Bull. Egypt. Soc. Physiol. Sci. Vol. (42). Issue (2), 202-212

Bull. of Egyp. Soc. Physiol. Sci.

(Official Journal of Egyptian Society for Physiological Sciences)

(pISSN: 1110-0842; eISSN: 2356-9514)

Autophagy promotion and fibrosis inhibition by combination of GLP1 analogue and metformin decreasing the progression of type II diabetic cardiomyopathy of albino rats: Immunohistochemical study

Hendawy, M.[1*], Ramy A. Abdelsalam[2], Mohie M. Ibrahim[1]

[1] **Department of Anatomy and Embryology, Faculty of Medicine, Mansoura University, Egypt**

[2] **Department of Pathology, Faculty of Medicine, Mansoura University, Egypt**

Submit Date: 30 October 2021

Accept Date : 11 Nov. 2021

Keywords

- Autophgy
- GLP1 analogue
- Diabetes
- cardiomyopathy

Abstract

Diabetic cardiomyopathy is one of the most serious chronic complications of type 2 diabetes. This study aimed to examine the therapeutic effect of GLP1 and metformin combination as oral antidiabetic drugs on diabetic cardiomyopathy through promotion of oxidative stress, improvement of autophagy of the cardiomyocytes and regression of cardiac fibrosis. Type 2 diabetes mellitus was induced by feeding the rats high fat diet for 12 week then injecting streptozotocin (30mg/kg) intraperitoneally after 4 weeks. One group of diabetic rats received metformin (30mg/kg), another group of diabetic rats received GLP1 analogue; liraglutide (75 µg/kg) and the last group of diabetic rats received combination of both drugs. After 24 hours of the experiment, the cardiac tissues were fixed in formalin and embedded in paraffin blocks to be examined histopathologically and immunohistochemically for autophogic markers (LC3 and P62). Also homogenate of heart tissues was made to measure oxidative stress markers (MDA, GSH) in the supernatant. Light microscope examination showed typical features of diabetic cardiomyopathy in diabetic group with increase in fibrous tissue interstitially and around blood vessels, which markedly improved in diabetic rats which received combination of both drugs, also combination of both drugs showed significant increase in early and late markers autophagy LC3 and P62 respectively when compared with diabetic rats, finally synergetic effect of both drug markedly improved oxidative stress (MDA,GSH activity). So we think that our study is the first study that discuss the therapeutic effect of combination of GLP1 analogue and metformin on diabetic cardiomyopathy through the antioxidative stress, antifibrotic and autophagic improving properties

Corresponding author: Hendawy, Mahmoud Mohammed, E-mail: hendawy016@mans.edu.eg, Mobile: 01008540444

.

INTRODUCTION

Diabetes mellitus (DM) is a worldwide trouble. In 2015, it was estimated that 415 million adults are suffering from diabetes and it is expected to jump to 642 million by 2040 [1,2,3]. Type 2 diabetes mellitus (T2DM) represents almost 90% of total prevalence of the epidemic of diabetes and this likely in parallel to the epidemic of obesity and metabolic syndrome [4,5]. T2DM is usually accompanied by many cardiovascular complications [6].

Diabetic cardiomyopathy (DCM) can be defined as a complicated case of diabetes associated with heart failure without coronary artery affection [7]. It accounts for mortality rate of 15%–20% annually [8]. It is a multifactorial complication which can be caused by increased reactive oxygen species (ROS), apoptosis, and insulin-like growth factor-1 (IGF-1), collagen deposition and fibrosis [9,10].

Autophagy is a normal cellular process for clearing the damaged organelles and keeping the normal cell functions. But increased autophagy leads to proteins and organelles damage [11,12]. So, the balanced autophagy is essential for cell survival. Recent studies have illustrated the relation between apoptosis and autophagy. Some proteins are vital in regulation of both apoptosis and autophagy. For example, Bcl-2 (anti-apoptotic protein) and Bim (pro-apoptotic protein) are the interacting partners of Beclin1, an autophagic protein that binds to Vps34 (a Class III PI-3 kinase) to form autophagosomes [13]. Bcl-2 and Bim can upset that bound between Beclin1 and Vps34 supressing Beclin1-dependent autophagy [14, 15]. Also there are many other signals the control the autophagy, such as AMP-activated protein kinase (AMPK) which was found to be affected by metformin administration [16]. Glucagon-like peptide-1 (GLP1) (exenatide and liraglutide) is widely used in treatment of type 2 diabetes [17,18]. It improves glycaemic control by stimulating insulin production and supressing glucagon secretion, besides retarding the gastric empting [19]. Both metformin and GLP1analogue have the ability to improve β-cell function, protecting them against glucolipotoxicity [20]. In this research work, we have assessed the impact of GLP1 analogue and metformin in decreasing the progression of type II diabetic cardiomyopathy of albino rats.

