genes associated with lipogenesis, such as ACC1 (56), as well as inactivates 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (57), a key enzyme in cholesterol synthesis, altogether leading to decreased lipid synthesis. Activation of AMPK in liver also leads to increased lipid oxidation via phosphorylation and subsequent inactivation of ACC2 (58). Thus, AMPK suppresses liver gluconeogenesis and lipid production.

In T2DM the insulin-mediated suppression of lipolysis in adipose tissue is impaired, leading to high circulating levels of FFAs (59). AMPK, like insulin, have been demonstrated to regulate lipolysis through inhibition of HSL, a rate-limiting enzyme controlling lipolysis (60). Also, activation of AMPK inhibits lipogenesis in adipocytes through phosphorylation and inactivation of ACC1 (61), the rate-limiting enzyme in de novo fatty acid synthesis, and by decreasing mRNA expression of several lipogenic enzymes, i.e. FAS, stearoyl CoA desaturase 1 (SCD-1) and glycerol-3-phosphate acyltransferase (GPAT) (62). Isolated adipocytes treated with AICAR have increased gene expression of PGC1α, peroxisome proliferator-activated receptor (PPAR)α and PPARγ, transcription factors involved in fatty acid oxidation and mitochondrial biogenesis (63). Overall, activation of

AMPK in adipose tissue leads to decreased lipolysis and lipogenesis, as well as increased fatty acid oxidation.

1.3.1.3 Animal models validating the AMPK pathway

Genetically modified animal models have played a key role in understanding the role of AMPK in regulation of whole body metabolism. Table 1 lists the selected transgenic and knock-out mouse models with associated phenotypes, which are considered to be most important to understand the metabolic function of AMPK.

Table 1

	Model	Phenotype	Reference
Whole body	AMPK α1 knock-out	No apparent phenotype observed	(64)
	AMPK α2 knock-out	Impaired glucose tolerance, insulin resistance, impaired glucose-stimulated insulin secretion, reduced insulin-stimulated whole body glucose utilisation	(65)
		Reduced skeletal muscle glycogen content	(64)
		Increased high-fat diet-induced body weight and fat mass	(66)
	AMPK γ3 knock-out	Impaired skeletal muscle glycogen resynthesis after exercise	(67)
		Impaired induction by fasting or exercise of skeletal muscle mRNA expression of metabolic genes	(68-70)
Liver	LKB1 depletion	Hyperglycaemia, glucose intolerance, increased hepatic gluconeogenic and lipogenic gene expression	(71)
	AMPK α2 knock-out	Hyperglycaemia, glucose intolerance, increased hepatic glucose production	(72)
Skeletal muscle	Overexpression of dominant negative AMPK α2	Impaired skeletal muscle glucose uptake	(73)
		Impaired skeletal muscle mitochondrial biogenesis	(74)
		Impaired skeletal muscle glycogen resynthesis after exercise	(75)
	Overexpression of AMPK γ3	No apparent phenotype observed	(67)
	Overexpression of AMPK γ3 R225Q mutant	Reduced high-fat diet-induced fat deposition and improved insulin sensitivity, increased glycogen content in skeletal muscle	(67)
		Increased expression of lipid metabolic genes	(68, 69)

1.3.1.4 Pharmacological activation of AMPK

Pharmacological agonists of AMPK have been widely used to map the metabolic effects of APMK activation. 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) is a cell permeable compound able to activate AMPK in various tissues. Once taken up by the cells, AICAR is converted to ZMP, which mimics the effects of AMP (76). Metformin is used extensively to treat T2DM. Although its primary mode of action is uncertain, it has been demonstrated that metformin inhibits complex I of the mitochondrial respiratory chain, increasing the cellular AMP:ATP ratio, which in turn activates AMPK (77). Another group of substances used for treatment of

T2DM that are also known to activate AMPK are the PPARγ agonists thiazolidinediones (TZDs). Although TZDs can indirectly activate AMPK similarly to metformin (78, 79), recent studies suggest that the primary effect of TZDs is due to an increase in the expression of adiponectin (80, 81). Adiponectin, in turn, is known to activate AMPK in liver and skeletal muscle (82). The A-769662 compound is a small molecule that directly binds to the β subunit of AMPK, and it activates the kinase via both allosteric activation and protection against Thr^{172} dephosphorylation (83, 84). Additionally, salicylate was shown to bind to the same site as A-769662, activating AMPK in the same manner (85).

1.3.1.5 AMPK in regulation of inflammation

While the role of AMPK as a key regulator of metabolic profile has long been recognised, the evidence of its anti-inflammatory actions has only recently started to emerge. Metformin has been reported to decrease the levels of inflammatory markers in plasma, such as C-reactive protein (CRP) (86). Moreover, *in vitro* experiments and animal studies show that AICAR inhibits acute and chronic colitis (87), autoimmune encephalomyelitis (88), inflammation in cystic fibrosis (89), and proinflammatory effects after lung injury (90). Additionally, the TZD rosiglitazone has been shown to cause a fall in plasma concentrations of inflammatory signals such as CRP and serum amyloid A (SAA) (91). In macrophages, endothelial cells, and neutrophils, it has been demonstrated that AMPK activation negatively regulates the inflammatory response via antagonism of the nuclear factor-kappaB (NF-κB) signalling, a key regulator of the immune system and inflammation (90, 92-96). AMPK indirectly antagonises the NF-κB subunits via inhibition of its downstream targets, such as IkappaB kinase (IKK) (92), sirtuin 1 (SIRT1) (95), or p300 (96). Interestingly, activation of AMPK by both metformin and AICAR attenuated the TNF-α-induced expression of proinflammatory and cell adhesion molecules in human endothelial cells (94). AICAR also decreased inflammatory mediator production in stimulated kidney mesangial cells in an AMPK-dependent manner (97). In different cell types, stimulation by inflammatory mediators such as macrophage migration inhibitory factor (MIF), ciliary neurotrophic factor (CNTF) and IL-6 correlates with AMPK activation (98-101), further supporting a possible cross-talk between these pathways.

1.3.2 Serine/threonine protein kinase 25

Serine/threonine protein kinase 25 (STK25) was shown to be differentially regulated in skeletal muscle comparing AMPKγ3 knock-out mice to wild-type littermates (69). This finding, that STK25 is regulated by metabolic master switch AMPK, led to the hypothesis that STK25 might also have a role in regulation of glucose metabolism and insulin sensitivity of the body.

1.3.2.1 Structure of STK25

STK25, also referred to as UK1, SOK1 or YSK1, belongs to the sterile 20 (Ste20) superfamily of kinases. The *Ste20* gene was first identified in yeast as a key molecule involved in pheromone signalling (102). In mammals, about thirty Ste20-related kinases, characterized by the presence of a conserved catalytic domain, have been described. Based on the location of this conserved kinase domain, Ste20-kinases have been divided into two large families; p21-activated kinases (PAKs), where the kinase domain can be found at the C-terminus, and germinal centre kinases (GCKs), where the kinase domain is positioned at the N-terminus (103). The GCK group can be further subdivided into GCKI to GCKVIII, and STK25 belongs to the GCKIII subgroup together with mammalian sterile 20-like (MST) 3 (also known as STK24) and MST4 (also known as MASK; Figure 2). Members of Ste20 superfamily have been implicated in the regulation of a large number of diverse cellular functions in mammalian cells, including cell polarization, cell differentiation and apoptosis (104). Most of these kinases activate mitogen-activated protein kinase (MAPK) cascades, which are crucial in a wide range of cellular events.

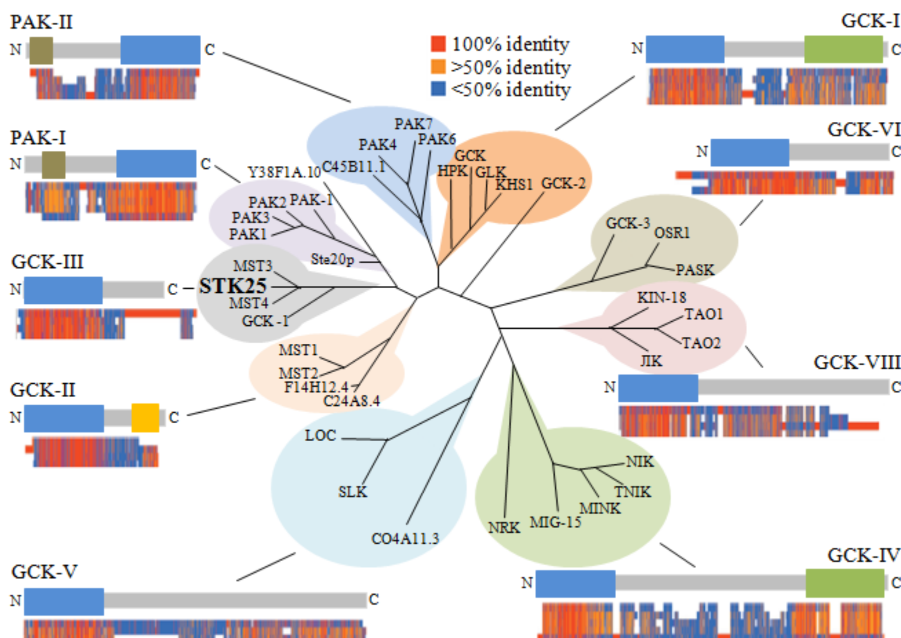

Figure 2. Phylogenetic tree, domain structure, and multiple sequence alignments of Ste20-kinases. For reference, yeast kinase Ste20p, the founding member of this family, is also shown. PAKs fall into two structurally similar subfamilies, PAK-I and PAK-II, whereas GCKs fall into eight subfamilies. Protein kinase domains are indicated by blue boxes. Citron-like domains (green box) may be involved in macromolecular interactions, particularly with small GTPases. The SARAH domain (yellow box) facilitates dimerization. PAK domains (brown box) allow the PAK family kinases to bind to members of the p21 and Rho families. In the alignments for each subfamily shown below the domain structures, red indicates 100%, orange >50%, and blue <50% identity. Adapted from (105).

STK25 was first described in 1996 as a 426 amino acids long kinase activated by oxidant stress (106). STK25 is ubiquitously expressed in rodent tissues, i.e. liver, skeletal muscle and intestine, although it is most highly expressed in the brain and testis (106, 107). The predicted size of STK25 is 48 kDa, however, on SDS-polyacrylamide gel electrophoresis, the apparent size of the kinase is approximately 55 kDa, suggesting that STK25 undergoes post-translational modifications (107). The kinase is activated by phosphorylation and partly inactivated by dephosphorylation, with auto-phosphorylation of the Thr^{174} residue being an important mechanism for activation of the kinase (106, 108). Furthermore, STK25 is negatively regulated by its C-terminal non-catalytic region (106).

