Metabolic utilization of free fatty acids and carbohydrates in diabetic hearts

Lem, Michael

UNIVERSITY OF SPLIT
SCHOOL OF MEDICINE

Michael Lem

METABOLIC UTILIZATION OF FREE FATTY ACIDS AND CARBOHYDRATES IN DIABETIC HEARTS

Diploma Thesis

Academic Year:

2018/2019

Mentor:

Prof. Marko Ljubković, MD, PhD

Split, July 2019

UNIVERSITY OF SPLIT
SCHOOL OF MEDICINE

Michael Lem

METABOLIC UTILIZATION OF FREE FATTY ACIDS AND CARBOHYDRATES IN DIABETIC HEARTS

Diploma Thesis

Academic Year:

2018/2019

Mentor:

Prof. Marko Ljubković, MD, PhD

Split, July 2019

Table of Contents

ACKNOWLEDGMENT

To My family who introduced me to my dream career and their never-ending support

To My friends all over the world who showed me the many wonders of life

To My mentor, Marko Ljubkovic for all the help and support for this thesis

LIST OF ABBREVIATIONS

DM1 – Type 1 Diabetes

DM2 – Type 2 Diabetes

GLUT4 – Glucose Transporter 4

eNOS - endothelial Nitric Oxide Synthase

PFK-1 - Phosphofructokinase-1

CAD – Coronary Artery Disease

CABG – Coronary Artery Bypass Graft

LVEF – Left Ventricular Ejection Fraction

NADH - Nicotinamide Adenine Dinucleotide

$FADH_2$ - Flavin Adenine Dinucleotide

1. INTRODUCTION

1.1. Diabetes mellitus

Millions of people in the United States are affected and have been diagnosed with diabetes mellitus (1). Of which, the two most common forms are type 1 diabetes (DM1) and type 2 diabetes (DM2). Type 1 diabetes is an autoimmune disease in which the pancreatic beta cells, which secretes insulin, are attacked and ultimately cause a decrease of circulating insulin (2). On the other hand, type 2 diabetes can be seen as a combination of pathophysiology in which there is both insulin resistance and insulin deficiency (3). The causes of DM 2 are multifactorial such as genetics and environmental factors. Environmental factors include, but not limited to: obesity, lack of exercise, stress, and aging (4). In terms of genetics, it is quite clear that past family history plays a role in the development of DM 2 (5).

The pathophysiology of DM 2 that was previously mentioned is due to a mixture of insulin resistance and insulin deficiency. Insulin resistance is defined as insulins inability to produce its physiological actions on its target cells at normal circulating concentrations (6). This insulin resistance is largely in part of metabolic syndrome. Metabolic syndrome is plethora of pathophysiology which greatly increase the risk of cardiovascular disease, DM2 and all causes of mortality (7). One of the major complications of DM 2 is heart failure.

1.2. Heart failure

Heart failure has been shown in clinical trials to have an increase prevalence in diabetic patients (8). Many of these diabetic patients do not have any history of other cardiac risk factors such as coronary heart disease, hypertension and significant valvular disease. Yet, there is still abnormal myocardial structure and performance in these aforementioned patients, and thus this phenomenon has been aptly named diabetic cardiomyopathy. Diabetic cardiomyopathy can be defined as the presences of an abnormal myocardial structure and performance in the absences of other cardiac risk factors which include but not limited to: coronary artery disease, hypertension and significant valvular disease, in individuals with diabetes mellitus.

The prevalence of heart failure in diabetic patients can range between 19 - 26% (9). The classic Framingham Heart Study showed that the incidence of heart failure was increased in both male and female diabetic patients in comparison with age-related individuals. This surprisingly was independent of obesity, hypertension, dyslipidemia and coronary heart

disease. Interestingly enough the incidence of heart failure was higher in diabetics as compared to nondiabetics. Further studies have shown that the differences in left ventricular mass and wall thickness and increased diastolic and systolic dysfunctions between diabetic patients and normal patients (10).

1.3. Diabetic cardiomyopathy

Some of the risk factors for diabetic cardiomyopathy are those seen in DM2 such as: hyperglycemia, systemic insulin resistance, and impaired cardiac metabolic signaling all of which are involved in the pathogenesis of diabetic cardiomyopathy (Figure 1). When looking at the earliest stages of diabetic cardiomyopathy many of the patients are asymptomatic, yet at this stage the heart is undergoing structural changes. This can be seen in changes in the left ventricle, in which it becomes hypertrophic and has a decrease compliance. These changes then cause: an impaired diastolic filling, increased atrial filling, and a prolong isovolumic relaxation (11).

It is important to note that patients start showing symptomatic heart failure once there has been there is impaired systolic function (12). On the molecular level, these structural changes are largely due to the impaired insulin metabolism. This impairment causes a decrease amount of glucose transporter 4 (GLUT4) on the target cells plasma membrane. When this occurs, a cascade of events will begin (13).

These events will result with glucose not being able to enter a targeted cell, in turn causing sarcoplasmic Ca^{2+} pump activity, which then allows an increase amount of Ca^{2+} intracellularly in a cardiomyocyte. Ultimately, this will case cardiomyocyte stiffness. Another abnormality due to impaired insulin metabolism is a decrease in insulin-stimulated coronary endothelial nitric oxide synthase activity (eNOS). The reduction of nitric oxide, known for its vasodilatatory effects, causes titin to stiffen and thus also causes cardiomyocyte stiffness. These structural abnormalities such as the aforementioned cardiac stiffness and impaired relaxation are the hallmark pathological changes of a diabetic heart (14).

