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Original Research

Elevated Carbohydrate Response Element-Binding Protein Beta (ChREBPβ) and Thioredoxin Interacting Protein (TXNIP) Levels in Human Adipocytes Differentiated in High Glucose Concentrations

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Key Messages

- ChREBPβ, but not ChREBPα, is selectively upregulated when human adipocytes are differentiated in high-glucose conditions.
- The level of TXNIP, a ChREBP target gene linked to glucose transport, is also elevated.
- Inhibition of insulin-stimulated glucose uptake is observed in high glucose-differentiated human adipocytes.

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ABSTRACT

Objectives: Obesity and type 2 diabetes often coexist. The effect of hyperglycemia on adipose tissue is, therefore, of interest. Although studies have shown that high glucose (HG) concentrations do not inhibit adipocyte differentiation, the resulting adipocyte phenotype has not been investigated. In particular, the levels of the glucose-responsive transcription factor carbohydrate-responsive response element binding protein (ChREBP) isoforms have not been assessed.
Methods: Human preadipocytes were differentiated into adipocytes in either normal glucose (NG) or HG conditions. RNA and protein analyses were used to measure the expression of ChREBP isoforms, thioredoxin interacting protein (TXNIP) and lipogenic genes. Insulin-stimulated glucose uptake was measured.
Results: HG- vs. NG-differentiated adipocytes expressed more ChREBPβ and more TXNIP at the mRNA and protein levels. There was no change in lipogenic gene expression. HG- vs. NG-differentiated adipocytes displayed an inhibition of insulin-stimulated glucose uptake.
Conclusions: HG-differentiated human adipocytes have distinct molecular differences and are insulin resistant. More studies are warranted to investigate potential mechanisms linking changes in ChREBPβ and TXNIP to insulin responsiveness.

Mots clés :
adipocyte
protéine ChREBP (*carbohydrate response element binding protein*)
glucose
résistance à l'insuline
protéine d'interaction avec la thiorédoxine (TXNIP)

RÉSUMÉ

Objectifs : La coexistence de l'obésité et du diabète de type 2 est fréquente. Les répercussions de l'hyperglycémie sur le tissu adipeux présentent donc un intérêt. Bien que les études aient démontré que des concentrations élevées de glucose (GÉ) n'inhibent pas la différenciation adipocytaire, le phénotype adipocytaire résultant n'a fait l'objet d'aucune étude. Notamment, les concentrations des isoformes ChREBP (*carbohydrate response element binding protein*), le facteur de transcription sensible au glucose, n'ont pas fait l'objet d'une évaluation.
Méthodes : La différenciation des préadipocytes humains en adipocytes s'est produite dans des conditions normales (GN) ou élevées (GÉ) de glucose. L'ARN et les analyses de protéines ont servi à mesurer l'expression des isoformes ChREBP, des gènes de la protéine d'interaction avec la thiorédoxine (TXNIP) et des gènes lipogènes. La captation du glucose stimulée par l'insuline a été mesurée.

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Résultats : Les adipocytes différenciés GÉ *vs* les adipocytes différenciés GN ont exprimé plus de ChREBPβ et plus de TXNIP aux concentrations d'ARNm et de protéines. Il n'y a eu aucun changement dans l'expression des gènes lipogènes. Les adipocytes différenciés GÉ *vs* les adipocytes différenciés GN ont montré une inhibition de la captation du glucose stimulée par l'insuline.
Conclusions : Les adipocytes humains différenciés GÉ présentent des différences moléculaires distinctes et une résistance à l'insuline. Plus d'études sont justifiées pour examiner les mécanismes potentiels reliant les changements dans ChREBPβ et TXNIP à la réceptivité à l'insuline.

Introduction

Cellular turnover within adipose tissue involves the death of mature adipocytes and the formation of new adipocytes from adipose progenitor cells (preadipocytes) (1). Disturbances in this process and adipose tissue dysfunction are associated with obesity (2). In the presence of diabetes, adipose tissue remodeling occurs in the context of elevated glucose levels. The extent of differentiation by preadipocytes in high glucose (HG) culture concentrations is not overtly altered (3–5). Little is known about any changes there may be to the molecular and cellular phenotype of the resulting HG-differentiated adipocytes.

Glucose can activate carbohydrate response element-binding protein (ChREBP), a basic helix-loop-helix transcription factor that targets genes possessing a carbohydrate response element (6). It is expressed in metabolic cells, such as adipocytes, hepatocytes, myocytes and pancreatic beta cells. Elevated ChREBP levels have been associated with an increase in lipogenic gene induction in mouse and human adipocytes as well as in insulin sensitivity (6). Thioredoxin-interacting protein (TXNIP), which is involved in oxidative stress and insulin resistance, is also induced by ChREBP (7).

