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The hexosamine biosynthetic pathway induces gene promoter activity of acetyl-CoA carboxylase beta

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ABSTRACT

The cardiac isoform of acetyl-CoA carboxylase (ACCβ) produces malonyl-CoA, a potent inhibitor of mitochondrial fatty acid (FA) uptake. Higher ACCβ activity decreases FA utilization, potentially leading to intracellular myocardial lipid accumulation and insulin resistance (IR). Since increased hexosamine biosynthetic pathway (HBP) flux is linked to IR onset, we hypothesized that HBP activation leads to the induction of ACCβ gene promoter activity. Rat H9c2 cardio-myoblasts were transiently transfected with a 1317 bp human ACCβ promoter-luciferase construct (pPIIβ−1317) ± an expression construct encoding the HBP rate-limiting step, i.e., glutamine:fructose 6-phosphate amidotransferase (GFAT) ± various HBP modulators. The administration of L-glutamine (HBP substrate) dose-dependently increased, while HBP inhibitors attenuated pPIIβ−1317 activity. Co-transfections with dominant-negative GFAT constructs diminished pPIIβ−1317 activity. To explore underlying transcriptional mechanisms, we co-transfected with upstream stimulatory factor (USF) expression constructs and found that USF2 induced pPIIβ−1317 activity vs. controls. Moreover, co-transfection of a GFAT expression construct + USF reporter-promoter construct (with consensus USF binding elements) led to induction of pPIIβ−1317 activity vs. controls. We next performed transfections with GFAT ± full length ACCβ and seven truncated promoter-luciferase constructs, respectively. Here GFAT-mediated ACCβ promoter induction was blunted when co-transfected with the pPIIβ−38/+65 deletion construct indicating that USF2 binds to the proximal ACCβ promoter region (near start codon). Our study demonstrates that HBP activation induces ACCβ gene promoter activity in H9c2 cells via USF2. We propose that such ACCβ induction may elicit serious downstream effects, e.g. the inhibition of FA β-oxidation and the onset of IR.

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1. Introduction

The incidence and prevalence of insulin resistance/type 2 diabetes is rapidly increasing and constitutes a major concern for both developed and developing countries [9,15]. These alarming projections therefore necessitate the delineation of underlying, molecular mechanisms that drive the onset of such pathophysiologic conditions, with the eventual aim to develop improved therapeutic interventions and diagnostic tools.

There are several biochemical and molecular pathways that contribute to the onset of insulin resistance and type 2 diabetes. However, our focus is on the muscle- and cardiac-enriched isoform of acetyl-CoA carboxylase (ACCβ), encoding a 280-kDa enzyme that plays a key role in controlling mitochondrial fatty acid oxidation (FAO) [1,22]. ACCβ catalyzes the ATP-dependent carboxylation of cytosolic acetyl-CoA to form malonyl-CoA, a potent inhibitor of the mitochondrial FA transfer enzyme carnitine palmitoyltransferase 1 (CPT1) [13]. As the molecular control of the ACCβ gene promoter is not well understood, we began to investigate its regulation and found that it can be activated in a glucose-responsive manner (depending on cell-type) [12]. Since four E-box consensus sequences (CAANTG) were previously identified on the human ACCβ gene promoter [11], upstream stimulatory factors (USFs) emerged as ideal candidate transcription factors regulating its activation. This rationale is based on USFs ability to bind consensus E-box elements located within the promoter regions of several metabolic enzyme-encoding genes [23,24]. Our earlier study data found that upstream stimulatory factor 1 (USF1) forms part of the transcriptional machinery orchestrating ACCβ promoter activation in neonatal cardiomyocytes and CV-1 fibroblasts [12]. We proposed that such an induction would increase malonyl-CoA levels, thereby inhibiting mitochondrial FAO and eventually contributing to the build-up of intracellular long-chain FA levels. In support,

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others have suggested that lower FAO rates found in some type 2 diabetic individuals may be attributed to increased ACCβ gene expression and the subsequent accumulation of malonyl-CoA [4].

For the present study, we further investigated ACCβ gene promoter regulation and evaluated whether its control is linked to hyperglycemia-activated metabolic pathways implicated in the onset of insulin resistance, i.e., the hexosamine biosynthetic pathway (HBP). The HBP usually acts as a fuel sensor that repartitions substrates into suitable storage depots within the body [18]. Here fructose-6-phosphate enters the HBP, forming glucosamine-6-phosphate in a reaction catalyzed by glutamine:fructose-6-phosphate amidotransferase (GFAT), the HBP rate-limiting enzyme [2]. Glucosamine-6-phosphate is ultimately converted to UDP-N-acetylglucosamine (UDP-GlcNAc), the HBP end product. UDP-GlcNAc is a substrate for *O*-linked β-*N*-acetylglucosaminyl transferase transferase (OGT) that catalyzes the *O*-linked transfer of GlcNAc moieties to specific serine/threonine residues of target proteins.