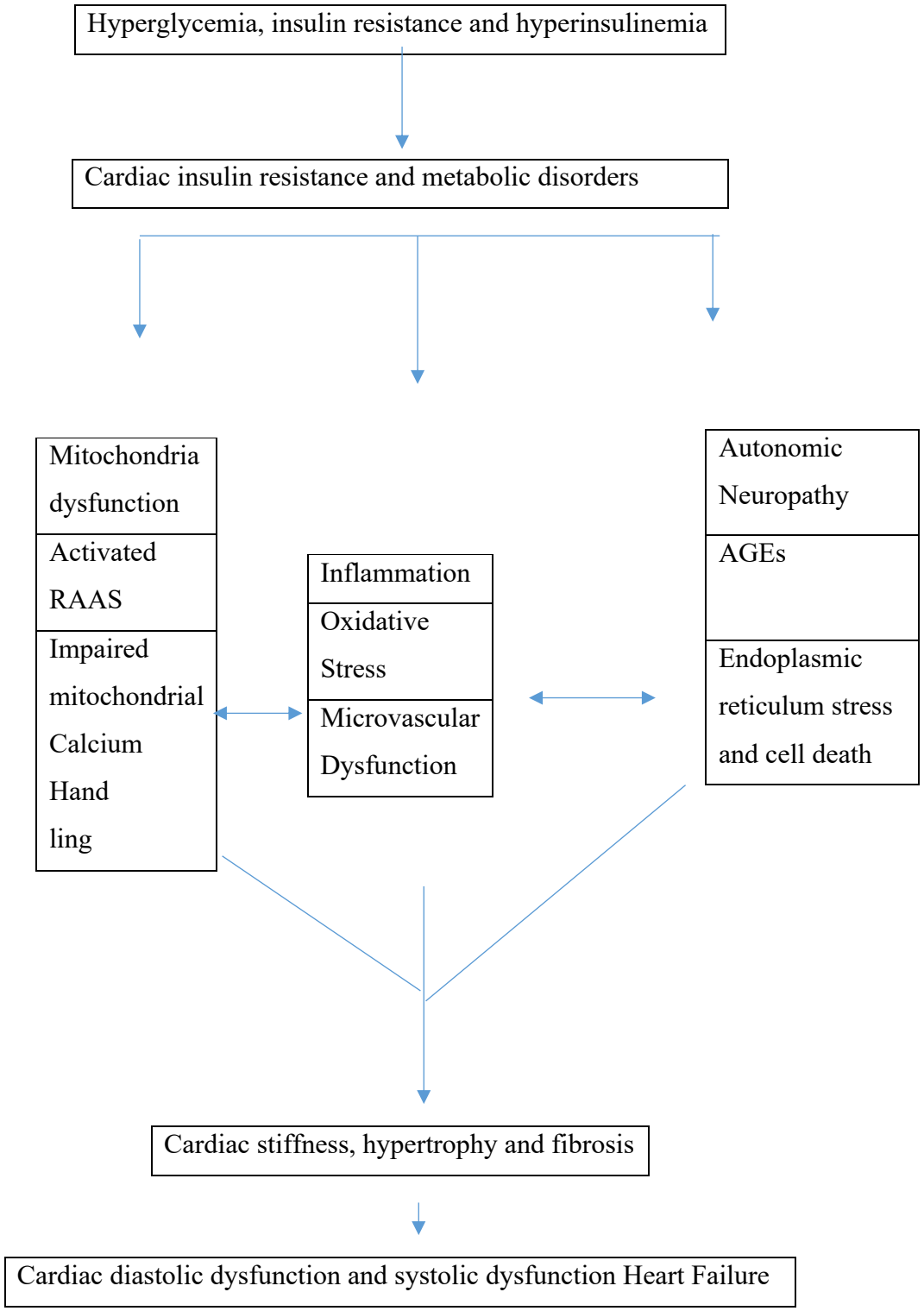

Figure 1. Pathophysiological mechanisms of diabetic cardiomyopathy (8)

1.4. Cardiac Metabolism

In diabetic patients one can notice the change of cardiac metabolism. In order to understand the complexities of the cardiac energy metabolism, one must understand the cellular standpoint of the creation of energy. ATP generation of the heart is largely due to oxidative phosphorylation (Figure 2) of a cardiomyocyte mitochondria (15). In a healthy heart, oxidative phosphorylation is uniquely correlated with ATP hydrolysis. In other words, the more ATP that is broken down, the more active oxidative phosphorylation becomes thus the allowing proper cardiac contractility.