It is now recognized that ChREBP is expressed as 2 isoforms. In mouse adipocytes, glucose stimulates ChREBPalpha (α) activity and leads to the induction of a novel, distinct ChREBPbeta (β) isoform located exclusively in the nucleus (8). The transcriptional activity of ChREBPβ is 20-fold greater than that of ChREBPα, augmenting the response of glucose target genes in mouse adipocytes.

The regulation of ChREBP isoform expression by HG in human adipocyte differentiation has not yet been investigated. Our objective was to determine the effect of HG on the regulation of ChREBP isoforms and ChREBP target genes in human preadipocytes and in differentiated human adipocytes.

Methods

Isolation of preadipocytes and differentiation into adipocytes

Human subcutaneous abdominal adipose tissue samples were obtained from 13 weight-stable women undergoing elective abdominal surgery, as approved by the Ottawa Health Science Network Research Ethics Board. The mean age (±SD) of patients was 57±7 years, and the mean body mass index (±SD) was 30±8.7 kg/m^2. Briefly, adipose tissue was dissected to remove connective tissue and blood vessels, then digested with collagenase type 1 (600 U/g of tissue; Worthington Biochemical, Lakewood, New Jersey, United States). The digested tissue was processed by progressive size filtration and centrifugation, and cells were exposed to an erythrocyte lysis buffer. Preadipocytes were grown in Dulbecco modified Eagle medium supplemented by 10% FBS, 100 U/mL penicillin, 0.1 mg/mL streptomycin and 50 U/mL nystatin (a designated growth medium). Cells were either passaged up to 3 times for direct use or were cryopreserved. For differentiation, preadipocytes were seeded at 3×10^4 cells/cm^2 in growth medium and grown to confluence. Differentiation was induced in growth medium containing 5 mmol/L normal glucose (NG), 25 mmol/L HG or 5 mmol/L glucose and 20 mmol/L mannitol (osmotic control) and supplemented with adipogenic inducers (850 nmol/L insulin, 0.25 mmol/L isobutylmethylxanthine, 100 μmol/L indomethacin and 0.5 μmol/L dexamethasone). Every 3 days, 50% of the medium was replaced for up to 14 days.

RNA preparation and real-time polymerase chain reaction

Differentiated human adipocytes were lysed in Qiazol (Qiagen, Toronto, Ontario, Canada). RNA was isolated and treated with DNase I (Life Technologies/ThermoFisher Scientific, Norristown, Pennsylvania, United States) according to the manufacturer's instructions. Total RNA (1.5 to 2 μg per sample) was heat-denatured, then reverse-transcribed using random primers (Life Technologies). Control reactions, without transcriptase, were performed for all samples. Real-time polymerase chain reaction (PCR) assays were performed using the QuantiTect SYBR Green real-time PCR kit (for target genes) or the QuantiTect Probe real-time PCR kit (for 18S rRNA) (both from Qiagen) according to the manufacturer's protocol. PCR was performed as previously described (9). Reactions were run on the Roche Light Cycler real-time PCR System (Roche, Laval, Quebec, Canada), and data were analyzed with Light Cycler Software, v. 3.0 (Roche) by relative quantification using 18S rRNA as a reference. Primer sequences were: ChREBPα: forward: 5′-AGTGCTTGAGCCTGGCCTAC-3′, reverse: 5′-TTGTTCAGGCGGATCTTGTC-3′; ChREBPβ: forward: 5′-AGCGGATTCCAGGTGAGG-3′, reverse: 5′-TTGTTCAGGCGGATCTTGTC-3′; acetyl-CoA carboxylase (ACC): forward: 5′-TTTAAGGGGTGAAGAGGGTGC-3′, reverse: 5′-CCAAAAAGACCTAGCCCTCAAG-3′: Fatty acid synthase (FAS): forward: 5′-CAGAGCAGCCATGGAGGAG-3′, reverse: 5′-AATCTGGGTTGATGCCTCCG-3′: stearoyl-CoA desaturase (SCD): forward: 5′-CCAGAGGAGGTACTACAAACCTG-3′, reverse: 5′-TGGTGGTAGTTGTGGAAGCC-3′: sterol regulatory element binding protein (SREBP-1c): forward: 5′-GACCGACATCGAAGACATGC-3′, reverse: 5′-GGCATGGACGGGTACATCTT-3′: TXNIP: forward: 5′-GCA GTG CAA ACA GAC TTC GG-3′, reverse: 5′-TAGCAGACA CAGGTGCCATT -3′.

