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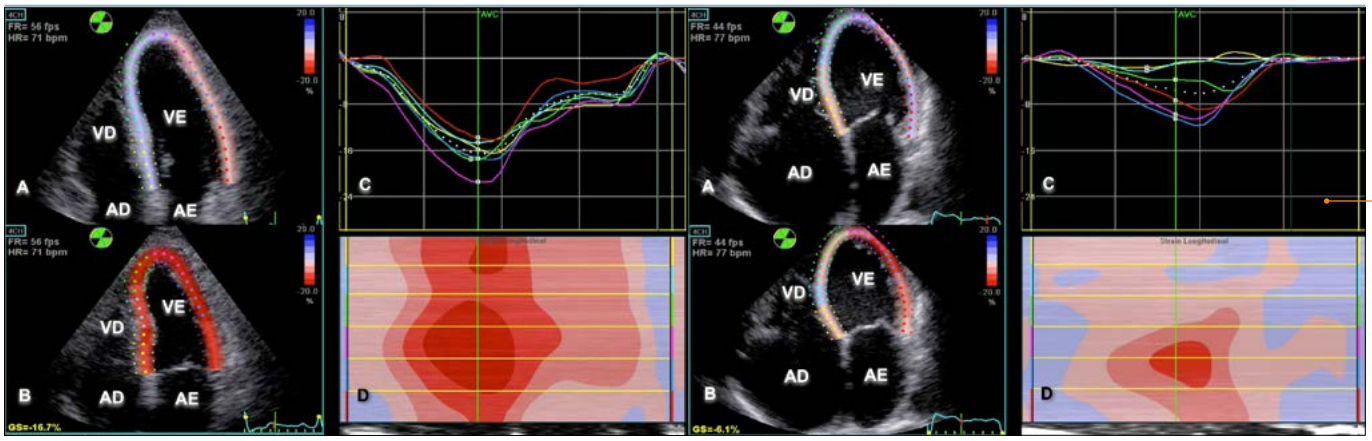

Thematic Issue

Cardiology

SECTIONS

EDITORIAL

ARTICLES

ORIGINAL ARTICLES

REVIEW ARTICLES

RAMB REVISTA DA ASSOCIAÇÃO MÉDICA BRASILEIRA
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SUMMARY

Thematic Issue: Cardiology

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SECTIONS

EDITORIAL

ARTICLES

ORIGINAL ARTICLES

Diabetes and premature death

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After overcoming, in the last century, the obstacle of a short life expectancy of about third years, humanity was faced with an amplitude of challenges associated to the several genetic vulnerabilities linked to lifestyle changes and aging. At the center of these dis-adjustments, is the type II diabetes mellitus (DM II). Despite the persistence of the unbalance in the calorie intake and lack of physical activity, according to estimates by the International Diabetes Foundation (IDF), the expansion curve for the incidence of DM2 shows a slight deceleration, at a ratio of 0.5%. However, the condition has already affected 7% of the world population of adults and is the cause of around 10% of all deaths[1-8]. Thus, a diagnosis of DM2 means a decrease of up to two decades in life expectancy, according to the type and prematurity of the DM2 found[9]. Besides, morbidities such as amaurosis, dementia, neuropathies, and chronic kidney disease make this condition a topic of absolute urgency and relevancy.

Since it started being recorded, cardiovascular disease (CV) has been responsible for 80% of deaths of individuals with DM2[10]. Thus, several observational and interventional studies sought to identify the primary mediators for CV risk in individuals with DM2 in order to improve the risk estimate and intervene slowing down the high mortality. Blood glucose control was the most significant intervention for reducing mortality. However, the intensive control of blood glucose levels to values close to those of healthy individuals did not present any benefits[11] and, in one of the studies, was even associated with an increase in mortality (+2.9 deaths for every 1,000 patients/year[12]. Likewise, the control of the arterial pressure (-3.2 deaths for every 1,000 patients/year per 10 mm Hg)[13] and the LDL cholesterol (-2.1 deaths for every 1,000 patients/year per 39 mg/dL)[14] were critical elements in this strategy for risk control. Similarly to the blood glucose control, the intensification of these interventions did not present any benefits regarding survival[15,16]. In addition, anti-diabetes therapies were associated with an increase in the incidence of cardiovascular diseases[17].

The high residual mortality and the uncertainty of the CV effects of the therapies led to a demand for new treatments systematically tested by cardiovascular safety studies. Despite having been outlined to asses safety, two classes of anti-diabetic medication have shown a reduction in mortality regardless of the effect on glucose: sodium-glucose cotransport-

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er-2 (SGLT-2i) inhibitors (-9 deaths for every 1,000 patients/year)[18] and Glucagon-like peptide-1 (GLP-1) agonists (-4 deaths for every 1,000 patients/year)[19]. Thus, over the last five years, not only the reduction of the possibility of prolonging the life of DM2 patients was demonstrated, but it was done as an additional effect to the control of traditional risk factors.

