

How Insulin Receptor Substrate Proteins Regulate the Metabolic Capacity of the Liver - Implications for Health and Disease

Louise Fritsche, Cora Weigert, Hans-Ulrich Häring and Rainer Lehmann*

Department of Internal Medicine 4, Div. of Clinical Chemistry and Pathobiochemistry, Central Laboratory, University Hospital Tuebingen, D-72076 Tuebingen, Germany

Abstract: The liver plays a key role in glucose homeostasis, lipid and energy metabolism. Its function is primarily controlled by the anabolic hormone insulin and its counterparts glucagon, catecholamines and glucocorticoids. Dysregulation of this homeostatic system is a major cause for development of the metabolic syndrome and type 2 diabetes mellitus. The features of the underlying dynamic molecular network that coordinates systemic nutrient homeostasis are less clear. But recently, considerable progress has been made in elucidating molecular pathways and potential factors involved in the regulation of energy and lipid metabolism and affected in diabetic states.

In this review we will focus on important stations in the complex network of molecules that control the balance between glucose production, glucose utilization and regulation of lipid metabolism. Special attention will be paid to the insulin receptor substrate (IRS) proteins with the two major isoforms IRS-1 and IRS-2 as a critical node in hepatic insulin signalling. IRS proteins act as docking molecules to connect tyrosine kinase receptor activation to essential downstream kinase cascades, including activation of the PI-3 kinase or MAPK cascade. IRS-1 and IRS-2 are complementary key players in the regulation of hepatic insulin signalling and expression of genes involved in gluconeogenesis, glycogen synthesis and lipid metabolism. The function of IRS proteins is regulated by their expression levels and posttranslational modifications. This regulation within the dynamic molecular network that coordinates systemic nutrient homeostasis will be outlined in detail under the following conditions: after feeding, during fasting and during exercise. Dysfunction of IRS proteins initially leads to post-prandial hyperglycemia, increased hepatic glucose production, and dysregulated lipid synthesis and is discussed as major pathophysiological mechanism for the development of insulin resistance and type 2 diabetes mellitus.

Understanding the molecular regulation and the pathophysiological modifications of IRS proteins is crucial in order to identify new sites for potential intervention to treat or prevent hepatic insulin resistance and type 2 diabetes mellitus.

Keywords: Liver, insulin resistance, diabetes, glucose metabolism, lipid metabolism, insulin signalling, insulin receptor substrate, gene regulation.

1. INTRODUCTION

Plasma protein synthesis, detoxification of endogenous and pharmaceutical substances, hemostasis, as well as energy storage and conversion are important functions of the liver. The central metabolic role of the liver in fuel homeostasis is underlined by the fact that it is the major organ with the ability to consume, store and produce glucose and lipids. Hepatic glucose metabolism includes the formation of glycogen (short-term energy storage), generation of glucose from non-sugar carbon substrates and intracellular energy supply *via* glycolysis [1]. Fatty acid oxidation, *de novo* synthesis of fatty acids, cholesterol and bile acid synthesis, as well as lipoprotein assembly are the essential roles of the liver in lipid metabolism. These metabolic pathways are coordinately regulated to maintain glucose and lipid homeostasis under physiological conditions [2]. Consequently, the liver is a key target for the anabolic hormone insulin and its catabolic counterpart glucagon. Insulin is released from the pancreatic β -cells in response to increased blood glucose concentrations and this is amplified in the presence of free fatty acids. Impaired insulin sensitivity and dysregulated insulin action in the liver contributes significantly to the pathogenesis of obesity, the metabolic syndrome and type 2 diabetes.

The pancreatic α -cells release glucagon in response to decreased blood glucose concentrations as it occurs during fasting and glucagon affects mainly the liver and adipose tissue. It induces the breakdown of glycogen and the mobilization of fatty acids. Most importantly it promotes gluconeogenesis, i.e. the formation of glucose from lactate, glycerol and glucogenic amino acids. During exercise and stress, when the organism has an increased demand for energy, other glucoregulatory hormones, the catecholamines epinephrine and norepinephrine and the glucocorticoids (most importantly cortisol) come to action and exert similar metabolic functions as glucagon.

The global regulation of the function of the liver in energy metabolism is well-investigated, however, the underlying molecular mechanisms are less clear. In this review we will focus on important crossroads in the complex network of molecules that control the balance between glucose production, glucose utilization and regulation of lipid metabolism. The emphasis will be on the molecular mechanisms underlying the hepatic regulation of energy metabolism in the fed state, during fasting and exercise paying particular attention to the role of IRS-1 and IRS-2. Furthermore, molecular mechanisms for the pathogenesis of hepatic insulin resistance and possible target molecules for therapeutic interventions to treat or prevent the dysregulation of hepatic insulin action and type 2 diabetes mellitus will be discussed.

2. THE INSULIN RECEPTOR SUBSTRATES (IRS)

On the molecular level insulin receptor substrates (IRS) play key roles in the orchestration of the complex hepatic metabolic responses. IRS are unique docking molecules whose actions are very tightly regulated by the phosphorylation at various sites [3-5]. The IRS bind to the activated insulin receptor, become phosphorylated, thereby providing docking sites for a multitude of signalling molecules, essential for the diversification and modulation of insulin action and hence for the tight regulation of the hepatic glucose and lipid metabolism. Dysregulation of this complex system leads to impaired signal transduction resulting in pathological states. Reduced IRS protein levels in the liver and hyperphosphorylation of IRS on serine/threonine residues are hallmarks in the development of insulin resistance and type 2 diabetes mellitus [6,7].

The insulin signalling cascade is initiated by the binding of insulin to the extracellular β -subunits of the dimerized receptor. This binding leads to the autophosphorylation of tyrosine residues at the intracellular β -subunit. IRS proteins bind to the phosphorylated receptor *via* their phosphotyrosine binding (PTB) domain and are in turn phosphorylated on multiple tyrosine residues thus creating docking sites for src homology 2 (SH2) domain containing proteins. The best studied SH2 proteins that bind to tyrosine phosphorylated

*Address correspondence to this author at the Div. of Clinical Chemistry and Pathobiochemistry, Central Laboratory, University Hospital Tübingen, Hoppe-Seyler-Str. 3, D-72076 Tübingen, Germany; Tel: ++49 7071 29 83193; Fax: ++49 7071 29 5348; E-mail: Rainer.Lehmann@med.uni-tuebingen.de

IRS proteins are the regulatory subunit of the phosphoinositide-3 (PI-3) kinase and the adaptor molecule growth factor receptor-binding protein 2 (Grb2). The PI-3 kinase catalyzes the formation of the lipid second messenger phosphatidylinositol-3,4,5-triphosphate, which is necessary to recruit downstream kinases, such as 3-phosphoinositide-dependent protein kinase 1 (PDK-1) and Akt (also called protein kinase B; PKB). Of note, IRS proteins do not contain kinase activities but they play a key role as branching point by transferring the extracellular signal (e.g. binding of insulin to its receptor) into multiple metabolic, mitogenic and anti-apoptotic signalling pathways. The IRS proteins regulate the glucose and lipid metabolism mainly *via* the PI-3 kinase-Akt pathway and through activation of the mitogen-activated protein kinase (MAPK) cascade gene expression, cell growth and differentiation. In addition, IRS proteins have a similar essential function in transducing the insulin-like growth factor (IGF)-I signal from the IGF-I-receptor into target cells. But although the ability of IGF-I to enhance insulin sensitivity and to stimulate insulin-like actions has been demonstrated (for review see reference [8]) insulin is the major physiological regulator of metabolism *via* IRS proteins.

There are 6 different IRS [9-13] with different tissue-specific distribution (for review see reference [4]). At least five of them are expressed in human tissues: IRS-1, -2 and -4, -5 and -6 whereas IRS-3 only appears in rodent adipose tissue and brain. Since IRS-1 and IRS-2 constitute the main IRS isoforms in the mammalian liver, we will focus on these molecules. Both isoforms appear to have a similar general architecture (Fig. (1)) [4,14,15]. They are composed of a N-terminal pleckstrin homology (PH) domain which enables their binding to phosphatidylinositol lipids within membranes. The PH-domain is followed by the PTB-domain that mediates the interaction with the tyrosine phosphorylated insulin receptor [14,15]. These two domains are highly conserved by 75% among IRS-1 and IRS-2 [16]. In contrast the C-terminal part is poorly conserved with only 35 % of matching amino acids [17]. The C-terminal part contains a large number of tyrosine phosphorylation motifs. In their phosphorylated state these sites represent binding motifs for many downstream partner proteins, such as adaptor proteins (Grb-2, Nck, Crk) [17], the SH2 domain-containing protein-tyrosine phosphatase (SHP)-2 and the p85 regulatory subunit of the lipid kinase PI-3 kinase [18] which is of great importance for the metabolic actions of insulin.

Despite their common features IRS-1 and -2 show a number of differences which renders them as point of diversification of the

insulin signal. This is accomplished *via* several mechanisms: both molecules appear in different subcellular compartments [19] with unequal frequency, IRS-2 being higher concentrated in the cytosol than in other intracellular compartments [20]. The IRS proteins also show different activation kinetics [21], probably due to structural differences. Only IRS-2 has a unique kinase regulatory loop binding domain (KRLB) (Fig. (1)), which interacts with the phosphorylated kinase activation loop of the insulin receptor [22,23]. This KRLB domain acts to limit IRS-2 tyrosine phosphorylation hereby regulating the extent of IRS-2 activation [24].

With these differences it seems likely that IRS-1 and -2 serve rather different than redundant functions. And indeed, data obtained in various knock out/knock down mouse models (for an overview see reference [25]) point towards complementary IRS functions. First studies with a global knock out of IRS-1 demonstrated that it is mainly involved in insulin-like growth factor (IGF)-1 signalling. IRS-1^{-/-} mice show growth retardation but only mild insulin resistance which never progresses to diabetes [25-27]. Abe *et al.* demonstrated that IRS-1 knock out mice have a metabolic syndrome-like phenotype with insulin resistance in fat and muscle tissue, increased blood pressure and elevated plasma triglycerides as well as impaired endothelial vascular relaxation [28]. All groups also reported a compensatory up-regulation of IRS-2, which maintains an almost normal PI-3 kinase activity in the liver [29,30]. Importantly, the restoration of hepatic IRS-1 expression *via* adenoviral infection is sufficient to normalize insulin sensitivity in the IRS-1^{-/-} mice [31].

In contrast the general IRS-2 knock out mouse is characterized by reduced β -cell mass and insulin resistance in liver and muscle without compensatory up-regulation of IRS-1, leading ultimately to type 2 diabetes mellitus [32,33]. Male IRS-2 knock out mice die from dehydration and hyperosmolar coma after 12 to 16 weeks of life [32]. Transgenic islets expressing IRS-2 in the IRS-2^{-/-} mice cured diabetes, demonstrating an essential function of IRS-2 for normal β -cell function [34]. Hence, based on the studies mentioned above IRS-1 and IRS-2 are not only alternative substrates for the insulin receptor [30] but are differentially regulated and exert different functions. As discussed later in this review (see chapter 6), the relative contribution of IRS-1 and IRS-2 to hepatic insulin action is still under investigation.

Of note, very recent studies provided novel aspects on the contribution of IRS proteins to aging and life span. Reduced insulin-like signalling, resulting in resistance to oxidative stress, has been

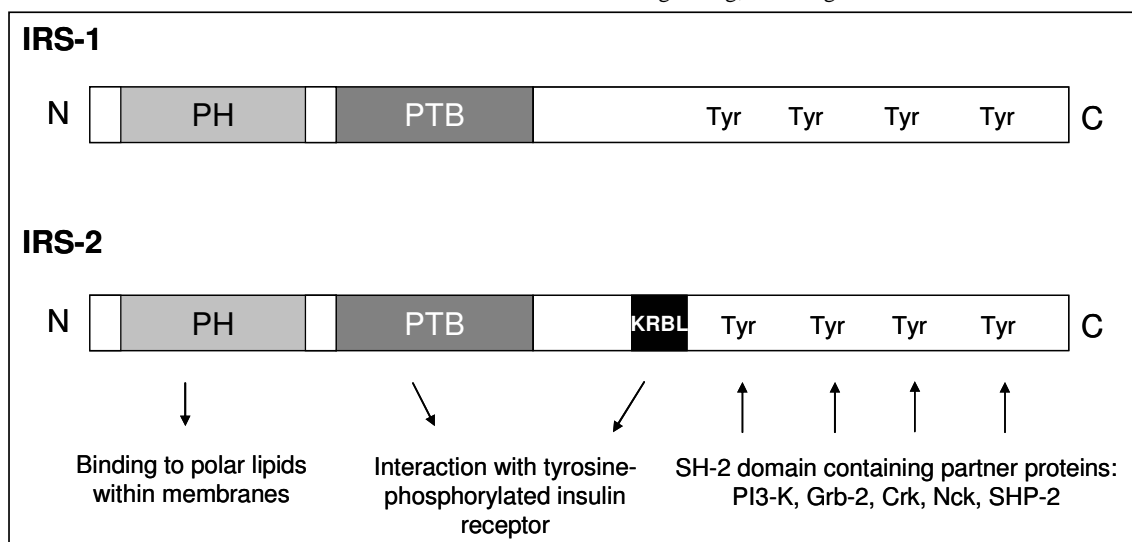


Fig. (1). Insulin receptor substrate (IRS) protein structure. Schematic diagram of IRS-1 and IRS-2 domain structure. Abbreviations: C, C-terminus; Grb-2, Nck, Crk, adaptorproteins; KRLB, kinase regulatory loop binding domain; N, N-terminus; PH domain, pleckstrin homolgy domain; PTB domain, phosphotyrosine binding domain; PI3K, phosphoinositide-3 kinase; SHP-2, SH2 domain-containing protein-tyrosine phosphatase 2.

associated previously with increased longevity in *C. elegans* and *D. melanogaster* (for review see reference [35]). In contrast, defects in insulin signalling in mammals leading to insulin resistance and diabetes reduce life span. Interestingly, female IRS-1^{-/-} mice have increased longevity [36]. Furthermore, reduced IRS-2 signalling in IRS-2^{+/-} mice and in brain specific IRS-2^{+/-} mice also increases the life span of these animals [37]. Thus certain aspects of insulin/IGF-I-dependent pathways *via* IRS in the brain are linked to the regulation of life span.

A number of polymorphisms in the human IRS genes had been described in the last 15 years (for an overview about the role of IRS proteins see reference [38]). Among these the change of Gly to Arg at codon 972 in IRS-1 and the Gly to Asp polymorphism at codon 1057 in IRS-2 are the best characterized ones.

The Gly to Arg972 polymorphism has been associated in early studies with an increased prevalence of type 2 diabetes [39] and obesity-linked insulin resistance [40]. Contrary to that, the polymorphism does not seem to determine clamp-derived insulin sensitivity and is therefore not suitable to predict insulin resistance [41]. This mutation has been associated with reduced insulin-stimulated phosphorylation of two flanking tyrosine residues in livers of transgenic mice overexpressing the IRS-1 Gly to Arg972 variant resulting in impaired downstream signal transduction [42,43]. The phosphorylation of these sites is important for the binding of the p85 subunit of the PI3K. *In vitro* studies using L6 skeletal muscle cells showed that the Arg972 polymorphism leads to defects in insulin-stimulated glucose transport and glycogen synthesis [44]. Human pancreatic islets of polymorphism carriers have reduced insulin secretion and increased β -cell apoptosis [45]. However, recent studies showed no clear association of this polymorphism and type 2 diabetes [46]. Several other polymorphisms have been described in human IRS-1 [47], but they appear with a lower frequency compared to the Gly to Arg972 variant [38].

The Gly to Asp1057 polymorphism in IRS-2 is of some importance for the relative risk to develop type 2 diabetes. Interestingly,

this polymorphism is associated with a lower risk for type 2 diabetes in lean polymorphism carriers but with a higher risk in obese humans [48]. The higher prevalence of obese homozygous carriers of the Asp1057 variant for type 2 diabetes has been also described for Pima indians [49], a population with a very high risk for obesity [50], and asian indians [51].

