Adipocyte 1:3, 157–160; July/August/September 2012; © 2012 Landes Bioscience

Evidence for tuning adipocytes ICER levels for obesity care

Saška Brajkovic,[1] Raphael Marenzoni,[2] Dimitri Favre,[1] Audrey Guérardel,[2] Roberto Salvi,[2] Nicole Beeler,[1] Philippe Froguel,[2] Peter Vollenweider,[1] Gérard Waeber[1] and Amar Abderrahmani[2,*]

[1]Service of Internal Medicine; Centre Hospitalier Universitaire Vaudois and University of Lausanne; Lausanne, Switzerland;
[2]University of Lille Nord de France; European Genomic Institute for Diabetes; UMR 8199; Lille, France

Abnormal adipokine production, along with defective uptake and metabolism of glucose within adipocytes, contributes to insulin resistance and altered glucose homeostasis. Recent research has highlighted one of the mechanisms that accounts for impaired production of adiponectin (ADIPOQ) and adipocyte glucose uptake in obesity. In adipocytes of human obese subjects and mice fed with a high fat diet, the level of the inducible cAMP early repressor (ICER) is diminished. Reduction of ICER elevates the cAMP response element binding protein (CREB) activity, which in turn increases the repressor activating transcription factor 3. In fine, the cascade triggers reduction in the ADIPOQ and GLUT4 levels, which ultimately hampers insulin-mediated glucose uptake. The c-Jun N-terminal kinase (JNK) interacting-protein 1, also called islet brain 1 (IB1), is a target of CREB/ICER that promotes JNK-mediated insulin resistance in adipocytes. A rise in IB1 and c-Jun levels accompanies the drop of ICER in white adipose tissues of obese mice when compared with mice fed with a chow diet. Other than the expression of ADIPOQ and glucose transport, decline in ICER expression might impact insulin signaling. Impairment of ICER is a critical issue that will need major consideration in future therapeutic purposes.

Keywords: inducible cAMP early repressor (ICER), CREB, adipocytes, obesity, JIP-1, JNK, insulin resistance, insulin signaling, GLUT4, ADIPOQ

Submitted: 02/27/12

Revised: 03/09/12

Accepted: 03/12/12

http://dx.doi.org/10.4161/adip.20000

*Correspondence to: Amar Abderrahmani; Email: amar.abderrahmani@univ-lille2.fr

Commentary to: Favre D, Le Gouill E, Fahmi D, Verdumo C, Chinetti-Gbaguidi G, Staels B, et al. Impaired expression of the inducible cAMP early repressor accounts for sustained adipose CREB activity in obesity. Diabetes 2011; 60:3169–74; PMID:21998402; http://dx.doi.org/10.2337/db10-1743

Systemic insulin resistance is one of the hallmarks of obesity that is associated with life-threatening complications.[1,2] In obesity, enlarged adipocytes have numerous abnormalities that contribute to overall insulin resistance such as defective insulin-mediated glucose uptake. The latter is caused by diminished production and translocation of the glucose transporter GLUT4 and impaired insulin signaling. As a consequence, adipocytes cannot properly store glucose and increase the release of free fatty acids (FFAs) into the blood.[3] Continuous overload of FFAs promotes an impairment in insulin signaling and glucose uptake in muscle cells.[3] Adipocytes produce several adipokines including the insulin sensitizer adipocyte-specific product adiponectin (ADIPOQ).[4] ADIPOQ is thought to decrease circulating FFAs by increasing their oxidation in skeletal muscle and liver.[5] Increased ADIPOQ levels reduce the triglyceride contents, leading thereby to improvement of insulin sensitivity.[5] Numerous studies report reduced adipose production and plasma circulating levels of the ADIPOQ in obese mice and human obese individuals.[4] A mechanism that accounts for this reduction of ADIPOQ and glucose uptake has been shown.[6] Adipocytes from obese subjects have a sustained increase in activity of the transcriptional activators cAMP response element (CRE) binding protein (CREB).[6] The rise in CREB activity elevates the activating transcription factor 3 (ATF3) levels, which in turn represses the expression of ADIPOQ and the glucose transporter GLUT4.[6]