1.3.2.2 Regulation pattern and function of STK25

Several inducers of oxidant stress have been shown to activate STK25, i.e. H_2O_2 and menadione. In contrast to activation of STK25 by oxidative stress, neither growth factors, osmolar stress, heat chock nor cytokines were shown to activate this kinase (106). Increase in the concentration of cytosolic free Ca^{2+} as a consequence of oxidant stress and chemical anoxia has also been shown to activate STK25 (109). Although these data clearly place STK25 on an oxidant stress response pathway, this kinase does not appear to activate any of the known stress-activated MAPK pathways, such as the ERK, JNK and p38 pathways, suggesting that STK25 defines a novel stress response pathway (106, 107).

STK25 has been shown to be localized to the Golgi apparatus, where it interacts with the Golgi matrix protein GM130, which has been demonstrated to activate STK25 via stimulation of auto-phosphorylation (108). STK25 is needed for correct localization of the Golgi apparatus within the cell, as well as cell migration (108). STK25 has also been identified as an interactor of cerebral cavernous malformation (CCM) 2 and 3, which are part of signalling pathways essential for vascular development and CCM pathogenesis (110). Furthermore, the interaction of STK25 and GM130 is stabilized by CCM3 to promote Golgi assembly, and ubiquitination and subsequent degradation of STK25 is increased in the absence of CCM3 (111). STK25 has also been implicated in nervous system development when in complex with LKB1 and GM130 (112). In response to chemical anoxia and increased reactive oxygen species (ROS) production, STK25 dissociates from the Golgi and translocates to the nucleus, where it induces apoptosis, a process at least in part dependent on caspase cleaving of STK25 (113). Thus, STK25 has distinctly different functions depending on the redox and energy status of the cell, regulating cell motility in non-stressed cells and cell death in the stressed cell (Figure 3).

There are only three known substrates for STK25. The first one described is myelin basic protein (MBP), and STK25 was shown to phosphorylate MBP on serine and threonine residues (106). STK25 also phosphorylates Ser^{58} of the Golgi-associated 14-3-3ζ, a protein important for cell polarity and cell migration (114). The third known substrate for STK25 is CCM3 (115).

To this point, the function of STK25 has mostly been characterized based on *in vitro* experiments. Matsuki *et al* electroporated a *Stk25* short hairpin RNA (shRNA)-expressing vector into the hippocampi of fetal mice, and demonstrated that STK25 is important for axons to mature (112). Except the studies by Matsuki *et al*, there were no published animal models investigating the effects of STK25 and demonstrating its *in vivo* significance before the start of this thesis work.

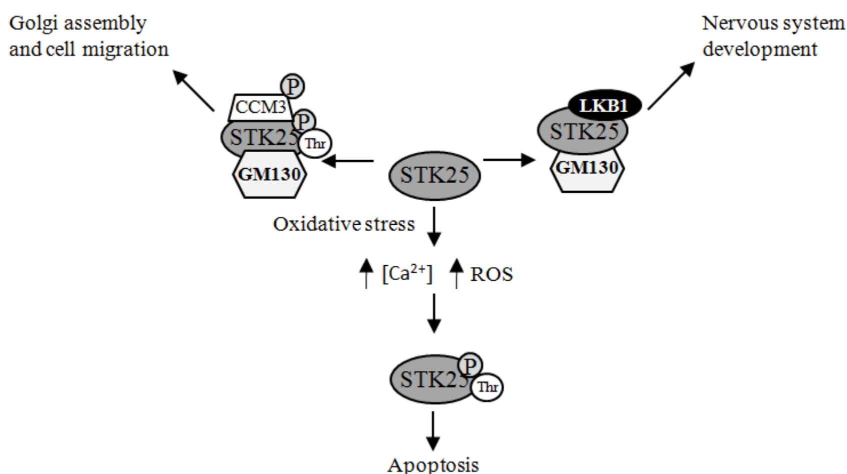

Figure 3. Cellular function of STK25.

1.3.2.3 STK25 in metabolism and inflammation

The role of STK25 in cell migration and as a stress-activated regulator of apoptosis has been well described, but before our recent studies, there were no reports of any effects of STK25 on metabolism and inflammation. However, Su *et al* suggested *Stk25* as a candidate gene for regulating high-density lipoprotein (HDL) levels in mice (116). Although the potential role of STK25 in regulation of inflammation had not been described prior to our experiments, proteins previously reported to associate with STK25 have been implicated in immune regulation. For instance, LKB1 has been shown to affect immunoglobulin genes (117) and CCM3 has been suggested to bind to CD8 and CD4 (118). Furthermore, kinases in GCK subgroups I and II have been implicated in regulation of inflammatory cytokines and NFκB signalling (118).

2 AIM

The general aim of this thesis was to characterize the anti-inflammatory effects of the metabolic master switch AMPK, and to elucidate the impact of a downstream target of AMPK – STK25 – in regulation of metabolism.

The specific aims of the five papers included in this thesis were:

Paper I. To elucidate the impact of AMPK on the regulation of the inflammatory response induced by cytokine action in hepatocytes.

Paper II. To evaluate the capacity of AMPK to repress liver inflammation *in vivo* and to further dissect the mechanism for AMPK to interfere with IL-6-mediated inflammatory signalling in liver cells.

Paper III. To elucidate if pharmacological activation of AMPK enables repression of IL-6-stimulated hepatic inflammation in a metabolically comprised state.

Paper IV. To investigate the role of STK25 in regulation of skeletal muscle metabolism.

Paper V. To evaluate the metabolic effect of STK25 at a whole body level *in vivo*.

3 METHODS

3.1 Ethical statement

The study involving human subjects was approved by the ethics committee of the University of Leipzig and conformed to the Declaration of Helsinki. All the participants provided written informed consent before taking part in the study.

All animal experiments were performed after prior approval from the local Ethics Committee for Animal Studies at the Administrative Court of Appeals in Gothenburg, Sweden.

3.2 Experiments in human subjects

Paper IV contains a study in human subjects. 41 consecutively recruited Caucasian men (n=23) and women (n=18) were categorized into groups of normal glucose tolerance (*n*=13, 9 males, 4 females), impaired glucose tolerance (*n*=14, 8 males, 6 females), and subjects with T2DM (*n*=14, 6 males, 8 females) based on a 75 g oral glucose tolerance test (GTT). Skeletal muscle biopsies were obtained under local anaesthesia from the right *vastus lateralis* muscle with a fine needle device, and were immediately snap-frozen in liquid nitrogen until further analysis. For subject characteristics and exclusion criteria, see Supplemental Material for *Paper IV*. These experiments were performed by our collaborator Prof. M. Blüher, University of Leipzig, Germany.

3.3 Animal experiments

A transgenic mouse model overexpressing STK25

Transgenic mice were created by the Norwegian Transgenic Centre by pronuclear injection using C57BL/6N strain of mice. For further details, see *Paper V*. Potential founders were screened for transgene integration by polymerase chain reaction (PCR) analysis from DNA isolated from tail biopsies. Subsequent breeding was done with C57BL/6N strain. The C57BL/6N mouse strain was used since it is known to be prone to diet-induced obesity and T2DM (119). Only male mice were used for

phenotyping. Animals were weaned at 3 weeks of age and housed 3–5 per cage in a temperature-controlled (21°C) facility with a 12-hour light-dark cycle with free access to chow and water. In the experimental setup in *Paper V*, age-matched male transgenic mice and wild-type littermates were fed pelleted high-fat diet (45 kcal% fat) from 6 weeks of age until the end of the study. In most experiments, male wild-type littermates of the same age, fed a chow diet, were included as a reference group.

Evaluation of glucose tolerance and insulin sensitivity

A GTT examines the systemic clearance of glucose. Briefly, a bolus dose of glucose is injected into the animal and plasma glucose values are monitored over a period of time. However, GTT does not account for endogenous insulin secretion and therefore, this method is limited in situations where pancreatic function is altered. An insulin tolerance test (ITT) examines the capacity of the body to respond to insulin at systemic level, and is a measurement of insulin sensitivity. After a bolus dose of insulin, plasma glucose levels are measured over a period of time. However, ITT has a risk of leading to severe hypoglycemia with systemic counter-regulatory responses affecting the results. Although both techniques have limitations, these methods are widely used to assess glucose tolerance and insulin sensitivity since they are easy to use and are considered non-invasive. GTT and ITT were used in *Papers III* and *V*.

Neither GTT nor ITT determines which organs are responsible for insulin resistant phenotype, a limitation that needs to be considered, because different degree of insulin resistance may develop in different tissues. A more sensitive method to assess insulin sensitivity is the euglycemic-hyperinsulinemic clamp (EHC) technique. The blood insulin concentration is raised and maintained by continuous intravenous infusion of insulin, while euglycemia is maintained by infusion of glucose at a variable rate. Insulin sensitive animals rapidly take up and utilize the glucose during the hyperinsulinemic condition, while utilization and clearance are impaired in insulin resistant animals. Correspondingly, the glucose infusion rate needed to reach a glucose steady-state equals whole body glucose uptake, a crude index of insulin sensitivity. Prior to the EHC, the system is primed with [^{3}H]glucose to be able to calculate glucose turnover rate, hepatic glucose production, as well as overall glucose uptake. The EHC technique was used in *Paper V*. Notably, the high-insulin infusion concentration used in our

study almost completely suppressed the clamp hepatic glucose output and therefore, any potential differences in hepatic insulin sensitivity cannot be evaluated by using this experimental protocol.

To assess the glucose uptake in individual tissues, the EHC approach can be further refined to include injections of [^{14}C]-labelled 2-deoxy-D-glucose (2-DG) when steady-state is reached. This radiolabelled glucose is a non-metabolizable glucose analogue that is taken up by the tissues, where it is phosphorylated and therefore trapped. Glucose uptake in individual organs can then be calculated from plasma 2-DG decay profile and tissue 2-DG-6-phosphate content (120). This method has been performed in our most recent studies, however, it is not included in the current thesis work.

Administration of test substances in mice

Proper restraint is the most important technique for performing injections in mice, to decrease stress and pain for the animal, and ensure repeatable and reliable results. There are different routs of systemic administration. Subcutaneous injections are easy to use and rarely painful, however, the rate of absorption is lower compared to intraperitoneal (IP) injections. IP administration is the most widespread route, being easy to perform and allowing quite large volumes to be administrated, although it requires pH to be within a physiological range. Intravenous administration has many advantages. Solutions with high or low pH, as well as irritating substances, can be injected this way. However, this technique requires technical expertise and skill, and often involves anaesthesia.