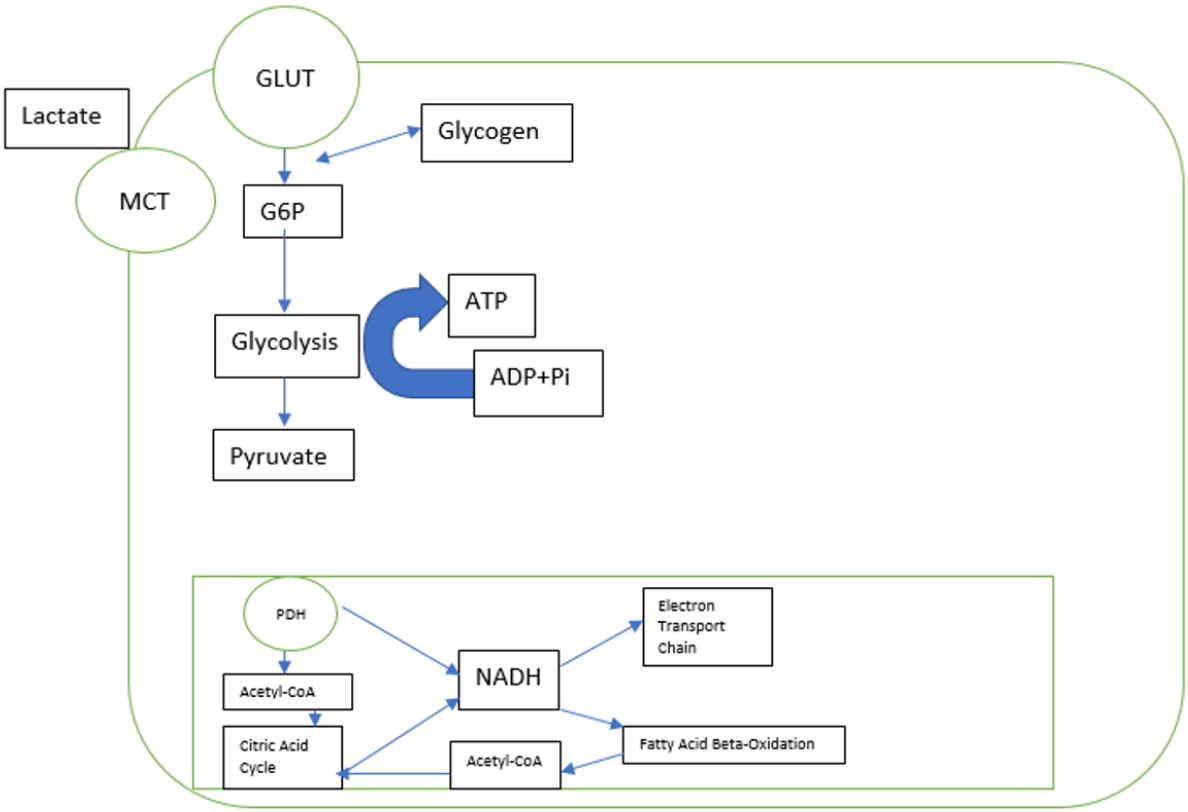

Figure 2. The pathways for and regulations myocardial substrate metabolism (15)

This allows the ATP storage constant even in times of increase cardiac energy usage such as exercise. The cardiomyocyte mitochondria oxidative phosphorylation is driven by electron transport of carbon which is ultimately due to the presences of reduced form of

nicotinamide adenine dinucleotide (NADH) and reduced form of flavin adenine dinucleotide ($FADH_2$) which are products of beta oxidation of free fatty acids (16).

1.5. Beta oxidation of free fatty acids

Beta oxidation of free fatty acids is a pinnacle in terms of cardiac metabolism and energy creation. The products that are created such as NADH, $FADH_2$, and acetyl-CoA. But, before beta oxidation in the mitochondria can occur, a precursor of acetyl-CoA, long-chain acyl-CoA, must go through changes in the cytosol before entering the mitochondria. Once it becomes its "proper" form to speak, beta oxidation can occur. The process of beta oxidation is a continuous cleaving mechanism that takes away two- carbon acetyl-CoA. Certain enzymes are need to cleave off certain lengths of fatty acid intermediates, but the end result is the much needed NADH, and $FADH_2$ (17).

1.6. Cardiac metabolism for carbohydrates

Cardiac metabolism can also be dependent on carbohydrates. The glycolytic pathway is driven from exogenous glucose and glycogen storage. For every glucose molecule a net of 2 ATP is created. The transport of a glucose molecule into a cardiomyocyte is regulated by not only a glucose gradient, but also due to the content of the glucose transporter (18). In this case, the transporter is mainly GLUT-4. The glycolytic pathway has enzymes that help regulate its forward movement. One of which is phosphofructokinase-1 (PFK-1). This enzyme is pertinent regulator and the first irreversible step in the glycolytic pathway. Without this enzyme the glycolytic pathway cannot move forward. Another key substrate created in this process is pyruvate (19).

Pyruvate has three main outcomes in terms of metabolism: help create pyruvate, decarboxylation to Acetyl-CoA, or carboxylation to oxalate or malate (20). The oxidation of glucose and pyruvate in conjunction with the activity of PDH are decreased when the rates of fatty acid oxidation are increased. Furthermore, the activity of pyruvate oxidation is enhanced by the decrease activity of free fatty acid oxidation (21). As one can see there is a continuing checks and balance between the two-metabolism cycle.

1.7. Changes in metabolic cycles in heart failure

The aforementioned metabolism cycles are changed in terms of heart failure. Though the actual causes and consequences of these abnormalities are not well documented. There is however some evidence that shows that these changes in the metabolism contributes towards the contractile dysfunction and to a chance in the left ventricular remodeling that are characteristic of heart failure (11). The idea behind this is the notion that myocardial substrate selection is somewhat normal during the beginning stages of heart failure. Eventually in the advanced stages there is a notable change in the downregulation in the fatty acid oxidation, an increased glycolysis and glucose oxidation, reduced respiratory chain activity and impaired reserve for mitochondrial oxidative flux (22).

The reducing equivalents generated from the intermediary metabolism is electron transport chain and oxidative phosphorylation. In heart failure there is a defect in the transfer of energy from mitochondrial ATP to the site of ATP hydrolysis and the use of the creatine phosphate system. There seems to be a decrease in tissue ATP content, a rise in ADP, and a decrease in the phosphorylation potential. This ultimately impairs the kinetics for ATP use for cell contraction and relaxation. Furthermore, heart failure hinders the potential for the creatine kinase system to transfer for mitochondrial ATP to myofibril (23).

A dysfunctional electron transport chain can also affect the mitochondrial and cytosolic redox state ($NADH/NAD^+$) and the pool of ATP, ADP, and Pi. When looking at the mitochondria of a failing heart there is a noticeable difference in membrane disruption and matrix depletion, a lower use for respiration, and decrease capacity for oxidative phosphorylation. (24). The activity of the complex III was reduced by 35% with no changes in the other complexes in patients with either idiopathic dilated cardiomyopathy or a previous histories of ischemic heart disease compared with donor hearts with normal cardiac function (25). This shows that in heart failure there is a major disruption in oxidative metabolism at the level of the electron transport system.

Unfortunately, there is not that much data of the effects of heart failure on myocardial substrate oxidation in patients with heart failure. The few data that has been obtain shows that in the early stages of heart failure, there is a slightly elevated rate of fatty acid oxidation. In one particular study (26), there is an increased uptake of plasma free fatty acid and a decrease in uptake in congestive heart patients. Now, if we combine that with a diabetic heart this can only further the demise of the patient. The purpose of this study is to find out what the direct

cause of diabetes in heart failure in terms of a dysfunctional myocardial substrate metabolism and fuel selection.

2. OBJECTIVES

The main goal of the present study was to investigate directly the ability of heart mitochondria to metabolize fatty acids and carbohydrates in patients suffering from DM2. Since DM2 is a metabolic disease which disturbs normal metabolic processes in the entire body, we wanted to explore the potential impact on utilization of the main substrates in metabolically very active organ, such as heart. Although some studies had been performed in animal models of diabetes, investigation conducted on the tissue derived from human DM2 patients enables a direct insight into clinically relevant aspects of the disease.

