Original Research Article

The impact of disease duration on sex differences in cardiometabolic risk factors in type 2 diabetic patients on metformin-glimepiride oral therapy

Comment [H1]: May be better to be changed to (gender) & anywhere in the paper.

ABSTRACT

Aims: Patients with type 2 diabetes mellitus (T2DM) are commonly treated with metformin-glimepiride combination therapy. The relationship between disease duration and cardiometabolic risk factors among T2DM patients on metformin-glimepiride combination therapy may be moderated by sex. This, however, has not been investigated in the Ghanaian population.

Study design: This was a cross-sectional study

Place and Duration of Study: The study was conducted from January to December 2019 at the Bolgatanga Regional Hospitalwhich is located in the Upper East region of Ghana.

Methodology: The study involved 163 patients with T2DM (Female=103 and Male=60) who were between the ages of 25 and 70 years. All the participants werereceiving metformin-glimepiride combination oral therapyat the time of sampling. The participants were matched by age, duration of disease and age at the onset of T2DM. The blood pressure and anthropometric variables were measured after which fasting venous blood samples were collected and analyzed for lipids, insulin, glucose and C-reactive protein.

Results: There was a significant interaction between sex and the duration of T2DM on fasting plasma triglycerides level (P=0.003). The fasting plasma triglyceride reduced with the duration of T2DM but the reduction was more marked in males ($adjR^2$=0.200) than females ($adjR^2$= 0.001).

Conclusion: Fasting plasma triglyceride levels may be reduced with the duration of T2DM among patients on metformin-glimepiride combination therapy.However, this reduction is more pronounced in males than in the female. There is a need for sex-specific protocols for the control of plasma triglyceride levels among patients with T2DM who are on metformin-glimepiride combination therapy.

Keywords: Glimepiride, Metformin, Triglycerides, Cardiovascular disease, Diabetes mellitus, Ghana

1.0 INTRODUCTION

Type 2 diabetes mellitus (T2DM) is characterized by a disorder of glucose metabolism in adulthood either due to insulin resistance and/or insulin insufficiency(Najim et al., 2013). Hyperglycemia is a cardinal feature of T2DM as the glucose uptake by tissues is reduced and this may be arising from the low insulin secretion due to β-cell dysfunction or tissue resistance to insulin action. Aside from hyperglycemia, T2DM patients also have increased odds of dyslipidemia, hypertension and other cardiometabolic risk factors that may lead to complications if left untreated (Najim et al., 2013). However, there are sex differences in the onset, pathophysiology and cardiometabolic risk factors in T2DM. Females seem to have better lipid homeostasis, insulin sensitivity and reduced risk of cardiovascular complications than similarly

matched males but may deteriorate faster than males after the onset of T2DM. It has been suggested that sex differences in enzyme expression may be dependent on variation in regulatory proteins which seem to be related to the presence of estrogen, acting through its receptors; Estrogen Receptor beta (ERβ), Estrogen Receptor alpha (ERα) and G-protein coupled Estrogen Receptor (GPER) (De Marinis et al., 2008, Wang et al., 2011, Palmisano et al., 2018).

There are several strategies in the management of T2DM ranging from dietary and lifestyle modification to medications. Metformin and glimepiride are among the most common antidiabetic drugs that may be used as monotherapies but are also regularly used in combination. Metformin is a biguanide insulin sensitizer that reduces hepatic glucose neogenesis and improves glucose uptake by cells(Bosi, 2009). Glimepiride, on the other hand, is sulphonylurea that stimulates insulin secretion from pancreatic β-cells. Metformin as a monotherapy or in combination with glimepiride has also been shown to have a pleiotropic effect on lipid metabolism and blood pressure homeostasis. Previous studies have shown significant relationships between disease duration with cardiometabolic risk factors among patients with T2DM, although there have been variabilities (Bosi, 2009, Wulffele et al., 2004, Shimpi et al., 2009).