Material and methods

2.1. Animals: Our study was performed at the animal house of medical experimental research center "MERC", Mansoura University. According to general guidelines of experimental animals, 40 male albino rats, 2-3 month, weighting 200-220 gm was used. Rats were randomly subdivided into 5 groups, 8 in each group; each group was housed in separate cage under standard environmental conditions (12h/12h day and night 24°c temperature with free food and water accessability).

2.2 Experimental design:

1. Group1 (negative control group): given distilled water via oral gavage.

2. Group 2 (DM positive control): according previous study done by [21] we can make a model of type 2 diabetes as each rat received high fat diet for 4 weeks then streptozotocin (STZ) (35 mg/Kg) was injected intraperitoneally. These rats were considered diabetic when rats tail blood sugar > 200 mg/dL after 48 h after injection.

3. Group 3 (DM treated with metformin): Including type 2 diabetic rats treated with metformin in a dose (30mg/kg/d) dissolved in distilled water by oral gavage for 12 weeks [22].

4. Group 4 (DM treated with GLP1 analogue): Including type 2 diabetic rats treated with GLP 1 (liraglutide) in a dose (75 μg/kg) dissolved in distilled water by oral gavage for 12 weeks [23].

5. Group 5 (DM treated with GLP1 analogue and metformin): Each type 2 diabetic rat was given metformin at the same dose and route of administration as group 3 in conjugation with GLP 1 (liraglutide) as in group 4.

After 24 hours, the rats were anaesthetized with 4% halothane and perfused with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4), left ventricle from each animal were dissected out, fixed in 10% buffered formalin, then left ventricle was placed in paraffin processed for light study.

2.3. Measurements of (MDA, GSH Activity) Oxidative Stress Markers in heart Tissues.

50-100mg of left ventricle was homogenised in cold buffer with using mortar and pestle then centrifugation was done at 4,000 rpm for 15 min at 4° C. The supernatant was stored at –20 ° C for measuring oxidative stress markers "malondialdehyde and reduced glutathione" using a colorimetric method according to the manufacturer's instructions (BioDiagnostics, Dokki, Giza, Egypt).

2.4. Histopathological examination of heart tissues: After dissecting the ventricle it was fixed into 10% neutral buffered formalin, then lodged into paraffin and sectioned at 3 μm thickness. Using light microscope (Leica DM500 LED Biological Microscope with ICC50W Camera Module – 5.0 Mega Pixel, haematoxylin and eosin (H&E) stained slides were studied for cardiomyopathy signs including disarrangement, hypertrophy and rupture of cardiomyocytes with irregularity of nuclei in addition to inflammatory cells infiltration [24]. Other slides were stained by masson trichrom for detection of fibrosis in interstitial tissues and around blood vessels.

2.5. Immunohistochemical Examination for LC3 and P62: The tissue section was deparaffinised, rehydrated, washed, immersed in 3% hydrogen peroxide and then digested with pepsin for antigen retrieval. the section was incubated with primary antibodies of Mouse anti-LC3B (sc-271625 for tissue), mouse anti-P62 (ab56416, Abcam), and horseradish peroxidase-(HRP-) labelled anti-mouse IgG antibodies (PV-9001; Zhongshan Golden Bridge Biotechnology Technology, Beijing, China) at 4 °C overnight, we use it for tissue immunostaining assays as previously described [25]. Brown colour immunstaining produced by counterstaining with Diaminobenzidine/peroxidase substrate, the region of interest is considered the area cardiac tissue filled with brown immunstaining (by calculating the average values from 10 fields at 10× magnification) by using image J software.

2.6. Statistical Analysis

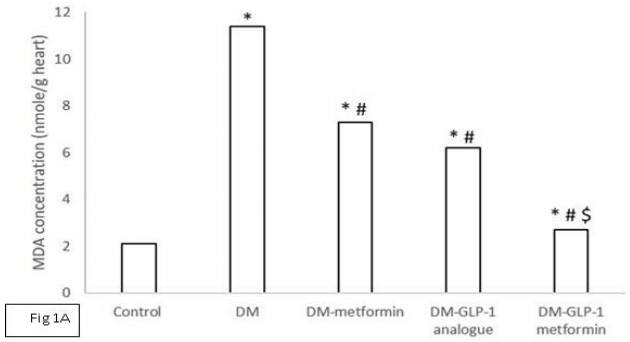
Data were analysed using SPSS by Student's t-test and expressed as mean+ SD the results were deemed non-significant when $p > 0.05$ and highly significant when $p < 0.001$.