CREB activity is tightly regulated by the level of the inducible cAMP early repressor (ICER), a natural antagonist that contains neither activating nor repressing domains.[7] ICER acts as a passive repressor that competes with CREB for binding to target genes promoters. In the normal situation, ICER activity is transiently induced by the same stimuli that induce CREB, but

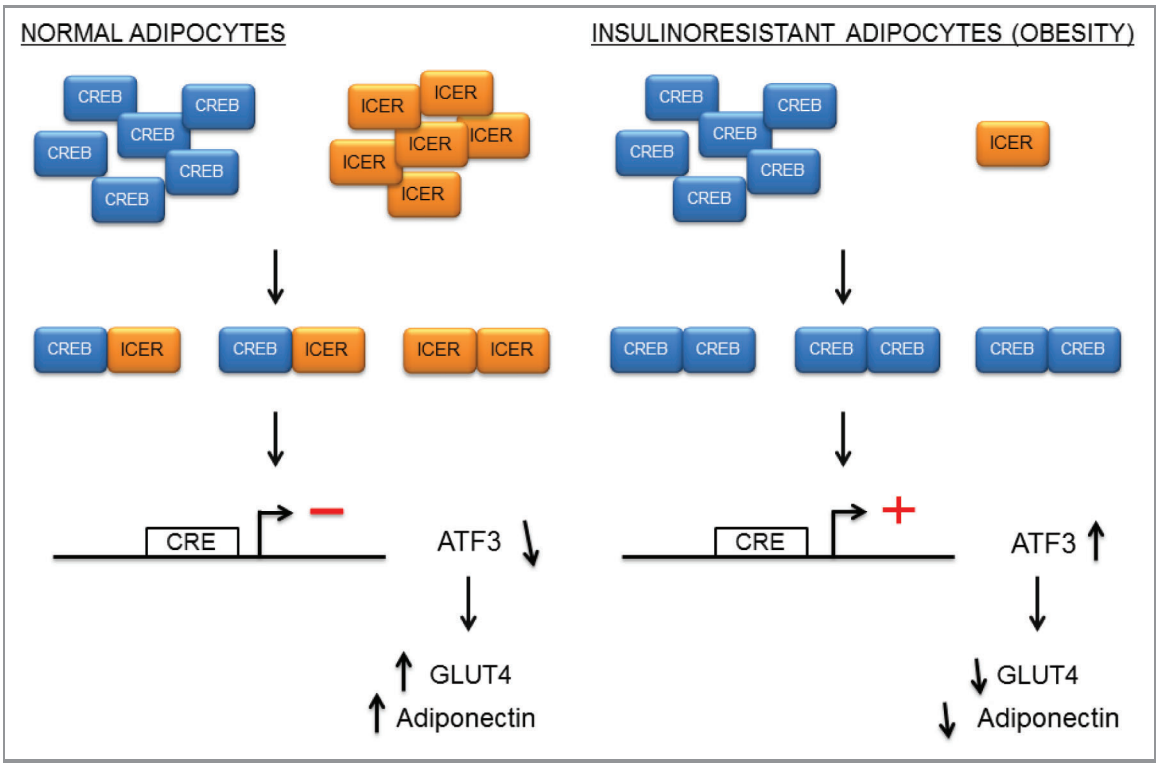

Figure 1. Schematic representation for the impaired expression of Adipoq and GLUT4 ruled by the unbalance of ICER and CREB in obesity. In normal adipocytes, there is enough production of ICER that permits the formation of homo- (ICER/ICER) and/or heterodimers (ICER/CREB) with CREB. These interactions often occur when ICER reaches a certain level upon stimulation and aim to prevent CREB activity. Thus decreased CREB activity reduces the expression of target genes with a cAMP response element (CRE) such as activating transcription factor 3 (ATF3). In turn ATF3 cannot repress the expression of adiponectin and GLUT4. In obesity, the level of ICER is constitutively reduced, fostering CREB homodimers and heterodimers with other activators. Increased CREB activity promotes the expression of ATF3, and therefore, inhibition of adiponectin and GLUT4.[14]