3. THE FED STATE – REPRESSION OF CATABOLIC AND ACTIVATION OF ANABOLIC PATHWAYS

Food intake leads to an increase of blood glucose concentration, which is a potent stimulus for the release of insulin from the pancreatic β -cells leading to an increased insulin plasma concentration. Consequently, anabolic pathways in the liver and in other target tissues (skeletal muscle, fat, kidney, brain) are activated. The hepatic action of insulin has three major targets: a) to activate glucose storage and to shutdown glucose production and output, b) to regulate lipid metabolism in the fed state, and c) to block all catabolic actions.

In the postprandial state, the excess of glucose is either immediately utilized by glycolysis or stored as glycogen. Insulin induces the transcription of glycolytic genes, such as glucokinase (GK) and pyruvate kinase (PK) and it up-regulates phosphofructokinase-1. The major function of hepatic glycolysis is thought to be the supply of carbons for the de-novo-synthesis of fatty acids rather than the generation of ATP [52]. This theory is supported by recent findings of Morral and co-workers who could show that the overexpression of GK in rats results in increased lipogenesis [53].

Glycogen synthesis is induced by the activation of the PI-3 kinase-Akt/PKB pathway (Fig. (2)). The serine/threonine kinase Akt/PKB phosphorylates and thereby inhibits glycogen synthase kinase (GSK)-3 β , which enables the activation of glycogen synthase the key enzyme responsible for the formation of glycogen. Simultaneously glycogenolysis is stopped by the action of Akt/PKB, which reduces the activity of glycogen phosphorylase [54,55].

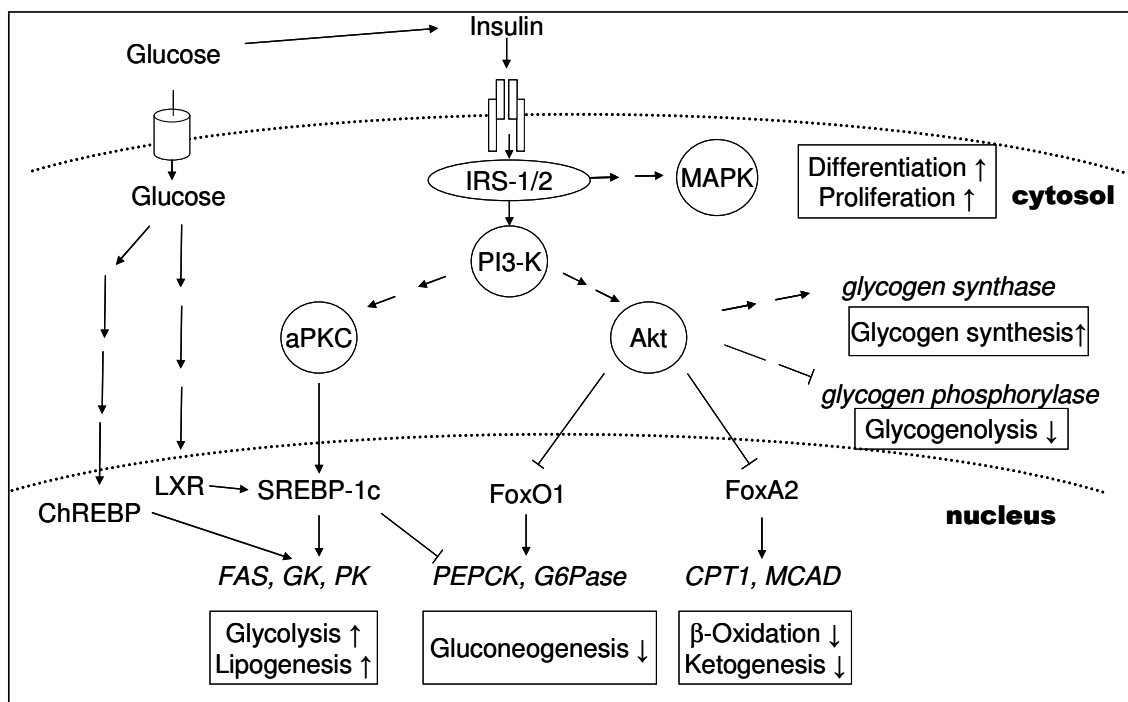


Fig. (2). The fed state. Scheme of hepatic signalling events in the fed state activating anabolic pathways in the liver. Abbreviations: aPKC, atypical protein kinase C; ChREBP, carbohydrate response element binding protein; CPT1, carnitine palmitoyltransferase 1; FAS, fatty acid synthase; FoxO1/FoxA2, forkhead box protein O1/A2; G6Pase, glucose-6-phosphatase; GK, glucokinase; IRS, insulin receptor substrate; LXR, liver X receptor; MAPK, mitogen-activated protein kinase; MCAD, medium-chain acyl-CoA dehydrogenase; PEPCK, phosphoenolpyruvate carboxykinase; PI3-K, phosphoinositide-3 kinase; PK, pyruvate kinase; SREBP-1c, sterol regulatory element binding protein 1c.

The major mediator of insulin action on GK and lipogenic gene expression is sterol regulatory element binding protein (SREBP)-1c. Together with the carbohydrate responsive element binding protein (ChREBP) SREBP-1c activates the transcription of key enzymes in fatty acid synthesis as acetyl-CoA carboxylase and fatty acid synthase (FAS) [56]. Moreover, it controls the rate of triglyceride synthesis and inhibits the expression of the gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) [57]. In contrast to other SREBPs, SREBP-1c activity is mainly controlled on the transcriptional level and less by the proteolytic cleavage and nuclear abundance [58,59]. Insulin activates SREBP-1c expression involving the activity of atypical protein kinase C (PKC) isoforms [60,61]. Recent work has shown that the insulin-induced expression of SREBP-1c requires the nuclear receptor liver X receptor (LXR) [62], whose ligands and activators have been identified as glucose and glucose-6-phosphate [63,64]. Hence, the regulatory role of insulin on hepatic gene expression to convert excess glucose into glycogen and lipids is mediated by the concerted action of SREBP-1c, ChREBP and LXR [56,65,66].

Feeding and subsequent insulin action not only induces anabolic pathways but it also leads to a down-regulation of catabolic pathways, most importantly gluconeogenesis. Insulin inhibits the expression of the rate limiting gluconeogenic enzymes, PEPCK and G6Pase. Again, the activation of the PI-3 kinase-Akt/PKB pathway *via* IRS-1 and IRS-2 plays hereby an important role. Akt/PKB is the key kinase responsible for the inhibition of the gluconeogenic program [67]: Akt/PKB phosphorylates the transcription factors forkhead box protein O1 (FoxO1) [68] and peroxisome proliferator-activated receptor γ co-activator 1 (PGC-1 α) [69], which prevents the binding to their regulatory sequences in the PEPCK and G6Pase promoters and subsequently blocks the transcription of these genes [70,71]. Insulin also represses the transcription of PGC-1 α *via* the Akt/PKB-FoxO1-pathway [72]. Furthermore, the co-activator of cyclic AMP response element binding protein (CREB), CREB binding protein (CBP) is phosphorylated by insulin-dependent

pathways, probably by Akt/PKB itself, thereby controlling CBP recruitment [73]. CREB acts as transcription factor in concert with PGC-1 α and FoxO1 to activate the expression of gluconeogenic enzymes.

Of note, the downregulation of gluconeogenic enzymes does not lead to a rapid inhibition of gluconeogenesis by insulin action. This is due to the fact that the regulation occurs only at the transcriptional level and not by direct posttranslational modifications of the enzymes. Thus the time span until this catabolic pathway is shut off upon insulin action depends on the half-life of the corresponding proteins (e.g. 6 hours for PEPCK) [74].

4. FASTING – THE INDUCTION OF ENERGY PROVIDING PATHWAYS

To maintain the blood glucose concentration within normal range and to supply glucose to the central nervous system during times of starvation the liver has the ability to provide glucose from two sources: glycogen and synthesis of glucose using non-carbohydrate precursors such as lactate, glucogenic amino acids and glycerol. Glycogenolysis occurs in the post absorptive state, 2 – 6 hours after ingestion of a meal [75] and is sufficient for short term fasting. Gluconeogenesis, however, is important during prolonged periods of fasting (18 – 24 hours [1]) and it accounts for up to 90% of endogenous glucose production after 40 hours of fasting [74,76], when hepatic glycogen stores are completely exhausted [77]. Gluconeogenesis requires the action of two key enzymes: PEPCK, which catalyzes the conversion of oxalacetate to phosphoenolpyruvate and G6Pase, which dephosphorylates glucose-6-phosphate thus enabling glucose output (Fig. (3)). Conversely, glycolysis and glycogen synthesis have to be intimately regulated in order to avoid futile cycling of glucose.

Both glucose production and the inhibition of glycogen synthesis are controlled by glucagon. The binding of glucagon to its G-protein coupled receptor results in the activation of adenylate cyclase which catalyzes the formation of cAMP [78]. The regulatory

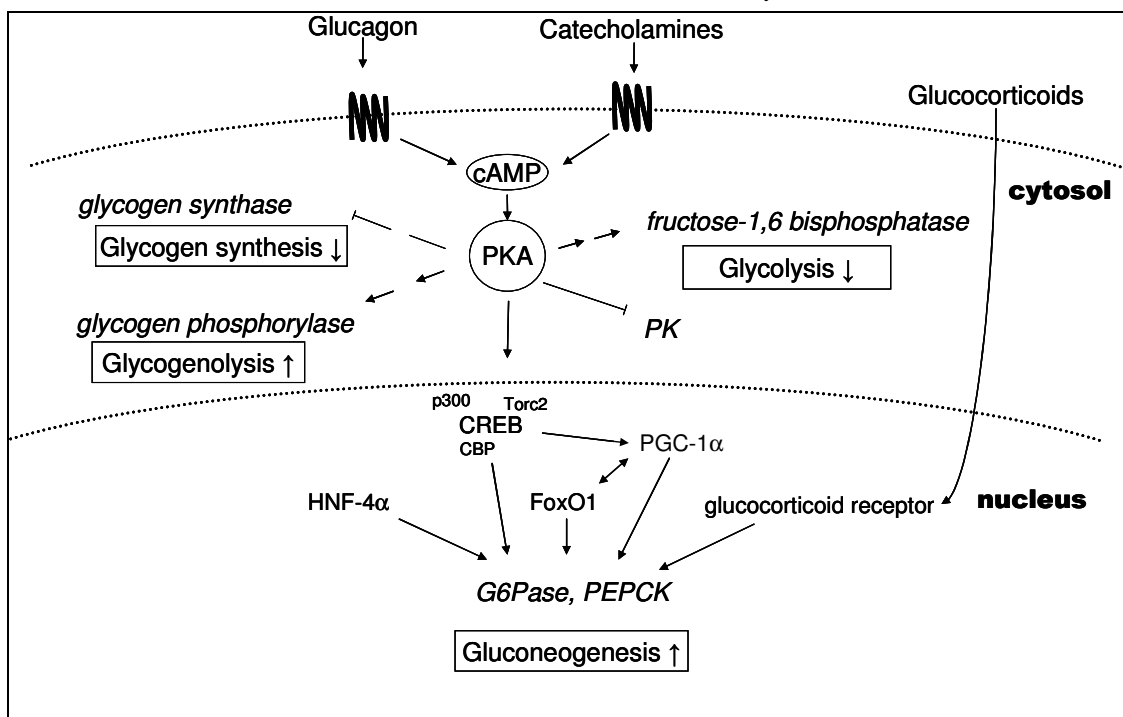


Fig. (3). Fasting. Scheme of hepatic signalling events during fasting resulting in an increased glucose output from the liver. Abbreviations: cAMP, cyclic adenosine monophosphate; CBP, CREB binding protein; CREB, cAMP response element binding protein; FoxO1, forkhead box protein O1; G6Pase, glucose-6-phosphatase; HNF-4 α , hepatocyte nuclear factor 4 α ; PEPCK, phosphoenolpyruvate carboxykinase; PGC-1 α , peroxisome-proliferator-activated receptor- γ co-activator 1 α ; PK, pyruvate kinase; PKA, protein kinase A; Torc2, transducer of regulated CREB activity 2.

subunits of the protein kinase A (PKA) bind cAMP resulting in activation of the enzyme (Fig. (3)). The serine/threonine kinase PKA plays a central role in glucagon signalling, comparable to the role of Akt/PKB for metabolic pathways in the insulin signalling network. It phosphorylates and activates glycogen phosphorylase kinase which in turn phosphorylates and activates glycogen phosphorylase resulting in increased glycogen breakdown [78]. At the same time glycogen synthase is phosphorylated by multiple kinases including PKA and GSK-3 β resulting in its downregulation [79].

Gluconeogenesis is potentiated by glucagon *via* enhanced expression of PEPCK and G6Pase and by regulation of the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase thus resulting in the activation of fructose-1,6-bisphosphatase [80]. Simultaneously, glucagon blocks glycolysis by inhibition of phosphofructokinase-1 and pyruvate kinase [81]. The glucagon-dependent activation of transcription is achieved by PKA-dependent phosphorylation of the transcription factor CREB on Ser-133 thus enabling its dimerization and interaction with several CREB binding proteins/co-activators (CBP [82], p300, Torc2 [83,84]) and the binding to CRE regions within promoters of several target genes. One of these targets is PGC-1 α [85], which is strongly induced by CREB [86] and FoxO1 [87] under fasting conditions. PGC-1 α could be considered as an important, but probably not essential co-factor for the regulation of hepatic glucose production. It interacts with the forkhead transcription factor FoxO1 and co-activates the transcription of the gluconeogenic enzymes [88]. The interdependency of FoxO1 and PGC-1 α has been controversially discussed [89] but recently it was shown that gluconeogenesis is not inducible by PGC-1 α in mice with liver specific knockout of FoxO1 [71]. The importance of PGC-1 α is further questioned since mice lacking PGC-1 α are still capable of inducing the gluconeogenic program [90] and in H4IIE rat hepatoma cells, which do not express PGC-1 α the induction of PEPCK and G6Pase is possible, which leads to the consideration of PGC-1 α as "transcriptional amplifier" [91]. Further studies indicate that not only FoxO1, but also hepatocyte nuclear factor (HNF)-4 α is required for activation of the gluconeogenic program [92]. Although PGC-1 α is increased in mice lacking HNF-4 α when compared with controls this compensatory counter-regulation is not sufficient to induce the gluconeogenic enzymes under fasting conditions [92].

The above mentioned FoxO1 seems to be the crucial factor for controlling the gluconeogenic enzymes. This factor belongs to the forkhead transcription factors, which are involved not only in energy metabolism but also in cell differentiation, proliferation and survival [93]. In the unphosphorylated state, which is the case during fasting, FoxO1 resides in the nucleus [94] and binds to insulin response elements (IRE) [95] thus enhancing the transcription of the respective genes [96]. FoxO1 has been intensively investigated in several gain of function and loss of function experiments. Mice expressing a constitutively nuclear, i.e. permanently active form of FoxO1 in the liver and transgenic mice that overexpress FoxO1 in the liver showed increased G6Pase and PEPCK mRNA levels, augmented glucose production and impaired glucose tolerance [97-100]. On the other hand, reduction of FoxO1 activity in obese mice and diabetic mouse models reduces fasting hyperglycemia and ameliorates insulin resistance [97,101,102]. Mice with a liver specific deletion of FoxO1 show suppressed hepatic glucose production due to decreased gluconeogenesis and glycogenolysis [71].

Recently FoxO1 has been implicated not only in glucose but also in lipid metabolism. Mice expressing permanently active FoxO1 in the liver show severely increased hepatic triglyceride content [98] due to enhanced expression of SREBP, fatty acid synthase and acetyl-CoA carboxylase [97]. Altomonte *et al.* [103] could show that adenoviral overexpression of a wildtype FoxO1 leads to hypertriglyceridemia caused by increased expression of apoC-III, a apolipoprotein which is associated with the accumulation of VLDL and chylomicrons in plasma [104].