However, sex is not strictly a binary variable but a multiple of masculinities or femininities that may converge and even interact with some other important sociodemographic and environmental variables (Kautzky-Willer et al., 2016). Moreover, the definition of disease duration as equaling the time from diagnosis may be a misclassification since T2DM may exist subclinically before the actual diagnosis. It is suggested that any significant associations, should, however, be regarded as underestimates of the true results rather than false positive outcomes(Östgren et al., 2002). Variabilities between the sexes and disease duration in T2DM require population-specific studies regarding disease duration and cardiometabolic risk factors to inform the decision for sex-specific protocols for the management of cardiometabolic risk factors.

2.0 MATERIALS AND METHODS

2.1 Design and setting

This was a cross-sectional study from January to December 2019 at the Bolgatanga Regional Hospital (BRH). The BRH is the main referral hospital in the Upper East Region of Ghana including some referrals from health institutions from the border towns of Burkina Faso.

2.2 Study sample and selection

The study involved 163type 2 diabetic patients (F=103, M=60), aged from 25 to 70 years. The participants had a disease duration ranging from zero to 13 years and were receiving long-term metformin-glimepiride combined oral therapy per the Ghana Health treatment protocol for type 2 diabetes mellitus(GHS, 2017). Persons with known type 1 diabetes, maturity-onset diabetes of the young, gestational diabetes, chronic liver disease, chronic renal disease and/or hypertension were excluded.

2.3 Definitions

Type 2 diabetes mellitus was defined per the criteria proposed by the World Health Organization Diabetes Experts Committee as a fasting blood glucose level ≥7.0 mmol/L or a 2-hour post-load

Comment [H2]: It is an old definition.

glucose ≥11.1 mmol/L (Organization, 1999). Disease duration was defined as the time from the diagnosis of T2DM (Östgren et al., 2002).

2.4 Data sources/Measurements

The sociodemographic and clinical data were collected using a pretested questionnaire. Blood pressure was measured twice with a mercurial sphygmomanometer according to the fifth Korotkoff sound and mean systolic (SBP) and diastolic (DBP) blood pressure were recorded. The standing height and body weight were measured following recommended guidelines. The body mass index (BMI) was derived by dividing the body weight (Kg) by the standing height (m2). Fasting venous blood samples were then collected and analyzed. The serum/plasma fasting blood glucose, total cholesterol (TCHOL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (TRIG)were measured using a routine biochemistry test. The serum/plasma insulin and human serum C-reactive protein (CRP) were determined using the ELISA technique. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using the formula below where FBG is fasting blood glucose in mmol/L and insulin was measured in mIU/L(Horáková et al., 2019).

$$\text{HOMA-IR} = \frac{FBG\ x\ Insulin}{22.5}$$

2.5 Statistical analysis

The data were collected using a Microsoft Excel Spreadsheet before statistical analysis in SPSS (v23) and GraphPad Prism (v8). The Shapiro-Wilk Test was used to test for the normality of the data and the presence of outliers. Descriptive statistics were then performed and each variable was summarized as mean ± SD or median (interquartile range-IQR) for parametric and nonparametric variables respectively. Linear regression models with interaction effects were formulated. To reduce multicollinearity in the regression models, the disease duration variable was centered on its mean by subtracting the mean from each score of the variable. An interaction variable was then created between sex (Coded as female=0, male=1) and disease duration by multiplying the sex variable by the centered disease duration variable (SEX*Duration of T2DM). Linear regression models were then created with a cardiometabolic risk factor as the dependent variable and sex, disease duration and interaction terms as the predictors. To reduce confounding, age, BMI and WHR were included in each model as covariates. The regression standardized predicted values and residuals were used for goodness-of-fit testing using a probability-probability (P-P) plot for normalitywhile a scatter plot was used to test for homoscedasticity. Where the assumption of homoscedasticity was violated, a weight was created using auxiliary regression analysis of the regression residuals. The regression analysis was then repeated with the addition of the weight in a weighted regression analysis. All statistical analyses were two-tailed at a significant level of $P<0.050$.

