The role of adipocyte CREB in insulin resistance

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Abstract

Obesity is a major public health problem. Dysregulation of lipid metabolism can result in an increase in plasma FFAs and lipid accumulation. Eventually these changes could lead to dyslipidemia, impaired glucose tolerance and insulin resistance, ultimately contributing to the development of type 2 diabetes mellitus. However, the role of the adipose tissue in insulin resistance is unclear. Therefore we would like to discuss the role of adipocyte CREB in insulin resistance. CREB is a cAMP-responsive activator which promotes cellular gene transcription. Together with its coactivators CBP/p300 and TORC2/CRTC2, CREB induces gluconeogenic gene expression in the adipose tissue and the liver. Under obese conditions, CREB is activated, leading to a decrease in the glucose uptake in the adipocytes and a decrease in adiponectin levels, resulting in hyperglycaemia and an increase in FFA levels, enhancing insulin resistance. Further analysis of the CREB pathway and studies of the CBP/p300 and the TORC2/CRTC2 pathway could provide more insight into the hormone regulated pathways in adipocytes under obese conditions. This could eventually lead to the development of a specifically targeted medicine and a better treatment of type 2 diabetes mellitus in obesity.

Introduction

Overweight and obesity are a major public health problem and the prevalence of overweight and obesity increases worldwide. This leads to an increase in obesity-associated disorders, such as hypertension, dyslipidemia, impaired glucose tolerance and insulin resistance[1,2]. These disturbances are also known as components of the metabolic syndrome and they are risk factors for the development of type 2 diabetes mellitus and cardiovascular disease[2,3]. Mainly abdominal obesity or an increase in visceral fat mass is strongly associated with the metabolic risk factors[2,4].

Lipid metabolism in adipose tissue

In mammals, two types of adipose tissue can be found, brown adipose tissue (BAT) and white adipose tissue (WAT). The main function of BAT is heat production and the main function of WAT is energy storage[5]. In this work we would like to focus on WAT.

WAT has an endocrine and metabolic function. The tissue contains several cell types, like fibroblasts and macrophages, but it is primarily composed of adipocytes. They synthesize and release adipocytokines, like adiponectin, resistin, plasminogen activator inhibitor-1 (PAI-1), tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6), leptin and monocyte chemotactic protein-1 (MCP-1)[2-4,6,7].

The main metabolic role of the adipose tissue is the storage of lipids in the body. Lipid metabolism involves the uptake of free fatty acids (FFA) by the adipocyte and the subsequential triacylglycerol (TAG) synthesis. In times of energy demand the process of lipolysis is initiated. The hydrolysis of TAGs occurs, leading to the release of FFA and glycerol from the adipocyte. Lipid metabolism is well regulated by hormones like catecholamines, glucagon and insulin[4].

Hormone regulated lipid metabolism and CREB activity in adipocytes

Insulin is an antilipolytic hormone and has its effect on adipocytes in the adipose tissue. The hormone is synthesized in the pancreas within the beta cells of the islets of Langerhans. It is able to bind the insulin receptor, expressed on the plasma membrane of the adipocyte. This binding leads to the phosphorylation of a serine/threonine protein kinase, protein kinase B/Akt2 (PKB/Akt2)[8,9]. This leads to a decrease in cyclic AMP (cAMP) levels and reduced activity of the cAMP-dependent protein kinase A (PKA). Ultimately, TAG hydrolysis is inhibited and there is a decrease in the release of FFAs and glycerol from the adipocyte. The phosphorylation of PKB/Akt2 also stimulates the translocation of the glucose transporter type 4 (GLUT4) from intracellular storage sites to the plasma membrane inducing glucose transport. These signalling processes, present in adipocytes, play a key role in glucose homeostasis and lipid metabolism[4] (figure 1).

Catecholamines, such as epinephrine and norepinephrine stimulate lipolysis. The hormones bind the beta-adrenergic receptor on the adipocyte membrane. This receptor is coupled to a stimulatory G-protein complex (Gs-coupled receptor), which is able to stimulate adenylate cyclase, leading to an increase of cAMP levels in the adipocyte[10,11]. Eventually, this results in the hydrolysis of TAGs and the release of FFAs and glycerol from the adipocytes[4,12].

Glucagon is a counter-regulator to insulin and is the second major glucose related hormone. It is produced by the pancreas within the alpha cells of the islets of Langerhans. Glucagon is able to bind to the Gs-coupled receptor in the plasma membrane, activating adenylate cyclase. This leads to an increase in cAMP levels and the subsequential lipolysis in adipocytes[13].

The activation of PKA in the adipocyte also induces the phosphorylation of the cAMP response element binding protein (CREB), enhancing CREB-dependent transcription.