In this edition, we bring a selection of original studies and reviews dedicated to DM2 grouped with the purpose of emphasizing the enormous mortality of this disease, but also to the substantial advancements achieved for its control.

REFERENCES:

1. IDF Diabetes Atlas. 1st ed. ed. Brussels, Belgium: International Diabetes Federation; 2000.
2. IDF Diabetes Atlas. 2nd edition ed. Brussels, Belgium: International Diabetes Federation; 2003.
3. IDF Diabetes Atlas. 3rd edition ed. Brussels, Belgium: International Diabetes Federation; 2006.
4. IDF Diabetes Atlas. 4th edition ed. Brussels, Belgium: International Diabetes Federation; 2009.
5. IDF Diabetes Atlas. 5th edition ed. Brussels, Belgium: International Diabetes Federation; 2011.
6. IDF Diabetes Atlas. 6th edition ed. Brussels, Belgium: International Diabetes Federation; 2013.
7. IDF Diabetes Atlas. 7th edition ed. Brussels, Belgium: International Diabetes Federation; 2015.
8. Bertolami A, de Lima-Junior JC, Cintra RM, et al. Adiponectin concentration data improve the estimation of atherosclerotic risk in normal and in overweight subjects. Clin Endocrinol (Oxf) 2018;88:388-96.
9. Emerging Risk Factors C, Di Angelantonio E, Kaptoge S, et al. Association of Cardiometabolic Multimorbidity With Mortality. JAMA 2015;314:52-60.
10. Wright AK, Kontopantelis E, Emsley R, et al. Life Expectancy and Cause-Specific Mortality in Type 2 Diabetes: A Population-Based Cohort Study Quantifying Relationships in Ethnic Subgroups. Diabetes Care 2017;40:338-45.
11. Fang HJ, Zhou YH, Tian YJ, Du HY, Sun YX, Zhong LY. Effects of intensive glucose lowering in the treatment of type 2 diabetes mellitus on cardiovascular outcomes: A meta-analysis of data from 58,160 patients in 13 randomized controlled trials. Int J Cardiol 2016;218:50-8.
12. Action to Control Cardiovascular Risk in Diabetes Study G, Gerstein HC, Miller ME, et al. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 2008;358:2545-59.
13. Emdin CA, Rahimi K, Neal B, Callender T, Perkovic V, Patel A. Blood pressure lowering in type 2 diabetes: a systematic review and meta-analysis. JAMA 2015;313:603-15.
14. Cholesterol Treatment Trialists C, Kearney PM, Blackwell L, et al. Efficacy of cholesterol-lowering therapy in 18,686 people with diabetes in 14 randomized trials of statins: a meta-analysis. Lancet 2008;371:117-25.
15. Brouwer TF, Vehmeijer JT, Kalkman DN, et al. Intensive Blood Pressure Lowering in Patients With and Patients Without Type 2 Diabetes: A Pooled Analysis From Two Randomized Trials. Diabetes Care 2018;41:1142-8.
16. Sabatine MS, Leiter LA, Wiviott SD, et al. Cardiovascular safety and efficacy of the PCSK9 inhibitor evolocumab in patients with and without diabetes and the effect of evolocumab on glycaemia and risk of new-onset diabetes: a prespecified analysis of the FOURIER randomised controlled trial. Lancet Diabetes Endocrinol 2017;5:941-50.
17. Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. N Engl J Med 2007;356:2457-71.
18. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. N Engl J Med 2015;373:2117-28.
19. Marso SP, Daniels GH, Brown-Frandsen K, et al. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med 2016;375:311-22.

ORIGINAL ARTICLE

Prevalence, treatment, and control of dyslipidemia in diabetic participants of two brazilian cohorts: a place far from heaven

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SUMMARY

OBJECTIVE: *Diabetes is one of the leading causes of cardiovascular mortality. Over the last years, mortality has decreased significantly, more in individuals with diabetes than in healthy ones. That is mostly due to the control of other cardiovascular risk factors. The objective of our study was to analyze the dyslipidemia control in two diabetes cohorts.*

METHODS: *Patients from two distinct cohorts were studied, 173 patients from the BHS (Brasília Heart Study) and 222 patients from the BDS (Brazilian Diabetes Study). The data on dyslipidemia control were studied in both different populations. All patients had diabetes.*