Immunoblot analysis

Preadipocytes were differentiated as described above. On day 14 of differentiation, either whole-cell extracts were prepared using Laemmli buffer (10), which was 1 mmol/L sodium orthovanadate, 50 mmol/L sodium fluoride, 5 mmol/L sodium pyrophosphate and 5 mmol/L EGTA, or nuclear/cytosolic fractions were prepared using a Nuclear/Cytosol Fractionation Kit (BioVision, Milpitas, California, United States). Total protein (5 to 20 μg) was resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (Bio-Rad, Mississauga, Ontario, Canada). Nonspecific binding sites were blocked by 5% nonfat milk, and the membranes were probed, with the following antibodies directed against ChREBP (1:1000; ThermoFisher Scientific); Annexin A1 (1:1000; Cell Signaling

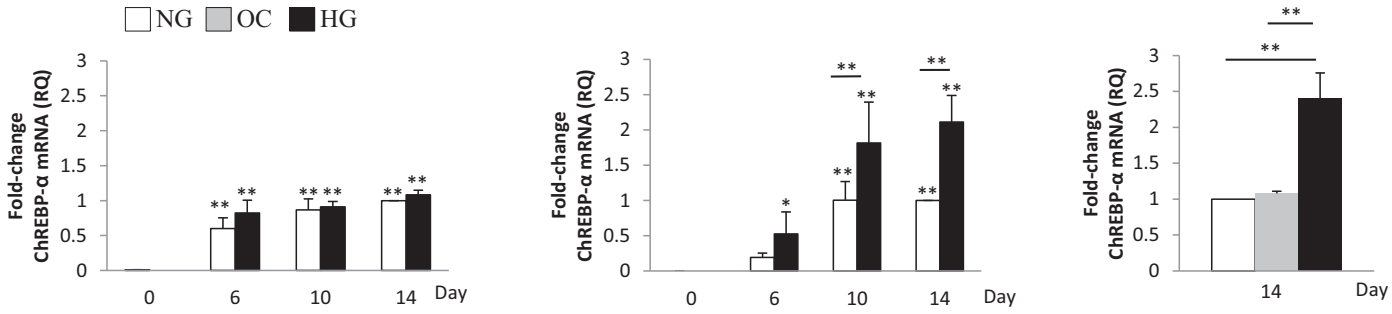

Figure 1. HG enhances ChREBPβ mRNA expression in differentiated adipocytes. Human subcutaneous abdominal preadipocytes were induced to differentiate in 5 mmol/L (NG), 5 mmol/L glucose/20 mmol/L mannitol (OC) or 25 mmol/L (HG) glucose for up to 14 days. RNA was isolated, then quantified by real-time PCR, using indicated primers. Levels were normalized to endogenous 18S RNA and expressed as a function of the NG control condition at day 14. Results from 3 to 5 separate patient samples are graphically presented as mean ± SEM. Means were compared by either 2-way ANOVA for time course of NG vs. HG or by 1-way ANOVA for NG, HG and osmotic control at day 14, with Tukey post hoc tests. *p<0.05; **p<0.01 compared to day 0 or between indicated pairs. *HG*, high glucose; *NG*, normal glucose; *OC*, osmotic control; *PCR*, polymerase chain reaction.

Technology, Danvers, Massachusetts, United States); and TXNIP (1:1000) (Cell Signaling Technology). The membranes were then incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies. Signal intensity was visualized by chemiluminescence (EMD Millipore, Billerica, Massachusetts, United States) and quantified using the AlphaImager imaging system (Alpha Innotech, San Leandro, California, United States).

Glucose uptake assay

Uptake of 2-deoxyglucose in human adipocytes, differentiated under NG or HG conditions, was measured as previously described (11). Briefly, differentiated adipocytes were incubated in NG Dulbecco modified Eagle medium +1% PS for 2 h, followed by stimulation with 100 nmol/L insulin or vehicles for an additional 30 min. The reaction was terminated by washing twice with HEPES buffer. Subsequently, the cells were incubated for 20 min in HEPES-buffered solution containing 10 μmol/L unlabeled 2-deoxyglucose and 10 μmol/L D-2-deoxy-(3H) glucose (0.05 mCi/mL). Incorporated radioactivity was determined by lysing the cells with 50 mmol/L NaOH, followed by liquid scintillation counting.

Statistical analysis

Statistical analysis was performed with either 1- or 2-way ANOVA followed by the Tukey post hoc test or the Student t test, as appropriate. Significance was defined as $p<0.05$.

