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RESEARCH ARTICLE

Gene Expression of *GSK3* in Type II Diabetics Compared to Non-Diabetics (*ex vivo*)

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Abstract:

Background:

GSK3 is a serine/threonine kinase that is involved in the storage of glucose into glycogen through the negative regulation of glycogen synthase. Defects in *GSK3* and glycogen synthase function are early stages of the development of insulin resistance, which may cause impaired glycogen synthesis in Type II diabetes.

Methods:

In this cross-sectional study, the gene expression level of *GSK3* from Type II diabetic and non-diabetic participants was compared *via* real-time RT-PCR. To investigate the relationships between *GSK3* expression and indicators of insulin resistance, Pearson's correlation analysis was performed. To compare the differences between *GSK3* expression levels based on BMI categories, one-way ANOVA was used.

Results:

Gene expression of *GSK3* was slightly higher in diabetic participants compared to non-diabetics, but it was statistically insignificant. Also, no significant difference was found based on BMI categories in the two groups. No significant association between *GSK3* expression and indicators of insulin resistance was observed in non-diabetic participants. There was only a positive significant correlation between *GSK3* expression and FBS in diabetic participants.

Conclusion:

These results indicate that the regulation of *GSK3* may occur at the translation level, as gene expression level was unaltered between diabetic and non-diabetic participants. Also, since circulating levels of both glucose and insulin regulate *GSK3* activity, tissue specificity for the expression and post-translation regulations of *GSK3* may exist, which cause hyperactivation or overexpression in some target tissues in diabetes. Furthermore, it is probable that glycogen synthase activity is also regulated by non-insulin mediated mechanisms like exercise or allosteric changes, independent of *GSK3* expression.

Keywords: *GSK3*, Gene expression, Type II diabetes, Insulin action, Metabolism, RT-PCR, Transcription.

1. INTRODUCTION

Impaired insulin regulation of glucose transport and utilization is associated with insulin-resistance and diabetes [1].

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Insulin-induced glycogen synthesis in elevated postprandial blood glucose levels is necessary to maintain glucose homeostasis and defective regulation of glycogen synthesis by insulin is an additional feature of the insulin-resistant condition [2]. Insulin receptors in target tissues stimulate a signaling cascade leading to phosphorylation/activation of insulin receptor substrates (*IRS1* and *IRS2*) and subsequent activation of

phosphatidylinositol-3 kinase (*PI3K*) cascade, which is the main pathway involved in glucose transport and glycogen synthesis [3]. By this signaling axis, insulin stimulates phosphorylation of phosphoinositide-dependent serine-threonine protein kinase (*AKT*) and Glycogen synthase kinase-3 (*GSK3*), which increases and decreases their activities, respectively [4]. The *PI3K/AKT* pathway is antagonized by various factors, including phosphatase and tensin homolog (*PTEN*) [5]. Glycogen synthase (GS) activity is activated by *PI3K/AKT* signaling cascade and inhibited by *GSK3* through phosphorylation. *AKT* phosphorylates/inactivates *GSK3* and increases GS activity [3]. *GSK3* is a serine/threonine-protein kinase and a negative regulator of glycogen synthesis and lipogenesis [6]. Previous *in vitro* studies have demonstrated the role of *GSK3* in the regulation of glycogen synthase phosphorylation and its function [7]. A specificity of *GSK3* regulation is that it is constitutively activated [8]; thus, the negative regulation of *GSK3* through the *PI3K/AKT* pathway keeps *GSK3* activity at low stages [9]. Several disease conditions, such as insulin resistance, can break cellular homeostasis and lead to increased *GSK3* activity [10]. Overly activated *GSK3* in disease