2.6 Ethical statement

The study complied with the guidelines of the 1964 Declaration of Helsinki and its later amendments regarding human subject studies. The study was approved by the institutional review board of the Institutional Review Board, Navrongo Research Center, Navrongo. Informed consent was obtained from each participant before the study.

3.0 RESULTS AND DISCUSSION

3.1 Results

3.1.1 General characteristics

The male and female participants were matched by chronological age, age at onset and the duration of disease (T2DM). However, females had significantly higher BMI, fasting TCHOL and LDL than males (P<0.050) as shown in Table 1.

Table 1. General characteristics of the study population

Variable	Female	Male	P-value
Age (years)	51.5±8.37	53.4±10.41	0.265
Age at onset (years)	47.9±8.94	49.6±10.46	0.321
Disease duration (years)	3.6(0.0-5.0)	3.7(0.3-6.0)	0.700
BMI (Kg/m^2)	29.1±4.93	24.8±4.55	<0.001
WHR	0.9±0.06	0.9±0.08	0.171
TCHOL (mmol/L)	4.8±1.37	4.3±1.39	0.049
HDL (mmol/L)	1.5±0.58	1.4±0.64	0.515
LDL (mmol/L)	4.3±1.22	3.9±1.26	0.047
TRIG (mmol/L)	1.5±0.91	1.5±0.89	0.861
CRP (mg/L)	6.6(2.3-10.8)	8.0(3.2-12.2)	0.585
SBP mmHg	140±21	136±21	0.177
DBP mmHg	90±12	86±14	0.184
HOMA-IR (mUI/mL)	2.7(1.85-3.75)	3.0(1.7-3.6)	0.862

The results are summarized as either mean ± SD or median (IQR) for parametric and nonparametric variables respectively. The differences in means and medians were determined using the student t-test (unpaired, 2-tailed) and the Mann-Whitney U test (unpaired, 2-tailed) respectively.

Comment [H3]: What is it mean?Waist to Hip Ratio? Need to define it at the foot of the table

3.1.2 Disease duration and cardiometabolic risk factors

The sex-moderated regression analysis with assumption testing between disease duration and cardiometabolic risk factors are summarized in Tables(2 and 3) and Figure 1. There was heteroscedasticity in the first model for fasting triglycerides (Supplementary Figure S1). To reduce the heteroscedasticity, weighted regression analysis was then performed for the same variable, triglycerides. There was a significant interaction between sex and disease duration on fasting triglyceride levels. There was a reduction in fasting plasma triglycerides with the duration of T2DM, which was independent of age, BMI, and WHR. Disease duration accounted for about 4.8% of the variability in fasting plasma triglycerides in the total study sample(adjR^2=0.048). However, there was a sex difference in the relationship between disease duration and fasting plasma triglycerides in T2DM. The relationship was negative in males where disease duration accounted for about 20% of the variability in fasting plasma triglycerides (adjR^2=0.200). In females, the relationship was almost negligible (adjR^2=0.001).

Table 2. The relationship between sex and T2DM duration on fasting lipid variables