However, chronically activated HBP is maladaptive contributing to pathophysiologic phenotypes, e.g., insulin resistance, mitochondrial impairment, inflammation and cell death [3,6,8,16,17]. Furthermore, *O*-GlcNAc modification is implicated in the modulation of transcriptional mechanisms, thus also contributing to the regulation of gene expression [5]. In light of this, we here hypothesized that HBP activation results in the transactivation of the ACCβ gene promoter in cardio-myoblasts.

2. Materials and methods

2.1. Cell culture

H9c2 rat cardiac-derived myoblasts (ECACC No. 88092904) were maintained at 37 °C (5% CO_2 and 95% humidity) in 5.5 M glucose DMEM (Sigma–Aldrich, St. Louis MO) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad CA) and 4 mM L-glutamine (Invitrogen, Carlsbad CA). Cells were not allowed to grow to a confluency greater than 70–80% and were cultured for a maximum of 8 passages before growing new cells. We routinely employed passages #9–15 for transfection experiments.

2.2. Transfection studies

Myoblasts were cultured as described above and plated at 35,000 cells per well on 12-well culture plates for 24 h prior to transfection. The next day, cells were transiently transfected for 48 h using FuGene 6 reagent (Roche, Mannheim, Germany) according to the supplier's instructions. Here we employed 0.25 μg of the full-length 1317 bp human ACCβ promoter-luciferase reporter construct (pPIIβ−1317) as previously described [12] ±various co-transfection combinations: (a) 0.25 μg of a human pcDNA3-GFAT expression vector; (b) 0.25 μg pcDNA3-GFAT577 dominant negative expression vector; (c) 0.25 μg pcDNA3-GFAT667 dominant negative expression vector; (d) 0.25 μg of the pUC-SRα-USF1 expression construct; (e) 0.25 μg of the psv-USF2 expression construct; and (f) 0.25 μg of the TransLucent USF Reporter Vector (USF-L) that contains promoter recognition sites for both USF1 and USF2 (Panomics, Redwood City CA). The pPIIβ−1317 construct was generously provided by Dr. Kyung-Sup Kim (Yonsei University College of Medicine, Seoul, Korea) [11]. The GFAT constructs were donated to us by Dr. Cora Weigert (University of Tubingen, Germany) [26], the pUC-SRα-USF1 expression construct provided by Dr. Tetsuya Kamataki (Hokkaido University, Japan) [21], and the psv-USF2 expression construct received from Dr. Michele Sawadogo (University of Texas TX) [14].

In additional experiments, promoter deletion constructs pPIIβ−1090/+65, pPIIβ−800/+65, pPIIβ−569/+65, pPIIβ−349/+65, pPIIβ−93/+65, pPIIβ−38/+65 and pPIIβ−18/+65 were transfected ± pcDNA3-GFAT as earlier described. Here we employed 0.25 μg of each deletion construct, respectively, for co-transfection experiments. The serial deletion human ACCβ promoter-reporter luciferase constructs have been previously described [12] and were kindly provided by Dr. Kyung-Sup Kim (Yonsei University College of Medicine, Seoul, Korea). The total amount of DNA transfected for each experiment was 0.75 μg and DNA concentrations were equalized with pGL3-Basic (vector only) (Promega, Madison WI) to normalize results according to cell number and transfection efficiency.

Twenty-four hours after transfection, media was changed and various drugs added (in separate experiments) to further test our hypothesis. We employed the following agents: (a) 0.1 mM, 1 mM and 2 mM alloxan, respectively, to inhibit OGT; (b) 40 μM azaserine to inhibit GFAT; and (c) 40 μM 6-diazo-5-oxo-L-norleucine (DON) as a GFAT inhibitor. We performed several experiments in our laboratory to test different doses for DON and azaserine (data not shown). These studies allowed us to determine inhibitor concentrations that actually resulted in the predicted effects, i.e., to inhibit GFAT. Thus we eventually employed similar concentrations as also found by others, i.e., 40 μM DON [9] and 40 μM azaserine [7]. For the alloxan doses, we based the concentrations on what was previously reported [10]. We also evaluated the effects of varying L-glutamine (HBP substrate) concentrations (0, 4 and 8 mM) on ACCβ ± GFAT transfections. It is important to note that the media used in culturing cells utilizes 4 mM L-glutamine for standard growing conditions, and that cells that were cultured at 0 mM L-glutamine grew slower than those with higher L-glutamine concentrations. We therefore had to ensure that the L-glutamine concentration of our growing media was maintained at the same level for all experiments to ensure that results were reproducible. Transfections were performed in triplicate for each experiment and repeated to generate the necessary numbers for statistical analysis. At the end of the overall transfection period cells were lysed, protein extracted and expression of luciferase measured using a luminometer as before [12].