Results

3.1. Effect of combined GLP1 and metformin on stress markers (MDA, GHS):

MDA level in heart tissue is significantly increased in diabetic untreated group in

comparison with control group ($p < 0.001$). Using combined GLP1 and metformin in treatment significantly decreases the level of MDA regarding to diabetic untreated group ($p < 0.01$). On the other hand, there is a non-significant decrease in MDA level in separate GLP1 and Metformin groups compared to diabetic untreated group (Fig 1A). In contrast, there was a significant decrease in GSH level in diabetic untreated group when compared with control group ($p < 0.001$). A significant increase in GSH when using combined GLP1 and metformin comparing with diabetic untreated group ($p < 0.01$). Also there is increase in GSH by the treatment of separate GLP1 and metformin group but not significant to diabetic untreated group (Fig 1B).



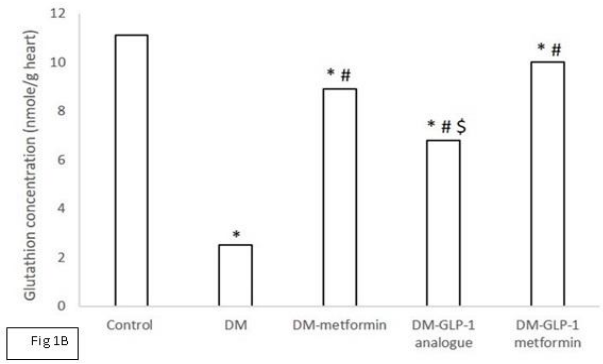


Fig 1. Oxidative stress markers in in cardiac tissues in different groups. (A) Malondialdehyde (MDA) concentration (nmol/gm cardiac tissues), (B) Glutathione enzyme activity (nmol/gm cardiac tissues). Results represent the mean ± SD (n = 4); Differences with P-value of < 0.05 were considered statistically significant.*Significant vs control group (group I), # significant vs T2DM group (group II), and $ significant vs T2DM +Metformin group (group III) & T2DM + GLP1 analogue (group IV).

3.2. Hitopathological effect of combined GLP1 analogue and metformin on cardiac tissues: Histological examination of the heart tissue of control rats showed normal myocardial architecture in the form of normal arranged cardiac muscles with normal surrounding interstitial tissue and blood vessels (Fig 2A). On contrast, the diabetic untreated group showing many of diabetic cardiomyopathy features in the form of widening of interstitial tissue, degeneration in cardiomyocytes nuclei with more eosinophilic sarcoplasm and congestion of blood vessels (Fig 2B,C). Metformin treated group shows some degenerative changes in the form of hyaline degeneration in cardiomyocytes and mild oedema in interstitial tissue (Fig 2D) and GLP1 treated group shows vaculations in cardiomyocytes (Fig 2E). Combined GLP1 analogue and metformin treated group was greatly improved and showed near normal structure of cadiomyocyte with near normal interstitial tissue and blood vessel (Fig 2F).

By staining of the cardiac tissue by masson trichrom stain for detection of fibrosis we found that control group haven't collagen fibres either in the interstitial tissue or around blood vessels (Fig 3A) while in diabetic untreated group there are abundant collagen deposition perivascular and interstitial tissue (Fig 3B,C) which significantly improved in combined GLP1 and metformin group with no fibrosis detected (Fig 3F) but in metformin group and GLP1 group there was mild perivascular fibrosis (Fig 3D,E respectively).

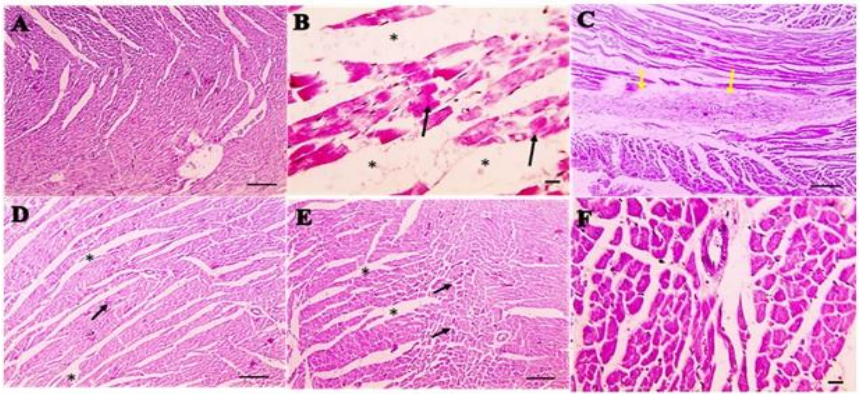


Fig 2. Histopathological examination of the heart tissues stained with H&E. (A) Heart specimen from negative control group showing normally arranged cardiac muscles, normal blood vessels and normal interstitial tissue, (B&C) wide interstitial tissue (asterisks) and severe hyaline degeneration in cardiac muscles (black arrows) characterized by more eosinophilic sarcoplasm and lost nuclei in diabetic untreated group and marked perivascular and interstitial fibrosis (yellow arrows), (D) heart specimen from the metformin treated group showing moderate hyaline degeneration in cardiac muscles (black arrow) and mild oedema in interstitial tissue (asterisks), (E) heart specimen from the GLP1 treated group showing mild hyaline degeneration in cardiac muscles (black arrows) and mild oedema in interstitial tissue (asterisks), (F) heart specimen from the combined GLP1 and metformin treated group showing normally arranged cardiac muscles, normal blood vessels and normal interstitial tissue. (100 bar 100)