Results

The extent of differentiation of human primary preadipocytes into adipocytes in HG vs. NG over 14 days was similar, as assessed by tryglyceride accumulation, as well as by protein expression levels of SREBP-1, FAS and PPARγ (data not shown). This agrees with what we have observed previously (5) and with other published reports (3,4).

The regulation of ChREBP isoforms during adipogenesis, in NG vs. HG conditions, was investigated (Figure 1). ChREBPα and ChREBPβ mRNA levels were not detectable in preadipocytes on day 0. ChREBPα mRNA was clearly evident on day 6 of the differentiation in NG and remained high on days 10 and 14. ChREBPβ levels rose more gradually than ChREBPα, with the peak increase on day 10 of differentiation in NG. Under HG conditions, there was a difference in isoform regulation. There was a significant ~2-fold enhancement in mRNA levels of ChREBPβ with HG vs. NG (no change with osmolar control) on days 10 and 14. In contrast, ChREBPα mRNA levels were not altered by HG.

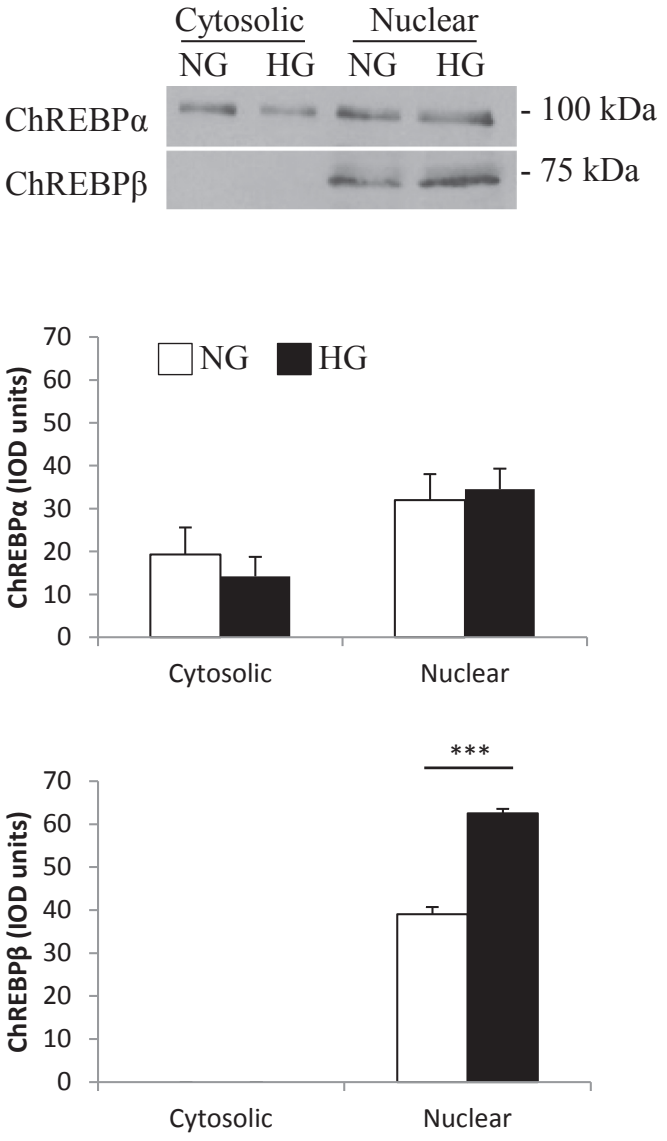

Figure 2. HG increases ChREBPβ protein levels in human differentiated adipocytes. Equal amounts of solubilized nuclear and cytosolic proteins were separated by SDS-PAGE and immunoblotted with the indicated antibodies. Immunoblots from 1 patient sample are shown. Densitometric data from 3 separate patient samples are expressed as mean ± SEM. Means were compared by 2-way ANOVA with Tukey post hoc tests. ***p<0.001 between indicated pairs. *HG*, high glucose; *NG*, normal glucose; *SDS-PAGE*, sulfate polyacrylamide gel electrophoresis.