conditions may occur when the *PI3K/AKT* pathway is either over-stimulated (*e.g.*, chronic over-nutrition) or repressed as a result of inhibitors or lack of stimuli. This "overstimulation-induced insensitivity" phenomenon is commonly present in almost all of the metabolic disorders [11]. Serine phosphorylation of *GSK3* by *AKT* results in inhibition of its kinase activity, whereas tyrosine phosphorylation of *GSK3* promotes the activity of the enzyme [11]. Also, *GSK3* phosphorylates *IRS* on serine residues and causes its proteolytic breakdown [12], which is one of the negative-feedback loops implicated in the *PI3K/AKT* signaling pathway that down-regulates insulin signal transduction. Furthermore, GSK3 plays the main role in *PTEN* phosphorylation, which acts as an antagonist factor of insulin signal transduction [13]. In addition, it has been demonstrated that *GSK3* is suppressed by physiological concentrations of insulin in human skeletal muscle [14, 15] as well as different cell lines [16 - 18]. As *GSK3* is inhibited by insulin (to stimulate the *PI3K/AKT* signalling pathway), phosphorylation and deactivation of *PTEN* by *GSK3* might be part of a negative feedback loop for the *PI3K/AKT* pathway [4] (Fig. **1**).
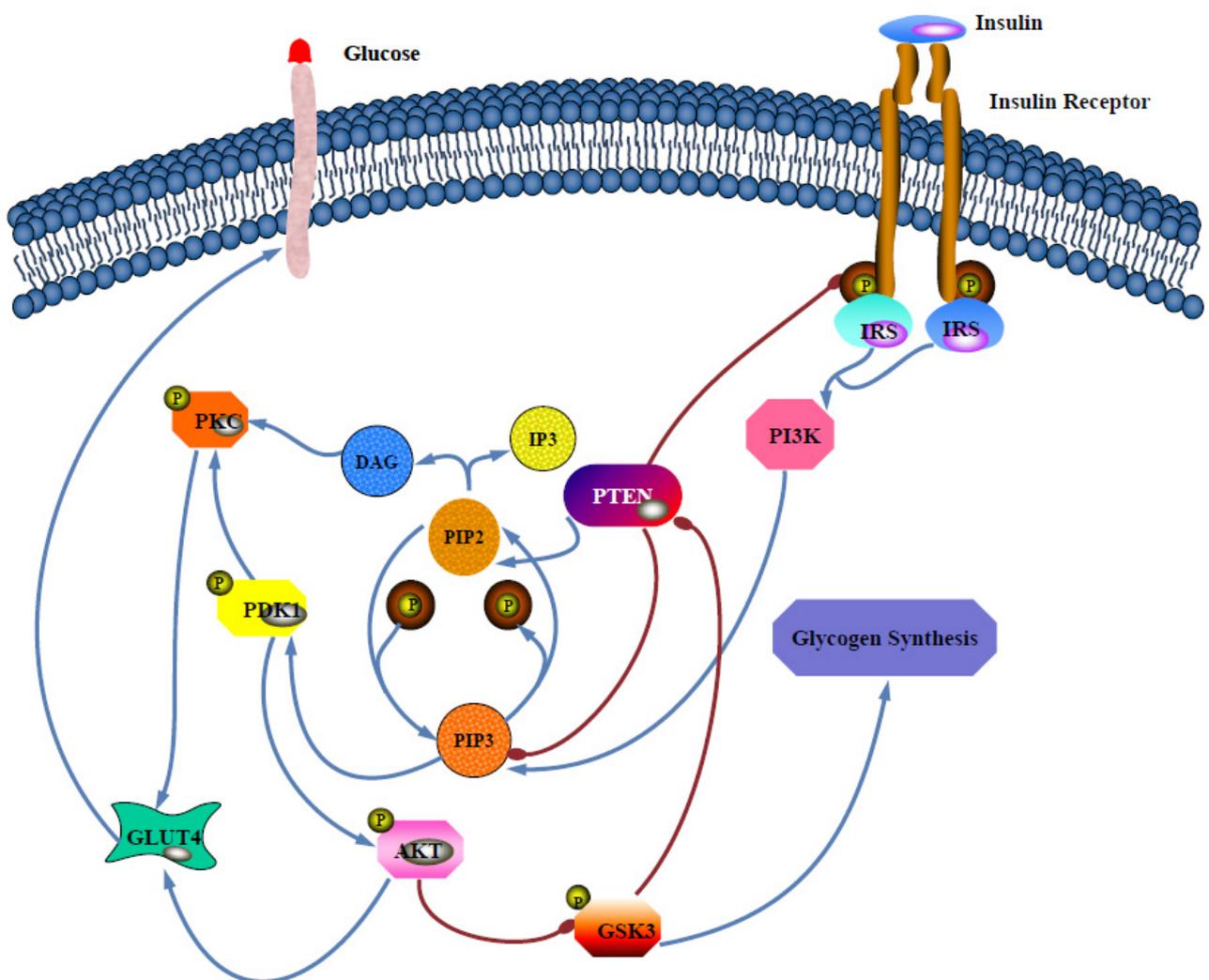

Fig. (1). Proposed negative feedback loop to regulate the *PI3K/AKT* pathway.

Administration of *GSK3* inhibitor increased glucose tolerance in diabetic mice [19, 20] and reduced blood glucose levels in patients with Type II diabetes as well as in Zucker diabetic fatty rats [21 - 24]. These findings demonstrate that inhibition of *GSK3* improves glucose regulation in diabetic animals. Consistent with this, overexpression of *GSK3* is enough to cause glucose intolerance [19, 25].

Since it has been suggested that *GSK3* is a ubiquitous kinase implicated in insulin function that induces insulin resistance and diabetes through different pathways, it was considered in the present study. The aim of this study was to investigate the relationships between *GSK3* gene expression levels and indicators of insulin resistance and compare those between diabetic and non-diabetic individuals.

In this schematic, insulin activates the insulin receptor and *PI3K*. The resulting increase in PIP3 level activates the *AKT* kinase that phosphorylates and inhibits *GSK3*. The loss of *GSK3* activity enhances *PTEN* activity, resulting in a negative feedback loop.

2. MATERIALS AND METHODS

2.1. Experimental Participants

The present work is a cross-sectional study that included 50 Type II diabetic and 50 non-diabetic individuals in the age group of 35-60 recruited from University Putra Malaysia and Serdang Hospital. The number of men and women were equal in each group and there was no statistically significant difference between the two groups with respect to age (P>0.05). Diabetic and non-diabetic participants were divided into two groups on the basis of body mass index (BMI<30 and BMI>30). In diabetic and non-diabetic groups, 50% were normal/overweight (BMI <30 kg/m2) and 50% were obese (BMI of >30 kg/m2). This weight distribution was not significantly different between the groups. All diabetic patients were administered to the anti-diabetic drug Metformin without insulin injection. Participants who had cancers, diabetic patients with diabetes complications, and pregnant women were excluded from this study. Ethical approval was obtained from the University Putra Malaysia Research Ethics Committee and the National Medical Research Register (Ministry of Health). The study was monitored according to the Declaration of Helsinki in its currently applicable version and the guidelines of the International Conference on Harmonization of Good Clinical Practice. Informed written consent was obtained from all participants.