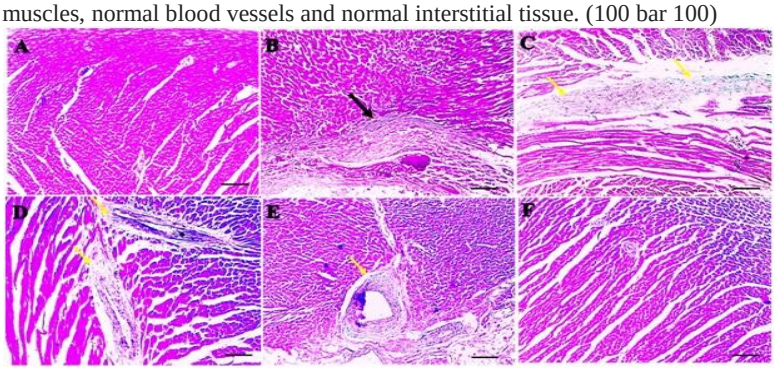


Fig 3. Histopathological examination for fibrosis by Masson trichrome. Interstitial fibrosis appears as blue colors. (A) Heart specimen from control group showing no interstitial fibrosis, (B&C) heart specimen from the diabetic untreated group showing marked interstitial fibrosis and marked perivascular fibrosis (D&E) heart specimen from the metformin treated and GLP1 treated groups respectively showing moderate interstitial fibrosis and collagen deposition, while (F) heart specimen from the combined GLP1 analogue and metformin treated group showing minimal interstitial fibrosis and collagen deposition (100 bar 100).

3.3. Combined GLP1 and metformin effect on autophagy marker (LC3) in cardiac tissue

There was a strong positive expression of early autophogic marker LC3 in control group (Fig 4B) and marked decrease in diabetic untreated rats (Fig 4C). By image analysis, the region of interest for immunexpression in diabetic untreated rats is significantly decreased compared to control group ($p \leq 0.001$) (Fig 4A). Combined GLP1 and metformin treated group showed great improvement in the process of the autophagy in the form of increasing in expression of LC3 (Fig 4E) this increase in expression is significant compared to diabetic untreated rats (Fig 4A). On the other hand metformin and GLP1 separate groups showing moderate increase in expression of LC3 in its cardiomyocytes (Fig 4D,E respectively) which is more or less significant to diabetic untreated rats (Fig 4A).

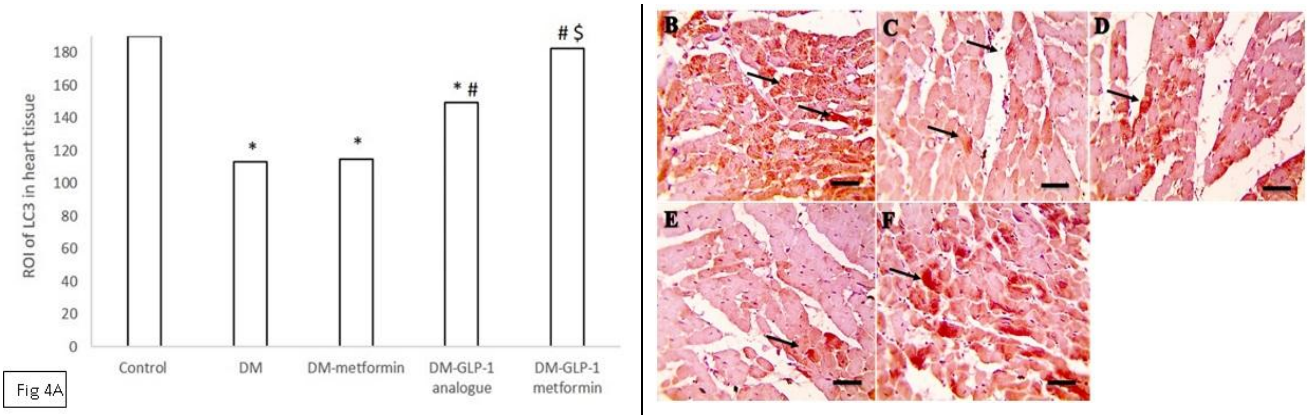


Fig 4. (A) Graphs of ROI of the expression of LC3 in different groups. Heart specimens showing (B) strong positive cytoplasmic expression of LC3 in control group, (C) marked decreased cytoplasmic expression level of LC3 in diabetic untreated group, (D&E) moderate cytoplasmic expression of LC3 (arrows) in metformin & GLP1 treated groups respectively, (F) markedly increased cytoplasmic expression of LC3 (arrows) in combined GLP1and metformin treated group. *Significant vs control group, # significant vs diabetic untreated group, and $significant vs GLP1 treated group.