LR Model	Dependent variable	B	95%CI		P-value
			Lower	Upper	
1	**TCHOL (mmol/L)**				
	(Constant)	0.51	-2.96	3.97	0.773
	AGE (years)	0.02	-0.01	0.04	0.198
	BMI (Kg/m^2)	0.06	0.01	0.11	0.019
	WHR	1.74	-1.81	5.29	0.334
	SEX	-0.31	-0.86	0.25	0.275
	SEX*Duration of T2DM	0.02	-0.10	0.14	0.763
2	**HDL (mmol/L)**				
	(Constant)	1.46	-0.10	3.02	0.066
	AGE (years)	-0.01	-0.02	0.01	0.427
	BMI (Kg/m^2)	0.02	-0.01	0.04	0.187
	WHR	-0.17	-1.77	1.44	0.839
	SEX	0.01	-0.24	0.26	0.964
	SEX*Duration of T2DM	0.00	-0.05	0.05	0.969
3	**LDL (mmol/L)**				
	(Constant)	1.07	-2.05	4.18	0.500
	AGE (years)	0.02	-0.01	0.04	0.200
	BMI (Kg/m^2)	0.05	0.00	0.10	0.033
	WHR	1.11	-2.08	4.30	0.492
	SEX	-0.29	-0.79	0.20	0.246
	SEX*Duration of T2DM	0.04	-0.07	0.15	0.457
4A	**TRIG (mmol/L)**				
	(Constant)	-1.82	-4.06	0.43	0.111
	AGE (years)	0.02	0.00	0.03	0.054
	BMI (Kg/m^2)	0.02	-0.01	0.06	0.180
	WHR	1.92	-0.38	4.22	0.101
	SEX	0.07	-0.29	0.43	0.704
	SEX*Duration of T2DM	-0.08	-0.16	-0.01	0.038
4B	**TRIG (mmol/L)**				
	(Constant)	-1.81	-3.34	-0.28	0.021
	AGE (years)	0.02	0.01	0.03	0.004
	BMI (Kg/m^2)	0.03	0.00	0.06	0.065
	WHR	1.82	-0.04	3.67	0.055
	SEX	0.03	-0.25	0.31	0.843
	SEX*Duration of T2DM	-0.06	-0.10	-0.02	0.003

Moderated regression analysis with interactions. Where the assumption of homoscedasticity was violated (A), weighted regression (B) was also performed.

Table 3. The relationship between sex and T2DM duration on non-lipid variables

LR Model	Dependent variable	B	95%CI		P-value
			Lower	Upper	
5	**CRP (mg/L)**				
	(Constant)	-9.90	-24.55	4.76	0.184
	AGE (years)	0.04	-0.07	0.15	0.487
	BMI (Kg/m^2)	-0.01	-0.23	0.20	0.901
	WHR	16.94	1.91	31.96	0.027
	SEX	0.09	-2.25	2.43	0.940
	SEX*Duration of T2DM	-0.25	-0.74	0.25	0.321
6	**SBP (mmHg)**				
	(Constant)	108.47	56.18	160.76	<0.001
	AGE (years)	0.79	0.40	1.19	<0.001
	BMI (Kg/m^2)	0.34	-0.43	1.11	0.380
	WHR	-19.74	-73.37	33.89	0.468
	SEX	-4.97	-13.32	3.39	0.241
	SEX*Duration of T2DM	-0.86	-2.63	0.91	0.337
7	**DBP (mmHg)**				
	(Constant)	69.66	36.80	102.53	<0.001
	AGE (years)	0.22	-0.03	0.47	0.081
	BMI (Kg/m^2)	0.35	-0.13	0.83	0.155
	WHR	-1.77	-35.48	31.94	0.917
	SEX	-2.03	-7.28	3.22	0.446
	SEX*Duration of T2DM	-0.20	-1.31	0.91	0.721
8	**HOMA-IR** (mUI/mL)				
	(Constant)	4.61	0.51	8.71	0.028
	AGE (years)	-0.01	-0.04	0.02	0.495
	BMI (Kg/m^2)	-0.01	-0.07	0.05	0.647
	WHR	-0.78	-4.98	3.43	0.716
	SEX	-0.01	-0.66	0.65	0.980
	SEX*Duration of T2DM	0.05	-0.09	0.19	0.495

Moderated regression analysis with interactions.