2.3. HBP assessment by flow cytometry

We evaluated HBP activation by employing a method previously described by us [20]. Cells were transfected with the GFAT overexpression ± the GFAT dominant negative (GFAT/577) constructs. The total amount of DNA transfected for each experiment was 8 μg. Following 48 h of transfection, cells were harvested, trypsinized, fixed and permeabilized with methanol at 20 °C for 10 min. Thereafter the cells were washed with ice cold PBS and blocked with 5% donkey serum in PBS for one hour at 4 °C. Cells were then incubated overnight at 4 °C in primary anti-*O*-GlcNAc antibody (RL-2 Santa Cruz Biotechnology, Dallas TX) made up in 5% donkey serum in PBS. Following this, the H9c2 cells were washed with ice-cold PBS and incubated for 30 min with fluorescent anti-mouse antibody at 4 °C. *O*-GlcNAc levels were analyzed using a flow cytometer (Becton–Dickinson, Franklin Lakes, NJ) and quantified by determining the mean of fluorescence for each treatment. We typically analyzed 10,000 cells per experiment and completed an $n = 4$ for these experiments.

2.4. Mitochondrial FA utilization

We employed a FAO flow cytometry kit (Abcam, Cambridge MA) that can measure the levels of several FA utilization enzymes. Since we added oleic acid as fuel substrate in these experiments, our analysis focused on acyl-CoA dehydrogenase very long-chain (ACADVL) since it metabolizes both long-chain and very long-chain FAs. Briefly, H9c2 myoblasts were cultured and transfected as

described before. Here cells were transfected with the GFAT overexpression ± the GFAT dominant negative (GFAT/577) constructs ± 0.25 mM oleic acid that was added during the last hour of the 48 h period following transfection. The total amount of DNA transfected for each experiment was 8 μg. Following 48 h of transfection, cells were harvested, fixed in 4% paraformaldehyde, permeabilized, a blocking step performed, and then incubated with primary and secondary antibodies (fluorescently labeled), respectively, with several washing steps in-between. ACADVL enzyme levels were thereafter measured using a flow cytometer (Becton–Dickinson, Franklin Lakes, NJ) and quantified by determining the mean of fluorescence for each treatment. We typically analyzed 10,000 cells per experiment and completed an $n = 3$ for these experiments.

2.5. Statistical analysis

Results are expressed as means ± SEM of experiments performed in triplicate and repeated until a minimum of $n = 6$ was achieved (unless otherwise indicated). Statistical analysis was performed by one-way analysis of variance (ANOVA) and the Student–Newman–Keuls *post hoc* test (GraphPad Prism v5, San Diego CA). Values were considered significant when $p < 0.05$.

3. Results and discussion

To evaluate the responsiveness of the ACCβ gene promoter to HBP activation, we transiently transfected rat H9c2 myoblasts with the full-length human ACCβ promoter-luciferase construct ± a GFAT expression vector. There was a significant increase in ACCβ promoter activity in response to GFAT overexpression, and this effect was abolished by co-transfecting with dominant negative constructs (GFAT/577 and GFAT/667) (Fig. 1A). To further assess the role of the HBP in ACCβ gene promoter regulation, we repeated GFAT co-transfection experiments with increasing concentrations of L-glutamine, a known HBP substrate. Our data demonstrated a dose-dependent rise in ACCβ gene promoter activity with increasing L-glutamine concentrations (Fig. 1B). These findings confirm a possible link – for the first time as far as we are aware – between HBP activation and the downstream stimulation of ACCβ promoter activity. Moreover, additional studies employing a downstream substrate such as glucosamine may also yield interesting data; however, this requires further investigation.