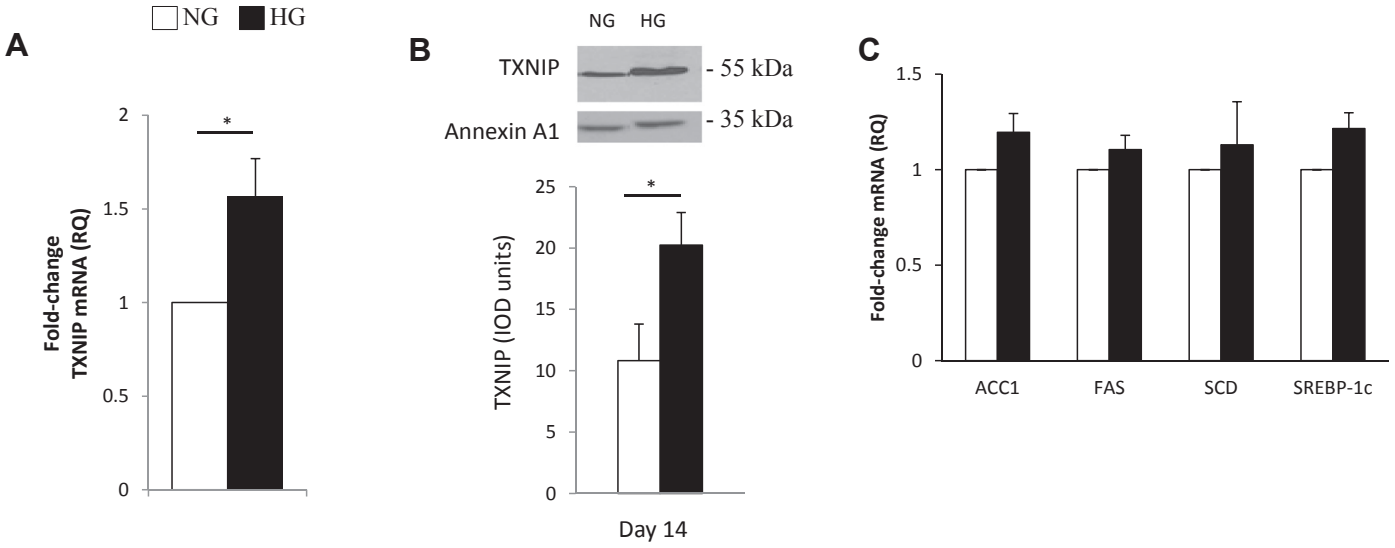

Figure 3. HG increases TXNIP gene expression. Human subcutaneous abdominal preadipocytes were induced to differentiate in 5 mM (NG) or 25 mM (HG) glucose for 14 days. A and C, RNA was isolated, then quantified by real-time polymerase chain reaction, using indicated primers. Levels were normalized to endogenous 18S RNA and were expressed as functions of the NG control condition at day 14. Results from 3 to 5 separate patient samples are graphically presented as mean ± SEM. B, Equal amounts of solubilized protein were separated by SDS-PAGE and immunoblotted with the indicated antibodies. Immunoblots from 1 patient sample are shown. Densitometric data from 3 separate patient samples are graphically presented as mean ± SEM. Means were compared either by Student t test (A, B) or by 1-way ANOVA with Tukey post hoc tests (C). *$p<0.05$ between indicated pair. *HG*, High glucose; *NG*, normal glucose; *SDS-PAGE*, sulfate polyacrylamide gel electrophoresis.

Protein levels of the ChREBP isoforms were assessed in the differentiated human adipocytes (Figure 2). ChREBPα protein was present in the cytosolic and nuclear cell fractions, whereas ChREBPβ was located exclusively in the nuclear fraction of adipocytes, whether differentiation was induced in HG or NG conditions. There was an ~50% increase in ChREBPβ protein in the nuclear fraction in HG- vs. NG-differentiated adipocytes. ChREBPα levels, in contrast, were the same in both nuclear fractions, i.e. were not altered by HG.

We next examined expression of genes that have been reported as downstream targets of ChREBP. TXNIP mRNA levels were significantly higher, by 50%, in adipocytes differentiated in HG vs. NG (Figure 3A). Similarly, TXNIP protein levels were twice as high in HG- vs. NG-differentiated adipocytes (Figure 3B). There was no change in mRNA levels of lipogenic genes ACC, SCD, FAS or SREBP1c in human adipocytes differentiated in HG vs. NG (Figure 3C).

Given these HG-associated increases in the level of ChREBPβ and TXNIP, we examined whether there might be a functional difference between the human adipocytes differentiated in HG vs. NG. Insulin-stimulated glucose uptake increased significantly, by 30%, in NG-differentiated adipocytes but was inhibited in HG-differentiated adipocytes (Figure 4).

Discussion

Given the frequent coexistence of obesity with type 2 diabetes, it is pertinent to examine the consequences of hyperglycemia on human adipocytes. Our strategy in this study was to examine the impact of high glucose concentrations on human adipocytes in culture, with a focus on ChREBP isoform responses.