The liver responses to fasting not only with the production of glucose, but with increased β -oxidation of free fatty acids (FFA) and, after prolonged fasting, ketogenesis. The oxidation of fatty acids yields high amounts of acetyl-CoA, which can be converted to ketone bodies. In case of starvation for several days most tissues, including the brain, can adapt to the utilization of ketone bodies instead of glucose. The key genes involved in the regulation of β -oxidation and ketogenesis are in part under control of the same factors as described for gluconeogenesis. PGC-1 α and its closely related homolog PGC-1 β induce by co-activation of SREBP [105,106] or by interaction with the forkhead transcription factor FoxA2 [107-109] medium-chain acyl-CoA dehydrogenase (MCAD), carnitine palmitoyl transferase 1 (CPT1) (β -oxidation), 3-ketothiolase and 3-hydroxy-3-methylglutaryl-CoA lyase (ketogenesis). FoxA2 is thereby regulated in a manner similar to FoxO1: during fasting this transcription factor resides in the nucleus and activates gene transcription. Its insulin-dependent phosphorylation at Thr-156 results in nuclear exclusion and inactivation [110], however, this mode of regulation is currently questioned by a paper of Zhang and co-workers [108], who showed that FoxA2 is constitutively nuclear.

In summary, the liver is under tight control of insulin and its catabolic counterparts to maintain carbohydrate and lipid homeostasis. The regulation of the gene transcription and activity of key enzymes in the postabsorptive state and during fasting involves the same key players such as the PI-3 kinase-Akt/PKB pathway, PGC-1 α , HNF-4 α , and transcription factors of the forkhead box protein and SREBP family (Fig. (3)). The importance of hepatic insulin signalling for glucose homeostasis is demonstrated by blockade of insulin signalling in the liver by liver-specific disruption of the insulin receptor in the LIRKO mice, which leads to severe glucose intolerance and impaired suppression of hepatic glucose production [111]. Indirect effects of insulin leading to inhibition of glucose output from the liver are also discussed, e.g. central action of insulin in the hypothalamus, reduction of plasma free fatty acids or inhibition of glucagon secretion. However, a recent publication demonstrates the dominance of insulin's direct effects, thus emphasizing the importance of hepatic IRS proteins [112].

5. EXERCISE

During exercise the working muscle is dependent on a constant supply with glucose. The main glucose source during early stages of exercise is muscle glycogen. But with increasing duration of exercise the liver becomes the main supplier for glucose, derived from glycogenolysis, and with decreasing glycogen stores gluconeogenesis is becoming more important. After several hours of exercise the hepatic gluconeogenesis accounts for 50 % of total glucose production [75]. Consistent with these data, several groups found that exercise induces the expression of PEPCK in livers of mice and rats [113-115]. Gluconeogenesis from lactate, amino acids and glycerol is not only important to supply glucose for the muscle but also to delay the depletion of muscle and liver glycogen stores.

With exercise the hepatic glucose production is enabled by enhanced glucagon and decreased insulin action not necessarily due to changes in their respective plasma concentrations, but e.g. increased glucagon receptor density [116,117]. Increased catecholamines levels are discussed to serve as a second defence line [118,119], but the exact contribution of catecholamines to hepatic glucose production during exercise is not completely clarified [120,121]. Furthermore, glucocorticoids, most importantly cortisol, are also induced by exercise which stimulate the hepatic gluconeogenesis *via* binding to its glucocorticoid receptor and activation of gene transcription [122-124] (Fig. (3)).

The molecular regulation of carbohydrate and fat metabolism during exercise has been mainly investigated in skeletal muscle of animals and humans. Only limited information is available on the

effects of exercise on these pathways in the liver, especially in humans.

The liver is highly sensitive to changes in metabolic demands and the hepatic energy charge is clearly decreased after acute exercise while the energy charge of skeletal muscle remained stable [125]. This results in activation of AMP-activated kinase (AMPK) in the liver. The AMPK is a key regulator in fuel metabolism and energy supply. It is activated by phosphorylation and by increases in the AMP/ATP ratio. Active AMPK stimulates glycogenolysis and prevents glycogen synthesis.

Furthermore, acute exercise has been shown to increase FoxO1 in the liver of healthy mice [126]. This is in line with the idea that exercise and fasting exert similar metabolic demands for the organism. A high level of FoxO1 supports the expression of gluconeogenic enzymes [88] thus enabling blood glucose homeostasis despite markedly increased glucose utilization of the working muscle.

Concerning lipid metabolism contradictory results emerged in relation to exercise. Fatty acid synthase is induced by insulin and glucose *via* binding of the respective transcription factors to the regulatory elements in the FAS promoter, e.g. the insulin responsive element (IRE) and the carbohydrate response element (CHoRE) [127-129]. During fasting the transcription of this gene is diminished and it seems likely that exercise would exert a similar effect on the FAS expression. Indeed, acute bouts of exercise as well as endurance exercise training are able to down regulate FAS mRNA or FAS activity in rats [130-132], but other studies reported an upregulation of lipogenic enzymes and a downregulation of genes involved in β -oxidation [133].

One study of Heled *et al.* [134] investigated the effect of a 4 week training protocol in combination with a high energy diet on the hepatic insulin signalling response in the desert gerbil *psammomys obesus*. The group reported an increased tyrosine phosphorylation of the insulin receptor and an increased association of IRS-2

but not of IRS-1 with PI-3 kinase. These results indicate that exercise has beneficial effects on insulin sensitivity in the liver, despite of the ingestion of a high fat diet, which induces insulin resistance in these animals [135]. Furthermore these findings support the theory that IRS-2 is the dominant IRS in hepatocytes [136]. Improved hepatic insulin sensitivity due to long term exercise training has also been suggested by findings of Chang and co-workers. They reported downregulation of hepatic PEPCK mRNA and protein in diabetes prone Zucker rats after 8 weeks of daily training on a treadmill [137].

In conclusion during exercise hepatic metabolism is controlled by insulin antagonists enabling glucose supply to the muscle. This is reflected by upregulation of FoxO1, which induces gluconeogenic enzymes and a downregulation of the anabolic enzyme FAS. Long term exercise training has beneficial effects on hepatic insulin sensitivity.

6. IRS-1 AND IRS-2 IN THE LIVER

Global knock out models and studies in IRS-2 deficient hepatocytes have suggested a dominant function for IRS-2 in hepatic metabolism [138-142]. In the last 2 years liver specific knock down and knock out of IRS proteins using RNAi and Crelox-technology allowed a more detailed view on the function of IRS-1 and IRS-2 in the liver [143-145].

The first report on liver specific IRS-1 and -2 knock down came from Taniguchi *et al.* [143], who used an adenovirus carrying short hairpin RNA (shRNA) to transiently knock down the expression of either IRS-1 or -2 or both together. Similar to the global IRS-1 knock out mouse IRS-2 expression and IRS-2 associated PI-3 kinase activity is increased in this IRS-1 liver specific knock down model. Furthermore, gluconeogenic enzymes and the transcription factor HNF-4 α are up-regulated. In contrast, the liver specific knock down of IRS-2 is characterized by an up-regulation of SREBP-1c and subsequently fatty acid synthase. IRS-1 expression

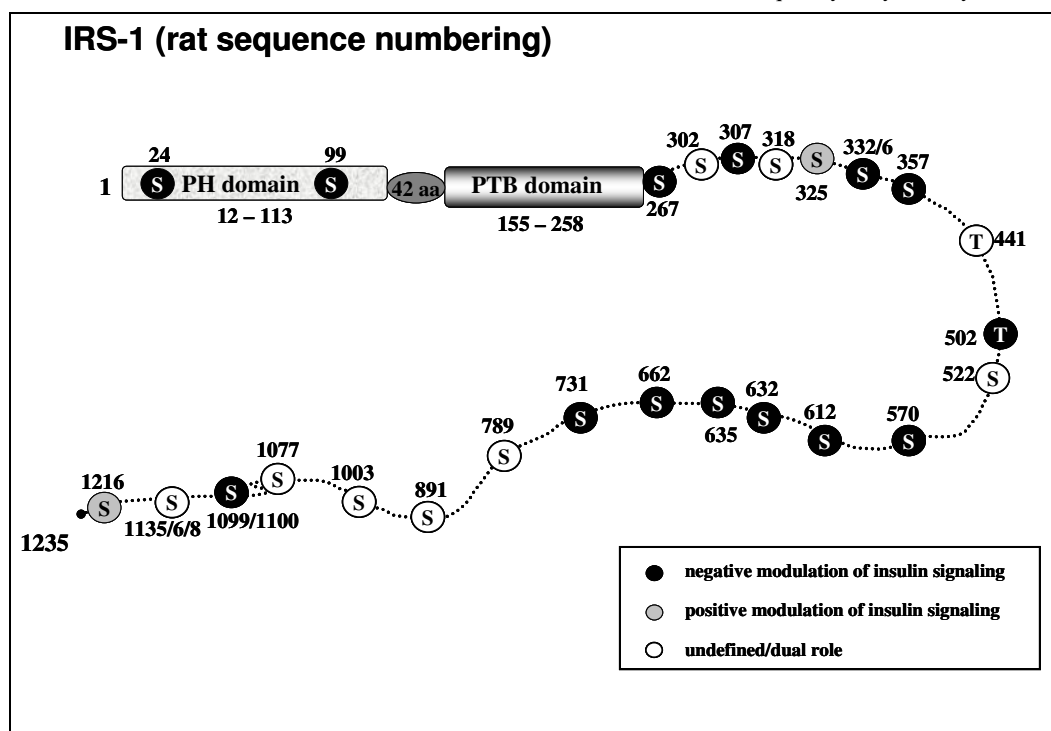


Fig. (4). Serine/threonine phosphorylation sites of insulin receptor substrate (IRS)-1. Scheme of insulin receptor substrate (IRS)-1 structure including serine/threonine phosphorylation sites confirmed by mass spectrometry or immunodetection *in vivo*. The numbering of the serine and threonine residues is based on the rat sequence. Abbreviations: aa, amino acids; PH domain, pleckstrin homology domain; PTB domain, phosphotyrosine binding domain; S, serine; T, threonine.

is not up-regulated but the IRS-1 associated PI-3 kinase activity is increased. The double knock down of both IRS isoforms in the liver leads to insulin resistance, glucose intolerance and hyperglycemia in the fasted and fed state [143]. The authors conclude that IRS-1 and -2 have complementary functions in the liver to maintain sufficient PI-3 kinase signalling, but that both IRS proteins also have distinct individual roles. IRS-1 is mainly involved in the regulation of gluconeogenesis and glucokinase expression while IRS-2 is required for the balanced activation of lipogenic genes. The relative lack of IRS-2 led to uncontrolled up-regulation of SREBP-1c probably due to insulin- and glucose-induced LXR activation [62]. Interestingly, the MAP kinase pathway was not affected by the double knock down of IRS-1 and -2 indicating the importance of alternative docking proteins, such as Shc and Grb2, for the transduction of the insulin signal [143].

However, in this study the IRS knockdown was transient and incomplete rendering 20 to 30% of functioning IRS isoforms in the liver. The group of M. White [144] generated mice with a targeted complete disruption of the IRS-2 gene in the liver, using the Crelox method. These mice showed slightly reduced insulin signalling events, i.e. reduced Akt/PKB Ser-473 and FoxO1 Ser-256 phosphorylation with subsequently elevated expression of gluconeogenic enzymes. The animals developed mild insulin resistance and glucose intolerance but never progressed to diabetes. The authors concluded that IRS-1 is sufficient to support an almost normal insulin signalling in the liver - as long as the β -cells are capable of producing enough insulin - and that at least in this model IRS-2 is not essential for hepatic insulin signalling [144].

Similar results came from a study of Simmgen and colleagues [145] who also used the Crelox-system to generate a liver specific IRS-2 knock out mouse. These mice showed only minimal abnormalities in hepatic glucose and lipid homeostasis and no insulin resistance, suggesting that functional glucose and lipid metabolism was not depending on the presence of IRS-2 in this study [145].

These data challenge the current view of IRS-2 as the most important insulin receptor substrate in the liver. Apparently the presence of IRS-1, presumably in cooperation with other insulin signalling molecules, is sufficient to maintain the insulin signalling when IRS-2 is lacking. However, an importance of IRS-2 action in early development could not be excluded from the shRNA and Crelox-studies [143-145]. Furthermore, as it will be outlined below, only IRS-2 is tightly regulated on the transcriptional level in the liver depending on the nutritional state while IRS-1 is not [146].

6.1. Regulation of IRS-1 and IRS-2 in the Liver

The regulation of both IRS-1 and IRS-2 isoforms occurs on different levels: expression, degradation, change of subcellular localization, and posttranslational modification such as tyrosine phosphorylation and serine/threonine phosphorylation (Fig. (4)), as well as S-nitrosation, O-linked β -N-acetylglucosamine-modification and acetylation (Fig. (5)).

6.1.1. Regulation of IRS-1

The major regulation of IRS-1 action in the liver appears not to be on the transcriptional level, however some information is available: a) short term insulin stimulation of rat hepatoma cells is reported to result in an upregulation of IRS-1 protein [147], b) stimulation with dexamethasone increases the amount of IRS-1 in Fao rat hepatoma cells [148] and also in the liver of rats [149]. Contrary to that, fasting led only to minimally increasing effects on the hepatic IRS-1 amount [150].

Targeted degradation has been verified as a regulator of IRS-1 protein levels: in *in vitro* studies demonstrated that long term (up to 24 h) insulin stimulation of cultured Fao hepatoma cells results in proteasomal degradation of the IRS-1 protein without a change of IRS-1 mRNA levels [151-154]. Other agents such as TNF α , INF γ ,

PDGF and phorbol esters also reduce the IRS-1 protein levels [155,156]. Suppressor of cytokine signalling (SOCS)-1 and -3 are reported to bind *via* their SH2 domains to IRS-1 and promote its ubiquitination and degradation [157]. Since several cytokines are inducers of SOCS expression this provides a mechanism for the interaction of cytokines with the insulin signalling pathway. Insulin-induced degradation of IRS proteins is thought to be mainly dependent on mammalian target of rapamycin (mTOR) [155], which is activated by the PI-3 kinase-Akt/PKB cascade. Serine phosphorylation of IRS-1 *via* the mTOR-S6K1 pathway is reported to release IRS-1 from intracellular complexes thereby enabling its degradation. The nutrient sensitive mTOR mediates the phosphorylation of Ser-636/639 in IRS-1 in muscle and adipose tissue [158,159] and this site is hyperphosphorylated in the liver during prolonged stimulation with insulin [160], palmitate [161] and in diet-induced obesity [162]. Rapamycin, the widely used mTOR inhibitor, prevents insulin-induced IRS-1 degradation in CHO cells [163] and 3T3-L1 adipocytes [153,164], stressing the importance of this kinase in the regulation of IRS-1. Accelerated degradation of IRS proteins stimulated by hyperinsulinemia and hyperlipidemia as it occurs in obesity is one of the mechanisms discussed in the development of insulin resistance [5].