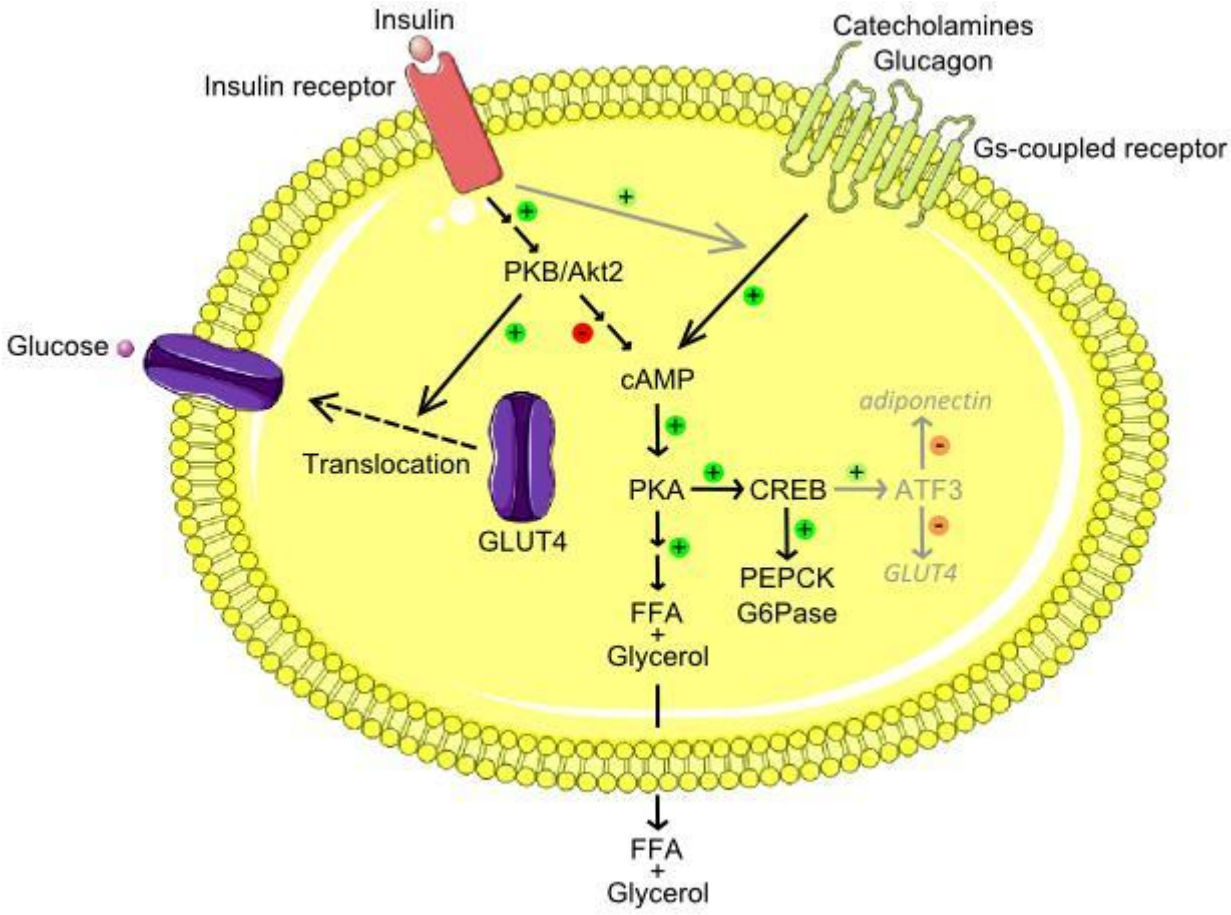

Figure 1. Schematic representation of hormone regulated lipid metabolism and CREB activity in an adipocyte.

Dysregulation of lipid metabolism can result in an increase in plasma FFAs and lipid accumulation in the liver, muscle and pancreatic islets. Eventually these changes could lead to dyslipidemia, impaired glucose tolerance and insulin resistance, ultimately contributing to the development of type 2 diabetes mellitus[2,6,14].

Obesity is associated with these metabolic disturbances, but loss of adipose tissue in patients with lipodystrophy also is accompanied by insulin resistance[15,16]. Therefore, adipose tissue plays a key role in the maintenance of systemic glucose metabolism.

However, the role of the adipose tissue in insulin resistance is unclear. CREB is a key protein present in the insulin signalling pathway and important for the regulation of gluconeogenesis. Qi *et al*. discovered that CREB in adipocytes promotes insulin resistance under obese conditions[17]. Therefore we would like to discuss the role of adipocyte CREB in insulin resistance.

Role of adipose tissue in insulin resistance

The CREB family

Qi *et al*. discovered that adipocyte CREB promotes insulin resistance in obesity[17]. CREB, a 43-kDa phosphoprotein, is a cAMP-responsive activator which promotes cellular gene expression[18].

Hormones, like glucagon and catecholamines are able to stimulate second messenger pathways such as the cAMP-dependent pathway[19,20]. cAMP controls the cAMP-dependent PKA. Upon phosphorylation by a cAMP-dependent protein kinase like PKA, CREB is able to bind to the cAMP response element (CRE) present in the promoter region of a target gene. The CRE is an eight-base-pair palindromic element, TGACGTCA and binding of CREB regulates the transcription of the downstream genes[19-21].

In addition to the characterization of CREB, two family members were characterized. Activating transcription factor-1 (ATF-1) and cAMP response element modulater (CREM) also belong to the CREB family[22]. The primary structure of ATF-1 is 65% identical to CREB and the genetic sequence of CREM is very similar to the CREB sequence[21]. CREB and ATF-1 are also expressed ubiquitously[23].

CREB

Several forms of CREB are known, but the main isoforms of CREB are CREB-alpha, CREB-delta and CREB-beta. CREB-alpha is the longest isoform with 341 residues. Due to alternative splicing, the CREB-alpha isoform contains an alpha-peptide consisting of 14 residues. CREB-delta lacks this insert, creating a shorter isoform, the 327-residue protein. These CREB isoforms are present in human, rat and mouse tissue, where they are uniformly expressed[20,24-26]. In 1996 a new isoform of CREB was identified in mice by Blendy *et al.*, CREB-beta. This isoform is also generated by alternative splicing and lacks the first 40 residues compared to CREB-alpha and CREB-delta. This isoform is also expressed ubiquitously[27]. The numbering of specific residues mentioned hereafter refers to the position in CREB-alpha.

The principal domains of CREB are the hydrophobic glutamine rich Q1 and Q2 constitutive activation domains, the kinase-inducible domain (KID) and the basic leucine zipper (bZIP) domain, which are shown in figure 2a[23,28].

As mentioned previously, CREB is regulated by phosphorylation. The KID of CREB contains a PKA phosphorylation site, a serine residue at position 133 (S133)[23]. Increased levels of cAMP lead to the activation of PKA. The cAMP-dependent kinase is able to phosphorylate CREB at the serine residue at position 133[18]. This phosphorylation is required for the binding of other regulatory proteins[20], since CREB requires the binding of other transcription factors to induce cellular gene expression in response to cAMP[18]. These regulatory proteins are discussed below.

The bZIP domain of CREB is required for DNA binding. The leucine zipper consists of a repeat of leucine residues at the C-terminus of CREB. The basic domain consists of a positively charged region (lysine- and arginine residues) amino-terminal to the leucine repeats or the leucine zipper. The leucine zipper in the bZIP domain at the C-terminus is essential for dimerization of CREB and the basic domain is required for specific DNA binding properties[20,29,30]. The basic domain and the leucine zipper together are the bZIP domain. This domain mediates the DNA interaction and the dimerization of CREB and the subsequent binding of CREB as a dimer to the CRE[20,23]. The bZIP domain also contains an arginine

residue at position 314 (R314). The importance of this residue will be discussed below, but interesting is that the amino acid sequence alignment of CREBs revealed that the serine at position 133 (S133) and the arginine at position 314 (R314) in CREB-alpha lies within a region that is highly conserved between species (figure 2a).

CBP/p300

The CREB binding protein (CBP) is one of the proteins that is able to bind to CREB, thereby enhancing the transcription activation by CREB.

CBP is encoded by the *Crebbp* gene[31] and p300 is a protein that is encoded by the *Ep300* gene. These proteins are highly related encoding a histone acetyltransferase domain and protein binding domains[32-34]. The histone acetyltransferase activity of CBP/p300 leads to a weaker binding of histones to DNA. DNA becomes more accessible for transcription factors, like CREB, thereby increasing the transcriptional activity.