In *Papers II*, *III* and *V*, mice received different treatments, all by IP administration. AICAR and metformin have been injected acutely to activate AMPK. Injections of human rIL-6 have been given to evoke an inflammatory response. Although the primary structures of human IL-6 and murine IL-6 exhibit only 42% amino acid sequence identity, human rIL-6 is shown to efficiently activate IL-6 signalling in both human and murine systems (121-123). Using human IL-6 in our experiments also allows us to distinguish between the basal plasma levels of endogenous IL-6 *vs.* the levels of exogenous IL-6 after the injection. To assess insulin sensitivity, glucose and insulin have been administered after 4 h of fasting. The fasting is used to achieve stable basal plasma glucose levels, mainly in order to reduce variability. Overnight fasting has not been used, since mice have nocturnal eating patterns, and food deprivation during this period might affect other

parameters, such as plasma glucagon, plasma glucocorticoids and plasma adrenaline (124, 125). For most treatments, saline solution has been administered to a different group of mice as a control.

Open field activity test

This test is a commonly performed as a qualitative and quantitative measure of general locomotor activity and willingness to explore the environment in rodents (126). Interestingly, this technique was originally designed to score defecation as a measure of the emotionality of animals (127). In the protocol used by us in *Paper V*, the activity test lasted for 23 hours, whereof 11 hours consisted of daylight (150 lux, 10.00-19.00 and 07.00-09.00) and 12 hours of nightlight (20 lux, 19.00-07.00). The equipment consisted of an opaque box (50 x 50 x 22.5 cm) that has a lower and a higher row of infrared sensors built into the walls connected to a control unit for tracking of the mouse. The mouse is placed in the centre of the box and the test is performed for 23 hours, allowing the mouse to acquaint itself with the open field test chamber for the first 3 hours. Several parameters can be recorded, such as horizontal activity (increased by 1 every time the animal breaks a beam in the lower plane), locomotion (increased by 1 every time the animal breaks a new beam), rearing activity (increased by 1 every time the animal breaks a beam in the higher plane), and rearing time (accumulated time, in seconds, that the animal breaks a beam in the higher plane).

3.4 Cell culture experiments

Cell lines

HepG2 is a human liver carcinoma cell line. It has been derived from the liver tissue of a 15-years-old Caucasian American male with a well-differentiated hepatocellular carcinoma (128). This immortalized cell line has been widely used as a model system for studies of liver metabolism. HepG2 cells were cultured as described in *Papers I* and *II*.

The rodent skeletal muscle cell line L6 was originally isolated from primary cultures of rat thigh muscle. Under normal growth conditions these cells propagate as mononucleated myoblasts. Once L6 cells become confluent, they start to differentiate into multinucleated myotubes (129). L6 cells were cultured as described in *Paper IV*.

Primary hepatocytes isolated from mouse liver are effective tools to confirm results gained using immortalized cell lines. Under optimal conditions, these cells will preserve both liver-specific functions and morphology over a substantial period of time (130). Primary hepatocytes were cultured and treated as described in *Papers I* and *III*.

In vitro stimulation

In *Papers I, II* and *III*, HepG2 cells and primary mouse hepatocytes were first treated with AICAR or metformin to activate AMPK, followed by treatment with human or murine recombinant cytokines, such as IL-6, IL-1β and TNF-α, to evoke an inflammatory response. To investigate the impact of insulin stimulation on IL-6-induced inflammatory response, primary mouse hepatocytes were treated with insulin for 10 min, prior to the treatment with IL-6.

In *Paper IV*, the activation of STK25 by different stress factors was tested by treating L6 cells with menadione, a potent intracellular generator of reactive oxygen intermediates (131), H_2O_2, a ROS (132), thapsigargin, a strong inducer of mammalian endoplasmic reticulum stress through perturbation of calcium homeostasis (133), or the cytokines TNF-α and IL-6. Stress was also induced by serum starvation.

Glucose transport and palmitate oxidation assay

In *Paper IV* the glucose uptake and β-oxidation in L6 cells were evaluated. The glucose transport rate into the cells was measured using [^{3}H]-labelled 2-DG. Briefly, cells were pre-treated with insulin to stimulate glucose uptake, followed by addition of 2-DG. The glucose uptake was stopped with phloretin, a glucose transporter inhibitor, and the amount of [^{3}H]glucose in the cells was quantified by scintillation counting.

β-oxidation is the process by which fatty acids are broken down in the mitochondria or peroxisomes to generate acetyl-CoA, which will be fed into the citric acid cycle to generate ATP. [^{3}H]palmitate, which is used as substrate for β-oxidation, was added to the media, and [^{3}H]-labelled H_2O was measured in the media as the product of β-oxidation.

3.5 Techniques for quantitative assessment of mRNA and protein level

Quantitative real-time PCR (qRT-PCR)

The PCR method was developed in 1983 and allows rapid amplification of DNA sequences. The PCR is based on cycles of heating and cooling through a defined series of temperature steps, for DNA melting and enzymatic replication of the DNA region of interest. Primers, designed to target the DNA sequence to be amplified, are used for selective amplification of the sequence of interest. Then, DNA polymerase in the reaction mixture will create a complementary DNA strand from nucleotides.

As with regular PCR, TaqMan qRT-PCR uses specific primers for the DNA sequence of interest, and new DNA sequences are synthesized with DNA polymerase. However, TaqMan-based detection also uses a probe with a fluorescence reporter, which is specific for the gene of interest. The probe also contains a quencher that will eliminate the fluorescence from the reporter as long as the probe is intact. When the DNA polymerase synthesizes the new DNA-strand, the quencher is separated from the reporter, and fluorescence can occur. The signal from the reporter is directly proportional to the amount of PCR product produced. Quantification of the amount of target can be performed using a standard curve or with relative quantification with a housekeeping gene for normalization.

The qRT-PCR technique has been performed in all papers of this thesis work, with relative quantification used for evaluation of the gene expression.

RNA extraction

RNA extraction is the first step in gene expression analysis. RNA is purified from cells or homogenized tissue samples, using commercially available kits. In this thesis, the RNeasy Mini Kit from Qiagen or the EZNA Total RNA kit from Omega Bio-Tek have been used. It is known that RNA purification from fibrous tissues, such as skeletal muscle, is difficult due to the abundance of collagen and contractile proteins. Therefore, proteinase K, which digests these proteins, was used when muscle samples were processed.

From extracted RNA, complementary DNA (cDNA) is synthesized using commercially available kits. The synthesized cDNA is used in qRT-PCR as template for expression analysis. RNA extraction and cDNA synthesis have been used in all papers of this thesis work.

Protein extraction and western blot analysis

Throughout this thesis work, protein expression has been evaluated using the western blot technique. Briefly, cells or homogenized tissue samples are lysed in lysis buffer optimized to maintain protein stability, to avoid protein degradation or inhibit phosphatase activity. Proteins are separated based on size using gel electrophoresis, and then transferred to a membrane. After the transfer of the proteins to the membrane, the membranes are coloured using Ponceau, to ensure the transfer procedure has been fully completed. The proteins of interest are then identified using specific antibodies. In most analyses, hybridization with antibody against housekeeping protein, such as actin or glyceraldehyde 3-phosphate dehydrogenase, has been included, as additional control for equal loading and complete transfer.

3.6 Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) is used to quantify proteins in samples. There are different examples of ELISAs, but most frequently a primary antibody binds to the protein of interest, followed by binding of a secondary antibody to the primary one. The secondary antibody carries an enzyme, and when a substrate for that enzyme is added to the working solution, it will lead to a shift in colour which is detectable by a spectrophotometer. The colour intensity is in proportion to the amount of the protein of interest in the sample, and is analyzed in relation to a standard curve.

4 RESULTS

Below is a brief summary of the main results of the five papers included in this thesis work. For further details, see the full papers at the end of the thesis.

Paper I
AMP-activated protein kinase inhibits IL-6-stimulated inflammatory response in human liver cells by suppressing phosphorylation of signal transducer and activator of transcription 3 (STAT3)

Human hepatocarcinoma cell line HepG2 was pre-treated with AICAR or metformin, followed by IL-6 stimulation, and the inflammatory response evoked by the cytokine was evaluated. The AMPK agonists markedly blunted the IL-6-stimulated expression of *SAA* cluster genes as well as haptoglobin *(HP)* in a dose-dependent manner. Additionally, the repression of AMPK expression by small interfering RNA (siRNA) significantly reversed the inhibition of *SAA* expression by both AICAR and metformin, indicating the effect of the agonists is dependent on AMPK. For the first time we show that AMPK regulates IL-6 signalling by directly inhibiting the activation of the main downstream target of IL-6, STAT3, in HepG2 cells. Furthermore, AICAR and metformin significantly decreased the IL-6-induced phosphorylation of STAT3 in mouse primary hepatocytes.

We provide evidence for a key function of AMPK in suppression of acute phase response caused by the action of IL-6 in hepatocytes, suggesting that AMPK is an important intracellular link between metabolic and inflammatory regulation in peripheral metabolic tissues.

Paper II
Pharmacological activation of AMPK suppresses inflammatory response evoked by IL-6 signalling in mouse liver and in human hepatocytes

Male mice of C57BL6/N strain were injected with AICAR 1 h prior to IL-6 administration. 15 or 45 minutes after the cytokine injection, livers were dissected and expression levels of *Saa1*, *Saa2* and suppressor of cytokine signalling 3 (*Socs3*), as well as phosphorylation of STAT3, were evaluated.

Similarly to the data obtained in hepatocytes, AMPK activation resulted in a blunted IL-6-induced inflammatory response *in vivo*. To further map the mechanism for AMPK to interfere with IL-6 signalling, we evaluated the effect of pharmacological activation of AMPK at total protein and phosphorylation level of all the components in the IL-6/STAT3 signalling pathway in HepG2 cell line. Our results suggest that AMPK inhibits IL-6 signalling by repressing IL-6-stimulated phosphorylation of several downstream components of the pathway, such as Janus kinase 1 (JAK1), SH2-domain containing protein tyrosine phosphatase 2 (SHP2), as well as STAT3 (Figure 4).