The short term regulation of IRS-1 action is provided by multiple posttranslational modifications of the molecule. The most intensively studied posttranslational modifications of IRS-1 up to now are the phosphorylation of tyrosine, serine and threonine residues (Fig. (4)). The IRS proteins contain several tyrosine phosphorylation sites, and more than 70 potential phosphorylation motifs for serine/threonine kinases [155]. While the phosphorylation of tyrosine residues is mandatory for the transduction of the insulin signal, serine/threonine phosphorylation appears to be the mechanism for the precise modulation and regulation and could either enhance or attenuate the effects of insulin [6,165] (Fig. (4)). The majority of the identified residues have been implicated in a negative regulation of insulin action, but it could not be excluded that based on a general interest in the elucidation of pathological mechanisms of insulin resistance the positive regulation by serine/threonine phosphorylation has yet been underestimated. The mechanisms involved in this regulation are the association of IRS-1 with other signalling molecules [166,167], the subcellular localization of IRS-1 [168] and regulation of its degradation [164]. These sites implicated in a negative regulation (corresponding to rat IRS-1 sequence) are Ser-24 [169], Ser-267 [170], Ser-307 [171,172], Ser-332 [173], Ser-357 [174], Ser-522 [175], Ser-612 [176-178], Ser-632 [179-181], Ser-662 [177,178], and Ser-1099/1100 [182] (Fig. (4)). The IRS-1 kinases responsible for phosphorylation of the serine residues of IRS-1 and for attenuation of insulin signalling are mTOR [159,183-185] [186] and its downstream kinase p70 ribosomal S6 kinase (p70S6K) 1 [181], c-jun-N-terminal kinase (JNK) [171], protein kinase C (PKC)- θ [187,188], PKC- δ [174], inhibitor of κ B (IkB) kinase [169,189], glycogen synthase kinase (GSK)-3 [173] and MAPKs [180]. These kinases help to turn off the insulin signal when activated under physiological conditions but when stimulated permanently they are implicated in the pathogenesis of insulin resistance. Chronically elevated concentration of insulin, free fatty acids (FFA) [190,191] and TNF α [171,192] are all reported to activate the above mentioned kinases resulting in decreased hepatic insulin sensitivity and glucose tolerance.

One of the best characterized sites is Ser-307 (Ser-312 in the human IRS-1 homolog). This site is responsible for the PI-3 kinase dependent downregulation of the insulin signal because it interferes with the IRS-1-insulin receptor interaction [193]. There are many kinases that phosphorylate Ser-307, with JNK being the first one reported [171]. The introduction of a dominant negative JNK isoform into the liver of obese diabetic mice led to an improved insulin sensitivity and also to a decreased hepatic glucose output due to decreased expression of gluconeogenic enzymes [6,194,195].

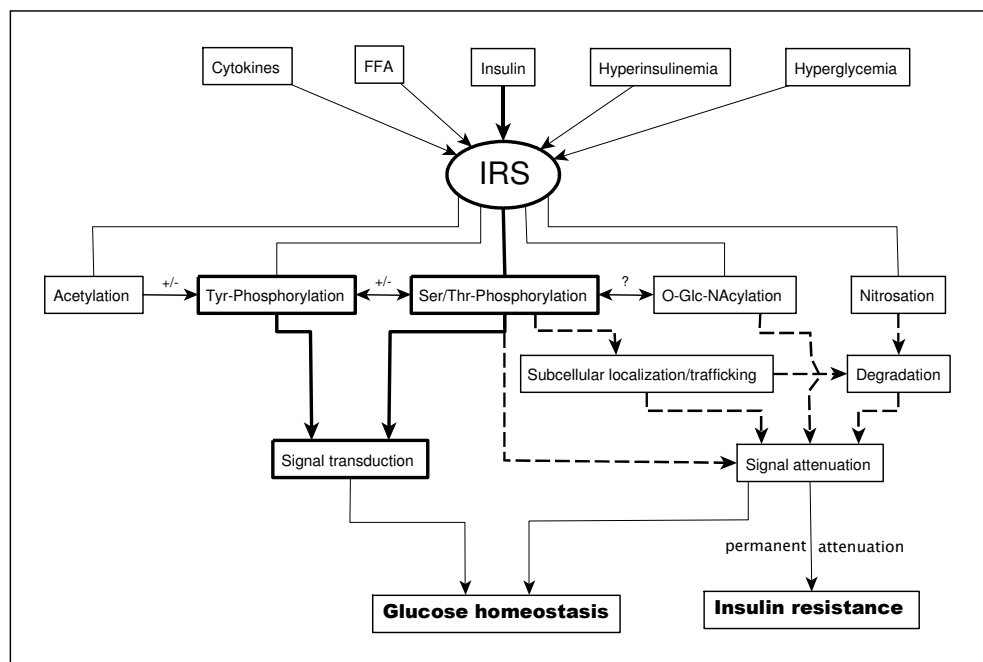


Fig. (5). Posttranslational modifications of insulin receptor substrate proteins and their interplay. IRS proteins are extensively post-translationally modified. Insulin induces tyrosine phosphorylation, which is the prerequisite for further signal transduction, and serine/threonine phosphorylation, leading to both activation and attenuation of the signalling. Other stimuli like acutely and chronically elevated cytokines, free fatty acids, glucose and insulin not only induce serine/threonine phosphorylation but also acetylation, *O*-Glc-NAcylation and nitrosation. The roles of these modifications are less clear than of the phosphorylations, but they have been implicated in positive as well as in negative regulation of signal transduction. Bold lines represent the activation of the system, dashed lines indicate an attenuation. Abbreviations: IRS, insulin receptor substrate; FFA, free fatty acids.

Studies performed to elucidate the physiological function of serine phosphorylation of IRS-1 revealed that serine/threonine phosphorylation is also involved in positive regulation of insulin signal transduction. The phosphorylation of Ser-302 [196,197], Ser-318 [198], Ser-325 [199], Ser-789 [200] and Ser-1216 [187] has been associated with improved insulin signalling (Fig. (4)), although the data are not consistent presumably due to the stimulus, kinetics and the cell type. Based on our recent data showing that the early phosphorylation of Ser-318 is involved in enhanced insulin action, but that phospho-Ser-318 is also necessary for the attenuation in the late phase [201], we hypothesize that the net result of serine phosphorylation of IRS-1 depends on the time course and the interdependency of phosphorylated serine sites rather than the phosphorylation of single residues.

Although not all above mentioned serine/threonine phosphorylation sites have been demonstrated in the liver, it is likely that their regulation and function is similar in the insulin-dependent tissues muscle, liver and fat. However, tissue-specific differences in serine/threonine phosphorylation of distinct residues may occur. As has been reported by our group, interleukin (IL)-6 treatment of C57Bl/6 mice induces PKC- δ -dependent IRS-1 phosphorylation at Ser 318 in muscle, but not in liver whereas Ser 307 is only phosphorylated in the liver but not in the muscle in the same animals [198]. This might provide a mechanism for a tissue-specific regulation of insulin signal transduction by the cytokine IL-6.

The function of IRS proteins is not only regulated by the activity of kinases, the dephosphorylation of IRS by phosphatases is a similar important but less intense studied mechanism. There are several phosphatases which terminate the insulin signal by dephosphorylating tyrosine residues, such as protein tyrosine phosphatase 1B (PTP1B) and SHP-2 [202]. These phosphatases modulate the insulin signal [160,203] and have been implicated in the development of insulin resistance. Increased PTP1B expression has been observed in insulin resistant human subjects [204] and in obese or insulin resistant animal models [205,206]. Mice lacking PTP1B have enhanced insulin sensitivity and do not develop diet-induced

obesity [207,208]. The importance of hepatic PTP1B expression for attenuation of insulin signalling has been demonstrated by liver-specific re-expression of the phosphatase in PTP1B^{-/-} mice which led to a marked decrease in the insulin sensitivity of these mice [209].

Recently the posttranslational modification on serine/threonine residues of IRS-1 with *O*-linked β -N-acetylglucosamine (*O*-GlcNAc) has been demonstrated in muscle and adipose tissue [210]. This modification, first identified in the 1980s in rat liver subcellular organelles [211,212], is enhanced by the increased activity of the hexosamine biosynthetic pathway, which generates UDP N-acetylglucosamine, the substrate for the addition of *O*-GlcNAc-moieties by *O*-GlcNAc-transferase [213]. Hyperglycemia and hyperlipidemia have been shown to increase the flux through this pathway, thus *O*-GlcNAc-modification is enhanced during insulin resistant states and has also been related to impaired insulin action [214,215]. In some proteins *O*-GlcNAc-modification occurs on the same sites as phosphorylations thereby inhibiting the proper phosphorylation. Ball *et al.* showed that in IRS-1 Ser-1036 is the major site of *O*-GlcNAc-modification and under conditions that model the diabetic state (high glucose, chronic insulin stimulation) the level of *O*-GlcNAc-modification was increased in human embryonic kidney 293 cells at this site [216]. The effect of the *O*-GlcNAc-modification on the interaction of IRS-1 with downstream signalling partners needs further clarification. Currently there are no data available on the *O*-GlcNAc-modification of IRS proteins in the liver but based on the data obtained in other tissues an important role is likely.

A further posttranslational modification of IRS-1 described in muscle tissue is the S-nitrosation *via* nitric oxide which has been implicated in the down-regulation of insulin action [217,218]. It appears that this modification induces the proteasomal down-regulation of IRS-1 in cultured skeletal muscle cells [219] as well as in rat liver [220]. The relevance of this modification for hepatic IRS-1 needs to be clarified further.

Furthermore, IRS-1 can also be modified by acetylation, i.e. the transfer of an acetyl group to a lysine residue. This modification is found on histones and it is important for chromatin regulation [221] but it is known that the histone acetyltransferases (HATs) and histone deacetylases (HDACs) also have non histone substrates [222], among them many proteins involved in the pathogenesis of diabetes [223]. Kaiser and James [224] demonstrated that IRS-1 is acetylated on lysine residues and this modification leads to improved insulin signalling, whereas the activity of a specific deacetylase (HDAC2) was associated with insulin resistance. We expect that more acetylation sites in IRS-1 will be found and the knowledge about acetylation/deacetylation may be potentially important for understanding the pathogenesis of insulin resistance.

In conclusion IRS-1 is mainly regulated *via* posttranslational modifications, most importantly phosphorylation. Tyrosine phosphorylation of IRS-1 enables the downstream signalling of insulin (and IGF-1). A complete spectrum of posttranslational modifications could further enhance or maintain insulin signalling, and appears to be mainly involved in attenuation of insulin action and under pathophysiological conditions in the development of insulin resistance. Currently, no analytical approach is capable to acquire the complete pattern of IRS modification at a given time point nor could follow the highly dynamic changes of the pattern of posttranslational modifications. This analytical challenge may be solved by interdisciplinary projects connecting analytical chemists, molecular biologists and medical researchers.

6.1.2. Regulation of IRS-2

Unlike IRS-1 IRS-2 is highly regulated at the transcriptional level: fasting strongly induces hepatic IRS-2 mRNA [225] and protein level [226]. IRS-2 protein was 3.5-fold increased after a 16 hour-fast in C57Bl6 wildtype mice (L. Fritsche, unpublished data). Furthermore signalling molecules known to be relevant during fasting induce IRS-2 mRNA and protein expression in different cell culture models. IRS-2 expression is induced by cAMP and the glucocorticoid dexamethasone in HeLa cells [227] and in Fao rat hepatoma cells (L. Fritsche, unpublished data). Dexamethasone alone induces IRS-2 protein in adult rat hepatocytes [228] and primary rat adipocytes [229] and it leads to an increased IRS-2 promoter activity as determined in a luciferase reporter assay [225]. The group of Montminy could demonstrate that cAMP induces IRS-2 expression *via* activation of CREB in murine β -cells [230] and Canettieri *et al.* showed that IRS-2 expression in the liver is stimulated by the CREB-Torc2 (transducer of regulated CREB activity 2) pathway [226]. Glucagon, the hormone which controls the hepatic glucose metabolism during fasting has been shown to induce IRS-2 in primary rat hepatocytes [139]. This induction of IRS-2 appears critical for glucose homeostasis and serves as a feedback response that limits glucose output from the liver during fasting.

In contrast, high insulin concentrations which emerge during the fed state lead to reduced IRS-2 concentrations in the liver [139]. This is accomplished through inhibition of transcription [231] and subsequently downregulation of IRS-2 mRNA [151] and increased proteosomal degradation of the IRS-2 protein. Rui and colleagues demonstrated that insulin mediates the ubiquitination and subsequent proteosomal degradation of IRS-2 *via* the PI-3 kinase – Akt – mTOR pathway [232]. The role of insulin as a suppressor of IRS-2 protein levels is eminent in LIRKO mice. The complete lack of insulin signalling in the liver of these mice results in 5-fold increased IRS-2 but not IRS-1 protein concentrations [111].

The proteosomal degradation of IRS-2 is also induced under pathophysiological conditions, such as insulin resistance. SOCS-1 and -3, negative regulators of cytokine signalling are not only reported to promote ubiquitination and degradation of IRS-1, but also of IRS-2 in mice [157]. SOCS-1 and -3 can inhibit IRS-2 function in an additional manner. Both SOCS isoforms can bind to different regions in the insulin receptor and inhibit thereby the interaction

and activation of the IRS proteins [233]. SOCS-1 has been also implicated as negative regulator of IRS-2 expression [234]: SOCS-1 knock out mice are reported to have increased IRS-2 protein levels and subsequently increased tyrosine phosphorylation of IRS-2 and enhanced Akt activation.

Studies investigating the promoter region of the IRS-2 gene revealed several response elements. It contains an insulin response element (IRE) [231] which is recognized by forkhead transcription factors like FoxO1 [225]. Guo and co-workers showed that FoxO1 and IRS-2 are regulated reciprocally, with FoxO1 increasing the expression of IRS-2, while the activation of IRS-2 results in FoxO1 down-regulation as described in the section about the fed state [235]. Liver specific FoxO1 knockout mice display a 50% decrease of IRS-2 gene expression [71] and the expression of a constitutively nuclear FoxO1 in mouse liver induces IRS-2 significantly [98]. Interestingly the IRS-2 promoter also contains a region which binds SREBP (named SRE), which partially overlaps with the IRE. SREBP is induced by insulin and it negatively regulates the expression of IRS-2, by replacing FoxO1 from the promoter [225].

Like IRS-1, IRS-2 also possesses multiple tyrosine residues responsible for the transmission of the insulin signal as well as serine and threonine residues, which are supposed to be involved in its regulation. However, to date only two sites are described as possible targets of JNK [236,237]. Solinas and colleagues propose that Thr 348 in IRS-2 is a functional homolog to Ser 307 in IRS-1, a site that is involved in negative regulation of the insulin signal [236]. Very recently Sharfi and Eldar-Finkelman could show that JNK phosphorylated IRS-2 on Ser-488 and that this is a prerequisite for the GSK-3 β -dependent phosphorylation of Ser-484. This sequential phosphorylation led to an inhibition of the insulin signal in hepatocytes and it could be speculated that it contributes to the development of insulin resistance [237]. Interestingly there appear to be some substrate specificity of serine kinases to IRS proteins: PKC- ζ phosphorylates IRS-1, -3 and -4 but not IRS-2 in *in vitro* kinase assays [238].

Furthermore, a study by Zhang demonstrated that IRS-2 is acetylated at several lysine residues and this modification interferes with insulin induced tyrosine phosphorylation [239]. Insulin has been shown to activate the deacetylase SirT1 which removes the acetyl residues and this facilitates the tyrosine phosphorylation of IRS-2. As for IRS-1, acetylation seems to be an additional important mode of IRS-2 regulation.

To summarize, hepatic IRS-2 is regulated mainly at the transcriptional level. High insulin concentrations lead to degradation of the protein and during conditions of fasting IRS-2 is strongly up-regulated. Although IRS-2 contains similar to IRS-1 multiple amino acid residues suitable for posttranslational modifications, less is known about the regulation of IRS-2 apart from changes in its protein levels.

7. IRS PROTEINS AS DRUG TARGETS AND THEIR IMPORTANCE FOR THE THERAPY OF INSULIN RESISTANCE

The best treatment to prevent insulin resistance and development of type 2 diabetes mellitus appeared to be as simple as a lifestyle intervention with increased physical activity and reduced calorie intake [240-244]. Moderate endurance exercise has been shown to improve glucose tolerance and insulin sensitivity in normal weight and obese subjects even if a profound loss of weight was not achieved (reviewed in [245]). Especially the reduction of visceral and liver fat is associated with improved glucose tolerance [246]. But adapting a new lifestyle for longer time periods in the every day life is often difficult. Given the epidemic number of patients diagnosed with type 2 diabetes mellitus the search for novel therapeutic approaches to prevent and treat diabetes is a global challenge.