Hypothesis

The main hypothesis of our investigation is that left ventricular mitochondria from patients suffering from DM2 will exhibit reduced capacity for oxidation of fatty acid.

3. SUBJECTS AND METHODS

3.1. Study design

In this study, we included thirty-seven patients who had been previously diagnosed with coronary artery disease (CAD), were hemodynamically stable and were scheduled for elective coronary artery bypass grafting (CABG) surgery. Emergency patients, patients with LV ejection fraction (LVEF) below 50%, patients with type 1 diabetes mellitus, concomitant valve replacement, and severe renal, hepatic or pulmonary disease were excluded from our investigation.

Furthermore, the included patients were subdivided into two categories. One group of nondiabetic patients (labelled as nonDM group) and the second the diabetic group (labelled DM). The diabetic group were patients defined on the clinical diagnosis of Type 2 Diabetes (DM2), chronic use of diabetic medication, having a fasting plasma glucose (> 7 mml/L) or a glycosylated hemoglobin (HbA1c >6.5%). Pre-, intra- and post-surgical procedures were performed according to the standard clinical routines of the Department of Cardiac Surgery at the University Hospital of Split. This experiment and all subsequent processes follow the Declaration of Helsinki and was approved by the Ethical Committees of the University Hospital of Split (2181-147-01). All patients were explained the planned procedures and study objectives in detail prior to giving the signed informed consent for their enrollment.

3.2. Left ventricular biopsies

Biopsy samples were obtained during the CABG procedure that was performed without the use of cardiopulmonary bypass and cardioplegia (“off-pump”). One cylinder-shaped sample (approximately 15 x 1 mm) was taken from the anteroseptal part of the left ventricle from each patient. No complications evidently related to the biopsy procedure was detected in any of the patients. Upon the biopsy, the sample was immediately immersed in an ice-cold storage solution, composed of 2.77 mmol/l CaK2EGTA, 7.23 mmol/l K2EGTA, 6.56 mmol/l MgCl2, 5.7 mmol/l Na2ATP, 15 mmol/l phosphocreatine, 20 mmol/l imidazole, 20 mmol/l taurine, 0.5 mmol/l dithiothreitol and 50 mmol/l K-methanesulfonate, pH 7.1 at 0°C. Afterwards, the samples were transferred within 15 minutes to the Laboratory of Cellular Physiology at the Medical School in Split for the purpose of performing the in vitro experiments.

3.3. Chemicals

All chemicals used for this study, unless otherwise noted, were purchased from Sigma-Aldrich (St Louis, MO, USA).

3.4. Mitochondrial respiration

Upon arrival to the Laboratory, the samples were first inspected under a magnifier and cleaned from excess fat and fibrous tissues. Afterwards, they were fine dissected using a pair of extra-fine scissors in order to mechanically separate bunches of myocardial fibers allow for better access of a permeabilizing agent. For the permeabilization purpose, a mild detergent saponin was used (50 μg/ml), which was added to the storage solution and in which the tissue was incubated for 30 min at 4 degrees Celsius with mild agitation. Saponin is used because it acts primarily on cholesterol-containing membranes – such as plasma membrane, but not mitochondrial membrane which contains minimal amounts of this sterol. This allows for selective permeabilization of the cellular membrane and generation of the so-called skinned fibers. Such samples can be used for measurement of mitochondrial respiration, since due to the plasmalemma permeabilization there is an easy access of substrate to the mitochondria, which remain intact after the described procedure.

Upon permeabilization, the samples were washed from the components of the storage solution by mild agitation at 4 degrees Celsius in the respiration buffer, that is composed of 2.77 mmol/l CaK2EGTA, 7.23 mmol/l K2EGTA, 1.38 mmol/l MgCl2, 3 mmol/l K2HPO4, 20 mmol/l imidazole, 20 mmol/l taurine, 0.5 mmol/l DTT, 90 mmol/l K-methanesulfonate, 10 mmol/l Na-methanesulfonate, 2 mg/ml BSA, pH 7.1). Upon washing, the samples were transferred to the metabolic chamber that was filled with 2 ml of the respiration buffer, under constant stirring. After the skinned fiber is placed into the chamber, the lid is closed and the oxygen amount in the chamber is constantly monitored using an oxygen-sensing Clark-type electrode (Oxygraph, Hansatech Instruments, Norfolk, UK). During the experiment, as mitochondria respire, the oxygen level in the chamber decreases, which is recorded by a specially designed software. The rate of oxygen decline in the chamber is later calculated and it is indicative of the rate of mitochondrial respiration, or in other words of mitochondrial ability to oxidize the substrate. For the latter, either fatty acids or carbohydrates may be used,

which enables the researcher to assess the mitochondrial capacity to utilize either type of metabolite.

3.5. Experimental Protocol

In order to evaluate the capacity of cardiac mitochondria to metabolize fatty acid, we used palmitoyl-carnitine was used as a substrate (40 μmol/l) in the metabolic chamber. Palmitate is the most commonly used fatty acid in cardiac metabolism. With the chemical formula CH3(CH2)14COOH it is categorized as a long fatty acid and in order to enter the mitochondrial matrix, where it is further metabolized in a series of enzymatic events named □-oxidation it needs to be conjugated to carnitine. In order to facilitate its entry into the mitochondria of the skinned fibers and eliminate the potential impact of the conjugation process, which may be dysfunctional in some patients, we used palmitoyl-carnitine in our system, which allowed a rapid delivery of a metabolite to mitochondria and investigation of the processes downstream. After palmitoyl-carnitine, we added ADP (2.5 mmol/l), in order to reconstitute mitochondrial respiration in vitro.