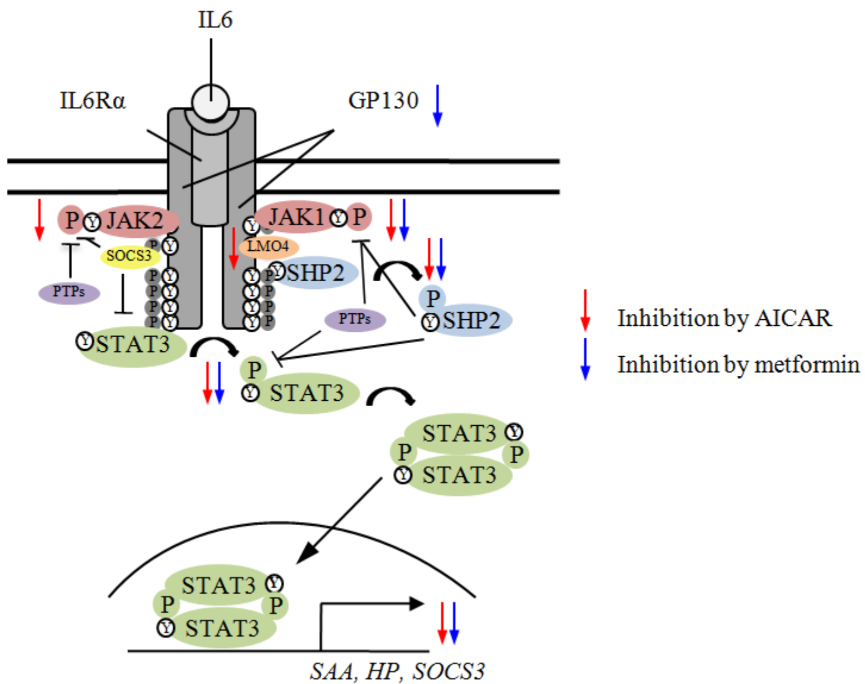

Figure 4. A schematic model of the effects of AICAR and metformin on the IL-6 signalling pathway. Red and blue arrows indicate targets which are regulated at the level of phosphorylation or protein expression by AICAR and metformin, respectively. IL6Rα, IL-6 receptor subunit α; GP130, glycoprotein 130; LM04, LIM domain only 4; PTPs, protein tyrosine phosphatases.

Our results of inhibition of hepatic IL-6 signalling cascade by AMPK *in vivo* further support the role of this kinase as a crucial point of convergence of metabolic and inflammatory signalling in liver.

Paper III

Partial hepatic resistance to IL-6-induced inflammation develops in type 2 diabetic mice, while the anti-inflammatory effect of AMPK is maintained

Male mice of C57BL6/N strain were fed a high-fat diet for 18 weeks, to induce insulin resistance and glucose intolerance. Lean male mice fed a chow diet were used as controls. At the end of the feeding period, the mice were injected with AICAR or metformin, followed by IL-6 treatment. Livers were dissected and expression levels of inflammatory markers were evaluated. Surprisingly, we found that the inflammatory response in liver evoked by IL-6 administration *in vivo* was markedly blunted in mice fed a high-fat diet, compared to lean metabolically normal mice. However, the anti-inflammatory action of AMPK agonists seen in lean control mice was still present in the high-fat diet-fed mice. Furthermore, we found that the inflammatory response to IL-6 treatment was identical in primary hepatocytes isolated from the mice fed a high-fat and chow diet, suggesting that systemic factors, rather than differences in intracellular signalling, account for the blunted IL-6 response observed *in vivo* in high-fat diet-fed mice.

In summary, our work reveals that partial hepatic IL-6 resistance develops in the mouse model of T2DM, while the anti-inflammatory action of AMPK is sustained, and that systemic factors are likely mediating the relative impairment in IL-6 effect. Our finding of changes in sensitivity to IL-6 in different metabolic context may give new insight into the understanding of the increased circulating levels of IL-6 seen in connection to insulin resistance and T2DM.

Paper IV

Stk25 – a novel negative regulator of lipid and glucose metabolism in skeletal muscle

The effect of decreasing STK25 levels in muscle cells was studied by reducing the mRNA and protein content of this target in the rodent muscle cell line L6 by siRNA approach. Expression of *STK25* was also evaluated in

skeletal muscle biopsies of 41 Caucasian men and women of normal and impaired glucose tolerance and T2DM. We demonstrated that partial depletion of STK25 increased the mRNA expression level of uncoupling protein (*Ucp)2* and *Ucp3*. Increased expression of UCP3 was also confirmed at protein level. Since UCP2 and UCP3 proteins are suggested to be involved in β-oxidation, we measured the rate of fatty acid oxidation after partial depletion of STK25 in L6 cells. β-oxidation was increased approximately 20%, reaching the same level as measured for the cells treated with positive control substance phenformin. In addition, reduced level of STK25 enhanced the mRNA and protein expression of genes involved in glucose metabolism, such as GLUT1, GLUT4 and hexokinase 2. Correspondingly, the insulin-stimulated glucose uptake in these muscle cells was improved. Consistent with these results, significantly higher *STK25* mRNA levels were observed in skeletal muscle of T2DM patients, compared to subjects with normal glucose tolerance.

This is the first study indicating a possible role for STK25 in regulation of glucose and lipid metabolism in skeletal muscle.

Paper V
Increased expression of STK25 leads to impaired glucose utilization and insulin sensitivity in mice challenged with a high-fat diet

Transgenic mice overexpressing STK25 and wild-type littermates were challenged with a high-fat diet for 18 weeks. During the feeding period, the *Stk25* transgenic carriers developed hyperinsulinemia, and impairments in glucose utilization and insulin sensitivity, compared to their wild-type siblings. In liver, expression levels of key enzymes regulating lipogenesis (*Fasn*), glycogen synthesis (*Gck*) and gluconeogenesis (*G6pc*, *Fbp1*) were increased in the transgenic carriers, while in skeletal muscle of transgenic mice, altered expression levels of genes controlling lipid synthesis and/or oxidation, *Cpt1*, *Acox1* and ACC, were seen, indicating reduced oxidative capacity in this tissue. Additionally, hepatic triglyceride and glycogen content, as well as adipocyte size, were increased in transgenic carriers. Despite increased spontaneous activity patterns, the mice overexpressing STK25 demonstrated decreased energy expenditure during the dark phase of observation (Figure 5).

Taken together, our findings suggest that overexpression of STK25, in conditions of excess dietary fuels, leads to a shift in the metabolic balance in peripheral tissues from lipid oxidation to storage, resulting in a systemic insulin resistance. The study suggests that STK25 is an interesting new target for therapeutic intervention in T2DM and related complications.

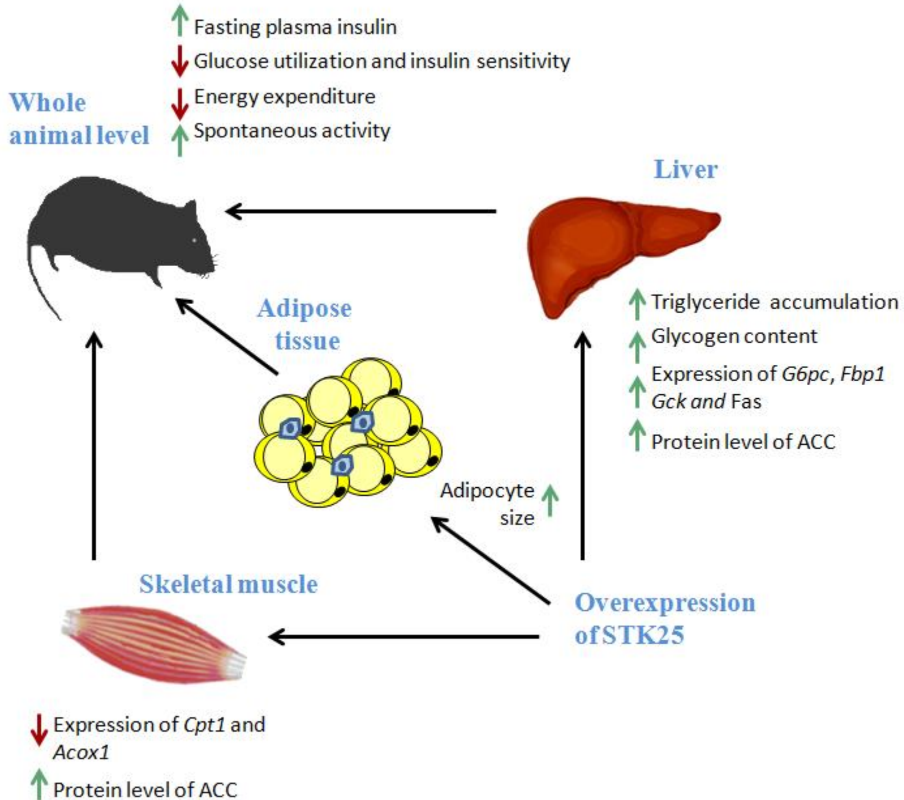

Figure 5. Effect of overexpression of STK25.

5 DISCUSSION

Evidence gained during the last two decades indicates that excessive caloric intake and obesity lead to a chronic low-grade inflammation in metabolic tissues, with elevations in markers and mediators of inflammation and the acute phase response. Chronic inflammation in metabolic tissues may be directly involved in the pathogenesis of insulin resistance and T2DM. Therefore, novel pharmacological treatments targeting both the metabolic and inflammatory disruptions seen in connection to insulin resistance and T2DM are warranted.

Of all the proinflammatory cytokines which are increased during chronic inflammation, circulating levels of IL-6 show the strongest correlation with insulin resistance (17, 18). IL-6 is highly produced by the adipose tissue, and IL-6 levels are increased two- to three-fold in patients with obesity, insulin resistance and T2DM (17, 134, 135). In adipose tissue, IL-6 treatment results in a decrease in insulin-mediated glucose uptake, as well as reduced insulin-stimulated tyrosine phosphorylation of insulin receptor substrate (IRS)-1, demonstrating that IL-6 promotes insulin resistance in this tissue (136). Moreover, it has been shown, both *in vivo* in mice and on a cellular level in human and mouse hepatocytes, that IL-6 induces hepatic insulin resistance (22, 25). However, in skeletal muscle, physical exercise promotes secretion of IL-6, which is shown to contribute to the increase in whole body glucose disposal rate and the metabolic clearance rate of glucose during muscle contraction (20, 137). Taken together, the role of IL-6 in the whole body energy homeostasis remains partly contradictory. This thesis does not address the function of IL-6 in whole body metabolism, but focuses on investigating the role of AMPK in regulation of the effects of IL-6 in liver.