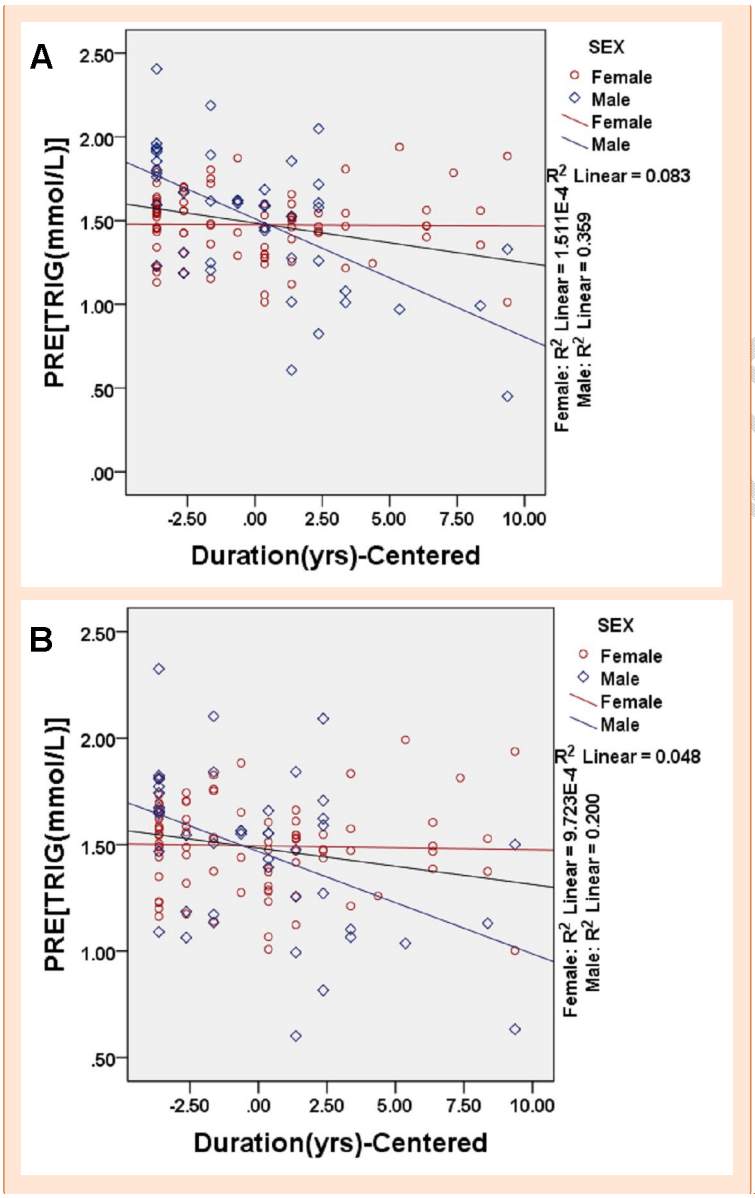


Figure 1. A graph showing the interactions between sex and the duration of type 2 diabetes on fasting plasma triglycerides. Moderated regression analysis (A); weighted regression analysis (B). PRE=regression predicted value of triglycerides.

Comment [H4]: Both A& B figure had the samrname ;need to be rename.

3.2 Discussion

The study aimed to determine the sex difference in the relationship between disease duration and cardiometabolic risk factors in T2DM patients on metformin-glimepiride oral therapy. It was observed that fasting plasma triglycerides reduced with disease duration which was independent of age, BMI and WHR. However, the reduction in fasting plasma triglycerides with the duration of T2DM was more pronounced in males than in females.

In this study, the average fasting plasma triglycerides decreased with increasing disease duration. However, this observation was more pronounced in males than females. Some previous studies have also reported decreased plasma triglyceride with disease duration among diabetics on metformin-glimepiride therapy (Ingle and Talele, 2011, Shimpi et al., 2009, Das et al.). However, some other studies found no significant difference or even the reverse(Najim et al., 2013).Metformin monotherapy has been found to have a significant effect on plasma triglycerides according to studies including meta-analysis(Wulffele et al., 2004). A reduction of about 0.19 mmol/L in plasma triglycerides, independent of BMI, was observed among patients with T2DM in a meta-analytic study that included 37 studies and involved 2891 patients (Wulffele et al., 2004). Similarly, metformin in combination with glimepiride has also been found to have a lowering effect on plasma triglycerides, but this observation has not been universal(Shimpi et al., 2009).