To strengthen these findings, we also employed three pharmacologic HBP inhibitors (40 μM DON, 40 μM azaserine – GFAT inhibitors; rising doses of alloxan – OGT inhibitor). Here DON and azaserine administration significantly attenuated the GFAT-induced elevation of ACCβ gene promoter activity (Fig. 1C and D). Moreover, alloxan treatment elicited a dose-dependent decrease in GFAT-mediated induction of the ACCβ gene promoter (Fig. 1E). Of note, the transfection experiments were not completed at the same time but spread out over a prolonged period and resulted in variations for ACCβ promoter activity at baseline. This likely occurred due to different passage number (for cell cultures) and varying conditions in terms of the media employed. However, relative changes remained the same for all these experiments. Together, the strength of these findings lies in the dominant vector data (Fig. 1A) and further complemented by the inhibitor data; since the inhibitors here employed may elicit additional side-effects. All in all these findings establish that increased HBP activation triggers ACCβ gene promoter activity in H9c2 cardio-myoblasts.

We next set out to confirm that our experimental model actually results in downstream changes following HBP modulation. GFAT overexpression resulted in higher *O*-GlcNAcylation while co-transfection with the dominant vector blunted this effect

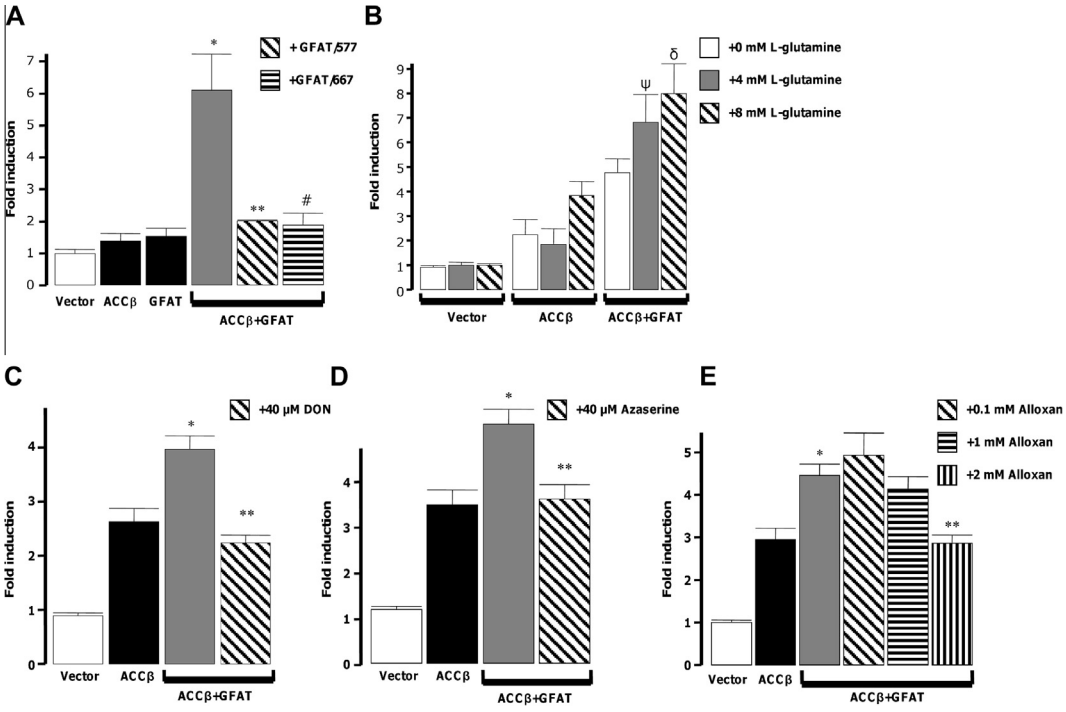

Fig. 1. The impact of HBP modulation on ACCβ gene promoter activity. H9c2 myoblasts were transiently co-transfected with the full-length 1317 bp human ACCβ promoter-luciferase reporter construct ± GFAT expression vector. After 24 h of transfection, cells were exposed to various HBP modulators for an additional 24 h. (A) GFAT overexpression ± dominant GFAT constructs. (B) Effects of L-glutamine on ACCβ gene promoter activity ± GFAT. (C and D) Pharmacologic inhibition of GFAT (40 μM DON, 40 μM azaserine) attenuates GFAT-mediated induction of ACCβ gene promoter activity. (E) Pharmacologic inhibition of OGT blunts GFAT-mediated activation of ACCβ gene promoter activity. The data represent the mean ± SEM of at least 6 independent experiments performed in triplicate. $^{*}p < 0.001$ vs. ACCβ, $^{**}p < 0.001$ vs. GFAT + ACCβ, $^{\#}p < 0.001$ vs. GFAT + ACCβ, $^{\Psi}p < 0.05$ vs. ACCβ + GFAT (0 mM L-glutamine), $^{\delta}p < 0.01$ vs. ACCβ + GFAT (0 mM L-glutamine). Vector: pGL3-Basic; ACCβ: pPIIβ−1317/+65; GFAT: glutamine:fructose-6-phosphate amidotransferase; DON: 6-diazo-5-oxo-L-norleucine.