repression occurs only when ICER reaches certain levels.[8] This interplay between CREB and ICER constitutes part of an adaptive mechanism to answer environmental cues. While CREB rapidly induces the expression of target genes in response to stimuli, the repressor ICER restores their initial expression levels and thereby permits transient induction.[7,8] Stimuli that evoke CREB and ICER activities include hypoglycemia, fasting, vagal and β-adrenergic response for example, and this typically occurs in response to an elevation of cAMP levels.[8] Thus, as a passive repressor, ICER activity is directly correlated with its abundance.[7,8] It is therefore predictable that inadequate levels of ICER impact to CREB activity, thereby leading to cells dysfunction and ultimately certain pathologies.[9,10] For example, upregulation of ICER is thought to contribute to the development of type 2 diabetes (T2D).[11-13] The disease manifests when islets β cells from endocrine pancreas fail to release sufficient insulin to compensate for insulin resistance in target tissues. Loss of β-cells function includes a decrease in insulin secretion and amounts, which is caused by micro-environmental diabetogenic factors including chronic excess of FFAs, prolonged hyperglycemia and possibly oxidized LDL.[11-13] Persistent induction of ICER couples these factors to β-cell failure.[11-13] The prevalence of T2D is increasing dramatically as a result of the obesity epidemic. Identification of FFAs as diabetogenic factors, points to a crosstalk between adipocytes and β cells in the pathogenesis of diabetes. For this reason, in the opposite of β cells, we postulate that the loss of ICER elicits an increase in CREB mediated-adipocytes dysfunction in obesity. Quantification indeed revealed a collapse in the levels of ICER in adipocytes of mice obese fed with a high fat diet (HFD) and human obese individuals.[14] Both constitutive and induced levels of ICER are affected in obesity.[14] The decrease in the production of ICER is found in adipocytes of obese mice that were either underfed or fasting conditions and correlated with an augmentation in CREB activity. In vitro experiments realized in mouse differentiated adipocytes showed that ICER deficiency increases Atf3, which in turn inhibit Adipoq and Glut4 expression and insulin mediated-glucose uptake (**Fig. 1**).[14]

Insulin signaling fosters glucose entry in adipocytes. In obesity insulin signaling is defective and contributes to impaired glucose metabolism. A mechanism that triggers inhibition of this signaling involves the c-Jun N-terminal kinase (JNK) pathway.[15] JNK achieves upstream inhibition of insulin signaling by phosphorylating the Ser-307 residue of the insulin receptor substrate 1 (IRS-1), thus suppressing signal transduction of insulin.[16,17] In obesity, JNK activity is increased and its inhibition can prevent obesity-induced insulin resistance. The

JNK interacting protein 1, also termed islet brain 1 (IB1) because of its high abundance in pancreatic islets cells and brain, is a scaffold protein that tethers kinases of the JNK pathway.[18] At present the exact mechanism though which IB1 regulates the JNK pathway is not understood. IB1 can either inhibit or activate JNK in response to stimuli and the function depends of the cells type. In adipocytes, IB1 promotes activation of JNK. Homozygous disruption of the gene coding for IB1 in mice prevents activation of JNK in adipose tissue.[19] As the consequence of the inhibition of JNK activity, the knockout mice have smaller adipose tissue, gain less weight and have higher systemic insulin sensitivity under a HFD compared with wild-type mice.[19] Interestingly the promoter of IB1 contains a CRE that is conserved between mammalians and the level of the scaffold protein is regulated by CREB and ICER in β-cells.[20] This observation leads to the hypothesis that the drop of ICER in obesity accounts for JNK activity and reduced insulin sensitivity in adipocytes in a mechanism that involve IB1. Phosphorylation of the transcription factor c-Jun by JNK is accompanied by an elevation of the c-Jun mRNA.[21] The gene coding for c-Jun contains a site that is regulated by activated c-Jun itself in a positive feedback loop. For this reason we quantified the expression of c-Jun by quantitative real-time PCR in parallel to ICER and IB1 to mirror JNK activity in adipose tissues of obese mice fed with HFD. We found that the diminution of ICER correlated with an augmentation of c-Jun mRNA and IB1 protein levels in adipose tissues (**Fig. 2A and B**), supporting the idea that induction of IB1 might trigger JNK activity and insulin resistance in obesity. Adipose tissue expansion, which could be the consequence of both adipocytes hyperplasia and hypertrophy, accompanies adipocytes dysfunction. Some genes involved in cell proliferation and apoptosis are also targets of CREB/ICER.[22,23] It is therefore possible that the rise in CREB activity promotes expansion of adipose cell mass in humans even though such role has been ruled out in mice.[6]