CBP/p300 contains the KIX domain. Upon S133 phosphorylation in the KID of CREB, the association with the KIX domain of CBP/p300 is enhanced[35]. This phosphorylation is required for the binding of the KIX domain of CBP/p300[28] (figure 2a/b).

TORC2/CRTC2

Another regulatory protein that is able to bind to CREB is transducer of regulated CREB 2/CREB regulated transcription coactivator 2 or TORC2/CRTC2. Transcription activation is enhanced by TORC2/CRTC2[36,37].

Phosphorylated TORC2/CRTC2 is sequestered in the cytoplasm. The stimulation of cAMP pathways by hormones like glucagon and catecholamines, induces dephosphorylation of TORC2/CRTC2 and the translocation into the nucleus. Here, it is able to bind the arginine residue at position 314 present in the bZIP domain of CREB with its coiled-coil domain (figure 2a/b)[36].

In contrast to CBP/p300, TORC recruitment does not modulate the interaction of CREB with DNA, leading to enhanced transcriptional activity. Conkright *et al*. has shown that TORCs enhance the interaction of CREB with the TATA-binding protein (TBP)-associated factor 130 (TAF130), a component of the TFIID complex[36]. These proteins are part of the basal transcription machinery, which will be discussed below.

TORC2/CRTC2 is also able to associate with CBP/p300 and the CBP/p300:TORC2/CRTC2 complex mediates CRE-dependent transcription by binding to CREB[38]. CBP/p300 is required for the recruitment of TORC2/CRTC2 to the promoter[35].

Basal transcription machinery

As mentioned above, hormonal stimuli lead to the increase of the intracellular level of cAMP. cAMP stimulates PKA causing the phosphorylation of S133 in the KID of CREB. The phosphorylation of S133 makes it possible for CBP/p300 to interact with CREB. This interaction occurs via the KIX domain of CBP/p300[20,28].

TORC2/CRTC2 is able to bind the R314 in the bZIP domain of CREB and it is able to associate with CBP/p300. Upon binding to CREB, the complex of CBP/300, TORC2/CRTC2 and CREB is recruited to the promoter region of the target gene. Here, the bZIP domain of CREB is able to bind to the CRE region on the promoter (figure 2b). Nevertheless, gene transcription requires the presence of other proteins[20,28].

To induce gene expression, CREB associates with a general multi-component transcription factor, TFIID. The TFIID complex consist of a TATA-binding protein (TBP) and TBP-associated factors (TAFs). TFIID is able to bind the TATA box on the promoter via the TBP. The Q2 domain of CREB associates with the TBP-associated factor 130 (TAF130), a component of the TFIID complex[39].

TFIID is also able to interact with TFIIB, another transcription factor. TFIIB is also able to associate with CREB via the Q2 domain[20,39].

The presence of RNA polymerase II (Pol II) is required for the gene transcription and the production of messenger RNA (mRNA). To stabilize the interaction with Pol II, CREB uses CBP and RNA helicase A (RHA)[21,39,40]. The relevant transcription machinery is shown in figure 2b.

Gluconeogenic genes

CREB promotes cellular gene expression. In 1987 it was discovered as a transcription factor regulating somatostatin gene transcription[19]. Now, many genes regulated by CREB are identified including c-Fos, cytochrome *c,* vasopressin and brain-derived neurotrophic factor[21].

CREB is also a key regulator of gluconeogenic gene expression. CREB binding sites are located on promoter regions of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase)[41]. Both PEPCK and G6Pase are important enzymes in the metabolic pathway of gluconeogenesis[42,43].