2.2. Assessment of Clinical and Metabolic Characteristics of Subjects

FBS (Cat.No:04657527) was tested by the colorimetric enzymatic method using Roche Diagnostics GmbH Cobas-c 311 Germany machine. HbA1c analysis was performed by the colorimetric method (Diazyme; cat. No. DZ168A, USA) and the C-peptide level was determined by ELISA Kit (Cloud Clone Corp; cat. no. CEA447Hu, UK).

2.3. RNA Extraction and Real-time *PCR*

Real-time RT-PCR method was employed to investigate the variability within non-diabetic and Type II diabetic participants in gene expression of *GSK3* with a set of two housekeeping genes (*GAPDH* and *β-ACTIN*). The analysis was performed using the three technical replicate samples from each of the participants. Briefly, RNA was extracted from 2.5 ml blood by using PAXgene Blood RNA Tubes (Qiagen; cat. no. 762165). RNA concentration and purity were measured using a NanoDrop ND1000 spectrophotometer at A260: A280 ratio (Thermo Fisher Scientific; USA). RNA quality and integrity were determined *via* agarose gel electrophoresis. The purified RNA sample was converted to cDNA, using the Qiagen cDNA Synthesis Kit, according to the manufacturer's instructions (Qiagen; cat. no. 205313), and then cDNA was used to perform RT-PCR *via* SYBR green technology with the QuantiTect Reverse Transcription Kit (Qiagen; cat. no. 204054). The final volume of the RT-PCR reaction mixture was 20 μl and contained 2 μl cDNA, 1 μM of each primer, 10 μl Master Mix, and RNase-free water up to 20 μl. *GAPDH* and *β-ACTIN* were chosen as suitable reference genes to normalize the mRNA expression. The specificity of the product was assessed from the melting curve analysis. According to manufacturer recommendation, real-time PCR quantification experiments should include a positive control containing all the components of the reaction except for the template (NTC) and negative control (template without reverse transcriptase) to ensure the quality of run and confirm the absence of contamination. In this study, primers were designed by Qiagen and cycling conditions were as follows: 95 ºC for 5 min (PCR initial activation step); 95 ºC for 10 s (denaturation); 60 ºC for 30 s (combined annealing/extension); followed by 40 cycles and it was performed on a Bio-Rad Real-Time PCR detection system (Bio-Rad; CFX96, USA). Average gene Ct values were normalized to the average of housekeeping genes Ct values of the same cDNA sample. Fold differences were determined using the comparative ΔΔCT method.

2.4. Statistical Analyses

Data are shown as means ± SE unless stated otherwise. Before statistical analysis, non-normally distributed parameters were logarithmically transformed to approximate a normal distribution. Statistical analysis was performed using independent-samples t-test with the SPSS 21.0 statistical software package (SPSS Inc., Chicago, IL, USA). The threshold of significance was defined as a P<0.05. Pearson's correlation was used as appropriate to analyze the relationship between measured biochemical markers and expression level of *GSK3*. One-way ANOVA was used to compare differences of *GSK3* expression between two groups based on BMI categories. Significance was accepted at a P<0.05. Levene's test was used to check significant differences revealed by the ANOVA.

3. RESULTS

The clinical and metabolic characteristic data of participants involved in this study are summarized in Table **1**. Mean age and BMI were not significantly different between non-diabetic and diabetic participants. Diabetic participants displayed elevated HbA1c and FBS and lower C-peptide levels.

Table 1. Serum CP, HbA1c, and glucose levels of the participants.

Biochemical Parameters	Non-diabetic Participants Mean ± SE	Diabetic Participants Mean ± SE	df	t	P-value
Glucose (mmol/L)	5.38±0.14	8.45±0.41	52.88	7.10	0.001*
HbA1c (%)	5.40±0.78	7.95±0.85	98	4.57	0.001*
C-peptide (ng/mL)	2.02±0.02	1.96±0.01	83.21	-1.92	0.05*

*$P<0.05$

Table 2. Relative expression levels for *GSK3* between diabetic and non-diabetic participants.

GOI	Non-diabetic Participants Mean ± SE	Diabetic Participants Mean ± SE	df	t	P-value
GSK3	0.49±0.03	0.46±0.02	98	-0.722	0.472

*$P<0.05$

Table 3. Relation between GSK3 and BMI categories in diabetic and non diabetic participants.

-		Sum of Squares	df	Mean Square	F	Sig.
BMI Categories	Between Groups	0.026	3	0.009	0.202	0.895
	Within Groups	4.142	96	0.043	-	-
	Total	4.169	99	-	-	-

*$P<0.05$

Table 4. The *GSK3* gene expression level in relation to study variables of the diabetic and non-diabetic participants.

Study Variables	Non-diabetic Participants		Diabetic Participants	
	Pearson Correlation (r)	P-value	Pearson Correlation (r)	P-value
C-Peptide	-0.045	0.762	-0.204	0.155
FBS	-0.034	0.817	0.358*	0.012
HbA1c	-0.122	0.408	0.181	0.210
Gender	0.159	0.285	0.151	0.299
Age	-0.208	0.161	-0.170	0.249
BMI	0.035	0.813	0.047	0.747
Family History	-0.139	0.357	0.071	0.640

**Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Gene expression level of *GSK3* was higher in Type II diabetic participants compared to the non-diabetics, but it was not statistically significant (Table **2**).