Constitutive reduction in the levels of ICER in obesity elicits a sustained rise in CREB activity, which in turn stimulates the expression of genes that contain a CRE. However not only the CREB transcriptional factors but also activators of the CRE modulators (CREM) regulate the expression of these CRE containing genes in response to stimuli.[7,8] In addition, like CREB, CREM acts by either forming homo- or heterodimers with basic leucine zipper transcription factors and can be antagonized by ICER.[7,8] Approximately 4,000 genes could be potentially regulated by CREB, CREM and ATF in mammals.[24] In obesity, with the exception of ADIPOQ, expression of many adipokines is overproduced in adipocytes.[2,25,26] The production of these adipose products could be affected by the reduced abundance of ICER in mechanisms that evolve CREB and CREM activators. If so,

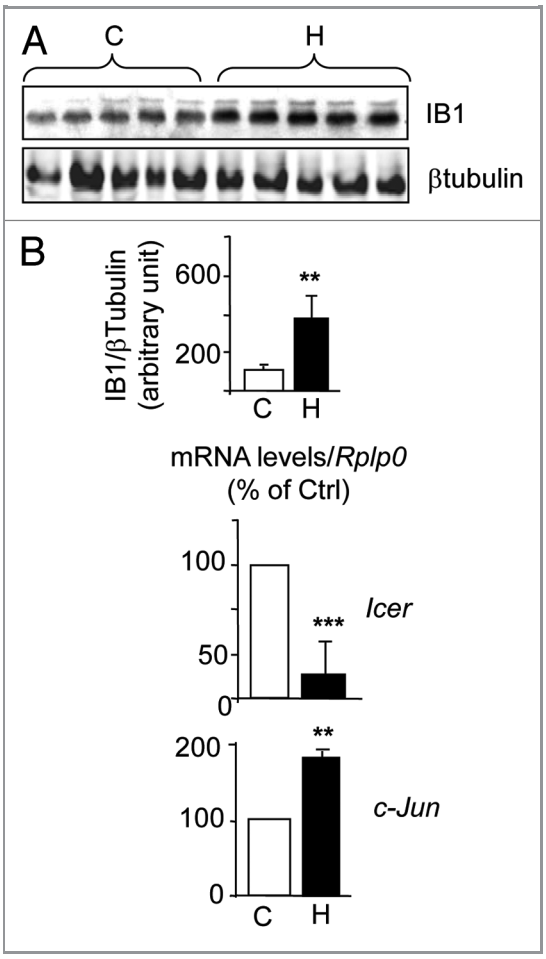

Figure 2. Examination of IB1, Icer and c-Jun levels in epididymal white adipose tissues (WAT) of obese mice. (A) Measurement of IB1 protein contents by western blotting experiments. Total proteins were prepared from WAT of C57Bl6-Rj male mice that either fed for 16 weeks with a high fat diet (obese, H) or chow diet (control, C). All procedures on mice were performed according to the Swiss legislation for animal experimentation. Total proteins (50 μg) were loaded into a SDS-polyacrylamide gel as described.[11,14] The figure shows the results of a representative experiment out of four. The corresponding quantitation is depicted below the blot. The values correspond to the ratio in band intensities of IB1 over β tubulin. Data are the mean ± SEM of four independent experiments. **$p < 0.01$. (B) Quantification of Icer and c-Jun mRNA levels. Total RNAs were prepared from WAT of obese mice (filled bars) and lean control mice (white bars) and were then subjected to quantitative real-time PCR. The primers sequence for *Icer* and *c-Jun* and conditions for PCR are those published elsewhere.[11,21] The levels of the two mRNAs were normalized against the housekeeping acidic ribosomal phosphoprotein P0 gene (*Rplp0*) gene and those of the lean mice (control) cells were set to 100%. Data are the mean ± SEM of five independent experiments. **$p < 0.01$, ***$p < 0.001$.

restoration in the adipose levels of ICER should be a major consideration for future therapeutic strategies to combat insulin resistance and its metabolic complications such as type 2 diabetes.