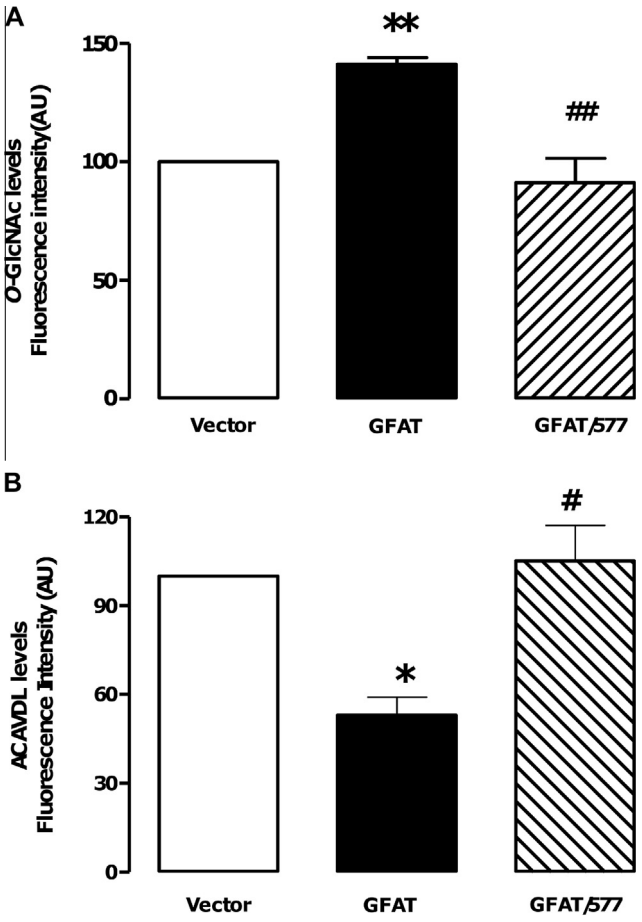

Fig. 2. The effects of HBP modulation on *O*-GlcNAcylation and fatty acid utilization. H9c2 myoblasts were transfected with a GFAT overexpression construct ± a dominant GFAT construct (577). After transfections, cells were harvested and assessed with flow cytometry in response to GFAT or dnGFAT overexpression to evaluate levels of (A) *O*-GlcNAc ($^{**}p < 0.01$ vs. control, $^{\#\#}p < 0.01$ vs. GFAT) and (B) acyl-CoA dehydrogenase targeting long- and very long-chain fatty acids (ACADVL), respectively. Data represent the mean ± SEM of at least 3 independent experiments (analysis of ~10, 000 cells per experiment) ($^{*}p < 0.05$ vs. control, $^{\#}p < 0.05$ vs. GFAT). AU: Arbitrary Units.

(Fig. 2A). The flow cytometry experiments revealed decreased ACADVL levels with GFAT overexpression, while the dominant negative GFAT construct was unable to do so (Fig. 2B). These data therefore indicate that HBP-mediated induction results in downstream effects to lower long-chain mitochondrial FA utilization in H9c2 cardio-myoblasts.

Although the HBP usually functions as an intracellular nutrient sensor, chronic activation (e.g., with hyperglycemia) is strongly linked to the onset of metabolic dysfunction and insulin resistance [3,6]. The implication of the present study is that HBP-induced ACCβ induction may also contribute to this phenomenon, i.e., by potentially attenuating FAO thereby leading to intracellular lipid accumulation and insulin resistance. In agreement, others found enhanced ACCβ gene expression together with increased malonyl-CoA levels with type 2 diabetes [4]. However, further studies are required to confirm this.