Each diabetic and non-diabetic group of participants was further divided into two groups based on the BMI (BMI<30 and BMI>30) to find out the possible role of obesity on the regulation of *GSK3* expression. Of the diabetics, 50% were normal/overweight (BMI <30 kg/m2) and 50% were obese (BMI of >30 kg/m2). In non-diabetic participants, 50% were normal/overweight and 50% were obese. This weight distribution was not significantly different between the four groups. To compare differences between *GSK3* expression levels based on BMI categories in diabetic and non-diabetic participants, one-way ANOVA was used in which no significant difference was found (Table **3**).

Diabetic and non-diabetic participants were divided into four groups based on family history of diabetes, and it was found that 25 (50%) non-diabetic participants and 29 (58%) diabetic participants had a family history of Type II diabetes.

Pearson's correlation analysis was performed to investigate the plausible relationship between *GSK3* expression and family history of Type II diabetes. No significant correlation was found in the gene expression of *GSK3* based on a family history of diabetes in diabetic and non-diabetic participants (Table **4**).

To investigate the relationships between *GSK3* gene expression and indicators of insulin resistance, Pearson's correlation analysis was performed, which indicated no significant association between *GSK3* expression and indicators of insulin resistance in two groups in except a significant positive correlation between *GSK3* expression and FBS in diabetic participants (Table **4**).

4. DISCUSSION

GSK3 is a serine/threonine kinase that is involved in the storage of glucose into glycogen. Defects in *GSK3* and GS function are early stages of the development of insulin resistance, which may cause impaired glycogen synthesis in

T2DM [26].

GSK3 activity has been shown to be increased in skeletal muscle and adipose tissue of insulin-resistant animals [26, 27]. In obese Zucker rats, reduced phosphorylation of GSK3 has been reported [27]. Constitutive activation of GSK3 in vitro [28] or muscle-specific overexpression of GSK3 [25] resulted in insulin resistance. Inhibition of GSK3 in Zucker diabetic fatty rats leads to improvement in insulin function and glucose uptake [7]. It has been demonstrated that PI3K/AKT pathway plays a key role in insulin-stimulated glucose transport [26, 29 - 31]. Reduced insulin stimulated glucose transport is one of the major metabolic defects of skeletal muscles in insulin-resistant and type II diabetic individuals [29 - 31]. Previous studies have demonstrated that the overexpression of GSK3 in skeletal muscle samples from insulin resistance states may negatively influence insulin signal transduction and cause its weakness, particularly at IR and IRS [32]. Also, overexpression of GSK3 in either cultured cells [28] or mice [28] has been shown to antagonize insulin signaling. One mechanism whereby GSK3 increases insulin resistance is by serine phosphorylation of IRS, resulting in reduced tyrosine phosphorylation of IRS by the insulin receptor and reduced PI3K/AKT signaling to downstream components [33]. Others have found that insulin resistance may be contributed to defects in glycogen synthesis [34]. GSK3 gene expression levels and function are increased in muscles of Type II diabetics patients and are inversely correlated with both GS function and insulin-stimulated glucose transport [10, 14]. Treatment of both human muscle cells and insulin-resistant rat with GSK3 inhibitors can increase insulin-stimulated glycogen synthesis and glucose transport [35, 36]. Transgenic mice with muscle-specific overexpression of human GSK3 developed glucose intolerance and hyperlipidemia [25].

In contrast to these findings, our study demonstrated no significant difference in GSK3 gene expression between diabetic and non-diabetic participants. Additionally, there was no significant difference in GSK3 gene expression based on BMI categories and family history of diabetes. Although correlations do not prove causal relationships, there was an expected positive correlation between GSK3 gene expression level and FBS only in diabetic participants. No association between GSK3 gene expression levels and indicators of insulin resistance was observed in non-diabetic participants. One possible interpretation of this observation is that higher gene expression levels of GSK3 in diabetics (although it was not enough to reach a significant amount) may predispose them to failure in glucose utilization and the development of insulin resistance. To understand more about the involvement of GSK3 in obesity and insulin resistance, the correlation between GSK3 gene expression level and BMI level was considered and no significant correlation was found. These results indicate that regulation of GSK3 may occur at the level of translation, as gene expression levels were unaltered between diabetic and non-diabetic as well as based on BMI categories or family history of diabetes. Whether disturbances in GSK3 expression or function are a cause of insulin resistance or a consequence of that, is unknown.

This study is consistent with previous studies which found no changes in GSK3 activity in samples from diabetic human or animals [20, 35, 37] and differ from those that found elevated GSK3 activity [38 - 40]. Information about the regulation of GSK3 in humans is limited. Acute bouts of exercise have been shown to both increase [15] and decrease [41] GSK3 activity in muscle. Differences in the duration and intensity of the exercise may be important variables. Prolonged treatment of obese individuals with impaired glucose tolerance with diet and exercise program caused reduced GSK3 gene expression in skeletal muscles [42].

Contradictions in literature could be due to the differences in experimental design, the method of tissue collection, and processing, which requires considerable optimization to overcome. Since this study merely considered the gene expression regulation without post-translation modification and tissue-specific differences, it is difficult to draw conclusions on the role of defective GSK3 function in the development of insulin resistance.

Our data indicate that it is probable that glycogen synthase activity is regulated by non-insulin mediated mechanisms such as exercise [43] or allosteric changes independent of GSK3 expression [44].