The primary function of metformin is to reduce hepatic glucose production as well as facilitate glucose uptake. However, metformin also has a pleiotropic effect on cardiometabolic processes including lipid homeostasis. Metformin action on lipid homeostasis has been suggested to be dependent onAMP-activated protein kinase activity in hepatocytes which results in reduced VLDL synthesis, increased fatty acid oxidation, emanating from the upregulation of downstream lipogenic genes as well as a reduction in sterol regulatory element-binding protein 1 expression and acetyl-coenzyme A carboxylase activity (Bosi, 2009). Glimepiride, when administered is said to stimulate insulin release and also induce hypoglycaemia. Glimepiride may also have an extra-pancreatic potential as an insulin-sensitizer but may also inhibit lipolysis and promote the accumulation of triacylglycerol, at least in the adipocytes of rats, although these have not been conclusive (Briscoe et al., 2010).

There was sexual dimorphism in the relationship between fasting plasma triglycerides and disease duration. While the reduction of triglycerides was marked in males, it was almost negligible in females. Previous studies have demonstrated that there is better lipid homeostasis, at least in premenopausal women, compared to similarly matched males. Premenopausal women tend to have lower fasting plasma triglycerides than males(Pradhan, 2014). This observation has been attributed to the hypolipidemic activity of estrogen which upregulates lipoprotein lipase activity and may also increase triglyceride and cholesterol clearance from circulation(Meyer et al., 2011, Tramunt et al., 2020, Pradhan, 2014). Moreover, sex differences in enzyme expression in lipid homeostasis may be dependent on variations in regulatory proteins which seem to be related to the presence of estrogen, acting through its receptors; Estrogen Receptor beta (ERβ), Estrogen Receptor alpha (ERα) and G-protein coupled Estrogen Receptor (GPER) (De Marinis et al., 2008, Wang et al., 2011, Palmisano et al., 2018).

Comment [H5]: Use different terms like gender variability or gender differences may be more acceptable

This study is among a few studies emanating from the northern part of Ghana to have examined the relationships between disease duration and cardiometabolic risk factors in T2DM patients on oral metformin-glimepiride therapy. The study also used moderated regression with interaction

terms while controlling for possible confounders (age, BMI and WHR). There was also, assumption testing for linear regression and where the assumption of homoscedasticity was violated, weighted regression was used to ensure homogeneity of variance in the regression model. The study was, however, limited by not including lifestyle modification factors such as diet, physical activity, smoking or alcohol consumption. Previous studies including clinical trials and meta-analyses have shown that the pathophysiology of T2DM and its management is impacted by lifestyle factors regardless of ethnicity (Hemmingsen et al., 2017, Haw et al., 2017). It is recommended that future studies should consider the effect of diet, physical activity, smoking and alcohol consumption on cardioembolic risk factors in patients with type 2 diabetes mellitus.

4.0 Conclusion

Fasting plasma triglyceride levels may be reduced with the duration of T2DM among patients on metformin-glimepiride combination therapy. However, this reduction is more pronounced in males than in the female. There is a need for sex-specific protocols for the control of plasma triglyceride levels among patients with T2DM who are on metformin-glimepiride combination therapy.

Data availability

The data supporting the findings of this study will be made available upon reasonable request from the corresponding author.

References

BOSI, E. 2009. Metformin–the gold standard in type 2 diabetes: what does the evidence tell us? *Diabetes, Obesity and Metabolism,* 11**,** 3-8.

BRISCOE, V. J., GRIFFITH, M. L. & DAVIS, S. N. 2010. The role of glimepiride in the treatment of type 2 diabetes mellitus. *Expert Opinion on Drug Metabolism & Toxicology,* 6**,** 225-235.

DAS, A., DATTA, S., CHAKRABARTY, S., BEGUM, S. A., DEY, S. K. & MUKHERJEE, A. K. An Open-Label, Prospective, Observational Study of Effects of Metformin versus Metformin Plus Glimepiride on Plasma Lipid Profile in Type II Diabetes Mellitus patients in a Tertiary Care Teaching Hospital In Kolkata.

DE MARINIS, E., MARTINI, C., TRENTALANCE, A. & PALLOTTINI, V. 2008. Sex differences in hepatic regulation of cholesterol homeostasis. *Journal of Endocrinology,* 198**,** 635-643.