3.4. Effect of combined GLP1 and metformin on autophagy marker (P62) in cardiac tissue

There was clear immunexpression of late autophogic marker P62 in negative control rats (Fig 5B), while diabetic untreated rats there was a marked decrease (Fig 5C) which is more significant in relation to control rats ($p \leq 0.001$) (Fig 5A). In combined GLP1 and metformin treated group, the immunexpression of P62 was markedly increased (Fig 5F) which is significantly increased in compare to diabetic untreated group ($p \leq 0.01$) (Fig 5A). When examining P62 immunexpression in cardiomyocytes of GLP1 and metformin treated groups, it was moderately increased (Fig 5D,E) this expression less significant to diabetic untreated group (Fig 4A).

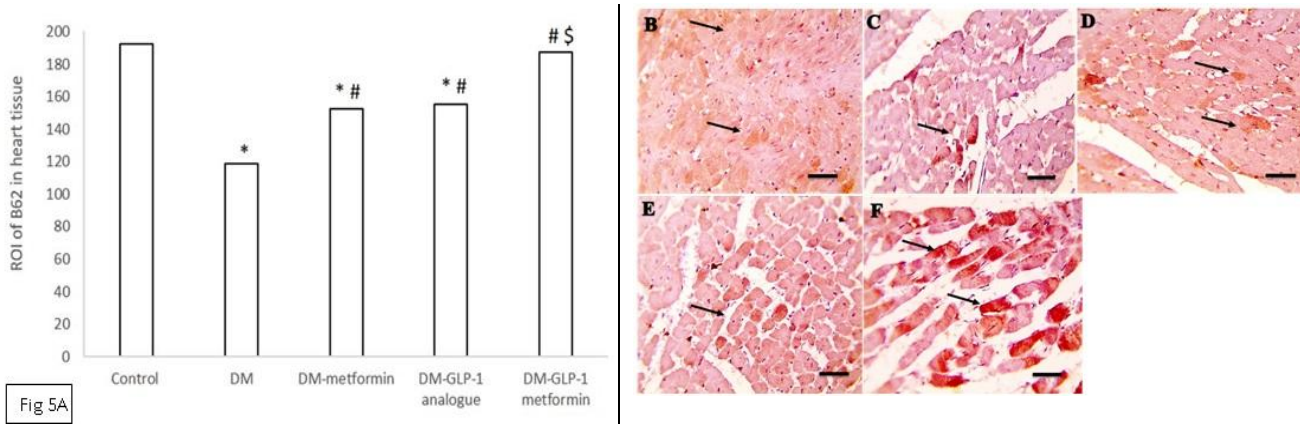


Fig 5. (A) Graphs of ROI of the expression of P62 in different groups. (B) negative cytoplasmic expression of P62 in cardiomyocytes of control group, (C) marked decreased expression level of P62 in diabetic untreated group, (D&E) moderate cytoplasmic expression of P62 (arrows) in GLP1& metformin groups respectively, (F) markedly increased expression of P62 level (arrows) in combined GLP1and metformin treated group. *Significant vs control group, # significant vs diabetic untreated group, and $significant vs GLP1 treated group.

4. Discussion

The finding of this study can be summarised in the following: a) T2DM was associated with increased cardiac enzymes, hyperglycaemia, insulin resistance, oxidative stress and distorted myocardial morphology with fibrosis. Also, up-

regulation of autophogic markers (LC3& P62) was considered. b) Using GLP1 significantly improved the blood sugar and autophogic markers.

In the recent study, T2DM rat model was established by using high-fat diet for 4 weeks then a single STZ injection. The model development was indicated by presence of high blood glucose level and insulin deficiency. Previous Studies go with these findings and explained hyperglycaemia by pancreatic β-cells dysfunction under the STZ cytotoxic effect and high fat diet-induced insulin resistance [26]. In our study, we noticed that using GLP1 for treatment significantly improved the impaired glucose homeostasis, insulin resistance and autophagy.

Significant cardiac damage in our model was demonstrated in the form of wide interstitial tissue, congestion, sever hyaline degeneration in cardiac muscles characterized by more eosinophilic sarcoplasm and nuclei loss. Sever perivascular and interstitial fibrosis by excess deposition of collagen fibres was observed in hearts of the diabetic untreated group. Nearby results were reported by other researchers [27]. In addition, we recorded moderate amelioration in morphology by separate GLP1 and metformin treatment but significant improvement was recorded in combined GLP1and metformin treatment. Consistent with these findings, Batchuluun et al. [28] found that combined liraglutide and metformin prevent hyperglycaemia-induced oxidative stress via inhibition of PKC-NAD(P)H oxidase pathway. As well as GLP1 has a protective influence against myocardial infarction [29] and cardiac remodelling in T2DM [30].