Since many functions are regulated by GSK3, the activity of GSK3 must be highly regulated. The main mechanisms of regulating the actions of GSK3 in a substrate-specific manner are [45]: regulation by GSK3 autophosphorylation, phosphorylation state of GSK3 substrates, translocation of GSK3, and formation of protein complexes containing GSK3 that direct, or inhibit, its actions toward specific substrates. The most well-defined regulatory mechanism is inhibition of the activity of GSK3 by phosphorylation of a regulatory serine [46]. The PI3K/AKT signaling pathway, activated in response to insulin, is one of the main regulators of GSK3. AKT and protein kinase C phosphorylates/inhibits GSK3 on serine residues [9]. Conversely, the enzymatic activity of GSK3 is stimulated by tyrosine-phosphorylation, but the mechanisms underlying this modification are not well-defined. This autoposhorylation of the tyrosine residue is probably intramolecularly mediated and not under the regulatory control of upstream modulators [9].

Since GSK3 is constitutively stimulated and activated [8], its regulation might be achieved mainly through the inhibitory serine-phosphorylation. Although normal suppression of GSK3 is controlled by insulin, the overstimulation of receptors may cause insensitivity of the GSK3 and result in uninhibited GSK3 activity [11]. Therefore, the release of GSK3 activity, a hallmark of PI3K/AKT/GSK3 pathway insensitivity, can happen under disease conditions as a consequence of overstimulation or inhibition of PI3K/AKT. These diversity mechanisms controlling the actions of GSK3 provide substrate-specific control of its functions, and exclusive capacity for an enzyme that can regulate numerous cellular functions.

Moreover, it has been found that the circulating levels of both glucose and insulin regulate GSK3 activity. This complicated relationship and collaboration between insulin and glucose may cause contradictions in literature as well. Also, as insulin depletion and hyperglycemia lead to tissue-specific

changes, the activity of *GSK3* might vary among different tissues [32, 47]. High fat diet induced-diabetes in mice caused a slight reduction in the activity of *GSK3* in the liver, enhancement in epididymal fat, and no changes in skeletal muscle [38], indicating that regulation of *GSK3* in insulin resistance conditions might be different among tissues [32, 48]. Also, it has been revealed that changes in *GSK3* phosphorylation due to insulin deficiency in diabetic mice brain are big and opposite to changes in peripheral tissues as phosphorylation level of *GSK3* in the brain is increased while reduced in epididymal fat [49]. This enhanced *GSK3* phosphorylation is ascribed to hyperglycemia associated with insulin depletion. Hyperglycemia, in the presence of normal or deficient insulin levels, caused increased *GSK3* phosphorylation level, whereas it was reduced by hypoglycemia with insulin administration [49]. Thus, both insulin and glucose contribute to regulating the activity of *GSK3*.

Also, it has been demonstrated that using *GSK3* knock-in mice, in which *AKT* phosphorylation sites on *GSK3α* and *GSK3β* were mutated, did not affect blood glucose level and insulin-induced hepatic glycogen accumulation were normal [50]. Also, a regulatory axis consisting of *AKT/PPP1R3G/PPP1R3B* that controls glycogen synthesis and glucose homeostasis in parallel to the *GSK3*-dependent axis has been revealed [51]. In that study, the knocking down of *PPP1R3G* inhibited insulin response while the wild type *PPP1R3G*, and not phosphorylation-defective mutants, increased glycogen deposition and insulin sensitivity. *PPP1R3G,* as a regulatory subunit of protein phosphatase1 (*PP1*), is phosphorylated by *AKT,* where its phosphorylation fluctuates with the fasting-feeding cycle and is necessary for insulin-stimulated glycogen synthase. Consequently, a *GSK3*-independent pathway has been proposed to link insulin signaling with glycogen synthesis.

CONCLUSION

Even though several lines of evidence support the involvement of *GSK3* in insulin resistance, the role of *GSK3* in diabetes is currently unknown and requires further investigations.

Although increased serine phosphorylation of *GSK3* represents a mechanism for the control of *GSK3* activity in response to hormones, such as insulin, it is probable that glycogen synthase activity is regulated by non-insulin mediated mechanisms as well, such as exercise or allosteric changes independent of *GSK3* expression or other insulin-dependent pathways. Taken together, it appears that there is tissue specificity for the expression and post-translation regulations of *GSK3,* which may cause hyperactivation or overexpression in some target tissues in diabetes, since circulating levels of both glucose and insulin regulate *GSK3* activity.

AUTHORS' CONTRIBUTIONS

SAHK[1], MSAM[1], and **HK[6]** were responsible for the study concept and design. **SAHK[1]** and **JAA[3]** contributed to data acquisition. **MFS[2]** assisted **SAHK[1]** with data analysis and interpretation of findings. **SAHK[1]** drafted the manuscript. **MSAM[1]**, **HK[6]**, **ZR[4],** and **RMA[5]** provided critical revision of the manuscript for important intellectual content and approved the final version for publication.

REFERENCES

[1]   Lorenzo C, Wagenknecht LE, D'Agostino RB Jr, Rewers MJ, Karter AJ, Haffner SM. Insulin resistance, beta-cell dysfunction, and conversion to type 2 diabetes in a multiethnic population: the Insulin Resistance Atherosclerosis Study. Diabetes Care 2010; 33(1): 67-72.
[http://dx.doi.org/10.2337/dc09-1115] [PMID: 19808919]

[2]   Jensen J, Rustad PI, Kolnes AJ, Lai Y-C. The role of skeletal muscle glycogen breakdown for regulation of insulin sensitivity by exercise. Front Physiol 2011; 2: 112.
[http://dx.doi.org/10.3389/fphys.2011.00112] [PMID: 22232606]

[3]   Alsadat S, Khorami H, Movahedi A, Kuzwah K, Mutalib A, Sokhini M. PI3K / AKT pathway in modulating glucose homeostasis and its alteration in Diabetes. Ann Med Biomed Sci 2015; 1: 46-55.