GHS. 2017. *Standard Treatment Guidlines, Ghana National Drugs Program, Ministry of Health* [Online]. Accra Ghana. Available: https://www.bing.com/search?q=Ghana+health+services+treatment+protocol+for+type+2+diabetes&cvid=880d3bcdcdbb4cc99b6422d6209217eb&aqs=edge.0.69i59j69i57j69i59j69i61j69i60l2j69i65.4142j0j4&FORM=ANAB01&PC=U531 [Accessed 10 May 2019].

HAW, J. S., GALAVIZ, K. I., STRAUS, A. N., KOWALSKI, A. J., MAGEE, M. J., WEBER, M. B., WEI, J., NARAYAN, K. V. & ALI, M. K. 2017. Long-term sustainability of diabetes prevention approaches: a systematic review and meta-analysis of randomized clinical trials. *JAMA internal medicine,* 177**,** 1808-1817.

HEMMINGSEN, B., GIMENEZ-PEREZ, G., MAURICIO, D., I FIGULS, M. R., METZENDORF, M. I. & RICHTER, B. 2017. Diet, physical activity or both for prevention or delay of type 2 diabetes mellitus and its associated complications in people at increased risk of developing type 2 diabetes mellitus. *Cochrane Database of Systematic Reviews*.

HORÁKOVÁ, D., ŠTĚPÁNEK, L., JANOUT, V., JANOUTOVÁ, J., PASTUCHA, D., KOLLÁROVÁ, H., PETRÁKOVÁ, A., ŠTĚPÁNEK, L., HUSÁR, R. & MARTINÍK, K. 2019. Optimal Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) Cut-Offs: A Cross-Sectional Study in the Czech Population. *Medicina (Kaunas),* 55.

INGLE, P. V. & TALELE, D. G. S. 2011. Comparative effects of metformin in combination with glimepiride and glibenclamide on lipid profile in indian patients with type 2 diabetes mellitus. *Age (year, Mean, SD),* 47**,** 45.

KAUTZKY-WILLER, A., HARREITER, J. & PACINI, G. 2016. Sex and gender differences in risk, pathophysiology and complications of type 2 diabetes mellitus. *Endocrine reviews,* 37**,** 278-316.

MEYER, M. R., CLEGG, D. J., PROSSNITZ, E. R. & BARTON, M. 2011. Obesity, insulin resistance and diabetes: sex differences and role of oestrogen receptors. *Acta Physiologica,* 203**,** 259-269.

NAJIM, H. D., MAJEED, I. A. & RAHMAH, A. M. 2013. Effects of Metformin, glimepiride and their combination on glycemia and lipid profile of NIDDM patients-A study in Iraqis. *Int J Adv Pharm Biol Chem,* 2**,** 2277-4688.

ORGANIZATION, W. H. 1999. Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part 1, Diagnosis and classification of diabetes mellitus. World health organization.

ÖSTGREN, C. J., LINDBLAD, U., RANSTAM, J., MELANDER, A. & RÅSTAM, L. 2002. Glycaemic control, disease duration and β-cell function in patients with Type 2 diabetes in a Swedish community. Skaraborg Hypertension and Diabetes Project. *Diabetic Medicine,* 19**,** 125-129.

PALMISANO, B. T., ZHU, L., ECKEL, R. H. & STAFFORD, J. M. 2018. Sex differences in lipid and lipoprotein metabolism. *Molecular metabolism,* 15**,** 45-55.