No difference in the differentiation of the human preadipocytes into adipocytes under HG conditions was observed, as assessed by triglyceride accumulation or by protein expression levels of SREBP-1, FAS and PPARγ. This is consistent with what we and others have reported previously (3,5), and it fits with a recent report of no difference in adipogenesis related to glucose in a longer, 21-day protocol (4). In this last study, an elevated inflammatory gene-response profile was noted in the differentiated adipocytes (4).

We investigated whether the resulting adipocytes, although differentiated normally, were altered in other ways by the HG-based differentiation process. Little is known about glucose-responsive ChREBP isoform expression in human adipocyte differentiation. A smaller and more potent isoform of ChREBP (75 kDa; ChREBPβ) was originally identified in 2012 in mouse adipocytes; the initially discovered 95 kDa ChREBP is now called ChREBPα (8).

ChREBPβ is localized to the nucleus because it does not have the N-terminal domain that directs binding to 14-3-3 proteins in the cytosol (12). It also lacks the low-glucose inhibitory domain and so is constitutively active. The extent of ChREBPβ expression may reflect the activity of ChREBPα, which induces it (8,13). ChREBP, along with SREBP-1c, stimulates lipogenesis in the adipocyte, inducing expression of genes such as ACC, FAS, and SCD-1 (14,15).

The liver is another major site of ChREBP expression, but its role differs from that in adipose tissue. For example, Liver X receptor is an upstream regulator of ChREBPβ in the liver but not in adipose tissue (16). Unlike in liver, where ChREBP is linked to insulin resistance, in adipocytes, it is associated with improved in vivo insulin sensitivity. This may be due to production of an insulin-sensitizing class of lipokines that are released from adipocytes (17,18).

ChREBPα mRNA and protein levels were reported to rise as human preadipocytes differentiate in NG conditions (19,20), extending studies initially done in rodent adipocytes (21). It is not yet known which adipogenic transcription factors positioned upstream of ChREBPα are responsible for its upregulation in the differentiated adipocyte. The ChREBPβ isoform was not evaluated in those human adipogenesis studies, nor was the effect of HG on its levels during differentiation.

Our data show that HG had a clear impact on the pattern and quantity of ChREBP isoform expression during differentiation. The increase in ChREBPβ protein levels in the nuclear fraction mirrored the higher level of mRNA expression in HG-differentiated adipocytes. The concomitant lack of change in ChREBPα protein can be considered to be a type of loading control in comparison to the increase in ChREBPβ. However, the use of classic loading

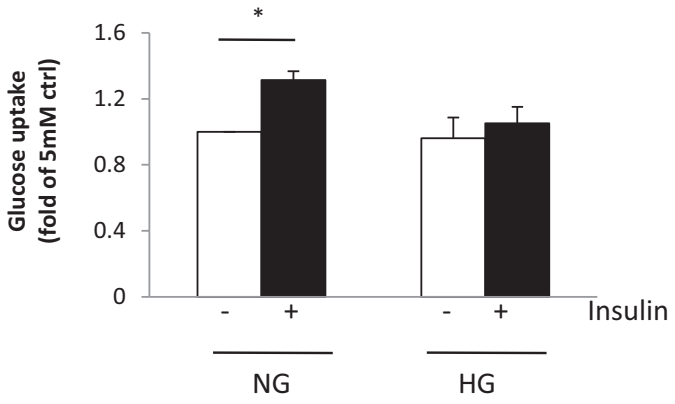

Figure 4. Insulin-stimulated glucose uptake is inhibited in HG-differentiated adipocytes. Human abdominal sc preadipocytes were maintained in growth medium or induced to differentiate in 5 (NG) or 25 (HG) mmol/L glucose. Cells were stimulated with vehicle or 100 nmol/L insulin for 30 min, and 3H-deoxyglucose uptake was determined. Results were normalized to 5 mmol/L glucose control condition and are the means ± SEM of 4 separate patient samples. Means were compared by 2-way ANOVA with Tukey post hoc tests. *$p<0.05$ between indicated pairs. *HG*, High glucose; *NG*, normal glucose.

controls, i.e. lamin A/C and tubulin A, would have strengthened our findings related to the effects of HG on the ChREBP protein levels.