[4]   Al-Khouri AM, Ma Y, Togo SH, Williams S, Mustelin T. Cooperative phosphorylation of the tumor suppressor phosphatase and tensin homologue (PTEN) by casein kinases and glycogen synthase kinase 3beta. J Biol Chem 2005; 280(42): 35195-202.
[http://dx.doi.org/10.1074/jbc.M503045200] [PMID: 16107342]

[5]   Vereshchagina N, Ramel MC, Bitoun E, Wilson C. The protein phosphatase PP2A-B' subunit Widerborst is a negative regulator of cytoplasmic activated Akt and lipid metabolism in Drosophila. J Cell Sci 2008; 121(Pt 20): 3383-92.
[http://dx.doi.org/10.1242/jcs.035220] [PMID: 18827008]

[6]   Mosser VA, Li Y, Quon MJ. PTEN does not modulate GLUT4

translocation in rat adipose cells under physiological conditions. Biochem Biophys Res Commun 2001; 288(4): 1011-7.
[http://dx.doi.org/10.1006/bbrc.2001.5876] [PMID: 11689011]

[7]   Cline GW, Johnson K, Regittnig W, *et al.* Effects of a novel glycogen synthase kinase-3 inhibitor on insulin-stimulated glucose metabolism in Zucker diabetic fatty (fa/fa) rats. Diabetes 2002; 51(10): 2903-10.
[http://dx.doi.org/10.2337/diabetes.51.10.2903] [PMID: 12351425]

[8]   Biondi RM, Nebreda AR. Signalling specificity of Ser/Thr protein kinases through docking-site-mediated interactions. Biochem J 2003; 372(Pt 1): 1-13.
[http://dx.doi.org/10.1042/bj20021641] [PMID: 12600273]

[9]   Beurel E, Grieco SF, Jope RS. Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. Pharmacol Ther 2015; 148: 114-31.
[http://dx.doi.org/10.1016/j.pharmthera.2014.11.016]   [PMID: 25435019]

[10]   Eldar-Finkelman H. Glycogen synthase kinase 3: An emerging therapeutic target. Trends Mol Med 2002; 8(3): 126-32.
[http://dx.doi.org/10.1016/S1471-4914(01)02266-3]   [PMID: 11879773]

[11]   Liu X. Overstimulation can create health problems due to increases in PI3K/Akt/GSK3 insensitivity and GSK3 activity. Springerplus 2014; 3: 356.
[http://dx.doi.org/10.1186/2193-1801-3-356] [PMID: 25089247]

[12]   Oliver Tschopp BAH Role of PKB/Akt in Liver Diseases. Berlin, Heidelberg: Springer Berlin Heidelberg 2010.

[13]   Vazquez F, Ramaswamy S, Nakamura N, Sellers WR. Phosphorylation of the PTEN tail regulates protein stability and function. Mol Cell Biol 2000; 20(14): 5010-8.
[http://dx.doi.org/10.1128/MCB.20.14.5010-5018.2000]   [PMID: 10866658]

[14]   Nikoulina SE, Ciaraldi TP, Mudaliar S, Mohideen P, Carter L, Henry RR. Potential role of glycogen synthase kinase-3 in skeletal muscle insulin resistance of type 2 diabetes. Diabetes 2000; 49(2): 263-71.
[http://dx.doi.org/10.2337/diabetes.49.2.263] [PMID: 10868943]

[15]   Wojtaszewski JF, Nielsen P, Kiens B, Richter EA, Wojtazsewski JF. Regulation of glycogen synthase kinase-3 in human skeletal muscle: effects of food intake and bicycle exercise. Diabetes 2001; 50(2): 265-9.
[http://dx.doi.org/10.2337/diabetes.50.2.265] [PMID: 11272135]

[16]   Cross DA, Watt PW, Shaw M, *et al.* Insulin activates protein kinase B, inhibits glycogen synthase kinase-3 and activates glycogen synthase by rapamycin-insensitive pathways in skeletal muscle and adipose tissue. FEBS Lett 1997; 406(1-2): 211-5.
[http://dx.doi.org/10.1016/S0014-5793(97)00240-8] [PMID: 9109420]

[17]   Moule SK, Welsh GI, Edgell NJ, Foulstone EJ, Proud CG, Denton RM. Regulation of protein kinase B and glycogen synthase kinase-3 by insulin and beta-adrenergic agonists in rat epididymal fat cells. Activation of protein kinase B by wortmannin-sensitive and -insensitive mechanisms. J Biol Chem 1997; 272(12): 7713-9.
[http://dx.doi.org/10.1074/jbc.272.12.7713] [PMID: 9065430]

[18]   Moule SK, Edgell NJ, Welsh GI, Diggle TA, Foulstone EJ, Heesom KJ, *et al.* Multiple signalling pathways involved in the stimulation of fatty acid and glycogen synthesis by insulin in rat epididymal fat cells. Biochem J 1995; 311(Pt 2): 595-601.
[http://dx.doi.org/10.1042/bj3110595]

[19]   Nabben M, Neumann D. GSK-3 inhibitors: Anti-diabetic treatment associated with cardiac risk?: Editorial to: "The impact of chronic glycogen synthase kinase-3 inhibition on remodeling of normal and pre-diabetic rat hearts." by Barbara Huisamen *et al.* Cardiovasc Drugs Ther 2016; 30(3): 233-5.
[http://dx.doi.org/10.1007/s10557-016-6669-y] [PMID: 27311575]