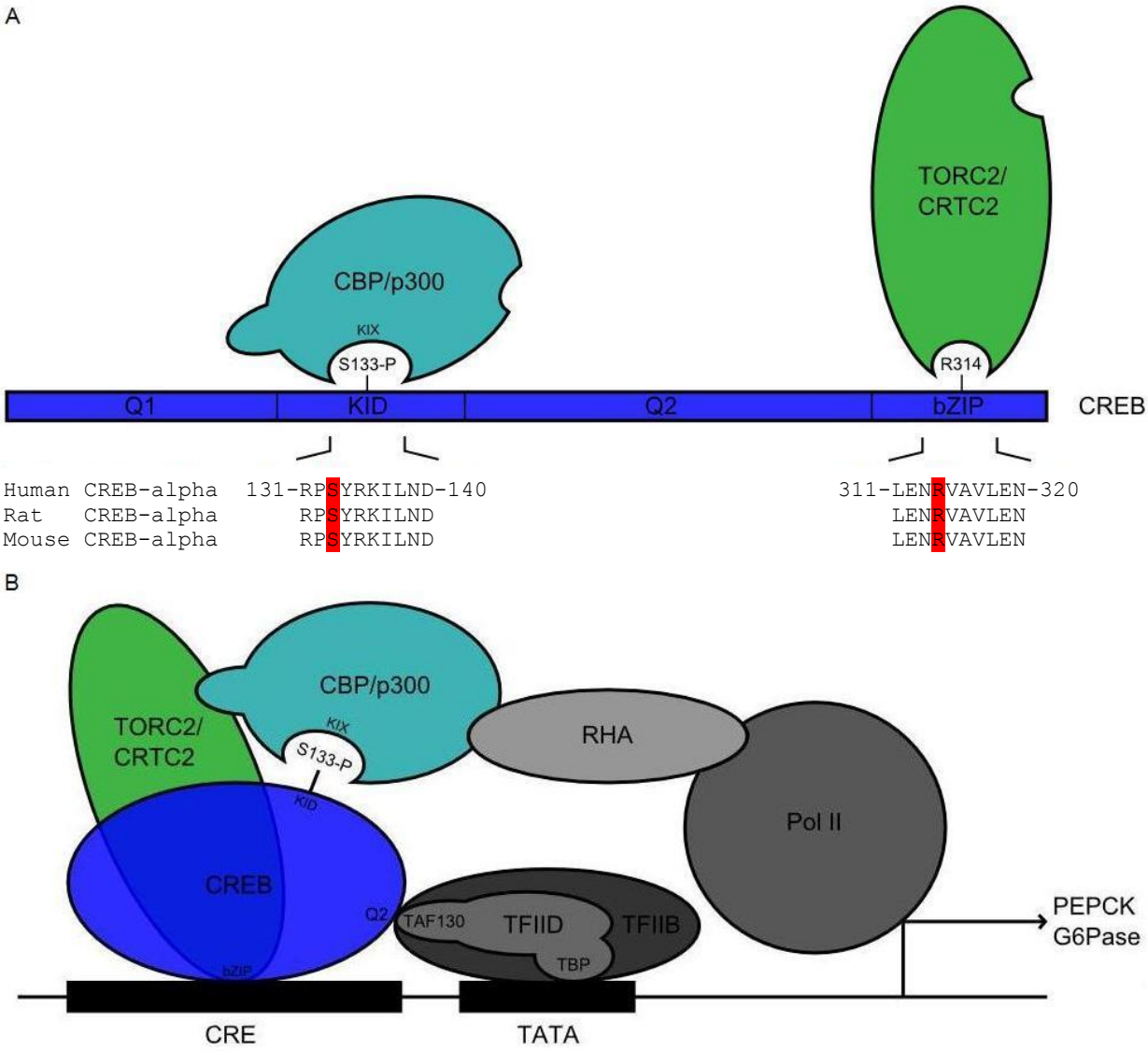

Figure 2. cAMP response element binding protein (CREB). (A) Schematic representation of the principal domains of CREB and the interaction with CBP/p300 and TORC2/CRTC2 together with the alignment of the amino acid sequence surrounding S133 and R314 in human CREB alpha, rat CREB alpha and mouse CREB alpha. **(B)** Schematic representation of the relevant transcription machinery.

Dysregulation lipid metabolism and CREB activity in adipocytes under obese conditions

Visceral obesity is strongly associated with insulin resistance. Weight gain leads to the increase of lipid stored in the adipocytes (an increase in adiposity). This causes dysregulation of lipid metabolism in the adipocytes, leading to an increase in circulating FFA. The increase in systemic FFA levels induces gluconeogenesis in the liver and leads to an overload of lipids in liver, muscle and pancreatic islets, also known as lipotoxicity. Another metabolic complication is the altered production of adipocytokines. Due to the increased release of TNF-alpha from the adipose tissue, the adipocytes become resistant to insulin[2,4].

During a fed state, insulin is produced by the beta cells in the islets of Langerhans. When the adipocytes become resistant to insulin, insulin is able to bind to the insulin receptor, but the response of the adipocyte to this binding is decreased. The insulin receptor pathway activation is impaired and PKB/Akt2 is not phosphorylated. This leads to impaired inhibition of cAMP. The cAMP levels increase, PKA is stimulated and the release of FFA and glycerol from the adipocyte increases, even though there is no energy requirement. These processes lead to insulin resistance in liver and muscle[4] and increased plasma levels of insulin. Since PKB/Akt2 is not phosphorylated , the translocation of GLUT4 in the adipocyte and in muscle does not occur and systemic glucose levels increase (figure 3)[2,3,14,44].

During chronic hyperinsulinaemic conditions, supersensitization of Gs-associated signalling occurs, enhancing cAMP production via insulin and the insulin receptor[45,46]. This leads to an increased stimulation of PKA and increased release of FFA and glycerol. In figure 4a (adapted from Qi *et al*., 2009) is in fact shown that CREB phosphorylation at S133 (P-CREB) is increased in adipocytes from high fat diet (HFD)-fed mice and genetically obese (*db/db*) mice compared to resp. normal chow (NC)-fed mice and lean controls[17].

The activation of the cAMP-dependent kinase also leads to increased phosphorylation of CREB, enhanced gene transcription and increased gluconeogenesis (figure 3).

Qi *et al*. also generated transgenic mice that express ACREB, the F-ACREB mice. This dominant-negative CREB inhibitor heterodimerizes with CREB leading to disrupted binding of CREB to DNA, thereby inhibiting CREB-mediated transcription. To determine the role of CREB activation under obese conditions, the transgenic mice were either fed a HFD or were bred onto a genetically obese (*ob/ob)* background. The F-ACREB mice blood glucose concentrations during fasting conditions were decreased compared to the control mice (figure 4b). The glucose levels during the glucose (GTT) and insulin (ITT) tolerance test were significantly decreased in the F-ACREB mice compared to the control mice after 9.5 weeks of HFD feeding (figure 4c). These results indicate that the insulin sensitivity is improved under obese conditions with reduced CREB activity in the adipocytes[17].

Qi *et al*. also discovered that the target gene of CREB, the gene of the transcriptional repressor ATF3, is upregulated in WAT from HFD-fed mice compared to NC-fed mice (figure 5a). *ATF3* mRNA and protein amounts were increased in WAT from HFD-fed and obese *ob/ob* mice (figure 5b)[17].

ATF3 is a member of the ATF/CREB family and it functions by binding to ATF/CRE sites[47]. The ATF3 promoter contains a conserved CRE site. However, ATF3 represses transcription from promoters with ATF/CRE sites[47].

The activation of CREB in adipose tissue under obese conditions leads to the induction of ATF3 and the subsequent downregulation of GLUT4 and adiponectin expression. The decrease in GLUT4 leads to a decrease in glucose uptake and further increase of systemic glucose levels occurs. The downregulation of adiponectin affects the gluconeogenic program

and beta-oxidation in the liver. Ultimately, CREB in adipocytes promotes insulin resistance under obese conditions[17].