Acute phase proteins SAA1, SAA2 and HP are synthesized in hepatocytes and upregulated in response to inflammatory signals, such as IL-6 (138, 139). Consistently, our studies demonstrate a markedly enhanced expression of *SAA* cluster genes as well as *HP* in HepG2, primary mouse hepatocytes as well as in mouse liver in response to IL-6 treatment. Interestingly, we found this acute phase gene expression evoked by IL-6 to be blunted by AICAR and metformin. Moreover, we show that IL-6-stimulated phosphorylation of key mediators in the IL-6 signalling pathway – JAK1 and its targets SHP2 and STAT3, is inhibited by both AICAR and metformin. AICAR and metformin are known to have off-target effects that are not AMPK-

dependent. However, the reduction in IL-6-induced phosphorylation of JAK1, SHP2 and STAT3 was observed in response to both AICAR and metformin, supporting the inhibition is likely mediated by AMPK activation caused by these substances. Thus, we have identified the point of interaction for AMPK activation and the IL-6 signalling pathway in liver. The anti-inflammatory effect of AMPK was previously shown in different cell types, such as L6 myocytes (140), microglial cells (141) and endothelial cells (93), however, our studies described in *Paper I* were the first report on anti-inflammatory function of AMPK in hepatocytes.

Recently, Kim *et al* showed that metformin represses IL-6 signalling in primary rat hepatocytes via induction of production of orphan nuclear receptor small heterodimer partner (NR0B2, also known as SHP), which directly interacts with STAT3, blocking its DNA binding on the *Socs3* gene promoter (21). Furthermore, the increase in STAT3 phosphorylation and SOCS3 content by IL-6 was markedly repressed by metformin-induced SHP protein production in wild-type mice *in vivo*, whereas metformin had no repressive effects on the IL-6 signalling pathway in *Shp* knock-out mice (21). Kim *et al* also showed that chronic IL-6 exposure caused hepatic insulin resistance in wild-type, but not in *Shp* knock-out mice, further supporting the role of proinflammatory cytokines in metabolic dysfunction (21). In another recent study, it was shown that treatment with adiponectin in HepG2 cells, similarly to AICAR and metformin, leads to reduced IL-6-induced phosphorylation of STAT3 (142). Since adiponectin is known to activate AMPK (82), one might speculate that the reduced STAT3 phosphorylation by adiponectin was AMPK-dependent, although further studies are needed to prove involvement of AMPK. Interestingly, it was recently reported that metformin upregulates the anti-inflammatory interleukin receptor antagonist IL1Rn in primary hepatocytes (143), which might provide an additional mechanism to regulate inflammatory gene expression in liver.

Since insulin resistance and T2DM are associated with increased levels of circulating IL-6, we wanted to evaluate the anti-inflammatory effects of AMPK activation under these conditions. Therefore, metabolic dysfunction was induced in mice via feeding of a high-fat diet. We showed that AMPK is able to repress hepatic IL-6-induced inflammatory response also in metabolically comprised state. Surprisingly, we found that the liver of high-fat diet-fed mice was less responsive to IL-6, compared to the lean controls, while this phenomenon was not present comparing primary hepatocytes

derived from the two diet groups. To our knowledge, our study was the first report that experimentally shows partial resistance of the liver to IL-6 signalling in a model of insulin resistance and T2DM. Previously, IL-6 resistance in muscle from T2DM subjects has been reported (144). Our study emphasizes the importance to interpret the evidence of increased circulating levels of IL-6 in connection to insulin resistance and T2DM in the light of changes in sensitivity to IL-6 signal in peripheral tissues, and studies further evaluating this partial IL-6 resistance are warranted.

We are the first, and so far the only research group, who has described a role of STK25 in metabolic regulation. We showed that partial depletion of STK25 in L6 muscle cells leads to altered expression of markers involved in glucose and lipid metabolism. Accordingly, insulin-stimulated glucose uptake and lipid oxidation were increased in these cells in response to reduced STK25 levels. Skeletal muscle is the major site for insulin-stimulated glucose uptake, accounting for over 70% of whole body glucose disposal after a meal, and a major consumer of lipids as oxidative fuel (145, 146), and therefore these findings were believed to be of relevance for the total metabolic homeostasis

To confirm the role of STK25 in whole body metabolism, we generated transgenic mice overexpressing STK25. We show that the phenotype of *Stk25* transgenic mice is the opposite compared to the results seen in L6 cells where STK25 is partially depleted. *Stk25* transgenic animals develop glucose intolerance and insulin resistance, when challenged with a high-fat diet. Furthermore, whole body insulin-mediated glucose uptake measured by EHC was significantly impaired. Recent unpublished experiments from our lab, using [^{14}C]-labelled 2-DG injections during the glucose steady-state of EHC, revealed that the impairment in whole body glucose uptake in transgenic mice is due to a specific reduction in skeletal muscle glucose uptake, mainly in gastrocnemius and quadriceps muscle, which is consistent with our results obtained in L6 cells. Taken together, our data, for the first time, show that STK25 is a negative regulator of glucose and lipid metabolism.

Hematoxylin and eosin stained liver sections from high-fat-fed STK25 overexpressing mice revealed increased level of inflammatory infiltration, compared to wild-type littermates (manuscript in preparation). We are currently investigating the type of inflammatory cells infiltrating the livers of transgenic carriers, using immunohistochemistry and fluorescence-activated

cell sorting (FACS) approach. These data suggests that, similarly to AMPK, STK25 might also regulate inflammatory and metabolic cross-talk in liver.

Currently, phenotypic characterization of *Stk25* knock-out mice, a kind gift from Prof. B. Howell, SUNY Upstate Medical University, Syracuse, NY, USA, is ongoing in our laboratory. The metabolic effects of a high-fat diet challenge will be investigated in *Stk25* knock-out mice and their wild-type littermates, while another cohort of mice will be evaluated after feeding of a regular chow diet. The findings from this study will further prove the metabolic relevance of STK25.

In summary, there is an increasing body of evidence suggesting that inflammation is one integrated part of development and aggravation of metabolic diseases, such as insulin resistance and T2DM, and therefore, understanding the molecular mechanisms governing cross-talk of inflammation and metabolic dysregulation is of high clinical relevance. With the new insight presented in this thesis, AMPK and STK25 are emerging as key regulators of both metabolic and inflammatory signalling in metabolic peripheral tissues prone to diabetic damage, and may provide targets for developing new pharmacological strategies for more comprehensive treatment regimen of metabolic abnormalities.

ACKNOWLEDGEMENT

During the time of this thesis work, I have come across a lot of people whom without this would never have been possible. I especially would like to thank:

My main supervisor, Margit Mahlapuu, who had the guts to give an inexperienced student the chance to discover the world of research. Thank you for always being there, for the good and the bad times. Your ability to move projects forward and coming up with new ideas is amazing, and I have never met anyone as driven as you. Thank you for believing in me.

My co-supervisor Ulf Smith. With his enormous experience he always knows what to do, even if we don’t always follow the suggestions.

My other co-supervisor, Christian Andersson. I always appreciate our talks. You are truly missed in the lab.

Annika Nerstedt, who thought me everything I needed to know in the lab. Without you I would never had made it. Thank you for always having time for me and my questions, no matter how busy you are.

Manoj Amrutkar and Esther Nuñez Durán, for your enthusiasm and great teamwork. You always managed to make being in the lab fun.

All the past and present co-workers at the Lundberg Laboratory for Diabetes Research. Maria João Pereira, for your friendship and for pink elephants. The day you left the lab was really though. Ida Sterner, for long talks about everything and nothing over coffee, which I really appreciate. John Grünberg and Jenny Hoffmann, if everything else fails, we can always be professional “innebandy” players instead. Ann Hammarstedt, Shahram Hedjazifar, Birgit Gustafson and Silvia Gogg, for all your help in scientific matters, as well as interesting talks in between all the lab work. Jenny Palming, Aino Johansson, Åsa Blixt and Lachmi Jenndahl, you were all important for making this possible. Carina Blomgren, who I call my “lab mommy” because of her loving and caring way. Lena Silberberg, who always is very helpful. Gunilla Lindell, Lisbeth Eriksson and Marie Magnusson, for all the administrative help during these years. Björn Eliasson, for computer help.

Louise Mannerås-Holm, thank you for all the help with the clamp technique, for sharing your experiences with me and for our interesting talks on life during the long hours at EBM.

All co-authors, for great collaborations and all your hard work, I could not have written this thesis without you.

I would also like to thank all the helpful people at the Wallenberg Laboratory. Marcus Ståhlman and Maria Johansson, thank you for all the help with lipid extractions and your intimidating machine. Marcus, perhaps one day I will also creepily pet the equipment. Elin Stenfeldt, thank you for all your help and always pleasant talks. Thank you Kristina Skålén for the help with animal techniques.

Thank you to all the staff at CPI, Anna Wickman, Katarina Rask, Caiza Wranning and Jing Jia, for your expertise and help during the years.

Thank you to Mia Hansson at EBM, who has helped me with the mice in the animal room, making sure everything was done correctly. I really appreciate our talks.

Thank you to all my friends who give me the strength to handle all difficulties. See you at Tidbloms.

And last but not least, a big thank you to Linus, my husband and best friend. Thank you for putting up with me working all the time and for being understanding. I am truly lucky to have met you. Words are not enough.

This is not the end, it's only the beginning.

REFERENCES

1. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract. 2011 Dec;94(3):311-21.
2. Pinhas-Hamiel O, Zeitler P. The global spread of type 2 diabetes mellitus in children and adolescents. J Pediatr. 2005 May;146(5):693-700.
3. Paulweber B, Valensi P, Lindstrom J, Lalic NM, Greaves CJ, McKee M, et al. A European evidence-based guideline for the prevention of type 2 diabetes. Horm Metab Res. 2010 Apr;42 Suppl 1:S3-36.
4. Smushkin G, Vella A. What is type 2 diabetes? Medicine (Abingdon). 2010 Nov 1;38(11):597-601.
5. Field AE, Coakley EH, Must A, Spadano JL, Laird N, Dietz WH, et al. Impact of overweight on the risk of developing common chronic diseases during a 10-year period. Arch Intern Med. 2001 Jul 9;161(13):1581-6.
6. Warram JH, Martin BC, Krolewski AS, Soeldner JS, Kahn CR. Slow glucose removal rate and hyperinsulinemia precede the development of type II diabetes in the offspring of diabetic parents. Ann Intern Med. 1990 Dec 15;113(12):909-15.
7. Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, et al. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. N Engl J Med. 1993 Dec 30;329(27):1988-92.
8. Cherrington AD, Edgerton D, Sindelar DK. The direct and indirect effects of insulin on hepatic glucose production in vivo. Diabetologia. 1998 Sep;41(9):987-96.
9. Bonadonna RC, Del Prato S, Bonora E, Saccomani MP, Gulli G, Natali A, et al. Roles of glucose transport and glucose phosphorylation in muscle insulin resistance of NIDDM. Diabetes. 1996 Jul;45(7):915-25.
10. Bergman RN, Ader M. Free fatty acids and pathogenesis of type 2 diabetes mellitus. Trends Endocrinol Metab. 2000 Nov;11(9):351-6.
11. Weir GC, Bonner-Weir S. Five stages of evolving beta-cell dysfunction during progression to diabetes. Diabetes. 2004 Dec;53 Suppl 3:S16-21.
12. Spector WG, Willoughby DA. The inflammatory response. Bacteriol Rev. 1963 Jun;27:117-54.

13. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science. 1993 Jan 1;259(5091):87-91.
14. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest. 1995 May;95(5):2409-15.
15. Saghizadeh M, Ong JM, Garvey WT, Henry RR, Kern PA. The expression of TNF alpha by human muscle. Relationship to insulin resistance. J Clin Invest. 1996 Feb 15;97(4):1111-6.
16. Krogh-Madsen R, Plomgaard P, Moller K, Mittendorfer B, Pedersen BK. Influence of TNF-alpha and IL-6 infusions on insulin sensitivity and expression of IL-18 in humans. Am J Physiol Endocrinol Metab. 2006 Jul;291(1):E108-14.
17. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. Am J Physiol Endocrinol Metab. 2001 May;280(5):E745-51.
18. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA. 2001 Jul 18;286(3):327-34.
19. Bastard JP, Maachi M, Van Nhieu JT, Jardel C, Bruckert E, Grimaldi A, et al. Adipose tissue IL-6 content correlates with resistance to insulin activation of glucose uptake both in vivo and in vitro. J Clin Endocrinol Metab. 2002 May;87(5):2084-9.
20. Febbraio MA, Pedersen BK. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. Faseb J. 2002 Sep;16(11):1335-47.
21. Kim YD, Kim YH, Cho YM, Kim DK, Ahn SW, Lee JM, et al. Metformin ameliorates IL-6-induced hepatic insulin resistance via induction of orphan nuclear receptor small heterodimer partner (SHP) in mouse models. Diabetologia. 2012 May;55(5):1482-94.
22. Senn JJ, Klover PJ, Nowak IA, Mooney RA. Interleukin-6 induces cellular insulin resistance in hepatocytes. Diabetes. 2002 Dec;51(12):3391-9.
23. Mukumoto H, Takahashi Y, Ando M, Nishikawa M, Takakura Y. Expression profile-dependent improvement of insulin sensitivity by gene delivery of interleukin-6 in a mouse model of type II diabetes. Mol Pharm. 2013 Oct 7;10(10):3812-21.

24. Park EJ, Lee JH, Yu GY, He G, Ali SR, Holzer RG, et al. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. Cell. 2010 Jan 22;140(2):197-208.
25. Klover PJ, Zimmers TA, Koniaris LG, Mooney RA. Chronic exposure to interleukin-6 causes hepatic insulin resistance in mice. Diabetes. 2003 Nov;52(11):2784-9.
26. Senn JJ, Klover PJ, Nowak IA, Zimmers TA, Koniaris LG, Furlanetto RW, et al. Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. J Biol Chem. 2003 Apr 18;278(16):13740-6.
27. Hunter T. Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. Cell. 1995 Jan 27;80(2):225-36.
28. Cohen P. The role of protein phosphorylation in human health and disease. The Sir Hans Krebs Medal Lecture. Eur J Biochem. 2001 Oct;268(19):5001-10.
29. Giamas G, Stebbing J, Vorgias CE, Knippschild U. Protein kinases as targets for cancer treatment. Pharmacogenomics. 2007 Aug;8(8):1005-16.
30. Carling D, Zammit VA, Hardie DG. A common bicyclic protein kinase cascade inactivates the regulatory enzymes of fatty acid and cholesterol biosynthesis. FEBS Lett. 1987 Nov 2;223(2):217-22.
31. Corton JM, Gillespie JG, Hardie DG. Role of the AMP-activated protein kinase in the cellular stress response. Curr Biol. 1994 Apr 1;4(4):315-24.
32. Carling D. The AMP-activated protein kinase cascade--a unifying system for energy control. Trends Biochem Sci. 2004 Jan;29(1):18-24.
33. Stapleton D, Mitchelhill KI, Gao G, Widmer J, Michell BJ, Teh T, et al. Mammalian AMP-activated protein kinase subfamily. J Biol Chem. 1996 Jan 12;271(2):611-4.
34. Quentin T, Kitz J, Steinmetz M, Poppe A, Bar K, Kratzner R. Different expression of the catalytic alpha subunits of the AMP activated protein kinase--an immunohistochemical study in human tissue. Histol Histopathol. 2011 May;26(5):589-96.
35. Mahlapuu M, Johansson C, Lindgren K, Hjalm G, Barnes BR, Krook A, et al. Expression profiling of the gamma-subunit isoforms of AMP-activated protein kinase suggests a major role for gamma3 in white skeletal muscle. Am J Physiol Endocrinol Metab. 2004 Feb;286(2):E194-200.

36. Hawley SA, Boudeau J, Reid JL, Mustard KJ, Udd L, Makela TP, et al. Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. J Biol. 2003;2(4):28.
37. Woods A, Dickerson K, Heath R, Hong SP, Momcilovic M, Johnstone SR, et al. Ca2+/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells. Cell Metab. 2005 Jul;2(1):21-33.
38. Momcilovic M, Hong SP, Carlson M. Mammalian TAK1 activates Snf1 protein kinase in yeast and phosphorylates AMP-activated protein kinase in vitro. J Biol Chem. 2006 Sep 1;281(35):25336-43.
39. Hawley SA, Davison M, Woods A, Davies SP, Beri RK, Carling D, et al. Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase. J Biol Chem. 1996 Nov 1;271(44):27879-87.
40. Scott JW, Hawley SA, Green KA, Anis M, Stewart G, Scullion GA, et al. CBS domains form energy-sensing modules whose binding of adenosine ligands is disrupted by disease mutations. J Clin Invest. 2004 Jan;113(2):274-84.
41. Cheung PC, Salt IP, Davies SP, Hardie DG, Carling D. Characterization of AMP-activated protein kinase gamma-subunit isoforms and their role in AMP binding. Biochem J. 2000 Mar 15;346 Pt 3:659-69.
42. Davies SP, Helps NR, Cohen PT, Hardie DG. 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. 1995 Dec 27;377(3):421-5.
43. Carling D, Clarke PR, Zammit VA, Hardie DG. Purification and characterization of the AMP-activated protein kinase. Copurification of acetyl-CoA carboxylase kinase and 3-hydroxy-3-methylglutaryl-CoA reductase kinase activities. Eur J Biochem. 1989 Dec 8;186(1-2):129-36.
44. Xiao B, Sanders MJ, Underwood E, Heath R, Mayer FV, Carmena D, et al. Structure of mammalian AMPK and its regulation by ADP. Nature. 2011 Apr 14;472(7342):230-3.
45. Woods A, Cheung PC, Smith FC, Davison MD, Scott J, Beri RK, et al. Characterization of AMP-activated protein kinase

beta and gamma subunits. Assembly of the heterotrimeric complex in vitro. J Biol Chem. 1996 Apr 26;271(17):10282-90.

46. DeFronzo RA, Gunnarsson R, Bjorkman O, Olsson M, Wahren J. Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. J Clin Invest. 1985 Jul;76(1):149-55.
47. Kurth-Kraczek EJ, Hirshman MF, Goodyear LJ, Winder WW. 5' AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle. Diabetes. 1999 Aug;48(8):1667-71.
48. Treebak JT, Glund S, Deshmukh A, Klein DK, Long YC, Jensen TE, et al. AMPK-mediated AS160 phosphorylation in skeletal muscle is dependent on AMPK catalytic and regulatory subunits. Diabetes. 2006 Jul;55(7):2051-8.
49. Winder WW, Wilson HA, Hardie DG, Rasmussen BB, Hutber CA, Call GB, et al. Phosphorylation of rat muscle acetyl-CoA carboxylase by AMP-activated protein kinase and protein kinase A. J Appl Physiol (1985). 1997 Jan;82(1):219-25.
50. Merrill GF, Kurth EJ, Hardie DG, Winder WW. AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. Am J Physiol. 1997 Dec;273(6 Pt 1):E1107-12.
51. Jager S, Handschin C, St-Pierre J, Spiegelman BM. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. Proc Natl Acad Sci U S A. 2007 Jul 17;104(29):12017-22.
52. Consoli A. Role of liver in pathophysiology of NIDDM. Diabetes Care. 1992 Mar;15(3):430-41.
53. Lochhead PA, Salt IP, Walker KS, Hardie DG, Sutherland C. 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. 2000 Jun;49(6):896-903.
54. Li Y, Xu S, Mihaylova MM, Zheng B, Hou X, Jiang B, et al. AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. Cell Metab. 2011 Apr 6;13(4):376-88.
55. Foretz M, Carling D, Guichard C, Ferre P, Foufelle F. AMP-activated protein kinase inhibits the glucose-activated expression of fatty acid synthase gene in rat hepatocytes. J Biol Chem. 1998 Jun 12;273(24):14767-71.

56. Woods A, Azzout-Marniche D, Foretz M, Stein SC, Lemarchand P, Ferre P, et al. Characterization of the role of AMP-activated protein kinase in the regulation of glucose-activated gene expression using constitutively active and dominant negative forms of the kinase. Mol Cell Biol. 2000 Sep;20(18):6704-11.
57. Henin N, Vincent MF, Gruber HE, Van den Berghe G. Inhibition of fatty acid and cholesterol synthesis by stimulation of AMP-activated protein kinase. Faseb J. 1995 Apr;9(7):541-6.
58. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest. 2001 Oct;108(8):1167-74.
59. Groop LC, Bonadonna RC, DelPrato S, Ratheiser K, Zyck K, Ferrannini E, et al. Glucose and free fatty acid metabolism in non-insulin-dependent diabetes mellitus. Evidence for multiple sites of insulin resistance. J Clin Invest. 1989 Jul;84(1):205-13.
60. Garton AJ, Campbell DG, Carling D, Hardie DG, Colbran RJ, Yeaman SJ. Phosphorylation of bovine hormone-sensitive lipase by the AMP-activated protein kinase. A possible antilipolytic mechanism. Eur J Biochem. 1989 Jan 15;179(1):249-54.
61. Daval M, Diot-Dupuy F, Bazin R, Hainault I, Viollet B, Vaulont S, et al. Anti-lipolytic action of AMP-activated protein kinase in rodent adipocytes. J Biol Chem. 2005 Jul 1;280(26):25250-7.
62. Orci L, Cook WS, Ravazzola M, Wang MY, Park BH, Montesano R, et al. Rapid transformation of white adipocytes into fat-oxidizing machines. Proc Natl Acad Sci U S A. 2004 Feb 17;101(7):2058-63.
63. Gaidhu MP, Fediuc S, Anthony NM, So M, Mirpourian M, Perry RL, et al. Prolonged AICAR-induced AMP-kinase activation promotes energy dissipation in white adipocytes: novel mechanisms integrating HSL and ATGL. J Lipid Res. 2009 Apr;50(4):704-15.
64. Jorgensen SB, Viollet B, Andreelli F, Frosig C, Birk JB, Schjerling P, et al. Knockout of the alpha2 but not alpha1 5'-AMP-activated protein kinase isoform abolishes 5-aminoimidazole-4-carboxamide-1-beta-4-ribofuranosidebut not contraction-induced glucose uptake in skeletal muscle. J Biol Chem. 2004 Jan 9;279(2):1070-9.