Diabetic cardiomyopathy is a complicated process affected by many factors such as persistent hyperglycaemia and insulin resistance that leads to myocarditis, apoptotic cell deaths and impaired cardiac autophagy. These changes finally lead to cardiac hypertrophy and fibrosis [31]. So, in the present study we searched for the influence of oxidative stress, collagen deposition and autophagy impairment in the myocardial fibrosis development.

Hyperglycaemia is the corner stone in the pathophysiology of diabetic cardiomyopathy as it causes increased oxygen free radicals production which leads to myocardial fibrosis. In this study, diabetic untreated rats showed significant rise in MDA and significant decrease in GSH going with results of Yang et al. [32] and Wilson et al. [33]. Furthermore, our study showed a significant amelioration in myocardial oxidative stress using metformin and GLP1 combination. This support the antioxidant actions for both agents as published in previous studies [34]. Milton proved that combined metformin and GLP1 improved the diabetic cardiomyopathy by inhibiting oxidative stress generated by NADPH oxidases. Olgar and Turan [35] also demonstrated that GLP1 decreased the oxidative stress and fibrosis.

Myocardial inflammation and fibrosis caused by stimulation of cytokines as TNFα and interleukins play a major role in diabetic cardiomyopathy [36]. In this study, Masson trichrome showed a significant deposition of collagen in myocardium of the diabetic untreated group, supposing the effect of TGF-beta in cardiac fibrosis [37]. Using combination of GLP1

and metformin leads a significant decrease in expressing TGF-β in cardiac tissue, suggesting their antifibrotic activity.

Wei et al [38] founded that autophagy suppression leads to increase of damaged mitochondria that releases ROS, accelerating the development of diabetic cardiomyopathy. Yi et al. [39] showed decreased expression of autophogic markers LC3, P62 in diabetic rats. In this study, we founded mild improvement of autophogic markers LC3, P62 in metformin group consonant with wang et al (40) how demonstrated the role of metformin in improving the autophagy related to its ability to act as an agonist of AMPK, but Daskalopoulos et al (41) showed that signalling through AMPK can improve oxidative stress and cardiac inflammation in ways that are independent of changes in autophagic flux. According to this study, metformin alone cannot improve the autophagy in diabetic cardiomyopathy but in combination with GLP1 there was a significant increase in the expression of LC3, P62 indicating the ameliorating effect of the combination on autophagy. Mima et al (42) founded that usage of both metformin with liraglutide prevents the increased GLP-1R degradation caused by PKCb2 activation. So to our knowledge, no previous studies studied the effect of GLP1 and metformin combination on the marker of the autophagy LC3 and P62 in diabetic rats. We think that our study is the first work to discuss the cardioprotective influence of combined GLP1 and metformin on diabetic cardiomyopathy.

5. Conclusions

The diabetic cardiomyopathy caused by complicated T2DM may be due to enhanced oxidative stress, deposition of collagen fibres and autophagy suppression. Usage of GLP1 (liraglutide) and metformin combination has more cardioprotective effects than metformin only. These agents may play this role though inhibiting the oxidative stress, fibrosis and stimulating the autophogic process.

References

1. **Marso, S.P..; Daniels, G.H..; Brown-Frandsen, K..; Kristensen, P..; Mann, J.F. Nauck.** Liraglutide and Cardiovascular outcomes in type 2 diabetes. N. Engl. J. Med., 2016, 375, 311–322.
2. **Kerner, W.; Brückel,** J. Definition, classification and diagnosis of diabetes mellitus. Exp. Clin. Endocrinol. Diabetes 2014, 122, 384–386.
3. **Ogurtsova, K.; da Rocha Fernandes, J.; Huang, Y.; Linnenkamp, U.; Guariguata, L.; Cho, N.** IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res. Clin. Pract. 2017, 128, 40–50.
4. **Association AD. 2**. Classification and diagnosis of diabetes. Diabetes Care, 2017, 40, S11-24
5. **Nolan C.J, Damm P, Prentki M,** Type 2 diabetes across generations: from pathophysiology to prevention and management, Lancet., 2011, 378, 169–181.
6. **Tuomilehto, J.; Lindström, J.** The major diabetes prevention trials. Curr. Diab. Rep. 2003, 3, 115–122, doi:10.1007/s11892-003-0034-9.