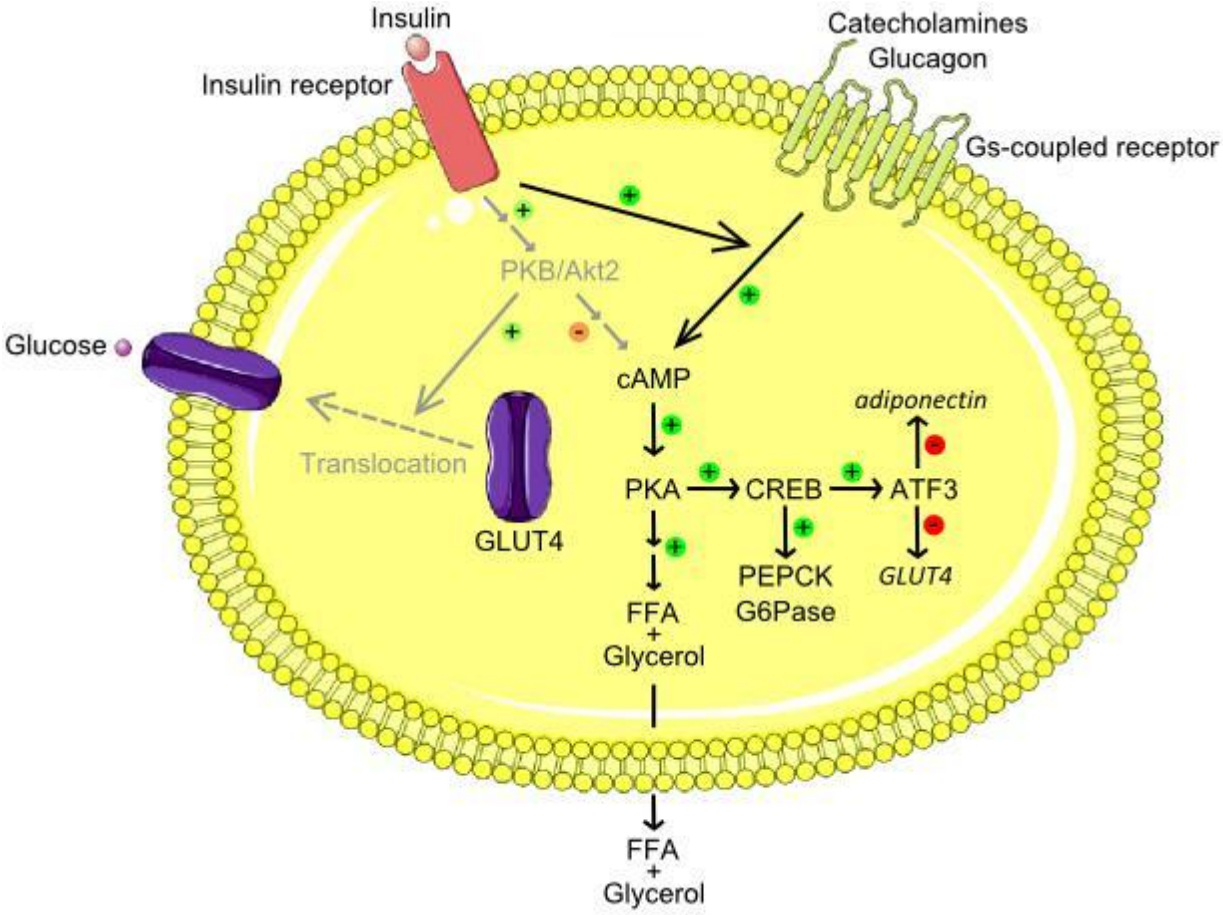

Figure 3. Schematic representation of dysregulated lipid metabolism and CREB activity in an adipocyte under obese conditions.

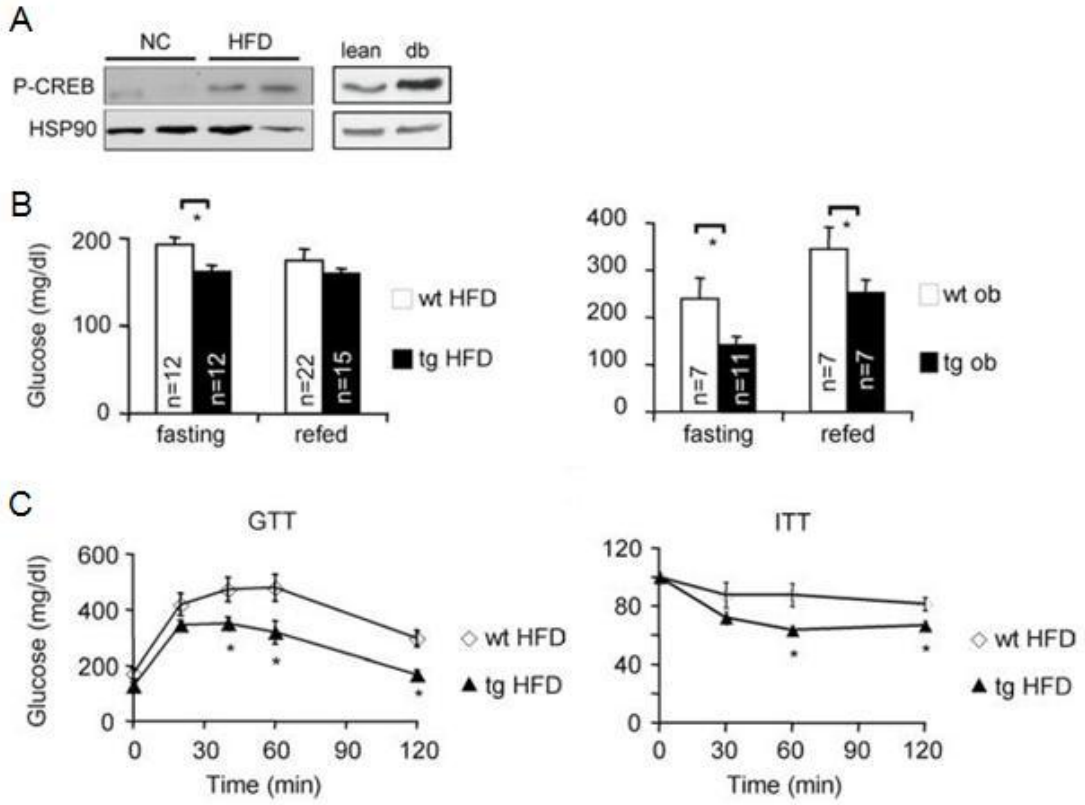

Figure 4. Mice with reduced adipocyte CREB activity remain insulin sensitive under obese conditions. (A) Left shows relative amounts of P-CREB in adipocytes from NC-fed mice and HFD-fed mice. Right shows relative amounts of P-CREB in adipocytes from lean or genetically obese (*db/db*) mice. **(B)** Relative circulating blood glucose concentrations during fasting or refed conditions in control (WT) and F-ACREB (tg) mice under HFD (left) and genetically obese (*ob/ob*) (right) conditions. **(C)** Relative blood glucose concentrations during glucose (GTT, left) and insulin (ITT, right) tolerance test in control (WT) and F-ACREB (tg) mice under HFD conditions for 9.5 weeks. Adapted from Qi *et al*., 2009.

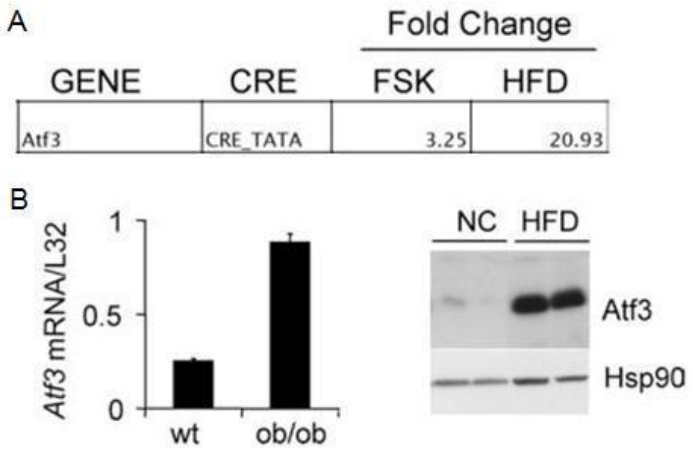

Figure 5. CREB stimulates the expression of ATF3 in adipose tissue under obese conditions. (A) Gene profiling study showing the upregulation of CREB target genes following exposure to FSK and in WAT harvested from HFD- compared to NC-fed mice. **(B)** Left, relative *ATF3* mRNA amounts in WAT from wt and ob/ob mice. Right, ATF3 protein amounts in WAT from NC- or HFD-fed wt mice. Adapted from Qi *et al*., 2009.