[20]   Plotkin B, Kaidanovich O, Talior I, Eldar-Finkelman H. Insulin mimetic action of synthetic phosphorylated peptide inhibitors of glycogen synthase kinase-3. J Pharmacol Exp Ther 2003; 305(3): 974-80.
[http://dx.doi.org/10.1124/jpet.102.047381] [PMID: 12626660]

[21]   Shen W, Taylor B, Jin Q, *et al.* Inhibition of DYRK1A and GSK3B induces human β-cell proliferation. Nat Commun 2015; 6: 8372.
[http://dx.doi.org/10.1038/ncomms9372] [PMID: 26496802]

[22]   Feng ZC, Donnelly L, Li J, Krishnamurthy M, Riopel M, Wang R. Inhibition of Gsk3β activity improves β-cell function in c-KitWv/+ male mice. Lab Invest 2012; 92(4): 543-55.
[http://dx.doi.org/10.1038/labinvest.2011.200] [PMID: 22249311]

[23]   Smith SA, Porter LE, Biswas N, Freed MI. Rosiglitazone, but not glyburide, reduces circulating proinsulin and the proinsulin:insulin ratio in type 2 diabetes. J Clin Endocrinol Metab 2004; 89(12): 6048-53.

[http://dx.doi.org/10.1210/jc.2004-0705] [PMID: 15579757]

[24]   Yue T-L, Bao W, Gu J-L, *et al.* Rosiglitazone treatment in Zucker diabetic Fatty rats is associated with ameliorated cardiac insulin resistance and protection from ischemia/reperfusion-induced myocardial injury. Diabetes 2005; 54(2): 554-62.
[http://dx.doi.org/10.2337/diabetes.54.2.554] [PMID: 15677515]

[25]   Pearce NJ, Arch JRS, Clapham JC, *et al.* Development of glucose intolerance in male transgenic mice overexpressing human glycogen synthase kinase-3β on a muscle-specific promoter. Metabolism 2004; 53(10): 1322-30.
[http://dx.doi.org/10.1016/j.metabol.2004.05.008] [PMID: 15375789]

[26]   Wang L, Wang Y, Zhang C, *et al.* Inhibiting glycogen synthase kinase 3 reverses obesity-induced white adipose tissue inflammation by regulating apoptosis inhibitor of macrophage /cd5l-mediated macrophage migration. Arterioscler Thromb Vasc Biol 2018; 38(9): 2103-16.
[http://dx.doi.org/10.1161/ATVBAHA.118.311363]   [PMID: 30026270]

[27]   Dokken BB, Sloniger JA, Henriksen EJ. Acute selective glycogen synthase kinase-3 inhibition enhances insulin signaling in prediabetic insulin-resistant rat skeletal muscle. Am J Physiol Endocrinol Metab 2005; 288(6): E1188-94.
[http://dx.doi.org/10.1152/ajpendo.00547.2004] [PMID: 15671078]

[28]   MacAulay K, Blair AS, Hajduch E, *et al.* Constitutive activation of GSK3 down-regulates glycogen synthase abundance and glycogen deposition in rat skeletal muscle cells. J Biol Chem 2005; 280(10): 9509-18.
[http://dx.doi.org/10.1074/jbc.M411648200] [PMID: 15632169]

[29]   Borges CC, Salles AF, Bringhenti I, Souza-Mello V, Mandarim-de-Lacerda CA, Aguila MB. Adverse effects of vitamin D deficiency on the Pi3k/Akt pathway and pancreatic islet morphology in diet-induced obese mice. Mol Nutr Food Res 2016; 60(2): 346-57.
[http://dx.doi.org/10.1002/mnfr.201500398] [PMID: 26446269]

[30]   Huang X, Liu G, Guo J, Su Z. The PI3K/AKT pathway in obesity and type 2 diabetes. Int J Biol Sci 2018; 14(11): 1483-96.
[http://dx.doi.org/10.7150/ijbs.27173] [PMID: 30263000]

[31]   Zhou J, Zhong J, Lin S, *et al.* Inhibition of PTEN Activity Aggravates Post Renal Fibrosis in Mice with Ischemia Reperfusion-Induced Acute Kidney Injury. Cell Physiol Biochem 2017; 43(5): 1841-54.
[http://dx.doi.org/10.1159/000484070] [PMID: 29049990]

[32]   Ciaraldi TP, Oh DK, Christiansen L, *et al.* Tissue-specific expression and regulation of GSK-3 in human skeletal muscle and adipose tissue. Am J Physiol Endocrinol Metab 2006; 291(5): E891-8.
[http://dx.doi.org/10.1152/ajpendo.00176.2006] [PMID: 16757548]

[33]   Liberman Z, Eldar-Finkelman H. Serine 332 phosphorylation of insulin receptor substrate-1 by glycogen synthase kinase-3 attenuates insulin signaling. J Biol Chem 2005; 280(6): 4422-8.
[http://dx.doi.org/10.1074/jbc.M410610200] [PMID: 15574412]

[34]   Højlund K. Metabolism and insulin signaling in common metabolic disorders and inherited insulin resistance. Dan Med J 2014; 61(7): B4890.
[PMID: 25123125]

[35]   Henriksen EJ, Kinnick TR, Teachey MK, *et al.* Modulation of muscle insulin resistance by selective inhibition of GSK-3 in Zucker diabetic fatty rats. Am J Physiol Endocrinol Metab 2003; 284(5): E892-900.
[http://dx.doi.org/10.1152/ajpendo.00346.2002] [PMID: 12517738]