7. **Chen Lin et al.,** FNDC5/Irisin attenuates diabetic cardiomyopathy in a type 2 diabetes mouse model by activation of integrin αV/β5-AKT signaling and reduction of oxidative/nitrosative stress**.** Journal of Molecular and Cellular Cardiology. 2021, 160, 27-41.
8. **Nunes, S.; Rolo, A.P.; Palmeira, C.M.; Reis, F. Diabetic Cardiomyopathy:** Focus on Oxidative Stress, Mitochondrial Dysfunction and Inflammation, Cardiomyopathies-Types and Treatments. Cardiomyopathies Types Treat. 2017, 12, 234-257.
9. **D'Souza, A.; Howarth, F.C.; Yanni, J.; Dobrzynski, H.; Boyett, M.R.; Adeghate, E.** Chronic effects of mild hyperglycaemia on left ventricle transcriptional profile and structural remodelling in the spontaneously type 2 diabetic Goto-Kakizaki rat. Heart Fail. Rev. 2014, 19, 65–74, doi:10.1007/s10741-013-9376-9.
10. **Eckhouse, S.R.; Spinale, F.G.** Changes in the myocardial interstitium and contribution to the progression of heart failure. Heart Fail. Clin. 2012, 8, 7–20, doi:10.1016/j.hfc.2011.08.012.
11. **B. Levine, D.J. Klionsky,** Development by self-digestion: molecular mechanisms and biological functions of autophagy, Dev Cell 6 (2004) 463-477.
12. **W. Martinet, M.W. Knaapen, M.M. Kockx, G.R. De Meyer,** Autophagy in cardiovascular disease, Trends Mol Med 13 (2007) 482-491.
13. **H. Zhu, L. He, Beclin 1** biology and its role in heart disease, Curr Cardiol Rev 11 (2015) 229-237.
14. **K.E. Ewings, C.M. Wiggins, S.J. Cook,** Bim and the pro-survival Bcl-2 proteins: opposites attract, ERK repels, Cell Cycle 6 (2007) 2236-2240.
15. **S. Mukhopadhyay, P.K. Panda, N. Sinha, D.N. Das, S.K. Bhutia,** Autophagy and apoptosis: where do they meet?, Apoptosis 19 (2014) 555-566.
16. **R.J. Shaw,** LKB1 and AMP-activated protein kinase control of mTOR signaling and growth, Acta Physiol (Oxf) 196 (2009) 65-80.
17. **Holst JJ.** The physiology of glucagon-like peptide-1. Physiological Reviews. 2007, 87, 1409–39.
18. **Meloni AR, DeYoung MB, Lowe C, Parkes DG.** GLP-1 receptor activated insulin secretion from pancreatic b-cells: mechanism and glucose dependence
diabetes obesity metabolism 2013, 15, 15–27.
19. **Graaf C, Donnelly D, Wootten D, Lau J, Sexton PM, Miller LJ, et al.** Glucagon-like peptide-1 and its Class B G protein–coupled receptors: a long march to therapeutic successes. Pharmacological Reviews.2016, 68, 954–1013.
20. **Ashrafizadeh M, Yaribeygi H, Atkin SL, Sahebkar A.** Effects of newly introduced antidiabetic drugs on autophagy. Diabetes & Metabolic Syndrome: Clinical Research & Reviews 2019, 13, 2445-2449.
21. **Hussein, A.A.; Omar, N.M.; Sakr, H.; Elsamanoudy, A.Z.; Shaheen, D.** Modulation of Metabolic & Cardiac

Dysfunctions by Insulin Sensitizers and Angiotensin Receptor Blocker in Rat Model of Type 2 Diabetes Mellitus. Can. J. Physiol. Pharmacol. 2011, 89, 216–226.

22. Zhang Q, Hu N. Effects of Metformin on the Gut Microbiota in Obesity and Type 2 Diabetes Mellitus. Diabetes Metab Syndr Obes. 2020;13:5003-5014. Published 2020 Dec 16. doi:10.2147/DMSO.S286430

23. Nagakubo, D.; Shirai, M.; Wakabayashi, K.; Nakamura, Y.; Kaji, N.; Asai, F. Therapeutic effects of liraglutide, a glucagon-like peptide-1 analog, in diabetic WBN/Kob-Leprfa rats with obesity and chronic pancreatitis. Integr. Obes. Diabetes 2016, 2, 265–270. doi:10.15761/IOD.1000162.

24. Yu, H.; Zhen, J.; Yang, Y.; Gu, J.; Wu, S.; Liu, Q. Ginsenoside Rg1 ameliorates diabetic cardiomyopathy by inhibiting endoplasmic reticulum stress-induced apoptosis in a streptozotocin-induced diabetes rat model. J. Cell. Mol. Med., 2016; 20, 623–631.

25. Girolamo F, Lia A, Amati A, Strippoli M, Coppola C, Virgintino D, et al. Overexpression of autophagic proteins in the skeletal muscle of sporadic inclusion body myositis. Neuropathol Appl Neurobiol 2013; 39:736–749.

26. Rouhi, S.Z.T.; Sarker, M.M.R.; Rahmat, A.; Alkahtani, S.A.; Othman, F., The effect of pomegranate fresh juice versus pomegranate seed powder on metabolic indices, lipid profile, inflammatory biomarkers, and the histopathology of pancreatic islets of Langerhans in streptozotocin-nicotinamide induced type 2 diabetic Sprague–Dawley rats. BMC Complementary Altern. Med. 2017, 17, 156.