High energy electrons, derived from the fatty acid, are shuttled to oxygen molecule via the mitochondrial electron transfer chain, with energy being transferred first into the proton gradient across the inner mitochondrial membrane and ultimately harvested in the form of ATP synthesis via the enzyme ATP synthase. Finally, we administered a chemical named trifluorocarbonylcyanide phenylhydrazone (FCCP, 1 μmol/l), which is an uncoupler of mitochondrial oxygen consumption from ATP synthesis. This yields even higher rates of O2 consumption in mitochondria, enabling the evaluation of the full capacity of a certain metabolic pathway.

In parallel, cardiac mitochondrial ability to metabolize carbohydrate was investigated by adding pyruvate (5 mmol/l) in place of palmitoyl-carnitine. Pyruvate is the main substrate of carbohydrate metabolism as it is derived from glucose via the glycolysis, which takes place in the cytosol. The example of the actual recordings evaluating the mitochondrial ability to oxidize palmitoyl-carnitine in the presence of ADP and FCCP is displayed in Figure A. As can also be seen in the figure, the level of available ambient oxygen was maintained above 210 μmol/l to avoid its diffusion limitation in the fibers (reoxygenation of the system performed

before addition of FCCP). Myocardial oxygen consumption rate was expressed in pmolO2/s per milligram of wet tissue weight (ww).

3.6. Statistical analysis

Normality of distribution was checked using D'Agostino-Pearson test and, in case of normal distribution, unpaired Student t test was conducted for comparison. Data in figures are presented as means ± SEM. Correlation analysis was performed using GraphPad Prism 6 software (San Diego, Ca, USA), with a two-sided p value < 0.05 considered as significant.

4. RESULTS

Examination of mitochondrial oxidation of the main energy substrates revealed significant alterations in the ability to process fatty acid in DM heart. Upon addition of palmitoyl-carnitine, respiration in the presence of ADP was significantly lower in LV myocardium from DM patients as compared to nonDM (Figure 3). The same effect was observed, albeit at the higher rates of oxygen consumption, upon addition of an uncoupling agent FCCP. On the other hand, ADP-supported mitochondrial oxidation of pyruvate, the specific substrate of carbohydrate metabolism, was not different between nonDM and DM group (Figure 4). Administration of FCCP accelerated the respiration in both groups to the same extent, suggesting that maximal electron transfer chain capacity is comparable between them. RCR values for palmitoyl- and pyruvate-fueled respiration also did not differ between nonDM and DM groups (Figure 5). Enzymatic activity of citrate synthase, a marker of mitochondrial content in the tissue, was not different between the two groups, indicating that the observed difference in mitochondrial palmitoyl oxidation is not due to decreased mitochondrial content in DM myocardium (not shown).

The rate of mitochondrial respiration driven by palmitoyl-carnitine was negatively correlated with blood levels of HbA1c ($r^2=0.21$) (Figure 6).

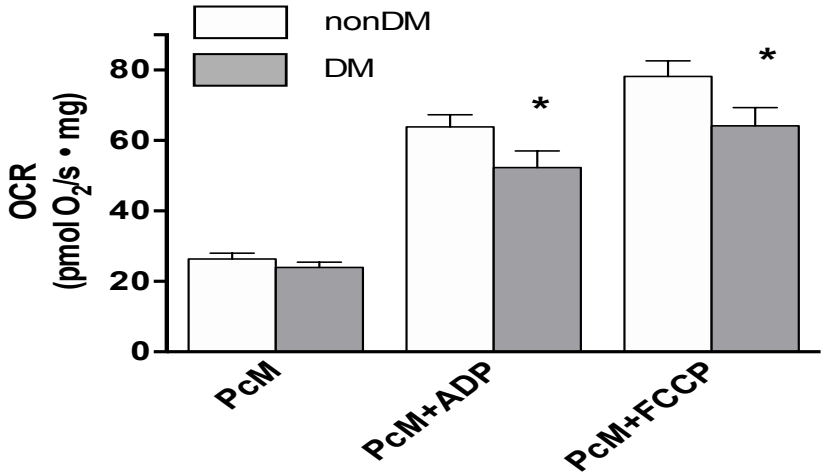

Figure 3. Oxygen Consumption Rate (OCR) for fatty acid oxidation is reduced in diabetic myocardium
*P<0.05 versus nonDM group Oxygen

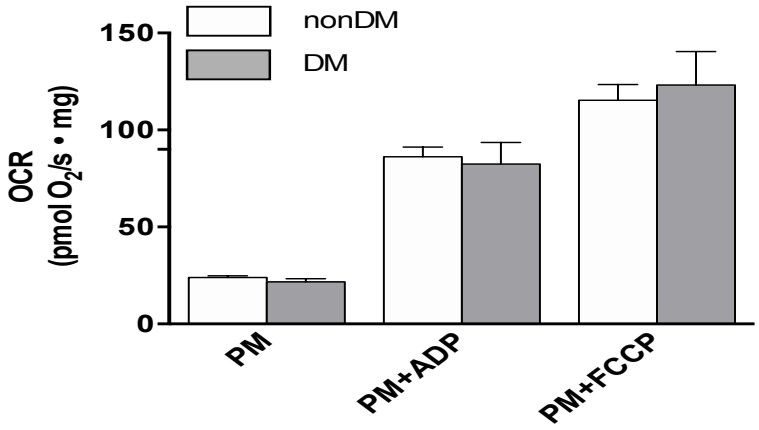

Figure 4. Oxygen Consumption Rate for carbohydrate oxidation is unaltered in diabetic myocardium

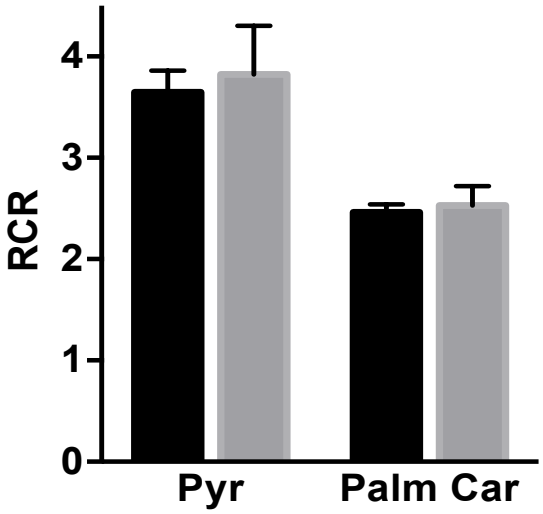

Figure 5. Respiratory Control Ratio (RCR) values for palmitoyl- and pyruvate-fueled respiration