Effect of dysregulation CREB activity in adipocyte on adiponectin signalling pathway in liver

The increase in adiposity also affects the levels of adiponectin. Adiponectin is expressed in adipocytes. It is able to bind to the Gs-protein coupled receptor in the membrane of the hepatocyte. Upon this binding AMP-activated protein kinase (AMPK) is activated. AMPK achieves its downstream effect by stimulating PKB/Akt. PKB/Atk is able to phosphorylate and inactivate glycogen synthase kinase 3-beta (GSK3-beta). This phosphorylation leads to reduced phosphorylation of CREB and reduced transcription of PEPCK and G6Pase[48]. AMPK also inhibits the transcriptional activity of CREB by phosphorylation of TORC2/CRTC2, inhibiting the nuclear entry of the CREB coactivator[49]. Adiponectin also mediates beta-oxidation through the activation of AMPK and peroxisome proliferator-activated receptor-alpha (PPAR-alpha)[50,51]. Adiponectin affects the gluconeogenesis through these signalling pathways (figure 6)[52-54].

It is also known that adiponectin increases insulin sensitivity[55-57] and the level of adiponectin is correlated to the severity of insulin resistance in liver and muscle. In obesity, the levels of adiponectin are decreased[4]. The activation of CREB in adipose tissue under obese conditions also leads to the induction of ATF3 and the subsequent downregulation of adiponectin expression[17]. This causes an increase in gluconeogenesis and a decrease in beta-oxidation leading to increased plasma glucose levels and FFA levels. Eventually, this results in insulin resistance.

Effect of insulin resistance on hormone regulated signalling pathways in hepatocytes

Insulin affects the hepatocytes too. Binding of insulin to the insulin receptor in the plasma membrane of the hepatocyte leads to the activation of PKB/Akt2 and the subsequent inhibition of cAMP. Eventually, this results in impaired CREB gene transcription and reduced gluconeogenesis (figure 7).

Insulin also antagonizes the transcriptional activity of CREB by inhibiting the binding of two coactivators, CBP[58,59] and TORC2/CRTC2[60]. Activation of the insulin receptor leads to the phosphorylation of CBP at the serine residue at position 436, inhibiting the CBP:CREB interaction and decreasing gluconeogenesis.
Furthermore, insulin stimulates the degradation of TORC2/CRTC2. PKB/Akt2 is able to phosphorylate a member of the AMPK family, salt-inducible kinase 2 (SIK2) at S358[60-63]. This Ser/Thr kinase phosphorylates S171 of TORC2/CRTC2 resulting in the ubiquitin-dependent degradation of the CREB coactivator[64-66], thereby inhibiting hepatic glucose production.

In the insulin resistant state, the PKB/Akt2 pathway is not stimulated, leading to decreased inhibition of cAMP and increased CREB activity. S358 of SIK2 does not become phosphorylated by PKB/Akt2 causing reduced TORC2/CRTC2 phosphorylation and increased CREB binding. CBP phosphorylation is also decreased, leading to increased CBP:CREB interaction. This results in increased CREB activity and a stimulation of gluconeogenic gene expression. The blood glucose concentrations levels rise, eventually leading to insulin resistance.

Glucagon is able to antagonize the effects of insulin by binding to the Gs-coupled receptor that activates PKA via cAMP. PKA is able to inactivate SIK2 by phosphorylation at S587, thereby reducing TORC2/CRTC2 phosphorylation and degradation[61]. Unphosphorylated TORC2/CRTC2 is then able to translocate to the nucleus, where it is able to bind to CREB[67,68]. Glucagon is also able to dephosphorylate TORC2/CRTC2 leading to translocation to the nucleus[60].