From the previous sections we can conclude that IRS proteins are central for the control of insulin signalling. Based on our knowledge of IRS protein regulation we can speculate that enhancing IRS function, half life and proper phosphorylation is beneficial for insulin sensitivity.

In the past years several molecules of the insulin signalling cascade have been evaluated as possible drug targets. Among these are phosphatases, SOCS proteins, IRS kinases, the ubiquitin proteasome system and the IRS proteins.

As outlined above, IRS-1 and 2 are negatively regulated by PTP1B, because this phosphatase dephosphorylates phosphotyrosine residues and attenuates the insulin signal [202,209]. Thus, inhibition of this phosphatase would be a good drug target and a number of studies have tested this possibility. Animal studies using PTP1B knock out mice in combination with diabetic models (high fat diet induced obesity, insulin receptor/IRS-1 heterozygous knock down) revealed beneficial effects of lowered PTP1B activity. These mice showed improved glucose tolerance and insulin sensitivity [208,247].

Several studies, reviewed in [248-250], reported on different chemical classes of molecules with PTP1B-specific inhibitory properties. Not only small molecule inhibitors were tested, but also antisense oligonucleotides (ASO) inhibitors directed against PTP1B. Treatment of obese diabetic (ob/ob) mice with ASOs normalized plasma glucose and insulin concentrations and increased tyrosine phosphorylation of the insulin receptor and of IRS-1 and IRS-2 in the liver [205]. In line with these findings downstream signalling was also enhanced shown as increased activation of PI-3 kinase, Akt/PKB and GSK-3 β . Interestingly, the increase of IRS-2 tyrosine phosphorylation was in part attributed to increased IRS-2 protein concentration. In two similar studies the administration of ASO against PTP1B in ob/ob mice resulted in decreased PEPCK and fructose-1,6-bisphosphatase gene expression [206] and decreased expression of genes involved in lipogenesis, such as SREBP-1 [251]. The PTP1B-ASOs are currently tested in phase II clinical trials [252]. Other protein phosphatases such as low molecular weight PTP were also successfully inhibited with the ASO technology resulting in similar positive effects in hepatic insulin signalling like PTP1B inhibition [253].

Apart from these new therapeutic approaches, already established drugs for the treatment of type 2 diabetes mellitus are demonstrated to act on impaired liver insulin signalling. Pioglitazone, which belongs to the class of thiazolidinediones is a so-called insulin sensitizer. It acts as PPAR γ agonist and increases the transcription of PPAR γ target genes, especially in adipose tissue. This is associated with enhanced insulin sensitivity and therefore the thiazolidinediones are widely used to improve glycemic control in diabetic patients [254]. Although the adipose tissue is the main target of the thiazolidinediones, other insulin sensitive tissues are affected as well [255]. Pioglitazone was shown to reduce the expression of SOCS-3 in adipose tissue and liver of db/db mice [256]. SOCS-3 is involved in the downregulation of IRS-1 and IRS-2 and SOCS-3 action is increased in insulin resistant states [257] therefore this molecule is an interesting target for the treatment of insulin resistant states. However, mice lacking hepatic SOCS-3 develop peripheral insulin resistance probably due to impaired downregulation of inflammatory processes [258]. Hence, a complete blockade of hepatic SOCS-3 might not be desirable in diabetes treatment but a moderate downregulation could provide a positive therapeutic outcome.

In the past years, glucagon like peptide (GLP)-1 has been studied for its application in the treatment of type 2 diabetes mellitus [259]. GLP-1 is an incretin, i.e. a gastrointestinal hormone which is released after ingestion of a meal. It potentiates the glucose-induced insulin secretion *in vivo* thereby enhancing insulin sensitivity and β -

cell function in diabetic subjects [260]. GLP-1 has been demonstrated to induce β -cell proliferation and function *in vitro* (reviewed in [261]).

Long acting GLP-1 analogs and dipeptidyl peptidase IV inhibitors have been developed [262] to circumvent the limitations of GLP-1 application due to its short half life of only a few minutes [263]. GLP-1 gene therapy of obese diabetic mice improved glucose tolerance and insulin sensitivity and reduced hyperinsulinemia [264]. In addition hepatic glucose and lipid synthesis, which is pathologically increased during insulin resistance and diabetes, was decreased by lower expression of PEPCK, G6Pase and fatty acid synthase. This effect might be mediated by an improvement of IRS-1 tyrosine-phosphorylation observed in the liver of these mice. But it remains to be demonstrated whether GLP-1 exerts primary effects on the liver or whether the improved hepatic IRS-1 phosphorylation is secondary to the improved insulin secretion.

Serine/threonine phosphorylation of IRS-1 is increased during insulin resistance and diabetes. An inhibition of this hyperphosphorylation is a possible drug target [265]. High doses of salicylates, long known for their plasma glucose lowering effects, have been shown to protect IRS-1 from Ser-307 phosphorylation [266]. As described in chapter 6.1, the action of JNK is accountable for the increased Ser-307 phosphorylation in insulin resistant states. The downregulation of JNK by use of JNK inhibitor could improve glucose tolerance in obese db/db mice [267]. Thus, modulation of the activity of IRS kinases will be of greater importance in the future treatment of insulin resistance.

In conclusion, IRS proteins seem to be a suitable target for the therapy of insulin resistance and type 2 diabetes mellitus. New as well as established drugs and treatment strategies engage IRS proteins and their posttranslational modifications in a way to improve insulin signalling by increased tyrosine phosphorylation (GLP-1, inhibition of PTPs), decreased degradation (targeting SOCS-3) and reduced serine/threonine phosphorylation (inhibition of IRS kinases). Enhancement of the expression of IRS proteins may provide beneficial effects on insulin signalling but to keep the balance is critical due to the involvement of IRS in mitogenesis and cell proliferation.

CONCLUSION

IRS proteins are key players in hepatic glucose and lipid metabolism and dysregulation of the function of IRS-1 and IRS-2 leads to disturbances in blood glucose and lipid homeostasis. Although considerable progress has been made in understanding the individual contribution of IRS-1 and IRS-2 to hepatic insulin action and their exact molecular regulation, the development of therapeutic approaches targeting IRS proteins to cure metabolic disorders such as diabetes mellitus type 2 still is a major challenge.

The future will probably yield more sophisticated treatment, e.g. an enhancement of positive posttranslational modifications, such as specific serine/threonine phosphorylation and acetylation. However, to accomplish this, a better understanding of IRS function in the liver and exact knowledge of posttranslational modification given by interdisciplinary projects of chemists, molecular biologists and medical researchers is required.

ACKNOWLEDGEMENTS

The current work on insulin receptor substrates is funded by grants from the Deutsche Forschungsgemeinschaft to L. Fritsche (GRK 1302/1) and the Landesstiftung Baden-Württemberg to R. Lehmann (P-LS-Prot/29). The authors like to thank E. Schleicher, R. S. Warach and M. Hoene for helpful discussion during preparation of this manuscript.

REFERENCES

- [1] Klover, P. J.; Mooney, R. A. *Int. J. Biochem. Cell. Biol.* **2004**, *36*, 753-758.
- [2] Raddatz, D.; Ramadori, G. Z. *Gastroenterology* **2007**, *45*, 51-62.
- [3] Zick, Y. *Sci. STKE* **2005**, *2005*, e4.
- [4] Taniguchi, C. M.; Emanuelli, B.; Kahn, C. R. *Nat. Rev. Mol. Cell. Biol.* **2006**, *7*, 85-96.
- [5] White, M. F. *Am. J. Physiol. Endocrinol. Metab.* **2002**, *283*, E413-E422.
- [6] Gual, P.; Marchand-Brustel, Y.; Tanti, J. F. *Biochimie* **2005**, *87*, 99-109.
- [7] Zick, Y. *Biochem. Soc. Trans.* **2004**, *32*, 812-816.
- [8] Clemmons, D. R. *Curr. Opin. Pharmacol.* **2006**, *6*, 620-625.
- [9] Sun, X. J.; Wang, L. M.; Zhang, Y.; Yenush, L.; Myers, Jr. M. G.; Glasheen, E.; Lane, W. S.; Pierce, J. H.; White, M. F. *Nature* **1995**, *377*, 173-177.
- [10] Sun, X. J.; Rothenberg, P.; Kahn, C. R.; Backer, J. M.; Araki, E.; Wilden, P. A.; Cahill, D. A.; Goldstein, B. J.; White, M. F. *Nature* **1991**, *352*, 73-77.
- [11] Lavan, B. E.; Lane, W. S.; Lienhard, G. E. *J. Biol. Chem.* **1997**, *272*, 11439-11443.
- [12] Fantin, V. R.; Sparling, J. D.; Slot, J. W.; Keller, S. R.; Lienhard, G. E.; Lavan, B. E. *J. Biol. Chem.* **1998**, *273*, 10726-10732.
- [13] Cai, D.; Dhe-Paganon, S.; Melendez, P. A.; Lee, J.; Shoelson, S. E. *J. Biol. Chem.* **2003**, *278*, 25323-25330.
- [14] Eck, M. J.; Dhe-Paganon, S.; Trub, T.; Nolte, R. T.; Shoelson, S. E. *Cell* **1996**, *85*, 695-705.
- [15] Dhe-Paganon, S.; Ottinger, E. A.; Nolte, R. T.; Eck, M. J.; Shoelson, S. E. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 8378-8383.
- [16] White, M. F. *Diabetologia* **1997**, *40* (Suppl 2), S2-17.
- [17] White, M. F. *Recent Prog. Horm. Res.* **1998**, *53*, 119-138.
- [18] Lee, Y. H.; White, M. F. *Arch. Pharm. Res.* **2004**, *27*, 361-370.
- [19] Anai, M.; Ono, H.; Funaki, M.; Fukushima, Y.; Inukai, K.; Ogihara, T.; Sakoda, H.; Onishi, Y.; Yazaki, Y.; Kikuchi, M.; Oka, Y.; Asano, T. *J. Biol. Chem.* **1998**, *273*, 29686-29692.
- [20] Inoue, G.; Cheatham, B.; Emkey, R.; Kahn, C. R. *J. Biol. Chem.* **1998**, *273*, 11548-11555.
- [21] Ogihara, T.; Shin, B. C.; Anai, M.; Katagiri, H.; Inukai, K.; Funaki, M.; Fukushima, Y.; Ishihara, H.; Takata, K.; Kikuchi, M.; Yazaki, Z.; Oka, Y.; Asano, T. *J. Biol. Chem.* **1997**, *272*, 12868-12873.
- [22] Sawka-Verhelle, D.; Tartare-Deckert, S.; White, M. F.; Van Obberghen, E. *J. Biol. Chem.* **1996**, *271*, 5980-5983.
- [23] Sawka-Verhelle, D.; Baron, V.; Mothe, I.; Filloux, C.; White, M. F.; Van Obberghen, E. *J. Biol. Chem.* **1997**, *272*, 16414-16420.
- [24] Wu, J.; Tseng, Y. D.; Xu, C. F.; Neubert, T. A.; White, M. F.; Hubbard, S. R. *Nat. Struct. Mol. Biol.* **2008**, *15*, 251-258.
- [25] Nandi, A.; Kitamura, Y.; Kahn, C. R.; Accili, D. *Physiol. Rev.* **2004**, *84*, 623-647.
- [26] Tamemoto, H.; Kadowaki, T.; Tobe, K.; Yagi, T.; Sakura, H.; Hayakawa, T.; Terauchi, Y.; Ueki, K.; Kaburagi, Y.; Satoh, S.; Sekihara, H.; Yoshioka, S.; Horikoshi, H.; Furuta, Y.; Ikawa, Y.; Kasuga, M.; Yazaki, Y.; Aizawa, S. *Nature* **1994**, *372*, 182-186.
- [27] Araki, E.; Lipes, M. A.; Patti, M. E.; Bruning, J. C.; Haag, B.; Johnson, R. S.; Kahn, C. R. *Nature* **1994**, *372*, 186-190.
- [28] Abe, H.; Yamada, N.; Kamata, K.; Kuwaki, T.; Shimada, M.; Osuga, J.; Shionoiri, F.; Yahagi, N.; Kadowaki, T.; Tamemoto, H.; Ishibashi, S.; Yazaki, Y.; Makuuchi, M. *J. Clin. Invest.* **1998**, *101*, 1784-1788.
- [29] Yamauchi, T.; Tobe, K.; Tamemoto, H.; Ueki, K.; Kaburagi, Y.; Yamamoto-Honda, R.; Takahashi, Y.; Yoshizawa, F.; Aizawa, S.; Akanuma, Y.; Sonenberg, N.; Yazaki, Y.; Kadowaki, T. *Mol. Cell. Biol.* **1996**, *16*, 3074-3084.
- [30] Patti, M. E.; Sun, X. J.; Bruening, J. C.; Araki, E.; Lipes, M. A.; White, M. F.; Kahn, C. R. *J. Biol. Chem.* **1995**, *270*, 24670-24673.
- [31] Ueki, K.; Yamauchi, T.; Tamemoto, H.; Tobe, K.; Yamamoto-Honda, R.; Kaburagi, Y.; Akanuma, Y.; Yazaki, Y.; Aizawa, S.; Nagai, R.; Kadowaki, T. *J. Clin. Invest.* **2000**, *105*, 1437-1445.
- [32] Withers, D. J.; Gutierrez, J. S.; Towery, H.; Burks, D. J.; Ren, J. M.; Previs, S.; Zhang, Y.; Bernal, D.; Pons, S.; Shulman, G. I.; Bonner-Weir, S.; White, M. F. *Nature* **1998**, *391*, 900-904.
- [33] Kubota, N.; Tobe, K.; Terauchi, Y.; Eto, K.; Yamauchi, T.; Suzuki, R.; Tsubamoto, Y.; Komada, K.; Nakano, R.; Miki, H.; Satoh, S.; Sekihara, H.; Sciacchitano, S.; Lesniak, M.; Aizawa, S.; Nagai, R.; Kimura, S.; Akanuma, Y.; Taylor, S. I.; Kadowaki, T. *Diabetes* **2000**, *49*, 1880-1889.
- [34] Hennige, A. M.; Burks, D. J.; Ozcan, U.; Kulkarni, R. N.; Ye, J.; Park, S.; Schubert, M.; Fisher, T. L.; Dow, M. A.; Leshan, R.; Zakaria, M.; Mossa-Basha, M.; White, M. F. *J. Clin. Invest.* **2003**, *112*, 1521-1532.
- [35] Katic, M.; Kahn, C. R. *Cell Mol. Life Sci.* **2005**, *62*, 320-343.
- [36] Selman, C.; Lingard, S.; Choudhury, A. I.; Batterham, R. L.; Claret, M.; Clements, M.; Ramadani, F.; Okkenhaug, K.; Schuster, E.; Blanc, E.; Piper, M. D.; Al Qassab, H.; Speakman, J. R.; Carmignac, D.; Robinson, I. C. A.; Thornton, J. M.; Gems, D.; Partridge, L.; Withers, D. J. *FASEB J.* **2008**, *22*, 807-818.
- [37] Taguchi, A.; Wartschow, L. M.; White, M. F. *Science* **2007**, *317*, 369-372.
- [38] Sesti, G.; Federici, M.; Hribal, M. L.; Lauro, D.; Sbraccia, P.; Lauro, R. *FASEB J.* **2001**, *15*, 2099-2111.
- [39] Almind, K.; Bjorbaek, C.; Vestergaard, H.; Hansen, T.; Echwald, S.; Pedersen, O. *Lancet* **1993**, *342*, 828-832.
- [40] Clausen, J. O.; Hansen, T.; Bjorbaek, C.; Echwald, S. M.; Urhammer, S. A.; Rasmussen, S.; Andersen, C. B.; Hansen, L.; Almind, K.; Winther, K.; Haraldsdottir, J.; Borch-Johnsen, K.; Pedersen, O. *Lancet* **1995**, *346*, 397-402.
- [41] Koch, M.; Rett, K.; Volk, A.; Maerker, E.; Haist, K.; Deninger, M.; Renn, W.; Haring, H. U. *Exp. Clin. Endocrinol. Diabetes* **1999**, *107*, 318-322.
- [42] McGettrick, A. J.; Feener, E. P.; Kahn, C. R. *J. Biol. Chem.* **2005**, *280*, 6441-6446.
- [43] Hribal, M. L.; Tornei, F.; Pujol, A.; Menghini, R.; Barcaroli, D.; Lauro, D.; Amoruso, R.; Lauro, R.; Bosch, F.; Sesti, G.; Federici, M. *J. Cell Mol. Med.* **2008**, Epub ahead of print.
- [44] Hribal, M. L.; Federici, M.; Porzio, O.; Lauro, D.; Borboni, P.; Accili, D.; Lauro, R.; Sesti, G. *J. Clin. Endocrinol. Metab.* **2000**, *85*, 2004-2013.
- [45] Federici, M.; Hribal, M. L.; Ranalli, M.; Marselli, L.; Porzio, O.; Lauro, D.; Borboni, P.; Lauro, R.; Marchetti, P.; Melino, G.; Sesti, G. *FASEB J.* **2001**, *15*, 22-24.
- [46] van Dam, R. M.; Hoebee, B.; Seidell, J. C.; Schaap, M. M.; Blaak, E. E.; Feskens, E. J. *Diabetes Med.* **2004**, *21*, 752-758.
- [47] Imai, Y.; Fusco, A.; Suzuki, Y.; Lesniak, M. A.; D'Alfonso, R.; Sesti, G.; Bertoli, A.; Lauro, R.; Accili, D.; Taylor, S. I. *J. Clin. Endocrinol. Metab.* **1994**, *79*, 1655-1658.
- [48] Mammarella, S.; Romano, F.; Di Valerio, A.; Creati, B.; Esposito, D. L.; Palmirotta, R.; Capani, F.; Vitullo, P.; Volpe, G.; Battista, P.; Della Loggia, F.; Mariani-Costantini, R.; Cama, A. *Hum. Mol. Genet.* **2000**, *9*, 2517-2521.
- [49] Knowler, W. C.; Bennett, P. H.; Hamman, R. F.; Miller, M. A. *J. Epidemiol.* **1978**, *108*, 497-505.
- [50] Stefan, N.; Kovacs, P.; Stumvoll, M.; Hanson, R. L.; Lehn-Stefan, A.; Permana, P. A.; Baier, L. J.; Tataranni, P. A.; Silver, K.; Bogardus, C. *Diabetes* **2003**, *52*, 1544-1550.
- [51] Bodhini, D.; Radha, V.; Deepa, R.; Ghosh, S.; Majumder, P. P.; Rao, M. R.; Mohan, V. *Int. J. Obes. (Lond.)* **2007**, *31*, 97-102.
- [52] Foufelle, F.; Ferre, P. *Biochem. J.* **2002**, *366*, 377-391.
- [53] Morral, N.; Edenberg, H. J.; Witting, S. R.; Altomonte, J.; Chu, T.; Brown, M. J. *Lipid Res.* **2007**, *48*, 1499-1510.
- [54] Aiston, S.; Coghlan, M. P.; Agius, L. *Eur. J. Biochem.* **2003**, *270*, 2773-2781.
- [55] Aiston, S.; Hampson, L. J.; Arden, C.; Iynedjian, P. B.; Agius, L. *Diabetologia* **2006**, *49*, 174-182.
- [56] Dentin, R.; Pegorier, J. P.; Benhamed, F.; Fougelle, F.; Ferre, P.; Fauveau, V.; Magnuson, M. A.; Girard, J.; Postic, C. *J. Biol. Chem.* **2004**, *279*, 20314-20326.
- [57] Yamamoto, T.; Shimano, H.; Nakagawa, Y.; Ide, T.; Yahagi, N.; Matsuzaka, T.; Nakakuki, M.; Takahashi, A.; Suzuki, H.; Sone, H.; Toyoshima, H.; Sato, R.; Yamada, N. *J. Biol. Chem.* **2004**, *279*, 12027-12035.
- [58] Hasty, A. H.; Shimano, H.; Yahagi, N.; Amemiya-Kudo, M.; Perrey, S.; Yoshikawa, T.; Osuga, J.; Okazaki, H.; Tamura, Y.; Iizuka, Y.; Shionoiri, F.; Ohashi, K.; Harada, K.; Godota, T.; Nagai, R.; Ishibashi, S.; Yamada, N. *J. Biol. Chem.* **2000**, *275*, 31069-31077.
- [59] Horton, J. D.; Bashmakov, Y.; Shimomura, I.; Shimano, H. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 5987-5992.
- [60] Matsumoto, M.; Ogawa, W.; Akimoto, K.; Inoue, H.; Miyake, K.; Furukawa, K.; Hayashi, Y.; Iguchi, H.; Matsuki, Y.; Hiramatsu, R.; Shimano, H.; Yamada, N.; Ohno, S.; Kasuga, M.; Noda, T. *J. Clin. Invest.* **2003**, *112*, 935-944.
- [61] Taniguchi, C. M.; Kondo, T.; Sajan, M.; Luo, J.; Bronson, R.; Asano, T.; Farese, R.; Cantley, L. C.; Kahn, C. R. *Cell Metab.* **2006**, *3*, 343-353.
- [62] Chen, G.; Liang, G.; Ou, J.; Goldstein, J. L.; Brown, M. S. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11245-11250.
- [63] Yamashita, H.; Takenoshita, M.; Sakurai, M.; Bruick, R. K.; Henzel, W. J.; Shillinglaw, W.; Arnot, D.; Uyeda, K. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 9116-9121.
- [64] Mitro, N.; Mak, P. A.; Vargas, L.; Godio, C.; Hampton, E.; Molteni, V.; Krensch, A.; Saez, E. *Nature* **2007**, *445*, 219-223.
- [65] Dentin, R.; Denechaud, P. D.; Benhamed, F.; Girard, J.; Postic, C. *J. Nutr.* **2006**, *136*, 1145-1149.
- [66] Towle, H. C. *Trends. Endocrinol. Metab.* **2005**, *16*, 489-494.
- [67] Cho, H.; Mu, J.; Kim, J. K.; Thorvaldsen, J. L.; Chu, Q.; Crenshaw, E. B., III; Kaestner, K. H.; Bartolomei, M. S.; Shulman, G. I.; Birnbaum, M. J. *Science* **2001**, *292*, 1728-1731.
- [68] Rena, G.; Guo, S.; Cichy, S. C.; Unterman, T. G.; Cohen, P. J. *Biol. Chem.* **1999**, *274*, 17179-17183.
- [69] Li, X.; Monks, B.; Ge, Q.; Birnbaum, M. J. *Nature* **2007**, *447*, 1012-1016.
- [70] Brunet, A.; Bonni, A.; Zigmond, M. J.; Lin, M. Z.; Juo, P.; Hu, L. S.; Anderson, M. J.; Arden, K. C.; Blenis, J.; Greenberg, M. E. *Cell* **1999**, *96*, 857-868.
- [71] Matsumoto, M.; Poci, A.; Rossetti, L.; DePinho, R. A.; Accili, D. *Cell Metab.* **2007**, *6*, 208-216.
- [72] Southgate, R. J.; Bruce, C. R.; Carey, A. L.; Steinberg, G. R.; Walder, K.; Monks, R.; Watt, M. J.; Hawley, J. A.; Birnbaum, M. J.; Febbraio, M. A. *FASEB J.* **2005**, *05*-3993fje.
- [73] Zhou, X. Y.; Shibusawa, N.; Naik, K.; Porras, D.; Temple, K.; Ou, H.; Kaihara, K.; Roe, M. W.; Brady, M. J.; Wondisford, F. E. *Nat. Med.* **2004**, *10*, 633-637.
- [74] Boden, G. *J. Investig. Med.* **2004**, *52*, 375-378.
- [75] Suh, S. H.; Paik, I. Y.; Jacobs, K. *Mol. Cell.* **2007**, *23*, 272-279.
- [76] Chandramouli, V.; Ekberg, K.; Schumann, W. C.; Kalhan, S. C.; Wahren, J.; Landau, B. R. *Am. J. Physiol. Endocrinol. Metab.* **1997**, *273*, E1209-E1215.
- [77] Wahren, J.; Ekberg, K. *Annu. Rev. Nutr.* **2007**, *27*, 329-345.
- [78] Jiang, G.; Zhang, B. B. *Am. J. Physiol. Endocrinol. Metab.* **2003**, *284*, E671-E678.