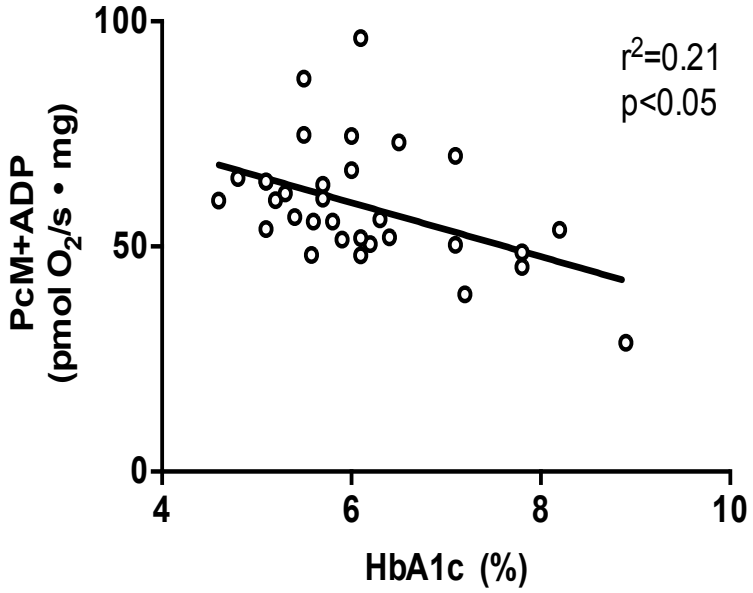

Figure 6. Rate of mitochondrial respiration driven by palmitoyl-carnitine has a negative correlation

5. DISCUSSION

To our knowledge, the current study represents the first direct assessment of the influence of type 2 diabetes mellitus on mitochondrial function in left ventricular myocardium of patients suffering from this condition, who have preserved cardiac contractile function. We found that the hearts of DM2 patients, even in the absence of contractile failure, a decreased mitochondrial capacity for oxidation of fatty acids and unchanged mitochondrial oxidative capacity for carbohydrates.

Mitochondrial substrate oxidation capacity in diabetic LV myocardium. Essentially, a disease of energy metabolism, diabetes mellitus causes significant alterations of myocardial metabolism of carbohydrates and fatty acids. Due to insulin resistance, there is an increased lipolysis in adipose tissue, with increased fatty acid delivery to the myocardium (16). This is coupled with decreased insulin-stimulated GLUT-4-mediated entry of glucose into the cardiac myocytes.

As a result, there is a change occurring in utilization of substrates, with augmented myocardial reliance on fatty acid uptake and metabolism for production of high-energy compounds and concomitantly decreased utilization of glucose. Most of the studies which investigated substrate utilization in patients with DM2 were performed at the level of whole heart, using positron emission tomography (PET), and were thus influenced by many variables, including plasma concentration of substrates.

In the current study, we investigated how ventricular mitochondria from diabetic patients process fatty acids and carbohydrates. By performing these experiments in permeabilized myocardial tissue supplied with a fixed amount of substrate (palmitoyl-carnitine), we have by-passed some of the rate-limiting steps in fatty acid utilization, such as sarcolemmal uptake by FAT/CD36 and mitochondrial translocation (CPT-1). By doing so, we were able to test the mitochondrial intrinsic ability for fatty acid oxidation. Also, in another set of experiments, by providing permeabilized tissue with pyruvate, we were able to test mitochondrial oxidation of carbohydrates independent of the insulin-mediated GLUT-4 uptake.

We found that mitochondrial respiration driven by palmitoyl-carnitine is decreased in DM as compared to nonDM, while the pyruvate-driven mitochondrial respiration is unaffected by diabetes. This indicates that cardiac mitochondria of diabetic patients have reduced capacity for oxidation of long chain fatty acids, while their capacity for carbohydrate oxidation is preserved.

There have been findings of altered mitochondrial oxidation of energy substrates were previously demonstrated, but the use of atrial tissue from DM2 patients (8,9) and animal models of the disease (18). Moreover, even data from a more recent PET study on LV substrate metabolism in diabetic patients agrees with our findings by showing that despite increased fatty acid esterification and utilization in diabetic myocardium, the percent fatty acid oxidation rate is lower (i.e. diabetic heart oxidizes proportionally less of the extracted fatty acids than nondiabetic heart) (17).

Examination of the main factors involved in metabolism of fatty acids and carbohydrates (activity and expression of pyruvate dehydrogenase) revealed no difference between nonDM and DM patients. These findings suggest that the observed reduction in mitochondrial oxidation of palmitoyl in DM myocardium is not a result of metabolic steps handling fatty acid intracellular or intramitochondrial uptake (function of FAT/CD36 and CPT1, respectively), nor the defects in mitochondrial respiratory chain activity. Rather, these findings suggest the main disruption leading to the decreased capacity for fatty acid oxidation might be at the level of beta-oxidation.

In diabetic LV myocardium there is a mismatch between increased fatty acid load to the cardiomyocytes and decreased capacity of mitochondria to oxidize their overwhelming amounts, causing an accumulation of lipids. Consequently, these conditions could lead to an increased accumulation of lipid in form of triglicerides, ceramides and diacyglycerols, which can lead to lipotoxic cardiac damage (21).

Limitations of the present study. Since the current study was performed on human LV myocardial tissue obtained during CABG surgery, we were able to obtain only a small size biopsy samples, which prevented us from performing all the measurements in tissues from all of the patients. Also, both of our patient groups (nonDM and DM) suffered from coronary artery disease requiring surgical revascularization.