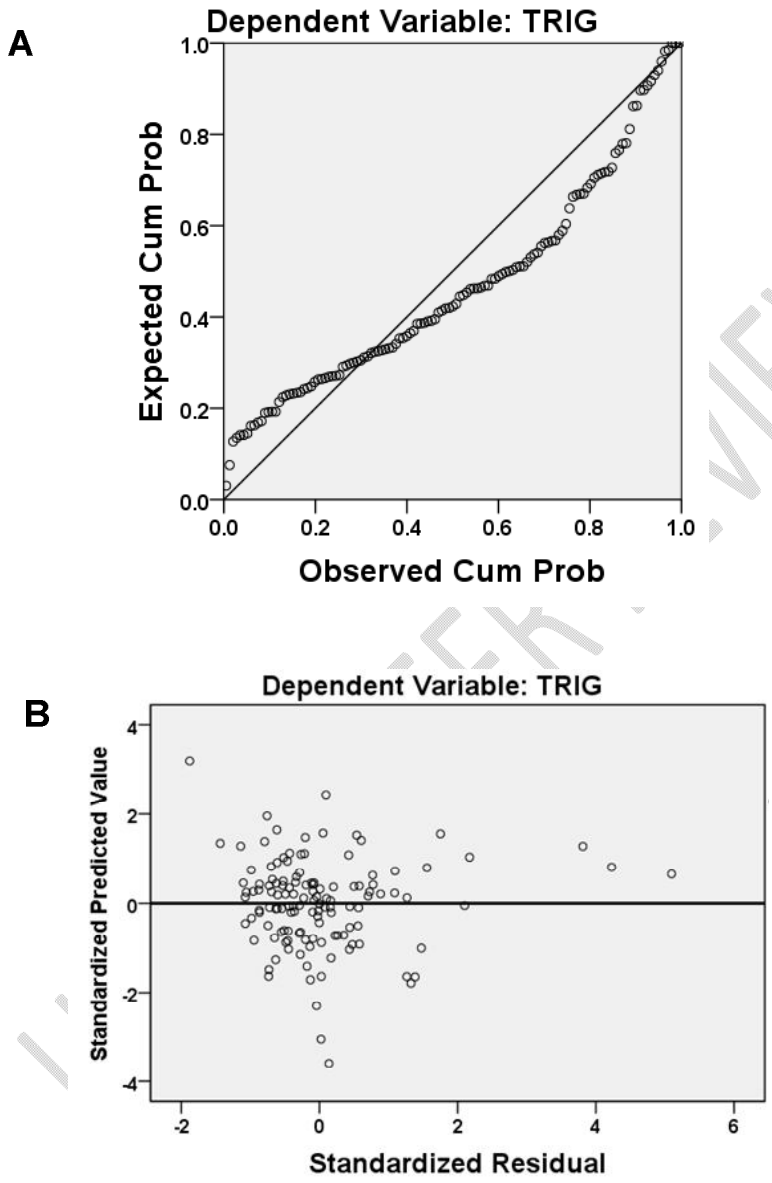
PRADHAN, A. D. 2014. Sex differences in the metabolic syndrome: implications for cardiovascular health in women. *Clinical chemistry,* 60**,** 44-52.

SHIMPI, R., PATIL, P., KUCHAKE, V., INGLE, P., SURANA, S. & DIGHORE, P. 2009. Comparison of effect of metformin in combination with glimepiride and glibenclamide on glycaemic control in patient with type 2 diabetes mellitus. *International Journal of Pharm. Tech. Research,* 1**,** 50-61.

TRAMUNT, B., SMATI, S., GRANDGEORGE, N., LENFANT, F., ARNAL, J.-F., MONTAGNER, A. & GOURDY, P. 2020. Sex differences in metabolic regulation and diabetes susceptibility. *Diabetologia,* 63**,** 453-461.

WANG, X., MAGKOS, F. & MITTENDORFER, B. 2011. Sex differences in lipid and lipoprotein metabolism: it's not just about sex hormones. *The Journal of Clinical Endocrinology & Metabolism,* 96**,** 885-893.

WULFFELE, E. M., KOOY, A., DE ZEEUW, D., STEHOUWER, C. & GANSEVOORT, R. 2004. The effect of metformin on blood pressure, plasma cholesterol and triglycerides in type 2 diabetes mellitus: a systematic review. *Journal of internal medicine,* 256**,** 1-14.



Supplementary Figure S1: Test of assumptions of multivariable regression analysis. Multivariable normality was tested using the probability-probability (P-P) plot (A). The homogeneity of variance or homoscedasticity was tested using the scatter plot from the regression residuals and predicted values (B).