- [79] Syed, N. A.; Khandelwal, R. L. *Mol. Cell Biochem.* **2000**, *211*, 123-136.
- [80] Wu, C.; Khan, S. A.; Peng, L. J.; Lange, A. J. *Adv. Enzyme Regul.* **2006**, *46*, 72-88.
- [81] Yamada, K.; Noguchi, T. *Biochem. J.* **1999**, *337*, 1-11.
- [82] Chrivia, J. C.; Kwok, R. P. S.; Lamb, N.; Hagiwara, M.; Montminy, M. R.; Goodman, R. H. *Nature* **1993**, *365*, 855-859.
- [83] Koo, S. H.; Flechner, L.; Qi, L.; Zhang, X.; Sreaton, R. A.; Jeffries, S.; Hedrick, S.; Xu, W.; Boussouar, F.; Brindle, P.; Takemori, H.; Montminy, M. *Nature* **2005**, *437*, 1109-1111.
- [84] Dentin, R.; Liu, Y.; Koo, S. H.; Hedrick, S.; Vargas, T.; Heredia, J.; Yates, J.; Montminy, M. *Nature* **2007**, *449*, 366-369.
- [85] Yoon, J. C.; Puigserver, P.; Chen, G.; Donovan, J.; Wu, Z.; Rhee, J.; Adelman, G.; Stafford, J.; Kahn, C. R.; Granner, D. K.; Newgard, C. B.; Spiegelman, B. M. *Nature* **2001**, *413*, 131-138.
- [86] Herzig, S.; Long, F.; Jhala, U. S.; Hedrick, S.; Quinn, R.; Bauer, A.; Rudolph, D.; Schutz, G.; Yoon, C.; Puigserver, P.; Spiegelman, B.; Montminy, M. *Nature* **2001**, *413*, 179-183.
- [87] Daitoku, H.; Yamagata, K.; Matsuzaki, H.; Hatta, M.; Fukamizu, A. *Diabetes* **2003**, *52*, 642-649.
- [88] Puigserver, P.; Rhee, J.; Donovan, J.; Walkey, C. J.; Yoon, J. C.; Oriente, F.; Kitamura, Y.; Altomonte, J.; Dong, H.; Accili, D.; Spiegelman, B. M. *Nature* **2003**, *423*, 550-555.
- [89] Schilling, M. M.; Oeser, J. K.; Boustead, J. N.; Flemming, B. P.; O'Brien, R. M. *Nature* **2006**, *443*, E10-E11.
- [90] Lin, J.; Wu, P. H.; Tarr, P. T.; Lindenberg, K. S.; St Pierre, J.; Zhang, C. y.; Mootha, V. K.; Jager, S.; Vianna, C. R.; Reznick, R. M.; Cui, L.; Manieri, M.; Donovan, M. X.; Wu, Z.; Cooper, M. P.; Fan, M. C.; Rohas, L. M.; Zavacki, A. M.; Cinti, S.; Shulman, G. I.; Lowell, B. B.; Krainc, D.; Spiegelman, B. M. *Cell* **2004**, *119*, 121-135.
- [91] Herzog, B.; Hall, R. K.; Wang, X. L.; Waltner-Law, M.; Granner, D. K. *Mol. Endocrinol.* **2004**, *18*, 807-819.
- [92] Rhee, J.; Inoue, Y.; Yoon, J. C.; Puigserver, P.; Fan, M.; Gonzalez, F. J.; Spiegelman, B. M. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4012-4017.
- [93] Accili, D.; Arden, K. C. *Cell* **2004**, *117*, 421-426.
- [94] Imae, M.; Fu, Z.; Yoshida, A.; Noguchi, T.; Kato, H. *J. Mol. Endocrinol.* **2003**, *30*, 253-262.
- [95] Ayala, J. E.; Streeper, R. S.; Desgrosellier, J. S.; Durham, S. K.; Suwanichkul, A.; Svitek, C. A.; Goldman, J. K.; Barr, F. G.; Powell, D. R.; O'Brien, R. M. *Diabetes* **1999**, *48*, 1885-1889.
- [96] Nakae, J.; Kitamura, T.; Silver, D. L.; Accili, D. *J. Clin. Invest.* **2001**, *108*, 1359-1367.
- [97] Qu, S.; Altomonte, J.; Perdomo, G.; He, J.; Fan, Y.; Kamagate, A.; Meseck, M.; Dong, H. H. *Endocrinology* **2006**, *147*, 5641-5652.
- [98] Matsumoto, M.; Han, S.; Kitamura, T.; Accili, D. *J. Clin. Invest.* **2006**, *116*, 2464-2472.
- [99] Zhang, W.; Patil, S.; Chauhan, B.; Guo, S.; Powell, D. R.; Le, J.; Klotsas, A.; Matika, R.; Xiao, X.; Franks, R.; Heidenreich, K. A.; Sajan, M. P.; Farese, R. V.; Stolz, D. B.; Tso, P.; Koo, S. H.; Montminy, M.; Unterman, T. G. *J. Biol. Chem.* **2006**, *281*, 10105-10117.
- [100] Nakae, J.; Biggs, W. H.; Kitamura, T.; Cavenee, W. K.; Wright, C. V. E.; Arden, K. C.; Accili, D. *Nat. Genet.* **2002**, *32*, 245-253.
- [101] Altomonte, J.; Richter, A.; Harbaran, S.; Suriawinata, J.; Nakae, J.; Thung, S. N.; Meseck, M.; Accili, D.; Dong, H. *Am. J. Physiol. Endocrinol. Metab.* **2003**, *285*, E718-E728.
- [102] Samuel, V. T.; Choi, C. S.; Phillips, T. G.; Romanelli, A. J.; Geisler, J. G.; Ghanot, S.; McKay, R.; Monia, B.; Shutter, J. R.; Lindberg, R. A.; Shulman, G. I.; Veniant, M. M. *Diabetes* **2006**, *55*, 2042-2050.
- [103] Altomonte, J.; Cong, L.; Harbaran, S.; Richter, A.; Xu, J.; Meseck, M.; Dong, H. H. *J. Clin. Invest.* **2004**, *114*, 1493-1503.
- [104] Shachter, N. S. *Curr. Opin. Lipidol.* **2001**, *12*, 297-304.
- [105] Lin, J.; Yang, R.; Tarr, P. T.; Wu, P. H.; Handschin, C.; Li, S.; Yang, W.; Pei, L.; Uldry, M.; Tontonoz, P.; Newgard, C. B.; Spiegelman, B. M. *Cell* **2005**, *120*, 261-273.
- [106] Lin, J.; Tarr, P. T.; Yang, R.; Rhee, J.; Puigserver, P.; Newgard, C. B.; Spiegelman, B. M. *J. Biol. Chem.* **2003**, *278*, 30843-30848.
- [107] Friedman, J. R.; Kaestner, K. H. *Cell Mol. Life Sci.* **2006**, *63*, 2317-2328.
- [108] Zhang, L.; Rubins, N. E.; Ahima, R. S.; Greenbaum, L. E.; Kaestner, K. H. *Cell Metab.* **2005**, *2*, 141-148.
- [109] Wolfrum, C.; Stoffel, M. *Cell Metab.* **2006**, *3*, 99-110.
- [110] Wolfrum, C.; Besser, D.; Luca, E.; Stoffel, M. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 11624-11629.
- [111] Michael, M. D.; Kulkarni, R. N.; Postic, C.; Previs, S. F.; Shulman, G. I.; Magnuson, M. A.; Kahn, C. R. *Mol. Cell* **2000**, *6*, 87-97.
- [112] Edgerton, D. S.; Lautz, M.; Scott, M.; Everett, C. A.; Stettler, K. M.; Neal, D. W.; Chu, C. A.; Cherrington, A. D. *J. Clin. Invest.* **2006**, *116*, 521-527.
- [113] Friedman, J. E. *Am. J. Physiol.* **1994**, *266*, E560-E566.
- [114] Nizielski, S. E.; Arizmendi, C.; Shteyngarts, A. R.; Farrell, C. J.; Friedman, J. E. *Am. J. Physiol.* **1996**, *270*, R1005-R1012.
- [115] Dohm, G. L.; Kasparek, G. J.; Barakat, H. A. *Am. J. Physiol. Endocrinol. Metab.* **1985**, *249*, E6-E11.
- [116] Drouin, R.; Lavoie, C.; Bourque, J.; Ducros, F.; Poisson, D.; Chiasson, J. L. *Am. J. Physiol.* **1998**, *274*, E23-E28.
- [117] Legare, A.; Drouin, R.; Milot, M.; Massicotte, D.; Peronnet, F.; Massicotte, G.; Lavoie, C. *Am. J. Physiol. Endocrinol. Metab.* **2001**, *280*, E193-E196.
- [118] Petersen, K. F.; Price, T. B.; Bergeron, R. J. *Clin. Endocrinol. Metab.* **2004**, *89*, 4656-4664.
- [119] Kjaer, M. *Adv. Exp. Med. Biol.* **1998**, *441*, 117-127.
- [120] Kreisman, S. H.; Mew, N. A.; Arsenault, M.; Nessim, S. J.; Halter, J. B.; Vranic, M.; Marliss, E. B. *Am. J. Physiol. Endocrinol. Metab.* **2000**, *278*, E949-E957.
- [121] Kreisman, S. H.; Halter, J. B.; Vranic, M.; Marliss, E. B. *Diabetes* **2003**, *52*, 1347-1354.
- [122] Cassuto, H.; Kochan, K.; Chakravarty, K.; Cohen, H.; Blum, B.; Olswang, Y.; Hakimi, P.; Xu, C.; Massillon, D.; Hanson, R. W.; Reshef, L. *J. Biol. Chem.* **2005**, *280*, 33873-33884.
- [123] Chakravarty, K.; Cassuto, H.; Reshef, L.; Hanson, R. W. *Crit. Rev. Biochem. Mol. Biol.* **2005**, *40*, 129-154.
- [124] Vander Kooi, B. T.; Onuma, H.; Oeser, J. K.; Svitek, C. A.; Allen, S. R.; Vander Kooi, C. W.; Chazin, W. J.; O'Brien, R. M. *Mol. Endocrinol.* **2005**, *19*, 3001-3022.
- [125] Camacho, R. C.; Donahue, E. P.; James, F. D.; Berglund, E. D.; Wasserman, D. H. *Am. J. Physiol. Endocrinol. Metab.* **2006**, *290*, E405-E408.
- [126] Huang, H.; Iida, K. T.; Sone, H.; Ajsaka, R. *Exp. Clin. Endocrinol. Diabetes* **2007**, *115*, 417-422.
- [127] Rufo, C.; Teran-Garcia, M.; Nakamura, M. T.; Koo, S. H.; Towle, H. C.; Clarke, S. D. *J. Biol. Chem.* **2001**, *276*, 21969-21975.
- [128] Adamson, A. W.; Suchankova, G.; Rufo, C.; Nakamura, M. T.; Teran-Garcia, M.; Clarke, S. D.; Gettys, T. W. *Biochem. J.* **2006**, *399*, 285-295.
- [129] Porstmann, T.; Griffiths, B.; Chung, Y. L.; Delpuech, O.; Griffiths, J. R.; Downward, J.; Schulze, A. *Oncogene* **2005**, *24*, 6465-6481.
- [130] Fiebig, R. G.; Hollander, J. M.; Ney, D.; Boileau, R.; Jeffery, E.; Ji, L. L. *Med. Sci. Sports Exerc.* **2002**, *34*, 1106-1114.
- [131] Griffiths, M. A.; Fiebig, R.; Gore, M. T.; Baker, D. H.; Esser, K.; Oscari, L.; Ji, L. L. *J. Nutr.* **1996**, *126*, 1959-1971.
- [132] Fiebig, R.; Griffiths, M. A.; Gore, M. T.; Baker, D. H.; Oscari, L.; Ney, D. M.; Ji, L. L. *J. Nutr.* **1998**, *128*, 810-817.
- [133] Colombo, M.; Gregersen, S.; Kruhoeffer, M.; Agger, A.; Xiao, J.; Jeppesen, P. B.; Orntoft, T.; Ploug, T.; Galbo, H.; Hermansen, K. *Metabolism* **2005**, *54*, 1571-1581.
- [134] Heled, Y.; Shapiro, Y.; Shani, Y.; Moran, D. S.; Langzam, L.; Barash, V.; Sampson, S. R.; Meyerovitch, J. *Metabolism* **2004**, *53*, 836-841.
- [135] Shafir, E.; Ziv, E.; Kalman, R. *ILAR J.* **2006**, *47*, 212-224.
- [136] Rother, K. I.; Imai, Y.; Caruso, M.; Beguinot, F.; Formisano, P.; Accili, D. *J. Biol. Chem.* **1998**, *273*, 17491-17497.
- [137] Chang, S. P.; Chen, Y. H.; Chang, W. C.; Liu, I. M.; Cheng, J. T. *Life Sci.* **2006**, *79*, 240-246.
- [138] Previs, S. F.; Withers, D. J.; Ren, J. M.; White, M. F.; Shulman, G. I. *J. Biol. Chem.* **2000**, *275*, 38990-38994.
- [139] Shimomura, I.; Matsuda, M.; Hammer, R. E.; Bashmakov, Y.; Brown, M. S.; Goldstein, J. L. *Mol. Cell* **2000**, *6*, 77-86.
- [140] Withers, D. J.; Burks, D. J.; Towery, H. H.; Altamuro, S. L.; Flint, C. L.; White, M. F. *Nat. Genet.* **1999**, *23*, 32-40.
- [141] Valverde, A. M.; Burks, D. J.; Fabregat, I.; Fisher, T. L.; Carretero, J.; White, M. F.; Benito, M. *Diabetes* **2003**, *52*, 2239-2248.
- [142] Suzuki, R.; Tobe, K.; Aoyama, M.; Inoue, A.; Sakamoto, K.; Yamauchi, T.; Kamon, J.; Kubota, N.; Terauchi, Y.; Yoshimatsu, H.; Matsuhisa, M.; Nagasaka, S.; Ogata, H.; Tokuyama, K.; Nagai, R.; Kadowaki, T. *J. Biol. Chem.* **2004**, *279*, 25039-25049.
- [143] Taniguchi, C. M.; Ueki, K.; Kahn, R. J. *Clin. Invest.* **2005**, *115*, 718-727.
- [144] Dong, X.; Park, S.; Lin, X.; Copps, K.; Yi, X.; White, M. F. *J. Clin. Invest.* **2006**, *116*, 101-114.
- [145] Simmgren, M.; Knauf, C.; Lopez, M.; Choudhury, M. A.; Charalambous, M.; Cantley, J.; Bedford, D. C.; Claret, M.; Iglesias, M. A.; Heffron, H.; Cani, P. D.; Vidal-Puig, A.; Barcelin, R.; Withers, D. J. *Diabetologia* **2006**, *49*, 552-561.
- [146] Murata, Y.; Tsuruzoe, K.; Kawashima, J.; Furukawa, N.; Kondo, T.; Moto-shima, H.; Igata, M.; Taketa, K.; Sasaki, K.; Kishikawa, H.; Kahn, C. R.; Toyonaga, T.; Araki, E. *Biochem. Biophys. Res. Commun.* **2007**, *364*, 301-307.
- [147] Ruiz-Alcaraz, A. J.; Liu, H. K.; Cuthbertson, D. J.; McManus, E. J.; Akhtar, S.; Lipina, C.; Morris, A. D.; Petrie, J. R.; Hundal, H. S.; Sutherland, C. *Biochem. J.* **2005**, *392*, 345-352.
- [148] Saad, M. J.; Folli, F.; Kahn, C. R. *Endocrinology* **1995**, *136*, 1579-1588.
- [149] Saad, M. J.; Folli, F.; Kahn, J. A.; Kahn, C. R. *J. Clin. Invest.* **1993**, *92*, 2065-2072.
- [150] Saad, M. J.; Araki, E.; Miralpeix, M.; Rothenberg, P. L.; White, M. F.; Kahn, C. R. *J. Clin. Invest.* **1992**, *90*, 1839-1849.
- [151] Hirashima, Y.; Tsuruzoe, K.; Kodama, S.; Igata, M.; Toyonaga, T.; Ueki, K.; Kahn, C. R.; Araki, E. *J. Endocrinol.* **2003**, *179*, 253-266.
- [152] Sun, X. J.; Goldberg, J. L.; Qiao, L. Y.; Mitchell, J. J. *Diabetes* **1999**, *48*, 1359-1364.
- [153] Haruta, T.; Uno, T.; Kawahara, J.; Takano, A.; Egawa, K.; Sharma, P. M.; Olefsky, J. M.; Kobayashi, M. *Mol. Endocrinol.* **2000**, *14*, 783-794.
- [154] Lee, A. V.; Gooch, J. L.; Oesterreich, S.; Guler, R. L.; Yee, D. *Mol. Cell. Biol.* **2000**, *20*, 1489-1496.
- [155] White, M. F. *Can. J. Physiol. Pharmacol.* **2006**, *84*, 725-737.
- [156] de Vente, J. E.; Carey, J. O.; Bryant, W. O.; Pettit, G. J.; Ways, D. K. *J. Biol. Chem.* **1996**, *271*, 32276-32280.