Therefore, we were not able to study directly diabetic cardiomyopathy, which is defined as myocardial dysfunction in absence of other cardiac risk factors (e.g. CAD, hypertension). Furthermore, although there was no obvious difference in severity of CAD between DM and nonDM patients, we cannot assess possible differences in microvascular function between the two groups. However, both groups of patients had preserved contractile performance, suggesting that they were well matched. Moreover, since our findings on reduced oxidation of fatty acid in diabetic mitochondria completely agree with previous work performed in human

atria of diabetic patients (tissue likely not affected by ischemia) (8, 9), we believe the differences observed here are primarily due to diabetes.

6. CONCLUSION

1. LV myocardium of DM2 patients undergoing CABG surgery has significantly altered mitochondrial function
2. There is a decrease capacity to oxidize long chain fatty acids in the LV myocardium mitochondria
3. Carbohydrate oxidation remains unaffected in LV myocardium mitochondria

7. REFERENCES

1. Centers for Disease Control and Prevention. National Diabetes Statistics Report, 2017. Atlanta, GA: Centers for Disease Control and Prevention, U.S. Dept of Health and Human Services; 2017.

2. Bullard KM, Cowie CC, Lessem SE, Saydah SH, Menke A, Geisset LS, et al. Prevalence of Diagnosed Diabetes in Adults by Diabetes Type — United States, 2016. Morb Mortal Wkly Rep. 2018;67:359–61.

3. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2019. Diabetes Care. 2019;42(Suppl 1):S13-28.

4. Khaku K. Pathophysiology of Type 2 Diabetes and Its Treatment Policy. Japan Med Assoc J. 2010;53(1):41-6.

5. Pyke DA. Diabetes: the genetic connections. Diabetologia. 1979;17(6):333-43.

6. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med. 2006;23(5):469-80.

7. Fox CS, Coady S, Sorlie PD, Levy D, Meigs JB, D'Agostino RB, Sr., et al. Trends in cardiovascular complications of diabetes. JAMA. 2004;292(20):2495-9.

8. Jia G, Hill MA, Sowers JR. Diabetic Cardiomyopathy: An Update of Mechanisms Contributing to This Clinical Entity. Circ Res. 2018;122(4):624-38.

9. Dayer M, Cowie MR. Heart failure: diagnosis and healthcare burden. Clin Med (Lond). 2004;4(1):13-8.

10. Kannel WB, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: the Framingham study. Am J Cardiol. 1974;34(1):29-34.

11. Montaigne D, Marechal X, Coisne A, Debry N, Modine T, Fayad G, et al. Myocardial contractile dysfunction is associated with impaired mitochondrial function and dynamics in type 2 diabetic but not in obese patients. Circulation. 2014;130(7):554-64.

12. Kuznetsov AV, Veksler V, Gellerich FN, Saks V, Margreiter R, Kunz WS. Analysis of mitochondrial function in situ in permeabilized muscle fibers, tissues and cells. Nat Protoc. 2008;3(6):965-76.

13. Saini V. Molecular mechanisms of insulin resistance in type 2 diabetes mellitus. World J Diabetes. 2010;1(3):68-75.

14. Chong CR, Clarke K, Levelt E. Metabolic Remodeling in Diabetic Cardiomyopathy. Cardiovasc Res. 2017;113:422-30.

15. Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. Physiol Rev. 2005;85(3):1093-129.

16. Abu-Erreish GM, Neely JR, Whitmer JT, Whitman V, Sanadi DR. Fatty acid oxidation by isolated perfused working hearts of aged rats. Am J Physiol. 1977;232(3):E258-62.

17. Abu-Elheiga L, Matzuk MM, Abo-Hashema KA, Wakil SJ. Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2. Science. 2001;291(5513):2613-6.

18. Bing RJ. The metabolism of the heart. Acta Cardiol. 1955;10(1):1-14.

19. Battiprolu PK, Lopez-Crisosto C, Wang ZV, Nemchenko A, Lavandero S, Hill JA. Diabetic cardiomyopathy and metabolic remodeling of the heart. Life Sci. 2013;92(11):609-15.

20. Nickel A, Loffler J, Maack C. Myocardial energetics in heart failure. Basic Res Cardiol. 2013;108(4):358.

21. Gnaiger E. Capacity of oxidative phosphorylation in human skeletal muscle: new perspectives of mitochondrial physiology. Int J Biochem Cell Biol. 2009;41(10):1837-45.

22. Carley AN, Severson DL. Fatty acid metabolism is enhanced in type 2 diabetic hearts. Biochim Biophys Acta. 2005;1734(2):112-26.

23. Herrero P, Peterson LR, McGill JB, Matthew S, Lesniak D, Dence C, et al. Increased myocardial fatty acid metabolism in patients with type 1 diabetes mellitus. J Am Coll Cardiol. 2006;47(3):598-604.

24. Marciniak C, Marechal X, Montaigne D, Neviere R, Lancel S. Cardiac contractile function and mitochondrial respiration in diabetes-related mouse models. Cardiovasc Diabetol. 2014;13:118.

25. Anderson EJ, Kypson AP, Rodriguez E, Anderson CA, Lehr EJ, Neufer PD. Substrate-specific derangements in mitochondrial metabolism and redox balance in the atrium of the type 2 diabetic human heart. J Am Coll Cardiol. 2009;54(20):1891-8.

26. Sharma S, Adrogue JV, Golfman L, Uray I, Lemm J, Youker K, et al. Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. FASEB J. 2004;18(14):1692-700.

8. SUMMARY

Objectives: The main goal of the present study was to investigate directly the ability of heart mitochondria to oxidize fatty acids and carbohydrates in patients suffering from DM2. Since DM2 is a metabolic disease which disturbs normal metabolic processes in the entire body, we wanted to explore the potential impact on utilization of the main substrates in metabolically very active organ, such as heart. Although some studies had been performed in animal models of diabetes, investigation conducted on the tissue derived from human DM2 patients enables a direct insight into clinically relevant aspects of the disease.