The elevation in ChREBPβ expression suggested the possibility of enhanced gene expression of ChREBP target genes. TXNIP was originally identified as a ChREBP target in islet cells (7). It is a glucose-responsive, 397 amino acid, 50 kDa protein that can bind thioredoxin, an antioxidant protein that reduces disulfide bonds in substrate proteins (22). TXNIP belongs to the alpha-arrestin family, and when it binds the reduced form of thioredoxin, it forms a mixed disulfide, and so inhibits the reducing action of thioredoxin. TXNIP-null mice show enhanced insulin responsiveness, despite promoting adiposity (23). TXNIP overexpression in mouse adipocyte cell lines inhibits basal and insulin-stimulated glucose uptake, and reduction in TXNIP expression enhances these processes (24). Mutant constructs of TXNIP indicate that this inhibitory effect on insulin responsiveness in adipocytes is independent of thioredoxin and oxidative stress (25,26). Studies in human skeletal muscle also suggested a redox-independent role for TXNIP; a reduction in its expression was tied to an increase in insulin sensitivity with caloric restriction (27). The nature of this redox-independent mechanism is not known; 1 group has proposed a direct inhibitory interaction of TXNIP with GLUT1 in cancer cell lines (28). It was because of these previous investigations that we selected TXNIP as a ChREBP target to investigate in our human adipose cell studies. It must be noted, however, that there are many other target genes of ChREBP that remain to be analyzed in human adipocytes.

Our data showing insulin-stimulated glucose uptake is inhibited in HG-differentiated adipocytes are, therefore, consistent with expected effects of TXNIP elevation. The NG insulin-stimulated glucose uptake response we observed is in line with what has previously been shown for human adipocytes in culture (11). A recent study using adipose-specific ChREBP knockout mice found that their adipocytes displayed a cell-autonomous reduction in insulin-stimulated glucose transport (29). It is possible that species-related and/or glycemic differences are the reason for the inhibition of insulin-stimulated glucose uptake we observed in HG-differentiated human adipocytes that express higher levels of ChREBPβ and TXNIP. It must be noted that our data showing an association between the expression of ChREBPβ and TXNIP with the inhibition of insulin-stimulated glucose uptake do not provide any evidence of causality between these events. Other studies, using strategies such as targeted gene silencing, will be required to address whether there is a mechanistic link.

We also examined the mRNA expression of several lipogenic genes that have been reported as ChREBP targets. However, under our conditions, using human differentiated adipocytes, we observed no upregulation of their expression in HG conditions. Induction of ChREBPβ has been associated with increased lipogenic gene expression and increased insulin sensitivity (17,18,30), but our experimental data from HG-differentiated adipocytes show neither lipogenic gene induction nor insulin sensitivity, suggesting distinct regulation in differing metabolic contexts. A limitation of our study is that the adipose cells used were all from females and included a wide BMI range. Further studies would be needed to determine whether the changes are BMI-dependent and whether similar findings would be observed using cells from male participants.

Conclusions

In summary, although human adipogenesis itself was unaffected by HG conditions, the resulting HG-differentiated adipocytes displayed differences in ChREBPβ, but not ChREBPα, expression. These adipocytes also express more TXNIP and are insulin resistant with respect to glucose uptake. More work will be needed to understand the glucose-dependent expression of the ChREBPβ isoform in this context and to examine potential mechanistic links between TXNIP and insulin-dependent glucose uptake in human adipocytes.

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Author Disclosures

Conflicts of interest: None.

Author Contributions

VP, AG, and AS participated in the design of the study. VP performed experimental work and data analysis with assistance from AG, AL, and LM; AS, VP, AG, and LM wrote the manuscript; and all authors were involved in reviewing and editing the manuscript.

References

1. Spalding KL, Arner E, Westermark PO, et al. Dynamics of fat cell turnover in humans. Nature 2008;453:783–7.
2. Arner E, Westermark PO, Spalding KL, et al. Adipocyte turnover: Relevance to human adipose tissue morphology. Diabetes 2010;59:105–9.
3. Collins JM, Neville MJ, Pinnick KE, et al. De novo lipogenesis in the differentiating human adipocyte can provide all fatty acids necessary for maturation. J Lipid Res 2011;52:1683–92.
4. Ronningen T, Shah A, Reiner AH, Collas P, Moskaug JO. Epigenetic priming of inflammatory response genes by high glucose in adipose progenitor cells. Biochem Biophys Res Commun 2015;467:979–86.
5. Peshdary V, Gagnon A, Sorisky A. Effect of high glucose concentration on human preadipocytes and their response to macrophage-conditioned medium. Can J Diabetes 2016;40:411–8.
6. Abdul-Wahed A, Guilmeau S, Postic C. Sweet sixteenth for ChREBP: Established roles and future goals. Cell Metab 2017;26:324–41.
7. Cha-Molstad H, Saxena G, Chen J, Shalev A. Glucose-stimulated expression of Txnip is mediated by carbohydrate response element-binding protein, p300,