Acknowledgments

This work was supported by the Swiss National Foundation, Chair of Excellence from the French National Agency for Research N°ANR-10-CEXC-005-01, the Regional Council Nord Pas de Calais and the European Regional Development Fund. We are grateful to Mr Raphael Boutry and Mrs Laure Rolland for editorial and technical assistance.

References

1. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988; 37:1595-607; PMID:3056758; http://dx.doi.org/10.2337/diabetes.37.12.1595
2. Reaven GM. Pathophysiology of insulin resistance in human disease. Physiol Rev 1995; 75:473-86; PMID:7624391
3. Shafrir E, Raz I. Diabetes: mellitus or lipidus? Diabetologia 2003; 46:433-40; PMID:12687345
4. Hotta K, Funahashi T, Bodkin NL, Ortmeyer HK, Arita Y, Hansen BC, et al. Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. Diabetes 2001; 50:1126-33; PMID:11334417; http://dx.doi.org/10.2337/diabetes.50.5.1126
5. Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med 2002; 8:731-7; PMID:12068289; http://dx.doi.org/10.1038/nm724
6. Qi L, Saberi M, Zmuda E, Wang Y, Altarejos J, Zhang X, et al. Adipocyte CREB promotes insulin resistance in obesity. Cell Metab 2009; 9:277-86; PMID:19254572; http://dx.doi.org/10.1016/j.cmet.2009.01.006
7. Molina CA, Foulkes NS, Lalli E, Sassone-Corsi P. Inducibility and negative autoregulation of CREM: an alternative promoter directs the expression of ICER, an early response repressor. Cell 1993; 75:875-86; PMID:8252624; http://dx.doi.org/10.1016/0092-8674(93)90532-U
8. Sassone-Corsi P. Coupling gene expression to cAMP signalling: role of CREB and CREM. Int J Biochem Cell Biol 1998; 30:27-38; PMID:9597751; http://dx.doi.org/10.1016/S1357-2725(97)00093-9
9. Peri A, Conforti B, Baglioni-Peri S, Luciani P, Cioppi F, Buci L, et al. Expression of cyclic adenosine 3',5'-monophosphate (cAMP)-responsive element binding protein and inducible-cAMP early repressor genes in growth hormone-secreting pituitary adenomas with or without mutations of the Gsalpha gene. J Clin Endocrinol Metab 2001; 86:2111-7; PMID:11344215; http://dx.doi.org/10.1210/jc.86.5.2111
10. Yehia G, Razavi R, Memin E, Schlotter F, Molina CA. The expression of inducible cAMP early repressor (ICER) is altered in prostate cancer cells and reverses the transformed phenotype of the LNCaP prostate tumor cell line. Cancer Res 2001; 61:6055-9; PMID:11507053
11. Favre D, Niederhauser G, Fahmi D, Plaisance V, Brajkovic S, Beeler N, et al. Role for inducible cAMP early repressor in promoting pancreatic beta cell dysfunction evoked by oxidative stress in human and rat islets. Diabetologia 2011; 54:2337-46; PMID:21547497; http://dx.doi.org/10.1007/s00125-011-2165-x
12. Abderrahmani A, Plaisance V, Lovis P, Regazzi R. Mechanisms controlling the expression of the components of the exocytotic apparatus under physiological and pathological conditions. Biochem Soc Trans 2006; 34:696-700; PMID:17052177; http://dx.doi.org/10.1042/BST0340696
13. Abderrahmani A, Cheviet S, Ferdaoussi M, Coppola T, Waeber G, Regazzi R. ICER induced by hyperglycemia represses the expression of genes essential for insulin exocytosis. EMBO J 2006; 25:977-86; PMID:16498408; http://dx.doi.org/10.1038/sj.emboj.7601008