Patients and Methods: Thirty-seven patients who had been previously diagnosed with Coronary Artery Disease (CAD), were hemodynamically stable and were scheduled for elective Coronary Artery Bypass Grafting (CABG) surgery. Emergency patients, patients with LV ejection fraction (LVEF) below 50%, patients with type 1 diabetes mellitus, concomitant valve replacement, and severe renal, hepatic or pulmonary disease were excluded from our investigation. The included patients were subdivided into two categories. One group of nondiabetic patients (labelled as nonDM group) and the second the diabetic group (labelled DM). The diabetic group were patients defined on the clinical diagnosis of Type 2 Diabetes (DM2), chronic use of diabetic medication, having a fasting plasma glucose (> 7 mml/L) or a glycosylated hemoglobin (HbA1c >6.5%). Pre, intra- and post-surgical procedures were performed according to the standard clinical routines of the Department of Cardiac Surgery. This experiment and all subsequent processes follow the Declaration of Helsinki and was approved by the Ethical Committees of the University Hospital of Split (2181-147-01). All patients were explained the planned procedures and study objectives in detail prior to giving the signed informed consent for their enrollment.

Results: Examination of mitochondrial oxidation of the main energy substrates revealed significant alterations in the ability to process fatty acid in DM heart. Upon addition of palmitoyl-carnitine, respiration in the presence of ADP was significantly lower in LV myocardium from DM patients as compared to nonDM. Cardiac mitochondrial ability to oxidize carbohydrates (pyruvate) was not affected in diabetic patients.

Conclusion: The study shows that LV myocardium of DM2 patients undergoing CABG surgery has significantly altered mitochondrial function, with decreased capacity to oxidize long chain fatty acids and unaffected capacity to oxidize carbohydrates.

9. CROATIAN SUMMARY

Naslov: Metaboličko korištenje slobodnih masnih kiselina i ugljikohidrata u dijabetičkom srcu

Ciljevi: Glavni cilj ove studije je bio ispitati sposobnost srčanih mitohondrija za oksidaciju masnih kiselina i ugljikohidrata kod pacijenata koji boluju od šećerne bolesti tipa 2. Obzirom da u toj bolest ujedno dolazi do poremećaja metabolizma u čitavom tijelu, namjera nam je bila ispitati njen mogući utjecaj na korištenje energetskih metaboličkih supstrata u metabolički vrlo aktivnom organu, poput srca. Iako su ranije napravljene neke slične studije na životinjskim modelima dijabetesa, ispitivanje sprovedeno na tkivu ljudskih pacijenata koji boluju od dijabetesa tipa 2 omogućuje izravni uvid u klinički relevantni aspekt bolesti.

Pacijenti i metode: Trideset i sedam pacijenata kojima je ranije dijagnosticirana ishemijska bolest srca (CAD), hemodinamski stabilni i na rasporedu za elektivni zahvat postavljanja aorto-srčane premosnice je bilo uključeno u studiju. Hitni pacijenti, oni s ejekcijskom frakcijom lijevog ventrikula ispod 50 %, s dijabetesom tip 1, pratećom zamjenom zaliska i teško oštećenom bubrežnom, jetrenom ili plućnom funkcijom su bili isključeni iz istraživanja. Uključeni pacijenti su podijeljeni u dvije skupine; pacijenti s dijabetesom tipa 2 (DM) i oni bez dijabetesa (nonDM). Dijabetičku su skupinu činili pacijenti s kliničkom dijagnozom dijabetesa tipa 2, kroničnom upotrebom antidijabetika, s koncentracijom glukoze natašte višom od 7 mml/l ili glikoziliranim hemoglobinom višim od 6.6 %. Svi postupci prije, za vrijeme i nakon operativnog zahvata su bili provedeni u skladu sa standardnim postupnikom Zavoda za kardijalnu kirurgiju. Svi eksperimentalni postupci su bili u sklada s Helsinškom deklaracijom i odobreni od strane Etičkog povjerenstva KBC-a Split (2181-147-01). Svim pacijentima je detaljno objašnjena planirana procedura i ciljevi studije prije nego im je dano na izbor potpisivanje obrasca informiranog pristanka.

Rezultati: Ispitivanje mitohondrijske oksidacije glavnih energetskih supstrata je otkrilo značajno smanjenu sposobnost srčanog mišića pacijenata s dijabetesom tipa 2 za procesuiranjem masnih kiselina. Nakon dodatka palmitoil-karnitina u sustav, mitohondrijska respiracija u prisutnosti ADP-a je bila značajno niža u miokardu lijevog ventrikula pacijenata s dijabetesom nego onih bez. Istovremeno, sposobnost srčanih mitohondrija za oksidaciju ugljikohidrata (piruvata) nije bila smanjena.

Zaključak: Istraživanje je pokazalo da miokard lijevog ventrikula kod pacijenata s dijabetesom tipa 2 koji su podvrgnuti operaciji postavljanja aortokoronarne premosnice ima značajno promijenjenu mitohondrijsku funkciju, koja se očituje u smanjenoj sposobnosti za

oksidaciju dugolančanih masnih kiselina, uz nepromijenjenu sposobnost za oksidaciju ugljikohidrata.

10. CURICULUM VITAE

Personal Information

Name: Michael Richard Lem

Date of Birth: 14.06.1993

Place of Birth: New York, New York U.S.A 10003

Nationality: American

Address: 10 Fairway Drive, Great Neck, New York, 11020

E-mail: mrlem61493@gmail.com

Education

October 2013-September 2019: University of Split School of Medicine, Split, Croatia

January 2013-May 2013: Emory University, Atlanta, Georgia, U.S.A

August 2011-December 2013: Oxford College of Emory University, Oxford, Georgia, U.S.A

September 2007-May 2011: Chaminade High School, Mineola, New York, U.S.A

Other Activities

October 2013 to October 2018: Representative, Student Government (Studentski Zbor) at the University of Split School of Medicine

October 2018 to June 2019: Vice President, Student Government (Studentski Zbor) at the University of Split School of Medicine

January 2015 to September 2017: Director of International Affairs and Communications, International Student Association of the University of Split School of Medicine (ISA-USSM)

September 2017 to July 2019: President, International Student Association of the University of Split School of Medicine (ISA-USSM)

March 2018 to July 2019: Founder and Head Coach, Split Legion Lacrosse Club

Language

English (Native Language)