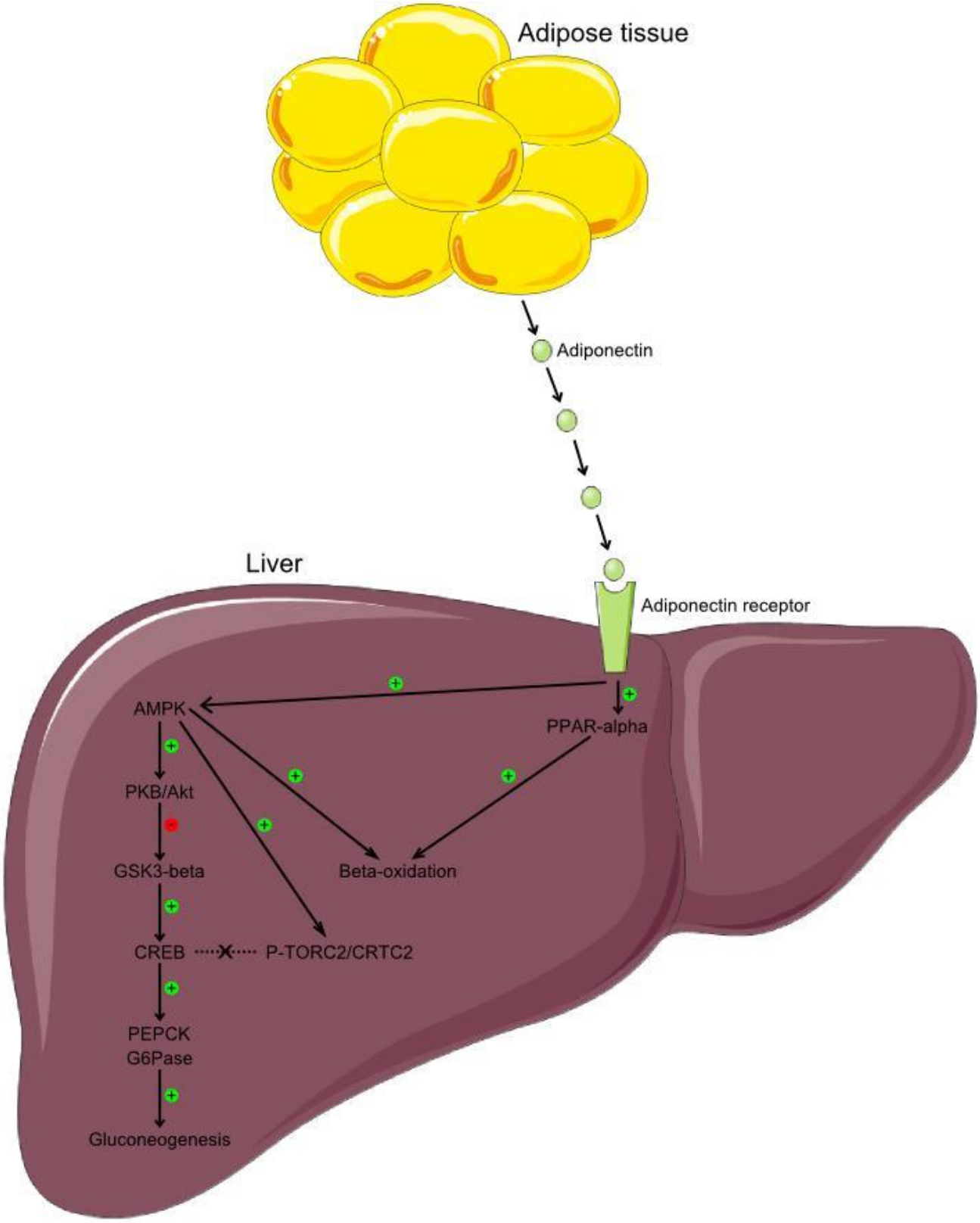

Figure 6. Schematic representation of the adiponectin signalling pathway in the liver.

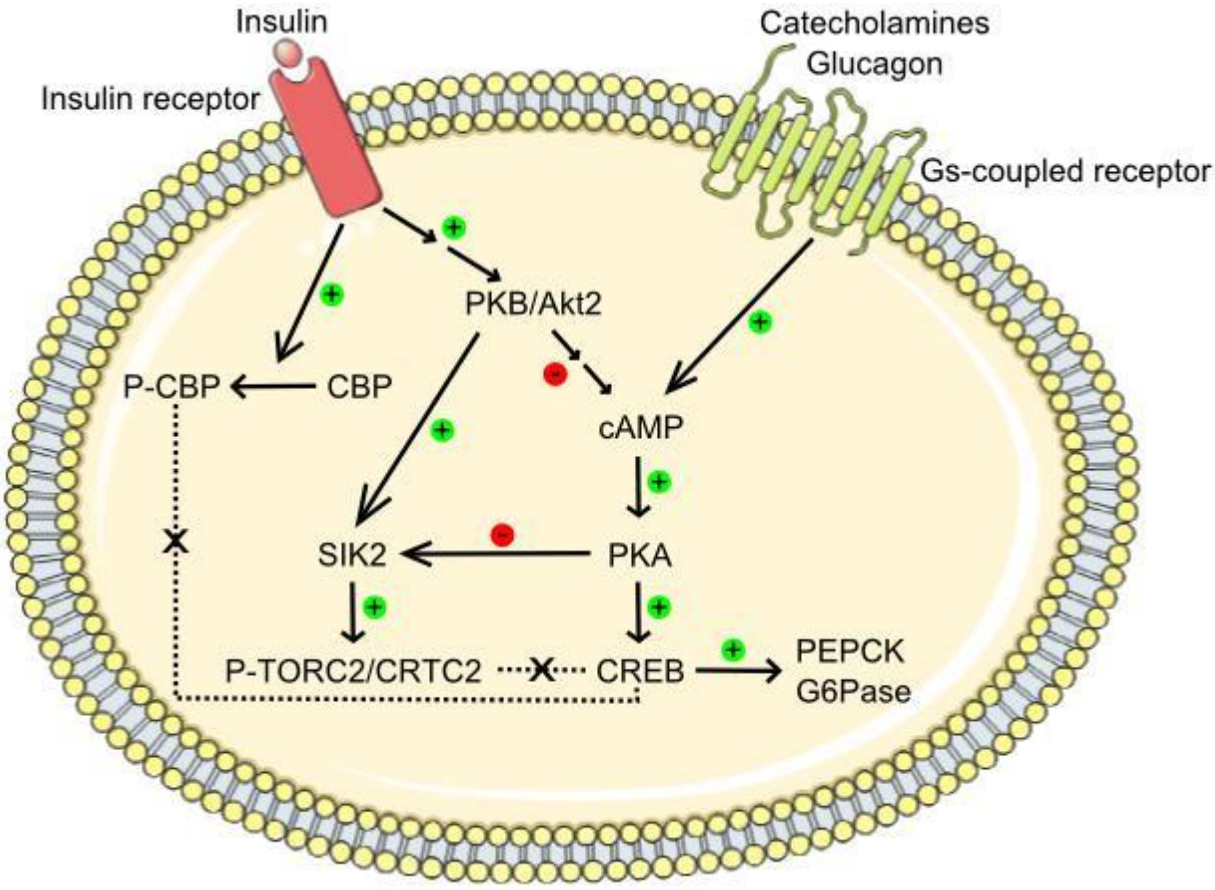

Figure 7. Schematic representation of hormone regulated signalling pathways in a hepatocyte.

Discussion

Insulin resistance is associated with obesity and a major risk factor for type 2 diabetes mellitus and cardiovascular diseases. CREB plays an important role in the development of insulin resistance in obesity. Therefore we would like to discuss the role of adipocyte CREB in insulin resistance.

CREB is present in the insulin signalling pathways in adipocytes and hepatocytes. It stimulates gluconeogenesis in adipocytes under non-obese conditions. Qi *et al.* showed that under obese conditions CREB is able to induce ATF3 gene transcription resulting in a decrease in GLUT4 and adiponectin expression. This decrease in GLUT4 expression leads to an impaired glucose uptake by the adipocyte resulting in hyperglycaemia. Adiponectin stimulates beta-oxidation and inhibits gluconeogenesis in the liver. Therefore, a decrease in adiponectin leads to an increase in FFA levels and an increase in plasma glucose levels. Due to the rise in plasma glucose levels, the pancreatic beta cells produce more insulin. However, the insulin levels are not sufficient to maintain normal glucose levels. Eventually, this could lead to dyslipidemia, impaired glucose tolerance and insulin resistance.

The activation of the PKB/Akt2 pathway in the adipocyte is reduced in the insulin resistant state leading to impaired inhibition of cAMP levels. This results in an increase in TAG hydrolysis and an increase in gluconeogenic gene expression. In contradiction, the catecholamine effects via the beta-adrenergic receptor in the adipocyte membrane are potentiated in the insulin resistant state. This leads to increased levels of FFA and enhanced CREB phosphorylation. The plasma glucose levels rise and eventually type 2 diabetes mellitus develops. However, the mechanism inducing this potentiation of the catecholamine effects on cAMP is unclear. It would be interesting to research the exact mechanism, since this affects the cAMP pathway and subsequently it affects lipid metabolism, gluconeogenic gene expression and the development of insulin resistance.