[36]   Nikoulina SE, Ciaraldi TP, Mudaliar S, Carter L, Johnson K, Henry RR. Inhibition of glycogen synthase kinase 3 improves insulin action and glucose metabolism in human skeletal muscle. Diabetes 2002; 51(7): 2190-8.
[http://dx.doi.org/10.2337/diabetes.51.7.2190] [PMID: 12086949]

[37]   Semiz S, Orvig C, McNeill JH. Effects of diabetes, vanadium, and insulin on glycogen synthase activation in Wistar rats. Mol Cell Biochem 2002; 231(1-2): 23-35.
[http://dx.doi.org/10.1023/A:1014437019586] [PMID: 11952162]

[38]   Ciaraldi TP, Nikoulina SE, Bandukwala RA, Carter L, Henry RR. Role of glycogen synthase kinase-3 alpha in insulin action in cultured human skeletal muscle cells. Endocrinology 2007; 148(9): 4393-9.
[http://dx.doi.org/10.1210/en.2006-0932] [PMID: 17569761]

[39]   Jope RS, Yuskaitis CJ, Beurel E. Glycogen synthase kinase-3 (GSK3): inflammation, diseases, and therapeutics. Neurochem Res 2007; 32(4-5): 577-95.
[http://dx.doi.org/10.1007/s11064-006-9128-5] [PMID: 16944320]

[40]   Lappas M. GSK3β is increased in adipose tissue and skeletal muscle from women with gestational diabetes where it regulates the inflammatory response. PLoS One 2014; 9(12)e115854
[http://dx.doi.org/10.1371/journal.pone.0115854] [PMID: 25541965]

[41]   Sakamoto K, Arnolds DE, Ekberg I, Thorell A, Goodyear LJ. Exercise regulates Akt and glycogen synthase kinase-3 activities in human skeletal muscle. Biochem Biophys Res Commun 2004; 319(2): 419-25.
[http://dx.doi.org/10.1016/j.bbrc.2004.05.020] [PMID: 15178423]

[42]   Venojärvi M, Puhke R, Hämäläinen H, *et al.* Role of skeletal muscle-fibre type in regulation of glucose metabolism in middle-aged subjects with impaired glucose tolerance during a long-term exercise and dietary intervention. Diabetes Obes Metab 2005; 7(6): 745-54.
[http://dx.doi.org/10.1111/j.1463-1326.2004.00466.x]   [PMID: 16219019]

[43]   Jensen J, Tantiwong P, Stuenæs JT, *et al.* Effect of acute exercise on glycogen synthase in muscle from obese and diabetic subjects. Am J Physiol Endocrinol Metab 2012; 303(1): E82-9.
[http://dx.doi.org/10.1152/ajpendo.00658.2011] [PMID: 22510711]

[44]   Bouskila M, Hunter RW, Ibrahim AFM, *et al.* Allosteric regulation of glycogen synthase controls glycogen synthesis in muscle. Cell Metab 2010; 12(5): 456-66.
[http://dx.doi.org/10.1016/j.cmet.2010.10.006] [PMID: 21035757]

[45]   Jope RS, Johnson GV. The glamour and gloom of glycogen synthase kinase-3. Trends Biochem Sci 2004; 29(2): 95-102.
[http://dx.doi.org/10.1016/j.tibs.2003.12.004] [PMID: 15102436]

[46]   McManus EJ, Sakamoto K, Armit LJ, *et al.* Role that phosphorylation of GSK3 plays in insulin and Wnt signalling defined by knockin analysis. EMBO J 2005; 24(8): 1571-83.
[http://dx.doi.org/10.1038/sj.emboj.7600633] [PMID: 15791206]

[47]   Patel S, Doble BW, MacAulay K, Sinclair EM, Drucker DJ, Woodgett JR. Tissue-specific role of glycogen synthase kinase 3beta in glucose homeostasis and insulin action. Mol Cell Biol 2008; 28(20): 6314-28.
[http://dx.doi.org/10.1128/MCB.00763-08] [PMID: 18694957]

[48]   Laviola L, Belsanti G, Davalli AM, *et al.* Effects of streptozocin diabetes and diabetes treatment by islet transplantation on *in vivo* insulin signaling in rat heart. Diabetes 2001; 50(12): 2709-20.
[http://dx.doi.org/10.2337/diabetes.50.12.2709] [PMID: 11723053]

[49]   Clodfelder-Miller B, De Sarno P, Zmijewska AA, Song L, Jope RS. Physiological and pathological changes in glucose regulate brain Akt and glycogen synthase kinase-3. J Biol Chem 2005; 280(48): 39723-31.
[http://dx.doi.org/10.1074/jbc.M508824200] [PMID: 16179343]

[50]   Wan M, Leavens KF, Hunter RW, *et al.* A noncanonical, GSK3-independent pathway controls postprandial hepatic glycogen deposition. Cell Metab 2013; 18(1): 99-105.
[http://dx.doi.org/10.1016/j.cmet.2013.06.001] [PMID: 23823480]

[51]   Li Q, Zhao Q, Zhang J, *et al.* The Protein Phosphatase 1 Complex Is a Direct Target of AKT that Links Insulin Signaling to Hepatic Glycogen Deposition. Cell Rep 2019; 28(13): 3406-3422.e7.
[http://dx.doi.org/10.1016/j.celrep.2019.08.066] [PMID: 31553910]