65. Viollet B, Andreelli F, Jorgensen SB, Perrin C, Geloen A, Flamez D, et al. The AMP-activated protein kinase alpha2 catalytic subunit controls whole-body insulin sensitivity. J Clin Invest. 2003 Jan;111(1):91-8.
66. Villena JA, Viollet B, Andreelli F, Kahn A, Vaulont S, Sul HS. Induced adiposity and adipocyte hypertrophy in mice lacking the AMP-activated protein kinase-alpha2 subunit. Diabetes. 2004 Sep;53(9):2242-9.
67. Barnes BR, Marklund S, Steiler TL, Walter M, Hjalm G, Amarger V, et al. The 5'-AMP-activated protein kinase gamma3 isoform has a key role in carbohydrate and lipid metabolism in glycolytic skeletal muscle. J Biol Chem. 2004 Sep 10;279(37):38441-7.
68. Long YC, Barnes BR, Mahlapuu M, Steiler TL, Martinsson S, Leng Y, et al. Role of AMP-activated protein kinase in the coordinated expression of genes controlling glucose and lipid metabolism in mouse white skeletal muscle. Diabetologia. 2005 Nov;48(11):2354-64.
69. Nilsson EC, Long YC, Martinsson S, Glund S, Garcia-Roves P, Svensson LT, et al. Opposite transcriptional regulation in skeletal muscle of AMP-activated protein kinase gamma3 R225Q transgenic versus knock-out mice. J Biol Chem. 2006 Mar 17;281(11):7244-52.
70. Barnes BR, Long YC, Steiler TL, Leng Y, Galuska D, Wojtaszewski JF, et al. Changes in exercise-induced gene expression in 5'-AMP-activated protein kinase gamma3-null and gamma3 R225Q transgenic mice. Diabetes. 2005 Dec;54(12):3484-9.
71. Shaw RJ, Lamia KA, Vasquez D, Koo SH, Bardeesy N, Depinho RA, et al. The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. Science. 2005 Dec 9;310(5754):1642-6.
72. Andreelli F, Foretz M, Knauf C, Cani PD, Perrin C, Iglesias MA, et al. 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. 2006 May;147(5):2432-41.
73. Mu J, Brozinick JT, Jr., Valladares O, Bucan M, Birnbaum MJ. A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle. Mol Cell. 2001 May;7(5):1085-94.
74. Zong H, Ren JM, Young LH, Pypaert M, Mu J, Birnbaum MJ, et al. AMP kinase is required for mitochondrial biogenesis in

skeletal muscle in response to chronic energy deprivation. Proc Natl Acad Sci U S A. 2002 Dec 10;99(25):15983-7.

75. Mu J, Barton ER, Birnbaum MJ. Selective suppression of AMP-activated protein kinase in skeletal muscle: update on 'lazy mice'. Biochem Soc Trans. 2003 Feb;31(Pt 1):236-41.

76. Corton JM, Gillespie JG, Hawley SA, Hardie DG. 5-aminoimidazole-4-carboxamide ribonucleoside. A specific method for activating AMP-activated protein kinase in intact cells? Eur J Biochem. 1995 Apr 15;229(2):558-65.

77. Owen MR, Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochem J. 2000 Jun 15;348 Pt 3:607-14.

78. Fryer LG, Parbu-Patel A, Carling D. The Anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. J Biol Chem. 2002 Jul 12;277(28):25226-32.

79. LeBrasseur NK, Kelly M, Tsao TS, Farmer SR, Saha AK, Ruderman NB, et al. Thiazolidinediones can rapidly activate AMP-activated protein kinase in mammalian tissues. Am J Physiol Endocrinol Metab. 2006 Jul;291(1):E175-81.

80. Nawrocki AR, Rajala MW, Tomas E, Pajvani UB, Saha AK, Trumbauer ME, et al. Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor gamma agonists. J Biol Chem. 2006 Feb 3;281(5):2654-60.

81. Kubota N, Terauchi Y, Kubota T, Kumagai H, Itoh S, Satoh H, et al. Pioglitazone ameliorates insulin resistance and diabetes by both adiponectin-dependent and -independent pathways. J Biol Chem. 2006 Mar 31;281(13):8748-55.

82. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med. 2002 Nov;8(11):1288-95.

83. Goransson O, McBride A, Hawley SA, Ross FA, Shpiro N, Foretz M, et al. Mechanism of action of A-769662, a valuable tool for activation of AMP-activated protein kinase. J Biol Chem. 2007 Nov 9;282(45):32549-60.

84. Sanders MJ, Ali ZS, Hegarty BD, Heath R, Snowden MA, Carling D. Defining the mechanism of activation of AMP-activated protein kinase by the small molecule A-769662, a member of the thienopyridone family. J Biol Chem. 2007 Nov 9;282(45):32539-48.

85. Hawley SA, Fullerton MD, Ross FA, Schertzer JD, Chevtzoff C, Walker KJ, et al. The ancient drug salicylate directly activates AMP-activated protein kinase. Science. 2012 May 18;336(6083):918-22.
86. Carter AM, Bennett CE, Bostock JA, Grant PJ. Metformin reduces C-reactive protein but not complement factor C3 in overweight patients with Type 2 diabetes mellitus. Diabet Med. 2005 Sep;22(9):1282-4.
87. Bai A, Ma AG, Yong M, Weiss CR, Ma Y, Guan Q, et al. AMPK agonist downregulates innate and adaptive immune responses in TNBS-induced murine acute and relapsing colitis. Biochem Pharmacol. 2010 Dec 1;80(11):1708-17.
88. Nath N, Giri S, Prasad R, Salem ML, Singh AK, Singh I. 5-aminoimidazole-4-carboxamide ribonucleoside: a novel immunomodulator with therapeutic efficacy in experimental autoimmune encephalomyelitis. J Immunol. 2005 Jul 1;175(1):566-74.
89. Myerburg MM, King JD, Jr., Oyster NM, Fitch AC, Magill A, Baty CJ, et al. AMPK agonists ameliorate sodium and fluid transport and inflammation in cystic fibrosis airway epithelial cells. Am J Respir Cell Mol Biol. 2010 Jun;42(6):676-84.
90. Zhao X, Zmijewski JW, Lorne E, Liu G, Park YJ, Tsuruta Y, et al. Activation of AMPK attenuates neutrophil proinflammatory activity and decreases the severity of acute lung injury. Am J Physiol Lung Cell Mol Physiol. 2008 Sep;295(3):L497-504.
91. Mohanty P, Aljada A, Ghanim H, Hofmeyer D, Tripathy D, Syed T, et al. Evidence for a potent antiinflammatory effect of rosiglitazone. J Clin Endocrinol Metab. 2004 Jun;89(6):2728-35.
92. Bess E, Fisslthaler B, Fromel T, Fleming I. Nitric oxide-induced activation of the AMP-activated protein kinase alpha2 subunit attenuates IkappaB kinase activity and inflammatory responses in endothelial cells. PLoS One. 2011;6(6):e20848.
93. Cacicedo JM, Yagihashi N, Keaney JF, Jr., Ruderman NB, Ido Y. AMPK inhibits fatty acid-induced increases in NF-kappaB transactivation in cultured human umbilical vein endothelial cells. Biochem Biophys Res Commun. 2004 Nov 26;324(4):1204-9.
94. Hattori Y, Suzuki K, Hattori S, Kasai K. Metformin inhibits cytokine-induced nuclear factor kappaB activation via AMP-activated protein kinase activation in vascular endothelial cells. Hypertension. 2006 Jun;47(6):1183-8.

95. Yang Z, Kahn BB, Shi H, Xue BZ. Macrophage alpha1 AMP-activated protein kinase (alpha1AMPK) antagonizes fatty acid-induced inflammation through SIRT1. J Biol Chem. 2010 Jun 18;285(25):19051-9.
96. Zhang Y, Qiu J, Wang X, Xia M. AMP-activated protein kinase suppresses endothelial cell inflammation through phosphorylation of transcriptional coactivator p300. Arterioscler Thromb Vasc Biol. 2011 Dec;31(12):2897-908.
97. Peairs A, Radjavi A, Davis S, Li L, Ahmed A, Giri S, et al. Activation of AMPK inhibits inflammation in MRL/lpr mouse mesangial cells. Clin Exp Immunol. 2009 Jun;156(3):542-51.
98. Miller EJ, Li J, Leng L, McDonald C, Atsumi T, Bucala R, et al. Macrophage migration inhibitory factor stimulates AMP-activated protein kinase in the ischaemic heart. Nature. 2008 Jan 31;451(7178):578-82.
99. Watt MJ, Dzamko N, Thomas WG, Rose-John S, Ernst M, Carling D, et al. CNTF reverses obesity-induced insulin resistance by activating skeletal muscle AMPK. Nat Med. 2006 May;12(5):541-8.
100. Carey AL, Steinberg GR, Macaulay SL, Thomas WG, Holmes AG, Ramm G, et al. Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. Diabetes. 2006 Oct;55(10):2688-97.
101. Kelly M, Keller C, Avilucea PR, Keller P, Luo Z, Xiang X, et al. AMPK activity is diminished in tissues of IL-6 knockout mice: the effect of exercise. Biochem Biophys Res Commun. 2004 Jul 23;320(2):449-54.
102. Leberer E, Dignard D, Harcus D, Thomas DY, Whiteway M. The protein kinase homologue Ste20p is required to link the yeast pheromone response G-protein beta gamma subunits to downstream signalling components. EMBO J. 1992 Dec;11(13):4815-24.
103. Dan I, Watanabe NM, Kusumi A. The Ste20 group kinases as regulators of MAP kinase cascades. Trends Cell Biol. 2001 May;11(5):220-30.
104. Pombo CM, Force T, Kyriakis J, Nogueira E, Fidalgo M, Zalvide J. The GCK II and III subfamilies of the STE20 group kinases. Front Biosci. 2007;12:850-9.
105. Strange K, Denton J, Nehrke K. Ste20-type kinases: evolutionarily conserved regulators of ion transport and cell volume. Physiology (Bethesda). 2006 Feb;21:61-8.