Transient co-transfection of the ACCβ promoter construct ± the GFAT overexpression vector, together with USF1 or USF2 expression constructs showed that USF2 plays a role regulating ACCβ promoter activity in H9c2 cardio-myoblasts (Fig. 3A). USF2 transactivated the ACCβ gene promoter at baseline and also when co-transfecting with the GFAT expression construct. However, when the ACCβ promoter was co-transfected with GFAT together with USF2, this did not show a further increase compared to sole USF2 co-transfections, indicating that USF2 effects were likely saturated in this case. We also found that USF1 did not exert any significant effects on ACCβ gene promoter activity in H9c2 cells. The USF1 data differs from our previous study where we established that USF1 is a novel transactivator of the human ACCβ gene promoter in neonatal cardiomyocytes and CV-1 fibroblasts [12]. We propose that USF-mediated induction of the human ACCβ gene promoter likely occurs in a cell-specific manner and may be strongly dependent on the specific intracellular milieu.

We next evaluated USF-mediated regulation of the ACCβ promoter by co-transfecting the GFAT expression construct together with a USF luciferase reporter construct that contains multiple promoter binding sites for USFs. This resulted in a marked induction of the USF reporter construct ($p < 0.001$, $n = 5$) versus control (Fig. 3B). These findings demonstrate that USF2 can transactivate the ACCβ gene promoter at baseline and with HBP activation. Moreover, HBP activation elicits increased USF binding to

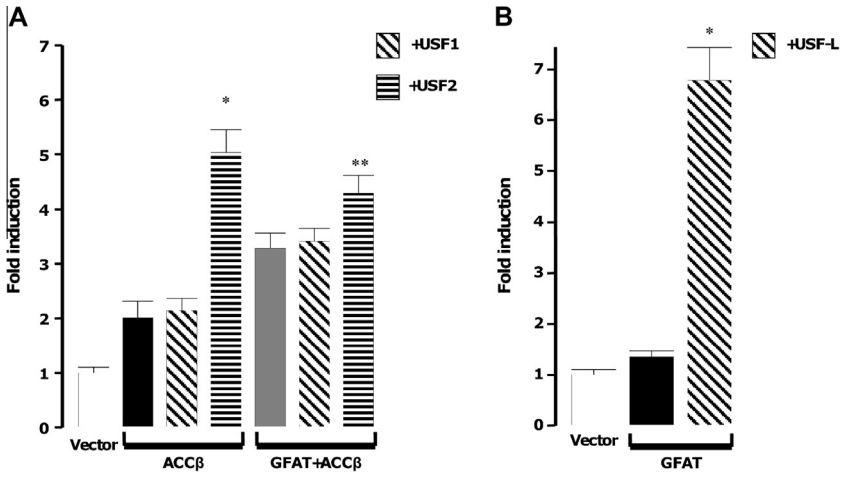

Fig. 3. Upstream stimulatory factor 2 regulates ACCβ gene promoter activity. H9c2 myoblasts were transiently transfected with either the full-length full-length 1317 bp human ACCβ promoter-luciferase reporter construct or the TransLucent USF Reporter Vector (USF-L) that contains promoter recognition sites for both USF1 and USF2. (A) USF2 overexpression activates ACCβ gene promoter activity. (B) GFAT overexpression induces USF luciferase reporter construct. The data represent the mean ± SEM of at least 6 independent experiments performed in triplicate. $^{*}p < 0.001$ vs. ACCβ, $^{**}p < 0.05$ vs. GFAT. Vector: pGL3-Basic; ACCβ: pPIIβ−1317/+65; GFAT: glutamine:fructose-6-phosphate amidotransferase; USF: upstream stimulatory factor.

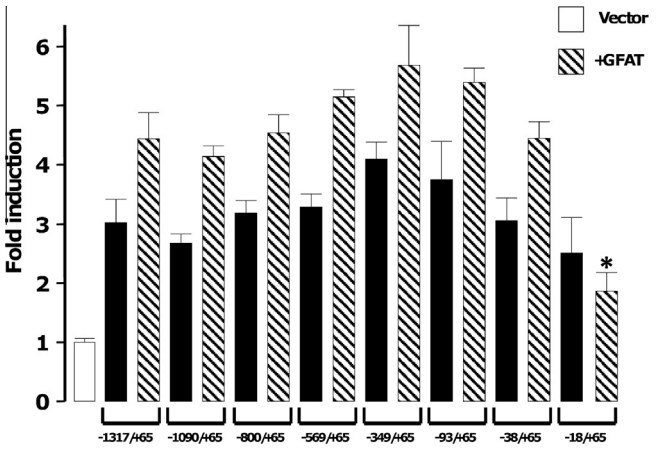

Fig. 4. Evaluation of GFAT-mediated transactivation of the ACCβ gene promoter using serial deletion constructs. H9c2 myoblasts were transiently transfected with serial deletion constructs of the human ACCβ gene promoter. The data represent the mean ± SEM of at least 6 independent experiments performed in triplicate. $^{*}p < 0.001$ vs. −1317/+65 and all constructs up to −38/+65, Vector: pGL3-Basic; ACCβ: pPIIβ−1317/+65; GFAT: glutamine:fructose-6-phosphate amidotransferase.