and histone H4 acetylation in pancreatic β cells. J Biol Chem 2009;284:16898–905.
8. Herman MA, Peroni OD, Villoria J, et al. A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. Nature 2012;484:333–8.
9. Gagnon A, Langille ML, Chaker S, Antunes TT, Durand J, Sorisky A. TSH signaling pathways that regulate MCP-1 in human differentiated adipocytes. Metabolism 2014;63:812–21.
10. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–5.
11. Tremblay F, Gagnon A, Veilleux A, Sorisky A, Marette A. Activation of the mammalian target of rapamycin pathway acutely inhibits insulin signaling to Akt and glucose transport in 3T3-L1 and human adipocytes. Endocrinology 2005;146:1328–37.
12. Ge Q, Huang N, Wynn RM, et al. Structural characterization of a unique interface between carbohydrate response element-binding protein (ChREBP) and 14-3-3β protein. J Biol Chem 2012;287:41914–21.
13. Dentin R, Langin D, Postic C. Hidden variant of ChREBP in fat links lipogenesis to insulin sensitivity. Cell Metab 2012;15:795–7.
14. Baraille F, Planchais J, Dentin R, Guilmeau S, Postic C. Integration of ChREBP-mediated glucose sensing into whole body metabolism. Physiology 2015;30:428–37.
15. Filhoulaud G, Guilmeau S, Dentin R, Girard J, Postic C. Novel insights into ChREBP regulation and function. Trends Endocrinol Metab 2013;24:257–68.
16. Beaven SW, Matveyenko A, Wroblewski K, et al. Reciprocal regulation of hepatic and adipose lipogenesis by liver x receptors in obesity and insulin resistance. Cell Metab 2013;18:106–17.
17. Yore MM, Syed I, Moraes-Vieira PM, et al. Discovery of a class of endogenous mammalian lipids with anti-diabetic and anti-inflammatory effects. Cell 2014;159:318–32.
18. Yilmaz M, Claiborn KC, Hotamisligil GS. De novo lipogenesis products and endogenous lipokines. Diabetes 2016;65:1800–7.
19. Pompei A, Toniato E, Innocenti P, et al. Cyanidin reduces preadipocyte differentiation and relative ChREBP expression. J Biol Regul Homeost Agents 2012;26:253–64.
20. Hurtado del Pozo C, Vesperinas-Garcia G, Rubio MA, et al. ChREBP expression in the liver, adipose tissue and differentiated preadipocytes in human obesity. Biochim Biophys Acta 2011;1811:1194–200.
21. He Z, Jiang T, Wang Z, Levi M, Li J. Modulation of carbohydrate response element-binding protein gene expression in 3T3-L1 adipocytes and rat adipose tissue. Am J Physiol Endocrinol Metab 2004;287:E424–30.
22. Lee S, Kim SM, Lee RT. Thioredoxin and thioredoxin target proteins: From molecular mechanisms to functional significance. Antioxid Redox Signal 2013;18:1165–207.
23. Chutkow WA, Birkenfeld AL, Brown JD, et al. Deletion of the alpha-arrestin protein Txnip in mice promotes adiposity and adipogenesis while preserving insulin sensitivity. Diabetes 2010;59:1424–34.
24. Parikh H, Carlsson E, Chutkow WA, et al. TXNIP regulates peripheral glucose metabolism in humans. PLoS Med 2007;4:e158.
25. Patwari P, Chutkow WA, Cummings K, et al. Thioredoxin-independent regulation of metabolism by the alpha-arrestin proteins. J Biol Chem 2009;284:24996–5003.
26. Anderson EJ. Cutting calories and TXNIP from the skeletal muscle to restore insulin sensitivity. Diabetes 2016;65:16–8.
27. Johnson ML, Distelmaier K, Lanza IR, et al. Mechanism by which caloric restriction improves insulin sensitivity in sedentary obese adults. Diabetes 2016;65:74–84.
28. Wu N, Zheng B, Shaywitz A, et al. AMPK-dependent degradation of TXNIP upon energy stress leads to enhanced glucose uptake via GLUT1. Mol Cell 2013;49:1167–75.
29. Vijayakumar A, Aryal P, Wen J, et al. Absence of carbohydrate response element binding protein in adipocytes causes systemic insulin resistance and impairs glucose transport. Cell Rep 2017;21:1021–35.
30. Eissing L, Scherer T, Todter K, et al. De novo lipogenesis in human fat and liver is linked to ChREBPβ and metabolic health. Nat Commun 2013;4:1528.