106. Pombo CM, Bonventre JV, Molnar A, Kyriakis J, Force T. Activation of a human Ste20-like kinase by oxidant stress defines a novel stress response pathway. EMBO J. 1996 Sep 2;15(17):4537-46.
107. Osada S, Izawa M, Saito R, Mizuno K, Suzuki A, Hirai S, et al. YSK1, a novel mammalian protein kinase structurally related to Ste20 and SPS1, but is not involved in the known MAPK pathways. Oncogene. 1997 May 1;14(17):2047-57.
108. Preisinger C, Short B, De Corte V, Bruyneel E, Haas A, Kopajtich R, et al. YSK1 is activated by the Golgi matrix protein GM130 and plays a role in cell migration through its substrate 14-3-3zeta. J Cell Biol. 2004 Mar 29;164(7):1009-20.
109. Pombo CM, Tsujita T, Kyriakis JM, Bonventre JV, Force T. Activation of the Ste20-like oxidant stress response kinase-1 during the initial stages of chemical anoxia-induced necrotic cell death. Requirement for dual inputs of oxidant stress and increased cytosolic [Ca2+]. J Biol Chem. 1997 Nov 14;272(46):29372-9.
110. Voss K, Stahl S, Schleider E, Ullrich S, Nickel J, Mueller TD, et al. CCM3 interacts with CCM2 indicating common pathogenesis for cerebral cavernous malformations. Neurogenetics. 2007 Nov;8(4):249-56.
111. Fidalgo M, Fraile M, Pires A, Force T, Pombo C, Zalvide J. CCM3/PDCD10 stabilizes GCKIII proteins to promote Golgi assembly and cell orientation. J Cell Sci. 2010 Apr 15;123(Pt 8):1274-84.
112. Matsuki T, Matthews RT, Cooper JA, van der Brug MP, Cookson MR, Hardy JA, et al. Reelin and stk25 have opposing roles in neuronal polarization and dendritic Golgi deployment. Cell. 2010 Nov 24;143(5):826-36.
113. Nogueira E, Fidalgo M, Molnar A, Kyriakis J, Force T, Zalvide J, et al. SOK1 translocates from the Golgi to the nucleus upon chemical anoxia and induces apoptotic cell death. J Biol Chem. 2008 Jun 6;283(23):16248-58.
114. Preisinger C, Short B, De Corte V, Bruyneel E, Haas A, Kopajtich R, et al. YSK1 is activated by the Golgi matrix protein GM130 and plays a role in cell migration through its substrate 14-3-3 zeta. Journal of Cell Biology. 2004 Mar 29;164(7):1009-20.
115. Voss K, Stahl S, Schleider E, Ullrich S, Nickel J, Mueller TD, et al. CCM3 interacts with CCM2 indicating common

pathogenesis for cerebral cavernous malformations. Neurogenetics. 2007 Nov;8(4):249-56.

116. Su Z, Cox A, Shen Y, Stylianou IM, Paigen B. Farp2 and Stk25 are candidate genes for the HDL cholesterol locus on mouse chromosome 1. Arterioscler Thromb Vasc Biol. 2009 Jan;29(1):107-13.

117. Sherman MH, Kuraishy AI, Deshpande C, Hong JS, Cacalano NA, Gatti RA, et al. AID-induced genotoxic stress promotes B cell differentiation in the germinal center via ATM and LKB1 signaling. Mol Cell. 2010 Sep 24;39(6):873-85.

118. Yin H, Shi Z, Jiao S, Chen C, Wang W, Greene MI, et al. Germinal center kinases in immune regulation. Cell Mol Immunol. 2012 Nov;9(6):439-45.

119. Surwit RS, Kuhn CM, Cochrane C, McCubbin JA, Feinglos MN. Diet-induced type II diabetes in C57BL/6J mice. Diabetes. 1988 Sep;37(9):1163-7.

120. Ferre P, Leturque A, Burnol AF, Penicaud L, Girard J. A method to quantify glucose utilization in vivo in skeletal muscle and white adipose tissue of the anaesthetized rat. Biochem J. 1985 May 15;228(1):103-10.

121. Van Snick J, Cayphas S, Szikora JP, Renauld JC, Van Roost E, Boon T, et al. cDNA cloning of murine interleukin-HP1: homology with human interleukin 6. Eur J Immunol. 1988 Feb;18(2):193-7.

122. Satoh T, Nakamura S, Taga T, Matsuda T, Hirano T, Kishimoto T, et al. Induction of neuronal differentiation in PC12 cells by B-cell stimulatory factor 2/interleukin 6. Mol Cell Biol. 1988 Aug;8(8):3546-9.

123. Taga T, Kawanishi Y, Hardy RR, Hirano T, Kishimoto T. Receptors for B cell stimulatory factor 2. Quantitation, specificity, distribution, and regulation of their expression. J Exp Med. 1987 Oct 1;166(4):967-81.

124. Ayala JE, Samuel VT, Morton GJ, Obici S, Croniger CM, Shulman GI, et al. Standard operating procedures for describing and performing metabolic tests of glucose homeostasis in mice. Dis Model Mech. 2010 Sep-Oct;3(9-10):525-34.

125. Jensen TL, Kiersgaard MK, Sorensen DB, Mikkelsen LF. Fasting of mice: a review. Lab Anim. 2013 Oct;47(4):225-40.

126. Walsh RN, Cummins RA. The Open-Field Test: a critical review. Psychol Bull. 1976 May;83(3):482-504.

127. Hall CS. Emotional behavior in the rat. I. Defecation and urination as measures of individual differences in emotionality. J Comp Psychol. 1934;18:385-403.
128. Aden DP, Fogel A, Plotkin S, Damjanov I, Knowles BB. Controlled synthesis of HBsAg in a differentiated human liver carcinoma-derived cell line. Nature. 1979 Dec 6;282(5739):615-6.
129. Klier FG, Schubert D, Heinemann S. The ultrastructural differentiation of the clonal myogenic cell line L6 in normal and high K+ medium. Dev Biol. 1977 Jun;57(2):440-9.
130. Guillouzo A. Liver cell models in in vitro toxicology. Environ Health Perspect. 1998 Apr;106 Suppl 2:511-32.
131. Hockenbery DM, Oltvai ZN, Yin XM, Milliman CL, Korsmeyer SJ. Bcl-2 functions in an antioxidant pathway to prevent apoptosis. Cell. 1993 Oct 22;75(2):241-51.
132. Freeman BA, Crapo JD. Biology of disease: free radicals and tissue injury. Lab Invest. 1982 Nov;47(5):412-26.
133. Wu J, Ruas JL, Estall JL, Rasbach KA, Choi JH, Ye L, et al. The unfolded protein response mediates adaptation to exercise in skeletal muscle through a PGC-1alpha/ATF6alpha complex. Cell Metab. 2011 Feb 2;13(2):160-9.
134. Fernandez-Real JM, Vayreda M, Richart C, Gutierrez C, Broch M, Vendrell J, et al. Circulating interleukin 6 levels, blood pressure, and insulin sensitivity in apparently healthy men and women. J Clin Endocrinol Metab. 2001 Mar;86(3):1154-9.
135. Kopp HP, Kopp CW, Festa A, Krzyzanowska K, Kriwanek S, Minar E, et al. Impact of weight loss on inflammatory proteins and their association with the insulin resistance syndrome in morbidly obese patients. Arterioscler Thromb Vasc Biol. 2003 Jun 1;23(6):1042-7.
136. Rotter V, Nagaev I, Smith U. Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. J Biol Chem. 2003 Nov 14;278(46):45777-84.
137. Febbraio MA, Hiscock N, Sacchetti M, Fischer CP, Pedersen BK. Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction. Diabetes. 2004 Jul;53(7):1643-8.
138. Castell JV, Gomez-Lechon MJ, David M, Hirano T, Kishimoto T, Heinrich PC. Recombinant human interleukin-6 (IL-6/BSF-2/HSF) regulates the synthesis of acute phase

proteins in human hepatocytes. FEBS Lett. 1988 May 23;232(2):347-50.
139. Selinger MJ, McAdam KP, Kaplan MM, Sipe JD, Vogel SN, Rosenstreich DL. Monokine-induced synthesis of serum amyloid A protein by hepatocytes. Nature. 1980 Jun 12;285(5765):498-500.
140. Pilon G, Dallaire P, Marette A. Inhibition of inducible nitric-oxide synthase by activators of AMP-activated protein kinase: a new mechanism of action of insulin-sensitizing drugs. J Biol Chem. 2004 May 14;279(20):20767-74.
141. Giri S, Nath N, Smith B, Viollet B, Singh AK, Singh I. 5-aminoimidazole-4-carboxamide-1-beta-4-ribofuranoside inhibits proinflammatory response in glial cells: a possible role of AMP-activated protein kinase. J Neurosci. 2004 Jan 14;24(2):479-87.
142. Sun H, Zhang Y, Gao P, Li Q, Sun Y, Zhang J, et al. Adiponectin reduces C-reactive protein expression and downregulates STAT3 phosphorylation induced by IL-6 in HepG2 cells. Mol Cell Biochem. 2011 Jan;347(1-2):183-9.
143. Buler M, Aatsinki SM, Skoumal R, Komka Z, Toth M, Kerkela R, et al. Energy-sensing factors coactivator peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1alpha) and AMP-activated protein kinase control expression of inflammatory mediators in liver: induction of interleukin 1 receptor antagonist. J Biol Chem. 2012 Jan 13;287(3):1847-60.
144. Jiang LQ, Duque-Guimaraes DE, Machado UF, Zierath JR, Krook A. Altered response of skeletal muscle to IL-6 in type 2 diabetic patients. Diabetes. 2013 Feb;62(2):355-61.
145. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. Diabetes. 1981 Dec;30(12):1000-7.
146. Andres R, Cader G, Zierler KL. The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in intact man in the basal state; measurements of oxygen and glucose uptake and carbon dioxide and lactate production in the forearm. J Clin Invest. 1956 Jun;35(6):671-82.