27. Attia, H.M.; Taha, M. Protective effect of captopril on cardiac fibrosis in diabetic albino rats: A histological and immunohistochemical study. Benha Med. J., 2018; 35, 378.

28. Batchuluun B., T. Inoguchi, N. Sonoda, et al., Metformin and liraglutide ameliorate high glucose-induced oxidative stress via inhibition of PKCNAD (P)H oxidase pathway in human aortic endothelial cells, Atherosclerosis 232 (2014) 156e164.

29. Noyan-Ashraf, M.H.; Abdul, M.M.; Ban, K.; Sadi, A.M.; Zhou, Y.Q.; Riazi, A.M. GLP-1R agonist liraglutide activates cytoprotective pathways and improves outcomes after experimental myocardial infarction in mice. Diabetes 2009, 58, 975–983.

30. Monji A, Mitsui T, Bando YK, Aoyama M, Shigeta T, Murohara T. Glucagon-like peptide-1 receptor activation reverses cardiac remodeling via normalizing cardiac steatosis and oxidative stress in type 2 diabetes. Am. J. Physiol. Heart C 2013, 305, 295–304.

31. Zhang L, Wen‐yuan D, Meng‐xiong T, Ming Z, and Wei Z: Early administration of trimetazidine attenuates diabetic cardiomyopathy in rats by alleviating fibrosis, reducing apoptosis and enhancing autophagy J Transl Med (2016) 14:109.

32. Yang, R.; Jia, Q.; Liu, X.; Gao, Q.; Wang, L.; Ma, S. Effect of hydrogen sulfide on oxidative stress and Endoplasmic reticulum stress in diabetic cardiomyopathy. Chin. J. Appl. Physiol. 2016, 32, 8–12.

33. Wilson, A.J.; Gill, E.K.; Abudalo, R.A.; Edgar, K.S.; Watson, C.J.; Grieve, D.J.; Ractive oxygen species signalling in the diabetic heart: Emerging prospect for therapeutic targeting. Heart 2018, 104, 293–299.

34. Milton P: Autophagy‐dependent and ‐independent Modulation of oxidative and organellar stress in the diabetic heart by glucose‐lowering drugs Cardiovasc Diabetol (2020) 19:62.

35. Olgar, Y.; Turan, B. A sodium-glucose cotransporter 2 (SGLT2) inhibitor dapaglilozin comparison with insulin exerts important effects on Zn2 + -transporters in cardiomyocytes from insulin-resistant metabolic syndrome rats through inhibition of oxidative stress. Can. J. Physiol. Pharmacol. 2019, 97, 528–535.

36. Fuentes-Antrás, J.; Picatoste, B.; Gómez-Hernández, A.; Egido, J.; Tuñón, J.; Lorenzo, Ó. Updating Experimental Models of Diabetic Cardiomyopathy. J. Diabetes Res. 2015, 2015, 15.

37. Li, C.J.; Lv, L.; Li, H.; Yu, D.M. Cardiac fibrosis and dysfunction in experimental diabetic cardiomyopathy are ameliorated by alpha-lipoic acid. Cardiovasc. Diabetol. 2012, 11, 73.

38. Wei Y, Wenliang Z and Jun R: Exendin-4 and Liraglutide Attenuate Glucose Toxicity-Induced Cardiac Injury through mTOR/ULK1-Dependent Autophagy Oxidative Medicine and Cellular Longevity Volume 2018, Article ID 5396806, 14 pages https://doi.org/10.1155/2018/5396806

39. Yi C, Yur-R.K., Ya C.W., Shih J.Y. andMei Y.L. Glucagon-Like Peptide-1 Receptor Agonist Attenuates Autophagy to Ameliorate Pulmonary Arterial Hypertension through Drp1/NOXand Atg-5/Atg-7/Beclin-1/LC3_ PathwaysInt. J. Mol. Sci. 2019, 20, 3435.

40. Wang Y, Yang Z, Zheng G, Yu L, Yin Y, Mu N, and Ma H. Metformin promotes autophagy in ischemia/reperfusion myocardium via cytoplasmic AMPKα1 and nuclear AMPKα2 pathways. Life Sci. 2019; 225:64–71.

41. Daskalopoulos EP, Dufeys C, Bertrand L, Beauloye C, Horman S. AMPK in cardiac fibrosis and repair: actions beyond metabolic regulation. J Mol Cell Cardiol. 2016; 91:188–200.

42. Mima A, Hiraoka-Yamomoto J, Li Q, et al. Protective effects of GLP-1 on glomerular endothelium and its inhibition by PKCb activation in diabetes. Diabetes 2012; 61:2967e79.