CREB is also present in the hepatocytes and similar to the adipocytes, the activation of the PKB/Akt2 pathway in the adipocyte is reduced in the insulin resistant state, leading to increased CREB dependent transcription of PEPCK and G6Pase.

In addition, insulin is able to inhibit the CREB coactivators CBP and TORC2/CRTC2 in the hepatocyte. Insulin induces phosphorylation of both proteins, inhibiting the interaction with CREB and the subsequential gluconeogenic gene transcription. In the insulin resistant state, the inhibition of the coactivators in the hepatocytes is decreased, leading to increased activation of CREB and increased transcription of gluconeogenic genes.

In this work we focused on the role of CREB in insulin resistance, but it would also be interesting to look at the role of TORCs and CBP in insulin resistance. They modulate the metabolic regulation via CREB, but it could be possible that they alter the function of other transcription factors in hormone regulated lipid metabolism. TORC2/CRTC2 is activated in diabetes inducing hyperglycemia[64]. It would be interesting to research the inhibition of SIKs, which inhibit TORCs or the TORC pathway by using a TORC knockout mouse. We have seen that SIK2 is present in hepatocytes, but it is also present in adipocytes and the phosphorylation of TORC2/CRTC2 by SIK2 in adipocytes is shown by Muraoka *et al.*

CBP is also phosphorylated in the hepatocytes and it could be that this process occurs in adipocytes too. If the insulin-induced phosphorylation of CBP occurs in adipocytes, CREB becomes even more activated in the insulin resistant state due to decreased inhibition of CREB, leading to a further increase in FFA levels and plasma glucose levels.

Qi *et al.* has shown that CREB is activated in adipocytes under obese conditions. The environmental cues inducing this activation are unknown. It is possible that obese conditions induce the dephosphorylation of the coactivators of CREB or stimulate the inhibition of phosphorylation of CBP and/or TORC2/CRTC2 in adipocytes leading to the activation of CREB and ultimately insulin resistance. It would be interesting to determine factors, like hormones or peptides, released by the adipose tissue, that can potentially mediate the CREB activation and subsequent CREB-regulated gene transcription. It could be that these factors stimulate the cAMP-dependent pathway, but other pathways might be involved too. Increased adiposity induces inflammatory pathways and the influx and accumulation of macrophages into the adipose tissue[2]. It would be interesting to determine if adipocytokines like TNF-alpha or IL-6 could be key players in the activation of CREB under obese conditions.

We have also seen that CREB requires the binding of the coactivators CBP/p300 and TORC2/CRTC2. The phosphorylation of S133 in the KID of CREB enhances the binding of CBP/p300. TORC2/CRTC2 is able to bind to the R314 in the bZIP domain of CREB. S133 and R314 lie within a region that is highly conserved between species. This could indicate that these positions are especially important for CREB activation. However, the exact role of these conserved residues is unknown. It would be interesting to determine the function of these two residues in CREB and the influence of these residues on the CREB-dependent transcription.

It is also known that TORC2/CRTC2 recruitment does not modulate the interaction between CREB and DNA. Conkright *et al*. has shown that TORCs enhance the interaction of the Q2 domain of CREB with TAF130, a component of the TFIID complex. Remarkable is that TORC2/CRTC2 binds CREB via the bZIP domain, the DNA binding domain of CREB. You would expect that TORC2/CRTC2 could influence the binding of CREB to DNA by binding to the bZIP domain. It would be interesting to determine the regulatory mechanisms of the TORC2/CRTC2 interaction with CREB and the subsequent enhanced interaction of the Q2 domain with TAF130.

Furthermore, the association of CBP/p300 and TORC2/CRTC2 is required for the recruitment of TORC2/CRTC2 to the promoter region of the target genes. The role of the association of these two coactivators and the influence on the target gene is unclear and it would be interesting to determine this role.

CREB and its coactivators CBP and TORC2/CRTC2 seem to be attractive therapeutic targets. Novel compounds that enhance CBP or TORC2/CRTC2 phosphorylation might be able to reduce CREB activation in adipocytes under obese conditions. This could lead to a decrease in plasma glucose levels and FFA levels, resulting in reduced insulin resistance and dyslipidemia. This could then decrease the development of type 2 diabetes mellitus in association with obesitas. However, many target gene promoters contain a CRE motif. CREB is able to bind this motif, thereby regulating the downstream genes. Blocking CREB activation will not only affect metabolic regulation in adipocytes or hepatocytes, but it will also affect the expression of neuropeptides, growth factors and structural proteins[21]. If you would like to use CREB as a therapeutic target, you would need to create a compound that is adipocyte specific or only expressed in adipocytes, where it could reduce CREB activation under obese conditions and thereby reduce insulin resistance. However, you need to determine all the target genes of CREB in the adipocytes. CREB could regulate the expression of other transcription factors, thereby stimulating or inhibiting genes involved in lipid metabolism or other metabolic pathways. For example, CREB induced hepatic gluconeogenesis through the nuclear receptor coactivator PGC-1[42]. This coactivator could be a direct target of CREB in adipocytes.

In summary, adipocyte CREB plays an in important role in the development of insulin resistance. Further analysis of the CREB pathway and studies of the CBP/p300 and the TORC2/CRTC2 pathway could provide more insight into the hormone regulated pathways in adipocytes under obese conditions. This could eventually lead to the development of a specifically targeted medicine and a better treatment of type 2 diabetes mellitus in obesity.

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Appendix

List of abbreviations

AMPK	AMP-activated protein Kinase
ATF-1	Activating Transcription Factor-1
BAT	Brown Adipose Tissue
bZIP	basic leucine Zipper
CBP	CREB Binding Protein
CREB	cAMP Response Element Binding protein
CREM	cAMP Response Element Modulater
FFA	Free Fatty Acids
GLUT4	Glucose Transporter type 4
GSK3-beta	Glycogen Synthase Kinase 3-beta
GTT	Glucose Tolerance Test
HFD	High Fat Diet
IL-6	Interleukin-6
ITT	Insulin Tolerance Test
KID	Kinase-Inducible Domain
MCP-1	Monocyte Chemotactic Protein-1
mRNA	messenger RNA
NC	Normal Chow
PAI-1	Plasminogen Activator Inhibitor-1
PKB/Akt2	Protein Kinase B/Akt2
PPAR-alpha	Peroxisome Proliferator-Activated Receptor-alpha
SIK2	Salt-Inducible Kinase 2
TAG	Triacylglycerol
TNF-alpha	Tumor Necrosis Factor-alpha
TORC2/CRTC2	Transducer Of Regulated CREB 2/ CREB Regulated Transcription Coactivator 2
WAT	White Adipose Tissue