target sequence elements. Interestingly, the USF-L construct was induced to a much greater extent by GFAT compared to co-transfection with GFAT together with USF2. These findings therefore suggest that other (unidentified) transcriptional modulators may act in concert with USF2 to induce ACCβ to a greater extent. For example, USF1 and USF2 can form either homo- or heterodimers [19,25] that may likely orchestrate ACCβ gene promoter activation in this instance.

Possible promoter target sites of HBP-mediated induction of the ACCβ gene promoter were assessed by transiently transfecting H9c2 cells with a GFAT expression vector ± the full length ACCβ and seven truncated promoter-luciferase constructs, respectively. The deletion constructs have been previously described [11] and are truncated versions of the full-length ACCβ gene promoter used in our experiments. We employed seven promoter-deletion constructs, with pPIIβ−1090/+65, pPIIβ−800/+65, and pPIIβ−569/+65 all containing four E-box motifs, while pPIIβ−349/+65, pPIIβ−93/+65, pPIIβ−38/+65, and pPIIβ−18/+65 each containing one E-box. This resulted in a loss of ACCβ promoter activity for the pPIIβ−18/+65 construct, indicating that this region may be a potential target for HBP-mediated promoter transactivation (Fig. 4). It is likely that an E-box element located near the transcription start site (E-box 4) may play a role in HBP-mediated transactivation of the ACCβ gene promoter. However, further studies are required why this particular E-box and not the others may be implicated in this instance.

In summary, the current study reveals a novel finding, i.e. that increased HBP activation activates USF2 thereby inducing ACCβ gene promoter activity and decreasing FA utilization in cardiomyoblasts. We propose that such an induction may have serious downstream effects such as the onset of insulin resistance.

Conflict of interest

No competing financial interests exist.

Author contributions

Conceived and designed study: M.F.E., J.I. (overall study and hypothesis). Performed experiments: J.I. and R.F.M. Analysis of data and wrote the paper: M.F.E., J.I., R.F.M.