- [157] Rui, L.; Yuan, M.; Frantz, D.; Shoelson, S.; White, M. F. *J. Biol. Chem.* **2002**, *277*, 42394-42398.
- [158] Ozes, O. N.; Akca, H.; Mayo, L. D.; Gustin, J. A.; Maehama, T.; Dixon, J. E.; Donner, D. B. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 4640-4645.
- [159] Tzatsos, A.; Kandrak, K. V. *Mol. Cell. Biol.* **2006**, *26*, 63-76.
- [160] Ueno, M.; Carvalheira, J. B.; Tambascia, R. C.; Bezerra, R. M.; Amaral, M. E.; Carneiro, E. M.; Folli, F.; Franchini, K. G.; Saad, M. J. *Diabetologia* **2005**, *48*, 506-518.
- [161] Mordier, S.; Iynedjian, P. B. *Biochem. Biophys. Res. Commun.* **2007**, *362*, 206-211.
- [162] Khamzina, L.; Veilleux, A.; Bergeron, S.; Marette, A. *Endocrinology* **2005**, *146*, 1473-1481.
- [163] Zhande, R.; Mitchell, J. J.; Wu, J.; Sun, X. *J. Mol. Cell Biol.* **2002**, *22*, 1016-1026.
- [164] Pederson, T. M.; Kramer, D. L.; Rondinone, C. M. *Diabetes* **2001**, *50*, 24-31.
- [165] Greene, M. W.; Garofalo, R. S. *Biochemistry* **2002**, *41*, 7082-7091.
- [166] Greene, M. W.; Morrice, N.; Garofalo, R. S.; Roth, R. A. *Biochem. J.* **2004**, *378*, 105-116.
- [167] Liu, Y. F.; Paz, K.; Herschkovitz, A.; Alt, A.; Tennenbaum, T.; Sampson, S. R.; Ohba, M.; Kuroki, T.; LeRoith, D.; Zick, Y. *J. Biol. Chem.* **2001**, *276*, 14459-14465.
- [168] Inoue, G.; Cheatham, B.; Emkey, R.; Kahn, C. R. *J. Biol. Chem.* **1998**, *273*, 11548-11555.
- [169] Kim, J. K.; Kim, Y. J.; Fillmore, J. J.; Chen, Y.; Moore, I.; Lee, J.; Yuan, M.; Li, Z. W.; Karin, M.; Perret, P.; Shoelson, S. E.; Shulman, G. I. *J. Clin. Invest.* **2001**, *108*, 437-446.
- [170] Gao, Z.; Zuberi, A.; Quon, M. J.; Dong, Z.; Ye, J. *J. Biol. Chem.* **2003**, *278*, 24944-24950.
- [171] Aguirre, V.; Uchida, T.; Yenush, L.; Davis, R.; White, M. F. *J. Biol. Chem.* **2000**, *275*, 9047-9054.
- [172] Rui, L.; Aguirre, V.; Kim, J. K.; Shulman, G. I.; Lee, A.; Corbould, A.; Dunaif, A.; White, M. F. *J. Clin. Invest.* **2001**, *107*, 181-189.
- [173] Liberman, Z.; Eldar-Finkelman, H. *J. Biol. Chem.* **2005**, *280*, 4422-4428.
- [174] Waraich, R. S.; Weigert, C.; Kalbacher, H.; Hennige, A. M.; Lutz, S.; Haring, H. U.; Schleicher, E. D.; Voelter, W.; Lehmann, R. *J. Biol. Chem.* **2008**, Epub ahead of print.
- [175] Giraud, J.; Haas, M.; Feener, E. P.; Copps, K. D.; Dong, X.; Dunn, S. L.; White, M. F. *Mol. Endocrinol.* **2007**, *21*, 2294-2302.
- [176] De Fea, K.; Roth, R. A. *Biochemistry* **1997**, *36*, 12939-12947.
- [177] Li, J.; DeFea, K.; Roth, R. A. *J. Biol. Chem.* **1999**, *274*, 9351-9356.
- [178] Mothe, I.; Van Obberghen, E. *J. Biol. Chem.* **1996**, *271*, 11222-11227.
- [179] Bouzakri, K.; Roques, M.; Gual, P.; Espinosa, S.; Guebre-Egziabher, F.; Riou, J. P.; Laville, M.; Marchand-Brustel, Y.; Tanti, J. F.; Vidal, H. *Diabetes* **2003**, *52*, 1319-1325.
- [180] Gual, P.; Gremeaux, T.; Gonzalez, T.; Marchand-Brustel, Y.; Tanti, J. F. *Diabetologia* **2003**, *46*, 1532-1542.
- [181] Um, S. H.; Frigerio, F.; Watanabe, M.; Picard, F.; Joaquin, M.; Sticker, M.; Fumagalli, S.; Allegrini, P. R.; Kozma, S. C.; Auwerx, J.; Thomas, G. *Nature* **2004**, *431*, 200-205.
- [182] Li, Y.; Soos, T. J.; Li, X.; Wu, J.; Degennaro, M.; Sun, X.; Littman, D. R.; Birnbaum, M. J.; Polakiewicz, R. D. *J. Biol. Chem.* **2004**, *279*, 45304-45307.
- [183] Carlson, C. J.; White, M. F.; Rondinone, C. M. *Biochem. Biophys. Res. Commun.* **2004**, *316*, 533-539.
- [184] Shah, O. J.; Hunter, T. *Mol. Cell Biol.* **2006**, *26*, 6425-6434.
- [185] Tremblay, F.; Marette, A. *J. Biol. Chem.* **2001**, *276*, 38052-38060.
- [186] Mussig, K.; Staiger, H.; Fiedler, H.; Moeschel, K.; Beck, A.; Kellerer, M.; Haring, H. U. *J. Biol. Chem.* **2005**, *280*, 32693-32699.
- [187] Luo, M.; Reyna, S.; Wang, L.; Yi, Z.; Carroll, C.; Dong, L. Q.; Langlais, P.; Weintraub, S. T.; Mandarino, L. *J. Endocrinology* **2005**, *146*, 4410-4416.
- [188] Liu, Y. F.; Herschkovitz, A.; Boura-Halfon, S.; Ronen, D.; Paz, K.; LeRoith, D.; Zick, Y. *Mol. Cell Biol.* **2004**, *24*, 9668-9681.
- [189] Gao, Z.; Hwang, D.; Bataille, F.; Lefevre, M.; York, D.; Quon, M. J.; Ye, J. *J. Biol. Chem.* **2002**, *277*, 48115-48121.
- [190] Boden, G. *Diabetes* **1997**, *46*, 3-10.
- [191] Anderwald, C.; Brunmair, B.; Stadlbauer, K.; Krebs, M.; Fornsinn, C.; Roden, M. *Eur. J. Clin. Invest.* **2007**, *37*, 774-782.
- [192] Hotamisligil, G. S. *J. Intern. Med.* **1999**, *245*, 621-625.
- [193] Aguirre, V.; Werner, E. D.; Giraud, J.; Lee, Y. H.; Shoelson, S. E.; White, M. F. *J. Biol. Chem.* **2002**, *277*, 1531-1537.
- [194] Nakatani, Y.; Kaneto, H.; Kawamori, D.; Hatazaki, M.; Miyatsuka, T.; Matsuoka, T. a.; Kajimoto, Y.; Matsuhisa, M.; Yamasaki, Y.; Hori, M. *J. Biol. Chem.* **2004**, *279*, 45803-45809.
- [195] Herschkovitz, A.; Liu, Y. F.; Ilan, E.; Ronen, D.; Boura-Halfon, S.; Zick, Y. *J. Biol. Chem.* **2007**, *282*, 18018-18027.
- [196] Danielsson, A.; Ost, A.; Nystrom, F. H.; Stralfors, P. *J. Biol. Chem.* **2005**, *280*, 34389-34392.
- [197] Giraud, J.; Leshan, R.; Lee, Y. H.; White, M. F. *J. Biol. Chem.* **2004**, *279*, 3447-3454.
- [198] Weigert, C.; Hennige, A. M.; Lehmann, R.; Brodbeck, K.; Baumgartner, F.; Schauble, M.; Haring, H. U.; Schleicher, E. D. *J. Biol. Chem.* **2006**, *281*, 7060-7067.
- [199] Paz, K.; Liu, Y. F.; Shorer, H.; Hemi, R.; LeRoith, D.; Quan, M.; Kanety, H.; Seger, R.; Zick, Y. *J. Biol. Chem.* **1999**, *274*, 28816-28822.
- [200] Jakobsen, S. N.; Hardie, D. G.; Morrice, N.; Tornqvist, H. E. *J. Biol. Chem.* **2001**, *276*, 46912-46916.
- [201] Weigert, C.; Hennige, A. M.; Brischmann, T.; Beck, A.; Moeschel, K.; Schauble, M.; Brodbeck, K.; Haring, H. U.; Schleicher, E. D.; Lehmann, R. *J. Biol. Chem.* **2005**, *280*, 37393-37399.
- [202] Stoker, A. W. *J. Endocrinol.* **2005**, *185*, 19-33.
- [203] Egawa, K.; Maegawa, H.; Shimizu, S.; Morino, K.; Nishio, Y.; Bryer-Ash, M.; Cheung, A. T.; Kolls, J. K.; Kikkawa, R.; Kashiwagi, A. *J. Biol. Chem.* **2001**, *276*, 10207-10211.
- [204] Qiu, W.; Avramoglu, R. K.; Dube, N.; Chong, T. M.; Naples, M.; Au, C.; Sidiropoulos, K. G.; Lewis, G. F.; Cohn, J. S.; Tremblay, M. L.; Adeli, K. *Diabetes* **2004**, *53*, 3057-3066.
- [205] Gum, R. J.; Gaede, L. L.; Koterski, S. L.; Heindel, M.; Clampit, J. E.; Zinker, B. A.; Trevillyan, J. M.; Ulrich, R. G.; Jirousek, M. R.; Rondinone, C. M. *Diabetes* **2003**, *52*, 21-28.
- [206] Zinker, B. A.; Rondinone, C. M.; Trevillyan, J. M.; Gum, R. J.; Clampit, J. E.; Waring, J. F.; Xie, N.; Wilcox, D.; Jacobson, P.; Frost, L.; Kroeger, P. E.; Reilly, R. M.; Koterski, S.; Oppenorth, T. J.; Ulrich, R. G.; Crosby, S.; Butler, M.; Murray, S. F.; McKay, R. A.; Bhanot, S.; Monia, B. P.; Jirousek, M. R. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11357-11362.
- [207] Klamon, L. D.; Boss, O.; Peroni, O. D.; Kim, J. K.; Martino, J. L.; Zabolotny, J. M.; Moghal, N.; Lubkin, M.; Kim, Y. B.; Sharpe, A. H.; Stricker-Krongrad, A.; Shulman, G. I.; Neel, B. G.; Kahn, B. B. *Mol. Cell Biol.* **2000**, *20*, 5479-5489.
- [208] Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Loy, A. L.; Normandin, D.; Cheng, A.; Himms-Hagen, J.; Chan, C. C.; Ramachandran, C.; Gresser, M. J.; Tremblay, M. L.; Kennedy, B. P. *Science* **1999**, *283*, 1544-1548.
- [209] Haj, F. G.; Zabolotny, J. M.; Kim, Y. B.; Kahn, B. B.; Neel, B. G. *J. Biol. Chem.* **2005**, *280*, 15038-15046.
- [210] Park, S. Y.; Ryu, J.; Lee, W. *Exp. Mol. Med.* **2005**, *37*, 220-229.
- [211] Holt, G. D.; Hart, G. W. *J. Biol. Chem.* **1986**, *261*, 8049-8057.
- [212] D'Onofrio, M.; Starr, C. M.; Park, M. K.; Holt, G. D.; Haltiwanger, R. S.; Hart, G. W.; Hanover, J. A. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 9595-9599.
- [213] Wells, L.; Vosseller, K.; Hart, G. W. *Cell Mol. Life Sci.* **2003**, *60*, 222-228.
- [214] Hart, G. W.; Housley, M. P.; Slawson, C. *Nature* **2007**, *446*, 1017-1022.
- [215] Patti, M. E.; Virkamaki, A.; Landaker, E. J.; Kahn, C. R.; Yki-Jarvinen, H. *Diabetes* **1999**, *48*, 1562-1571.
- [216] Ball, L. E.; Berkaw, M. N.; Buse, M. G. *Mol. Cell. Proteomics* **2006**, *5*, 313-323.
- [217] Carvalho-Filho, M. A.; Ueno, M.; Hirabara, S. M.; Seabra, A. B.; Carvalheira, J. B.; de Oliveira, M. G.; Velloso, L. A.; Curi, R.; Saad, M. J. *Diabetes* **2005**, *54*, 959-967.
- [218] Carvalho-Filho, M. A.; Ueno, M.; Carvalheira, J. B.; Velloso, L. A.; Saad, M. J. *Am. J. Physiol. Endocrinol. Metab.* **2006**, *291*, E476-E482.
- [219] Sugita, H.; Fujimoto, M.; Yasukawa, T.; Shimizu, N.; Sugita, M.; Yasuhara, S.; Martyn, J. A.; Kaneki, M. *J. Biol. Chem.* **2005**, *280*, 14203-14211.
- [220] Badal, S.; Brown, P. D.; Ragoobirsingh, D. *J. Biomed. Sci.* **2006**, *13*, 561-568.
- [221] Roth, S. Y.; Denu, J. M.; Allis, C. D. *Annu. Rev. Biochem.* **2001**, *70*, 81-120.
- [222] Chen, H.; Tini, M.; Evans, R. M. *Curr. Opin. Cell Biol.* **2001**, *13*, 218-224.
- [223] Gray, S. G.; De Meys, P. *Diabetes Metab. Res. Rev.* **2005**, *21*, 416-433.
- [224] Kaiser, C.; James, S. R. *BMC Biol.* **2004**, *2*, 23.
- [225] Ide, T.; Shimano, H.; Yahagi, N.; Matsuzaka, T.; Nakakuki, M.; Yamamoto, T.; Nakagawa, Y.; Takahashi, A.; Suzuki, H.; Sone, H.; Toyoshima, H.; Fukamizu, A.; Yamada, N. *Nat. Cell Biol.* **2004**, *6*, 351-357.
- [226] Canettieri, G.; Koo, S. H.; Berdeaux, R.; Heredia, J.; Hedrick, S.; Zhang, X.; Montminy, M. *Cell Metab.* **2005**, *2*, 331-338.
- [227] Vaßen, L.; Wegrzyn, W.; Klein-Hitpass, L. *Mol. Endocrinol.* **1999**, *13*, 485-494.
- [228] Klein, H. H.; Ullmann, S.; Drenckhan, M.; Grimmismann, T.; Unthan-Fechner, K.; Probst, I. *J. Hepatol.* **2002**, *37*, 432-440.
- [229] Buren, J.; Liu, H. X.; Jensen, J.; Eriksson, J. W. *Eur. J. Endocrinol.* **2002**, *146*, 419-429.
- [230] Jhala, U. S.; Canettieri, G.; Srean, R. A.; Kulkarni, R. N.; Krajewski, S.; Reed, J.; Walker, J.; Lin, X.; White, M.; Montminy, M. *Genes Dev.* **2003**, *17*, 1575-1580.
- [231] Zhang, J.; Ou, J.; Bashmakov, Y.; Horton, J. D.; Brown, M. S.; Goldstein, J. L. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 3756-3761.
- [232] Rui, L.; Fisher, T. L.; Thomas, J.; White, M. F. *J. Biol. Chem.* **2001**, *276*, 40362-40367.
- [233] Ueki, K.; Kondo, T.; Kahn, C. R. *Mol. Cell Biol.* **2004**, *24*, 5434-5446.
- [234] Jamieson, E.; Chong, M. M. W.; Steinberg, G. R.; Jovanovska, V.; Fam, B. C.; Bullen, D. V. R.; Chen, Y.; Kemp, B. E.; Proietto, J.; Kay, T. W. H.; Andrikopoulos, S. *J. Biol. Chem.* **2005**, *280*, 31516-31521.
- [235] Guo, S.; Dunn, S. L.; White, M. F. *Mol. Endocrinol.* **2006**, *20*, 3389-3399.
- [236] Solinas, G.; Naugler, W.; Galimi, F.; Lee, M. S.; Karin, M. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 16454-16459.
- [237] Sharfi, H.; Eldar-Finkelman, H. *Am. J. Physiol. Endocrinol. Metab.* **2008**, *294*, E307-E315.
- [238] Lee, S.; Lynn, E. G.; Kim, J. A.; Quon, M. J. *Endocrinology* **2008**, Epub ahead of print.
- [239] Zhang, J. *J. Biol. Chem.* **2007**, *282*, 34356-34364.
- [240] Costacou, T.; Mayer-Davis, E. *J. Ann. Rev. Nutr.* **2003**, *23*, 147-170.
- [241] Steyn, N. P.; Mann, J.; Bennett, P. H.; Temple, N.; Zimmet, P.; Tuomilehto, J.; Lindstrom, J.; Louheranta, A. *Public Health Nutr.* **2004**, *7*, 147-165.