14. Favre D, Le Gouill E, Fahmi D, Verdumo C, Chinetti-Gbaguidi G, Staels B, et al. Impaired expression of the inducible cAMP early repressor accounts for sustained adipose CREB activity in obesity. Diabetes 2011; 60:3169-74; PMID:21998402; http://dx.doi.org/10.2337/db10-1743
15. Hirosumi J, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, et al. A central role for JNK in obesity and insulin resistance. Nature 2002; 420:333-6; PMID:12447443; http://dx.doi.org/10.1038/nature01137
16. Aguirre V, Uchida T, Yenush L, Davis R, White MF. The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). J Biol Chem 2000; 275:9047-54; PMID:10722755; http://dx.doi.org/10.1074/jbc.275.12.9047
17. Lee YH, Giraud J, Davis RJ, White MF. c-Jun N-terminal kinase (JNK) mediates feedback inhibition of the insulin signaling cascade. J Biol Chem 2003; 278:2896-902; PMID:12417588; http://dx.doi.org/10.1074/jbc.M208359200
18. Beeler N, Riederer BM, Waeber G, Abderrahmani A. Role of the JNK-interacting protein 1/islet brain 1 in cell degeneration in Alzheimer disease and diabetes. Brain Res Bull 2009; 80:274-81; PMID:19616077; http://dx.doi.org/10.1016/j.brainresbull.2009.07.006
19. Jaeschke A, Czech MP, Davis RJ. An essential role of the JIP1 scaffold protein for JNK activation in adipose tissue. Genes Dev 2004; 18:1976-80; PMID:15314024; http://dx.doi.org/10.1101/gad.1216504
20. Ferdaoussi M, Abdelli S, Yang JY, Cornu M, Niederhauser G, Favre D, et al. Exendin-4 protects beta-cells from interleukin-1 beta-induced apoptosis by interfering with the c-Jun NH2-terminal kinase pathway. Diabetes 2008; 57:1205-15; PMID:18252896; http://dx.doi.org/10.2337/db07-1214
21. Abderrahmani A, Niederhauser G, Favre D, Abdelli S, Ferdaoussi M, Yang JY, et al. Human high-density lipoprotein particles prevent activation of the JNK pathway induced by human oxidised low-density lipoprotein particles in pancreatic beta cells. Diabetologia 2007; 50:1304-14; PMID:17437081; http://dx.doi.org/10.1007/s00125-007-0642-z
22. Inada A, Hamamoto Y, Tsuura Y, Miyazaki J, Toyokuni S, Ihara Y, et al. Overexpression of inducible cyclic AMP early repressor inhibits transactivation of genes and cell proliferation in pancreatic beta cells. Mol Cell Biol 2004; 24:2831-41; PMID:15024072; http://dx.doi.org/10.1128/MCB.24.7.2831-2841.2004
23. Jhala US, Canettieri G, Screaton RA, Kulkarni RN, Krajewski S, Reed J, et al. cAMP promotes pancreatic beta-cell survival via CREB-mediated induction of IRS2. Genes Dev 2003; 17:1575-80; PMID:12842910; http://dx.doi.org/10.1101/gad.1097103
24. Zhang X, Odom DT, Koo SH, Conkright MD, Canettieri G, Best J, et al. Genome-wide analysis of cAMP-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues. Proc Natl Acad Sci U S A 2005; 102:4459-64; PMID:15753290; http://dx.doi.org/10.1073/pnas.0501076102
25. Reaven GM. Banting Lecture 1988. Role of insulin resistance in human disease. 1988. Nutrition 1997; 13:65-, discussion 64, 66; PMID:9058458
26. Guerre-Millo M. Adipose tissue and adipokines: for better or worse. Diabetes Metab 2004; 30:13-9; PMID:15029093; http://dx.doi.org/10.1016/S1262-3636(07)70084-8