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References

[1] A. Bianchi, J.L. Evans, A.J. Iverson, A.C. Nordlund, et al., Identification of an isozymic form of acetyl-CoA carboxylase, J. Biol. Chem. 265 (1990) 1502–1509.
[2] M.G. Buse, Hexosamines, insulin resistance, and the complications of diabetes: current status, Am. J. Physiol. Endocrinol. Metab. 290 (2006) E1–E8.
[3] R.C. Cooksey, D.A. McClain, Transgenic mice overexpressing the rate-limiting enzyme for hexosamine synthesis in skeletal muscle or adipose tissue exhibit total body insulin resistance, Ann. N. Y. Acad. Sci. 967 (2002) 102–111.
[4] C. Debard, M. Laville, V. Berbe, E. Loizon, C. Guillet, B. Morio-Liondore, Y. Boirie, H. Vidal, Expression of key genes of fatty acid oxidation, including adiponectin receptors, in skeletal muscle of type 2 diabetic patients, Diabetologia 47 (2004) 917–925.
[5] X.L. Du, D. Edelstein, L. Rossetti, I.G. Fantus, H. Goldberg, F. Ziyadeh, J. Wu, M. Brownlee, Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 12222–12226.
[6] G.W. Hart, C. Slawson, G. Ramirez-Correa, O. Lagerlof, Cross talk between *O*-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease, Annu. Rev. Biochem. 80 (2011) 825–858.
[7] L.R. James, I.G. Fantus, H. Goldberg, H. Ly, J.W. Scholey, Overexpression of GFAT activates PAI-1 promoter in mesangial cells, Am. J. Physiol. Renal Physiol. 279 (2000) F718–F727.
[8] L.R. James, D. Tang, A. Ingram, H. Ly, K. Thai, J.W. Scholey, Flux through the hexosamine pathway is a determinant of nuclear factor κB-dependent promoter activation, Diabetes 51 (2002) 1146–1165.
[9] H. King, R.E. Aubert, W.H. Herman, Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections, Diabetes Care 21 (1998) 1414–1431.
[10] R.J. Konrad, F. Zhang, J.E. Hale, M.D. Knierman, G.W. Becker, J.E. Kudlow, Alloxan is an inhibitor of the enzyme *O*-linked *N*-acetylglucosamine transferase, Biochem. Biophys. Res. Commun. 293 (1998) 207–212.
[11] J. Lee, Y.A. Moon, J.H. Ha, D.J. Yoon, Y.H. Ahn, K.S. Kim, Cloning of human acetyl-CoA carboxylase beta promoter and its regulation by muscle regulatory factors, J. Biol. Chem. 276 (2001) 2576–2585.
[12] S.S. Makaula, T. Adam, M.F. Essop, Upstream stimulatory factor 1 transactivates the human gene promoter of the cardiac isoform of acetyl-CoA carboxylase (ACC), Arch. Biochem. Biophys. 446 (2006) 91–100.
[13] J.D. McGarry, S.E. Mills, C.S. Long, D.W. Foster, Observations on the affinity for carnitine, and malonyl-CoA sensitivity, of carnitine palmitoyltransferase I in animal and human tissues. Demonstration of the presence of malonyl-CoA in non-hepatic tissues of the rat, Biochem. J. 214 (1983) 21–28.
[14] J.L. Meier, X. Luo, M. Sawadogo, S.E. Straus, The cellular transcription factor USF cooperates with varicella-zoster virus immediate-early protein 62 to symmetrically activate a bidirectional viral promoter, Mol. Cell. Biol. 14 (1994) 6896–6906.
[15] K. Osei, D.P. Schuster, A.G.B. Amoah, S.K. Owusu, Diabetes in Africa. Pathogenesis of type 1 and type 2 diabetes mellitus in sub-Saharan Africa: implications for transitional populations, J. Cardiovasc. Risk 10 (2003) 85–96.
[16] U. Rajamani, M.F. Essop, Hyperglycemia-mediated activation of the hexosamine biosynthetic pathway results in myocardial apoptosis, Am. J. Physiol. Cell Physiol. 299 (2010) C139–C147.
[17] U. Rajamani, D. Joseph, S. Roux, M.F. Essop, The hexosamine biosynthetic pathway can mediate myocardial apoptosis in a rat model of diet-induced insulin resistance, Acta Physiol. 202 (2011) 151–157.
[18] E. Ravussin, Cellular sensors of feast and famine, J. Clin. Invest. 109 (2002) 1537–1540.
[19] M. Sirito, S. Walker, Q. Lin, M.T. Kozlowski, W.H. Klein, M. Sawadogo, Members of the USF family of helix-loop-helix proteins bind DNA as homo- as well as heterodimers, Gene Expr 2 (1992) 231–240.
[20] C. Springhorn, T.E. Matsha, R.T. Erasmus, M.F. Essop, Exploring leukocyte O-GlcNAcylation as a novel diagnostic tool for the earlier detection of type 2 diabetes Mellitus, J. Clin. Endocrinol. Metab. 97 (2012) 4640–4649.
[21] Y. Takahashi, K. Nakayama, S. Itoh, Y. Fujii-Kuriyama, T. Kamataki, Inhibition of the transcription of CYP1A1 gene by the upstream stimulatory factor 1 in rabbits. Competitive binding of USF1 with AhR.Arnt complex, J. Biol. Chem. 272 (1997) 30025–30031.
[22] K.G. Thampy, Formation of malonyl coenzyme A in rat heart. Identification and purification of an isozyme of acetyl coenzyme A carboxylase from rat heart, J. Biol. Chem. 264 (1989) 17631–17634.
[23] M. Van Bilsen, G.J. van der Vusse, R.S. Reneman, Transcriptional regulation of metabolic processes: implications for cardiac metabolism, Pflugers Arch. 437 (1989) 2–14.

[24] S. Vaulont, M. Vasseur-Cognet, A. Kahn, Glucose regulation of gene transcription, J. Biol. Chem. 275 (2000) 31555–31558.
[25] B. Viollet, A.M. Lefrançois-Martinez, A. Henrion, A. Kahn, M. Raymondjean, A. Martinez, Immunochemical characterization and transacting properties of upstream stimulatory factor isoforms, J. Biol. Chem. 271 (1996) 1405–1415.
[26] C. Weigert, K. Brodbeck, M. Sawadogo, H.U. Haring, E.D. Schleicher, Upstream stimulatory factor (USF) proteins induce human TGF-beta1 gene activation via the glucose-response element-1013/-1002 in mesangial cells: up-regulation of USF activity by the hexosamine biosynthetic pathway, J. Biol. Chem. 279 (2004) 15908–15915.