- [242] Lakka, T. A.; Laaksonen, D. E. *Appl. Physiol. Nutr. Metab.* **2007**, *32*, 76-88.
- [243] Astrup, A. *Public Health Nutr.* **2001**, *4*, 499-515.
- [244] Bonen, A.; Dohm, G. L.; van Loon, L. J. *Essays Biochem.* **2006**, *42*, 47-59.
- [245] Kelley, D. E.; Goodpaster, B. H. *Med. Sci. Sports Exerc.* **1999**, *31*, S619-S623.
- [246] Schafer, S.; Kantartzis, K.; Machann, J.; Venter, C.; Niess, A.; Schick, F.; Machicao, F.; Haring, H. U.; Fritsche, A.; Stefan, N. *Eur. J. Clin. Invest.* **2007**, *37*, 535-543.
- [247] Xue, B.; Kim, Y. B.; Lee, A.; Toschi, E.; Bonner-Weir, S.; Kahn, C. R.; Neel, B. G.; Kahn, B. B. *J. Biol. Chem.* **2007**, *282*, 23829-23840.
- [248] Goldstein, B. J. *J. Clin. Endocrinol. Metab.* **2002**, *87*, 2474-2480.
- [249] Lee, S.; Wang, Q. *Med. Res. Rev.* **2007**, *27*, 553-573.
- [250] Vats, R. K.; Kumar, V.; Kothari, A.; Mital, A.; Ramachandran, U. *Curr. Sci.* **2005**, *88*, 241-249.
- [251] Waring, J. F.; Ciurlionis, R.; Clampit, J. E.; Morgan, S.; Gum, R. J.; Jolly, R. A.; Kroeger, P.; Frost, L.; Trevillyan, J.; Zinker, B. A.; Jirousek, M.; Ulrich, R. G.; Rondinone, C. M. *Mol. Cell Endocrinol.* **2003**, *203*, 155-168.
- [252] Liu, G. *Curr. Opin. Mol. Ther.* **2004**, *6*, 331-336.
- [253] Pandey, S. K.; Yu, X. X.; Watts, L. M.; Michael, M. D.; Sloop, K. W.; Rivard, A. R.; Leedom, T. A.; Manchem, V. P.; Samadzadeh, L.; McKay, R. A.; Monia, B. P.; Bhanot, S. J. *J. Biol. Chem.* **2007**, *282*, 14291-14299.
- [254] Diamant, M.; Heine, R. J. *Drugs* **2003**, *63*, 1373-1405.
- [255] Yki-Jarvinen, H. *N. Eng. J. Med.* **2004**, *351*, 1106-1118.
- [256] Kanatani, Y.; Usui, I.; Ishizuka, K.; Bukhari, A.; Fujisaka, S.; Urakaze, M.; Haruta, T.; Kishimoto, T.; Naka, T.; Kobayashi, M. *Diabetes* **2007**, *56*, 795-803.
- [257] Ueki, K.; Kondo, T.; Tseng, Y. H.; Kahn, C. R. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 10422-10427.
- [258] Torisu, T.; Sato, N.; Yoshiga, D.; Kobayashi, T.; Yoshioka, T.; Mori, H.; Iida, M.; Yoshimura, A. *Genes Cells* **2007**, *12*, 143-154.
- [259] Gallwitz, B. *Rev. Diabet. Stud.* **2005**, *2*, 61-69.
- [260] Zander, M.; Madsbad, S.; Madsen, J. L.; Holst, J. J. *Lancet* **2002**, *359*, 824-830.
- [261] Drucker, D. J. *Mol. Endocrinol.* **2003**, *17*, 161-171.
- [262] Boyle, P. J.; Freeman, J. S. *J. Am. Osteopath. Assoc.* **2007**, *107*, S10-S16.
- [263] Deacon, C. F.; Nauck, M. A.; Toft-Nielsen, M.; Pridal, L.; Willms, B.; Holst, J. J. *Diabetes* **1995**, *44*, 1126-1131.
- [264] Lee, Y. S.; Shin, S.; Shigihara, T.; Hahm, E.; Liu, M. J.; Han, J.; Yoon, J. W.; Jun, H. S. *Diabetes* **2007**, *56*, 1671-1679.
- [265] Sykiotis, G. P.; Papavassiliou, A. G. *Mol. Endocrinol.* **2001**, *15*, 1864-1869.
- [266] Gao, Z.; Zuberi, A.; Quon, M. J.; Dong, Z.; Ye, J. *J. Biol. Chem.* **2003**, *278*, 24944-24950.
- [267] Bennett, B. L.; Satoh, Y.; Lewis, A. J. *Curr. Opin. Pharm.* **2003**, *3*, 420-425.

Received: February 03, 2008

Revised: March 01, 2008

Accepted: March 03, 2008