RESULTS: *There are significant differences concerning comorbidities between the LDL-C and BDS groups. The average glycated hemoglobin is of 8.2 in the LDL-C > 100 group in comparison with 7.7 and 7.5 in the 70-100 and < 70 groups, respectively (p = 0.024). There is a higher percentage of hypertensive patients with LDL between 70-100 (63.9%), when comparing the < 70 and > 100 groups (54.3% and 54.9%, respectively; p = 0.005). Diastolic pressure is higher in the group with LDL > 100, with an average of 87 mmHg, in comparison with 82.6 mmHg and 81.9 mmHg in the 70-100 and < 70 groups, respectively (p = 0.019). The group with LDL > 100 has the greatest percentage of smokers (8.7%) in comparison with the groups with LDL between 70-100 and < 70 (5.6% and 4.3%, respectively; p = 0.015). There is also a difference in the previous incidence of coronaropathy. In the group with LDL < 70, 28.3% of patients had already experienced a previous infarction, compared with 11.1% and 10.6% in the 70-100 and > 100 groups, respectively (p < 0.001).*

CONCLUSIONS: *The data in our study have shown that the dyslipidemia control in diabetic patients is inadequate and there is a tendency of direct association between lack of blood glucose control and lack of dyslipidemia control, in addition to the association with other cardiovascular risk factors, such as diastolic hypertension and smoking. This worsened control might be related to the plateau in the descending curve of mortality, and investments in this regard can improve the cardiovascular health in diabetic patients.*

KEYWORDS: *Dyslipidemias. Diabetes mellitus. Risk factors. Targets.*

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INTRODUCTION

Diabetes is a huge cause of morbidity, mortality and economic impact. It affects 415 million individuals worldwide[1], with an increase in prevalence over the past years, going from 4.7% in 1980 to 8.5% in 2014, most significantly in countries with low and medium income[2]. In Brasil, the estimated prevalence is of 8.1%. It is the direct cause of 6% of all deaths in the country, in addition to contributing to 31% of deaths attributed to cardiovascular disease[3].

Over the last 30 years, cardiovascular mortality was substantially reduced, more so in diabetic individuals than in healthy ones, reaching a plateau after 2008-2010.[4,5] In large part, this more favorable evolution of the cardiovascular prognosis in diabetic patients was due to an improved response to acute cardiovascular care in this population and also the improvement in the treatment of comorbidities such as hypertension and dyslipidemia.

The response to the lipid-lowering treatment has been widely demonstrated, and each reduction of 40 mg/dL (1 mmol/L) in LDL cholesterol translates into 20% less incidence of more serious cardiovascular events and 16% less cardiovascular mortality.[6] Interestingly, in a subanalysis of the Improve-IT study, only diabetic patients benefited from the addition of ezetimibe on top of the maximum dose of statin.[7]

Despite the relevancy of the subject, adherence to the lipid-lowering treatment is probably the most significant barrier and must represent part of the explanation for the plateau in the curve of mortality trend over the last decade. However, data on the control of LDL cholesterol in Brasil are very scarce and inconsistent. The most relevant data available on the subject comes from the Elsa study (Longitudinal Study of Adult Health) and suggests that the adequate con-

TABLE 1: CHARACTERISTICS OF PATIENTS ENROLLED IN THE BRAZILIAN DIABETES STUDY

	LDL-C, mg/dL			
	>100	**70-100**	**<70**	**p**
N (222)	104 (47%)	72 (32%)	46 (21%)	
Demographics				
Age, years	58.6±6.9	59.8±7.1	61.1±6.5	0.106
Schooling, years	10.8±4.1	10.61±4.7	9.96±4.5	0.538
Men, %	53.8	55.6	73.9	0.058
Medical history				
Previous AMI, %	10.6	11.1	28.3	<0.001
Smokers, % / Former smokers, %	8.7/31.7	5.6/30.6	4.3/28.3	0.015/ 0.011
Hypertension, %	54.8	63.9	54.3	0.005
Hemodynamics				
SBP, mmHg	149.47±22.0	143.75±20.4	145.71±20.6	0.177
SBP, mmHg	87.09±13.2	82.65±10.2	81.9±12.8	0.019
HR, bpm	78.13±12.5	75.01±11.53	74.33±11.0	0.114
ER, %	57.6±11.6	60.6±9.1	59.4±6.2	0.782
Biochemistry				
Blood glucose, mg/dL	172.6±69.6	163.8±58.4	159.1±54.8	0.439
HbA1c, %	8.2±2.0	7.7±1.4	7.5±1.3	0.024
Creatinine, mg/dL	0.89±0.22	0.88±0.18	0.94±0.20	0.316
HDL-C, mg/dL	42.5±12.4	44.2±13.4	43.0±14.3	0.681
LDL-C, mg/dL	135.4±32.1	88.1±8.0	58.0±10.5	<0.001
TG, mg/dL	234.5±138	158.7±92.6	153.7±156.5	<0.001
PCRus, mg/L	0.42±0.54	0.32±0.32	0.35±0.41	0.343
Treatment				
Simvastatin, %	21	38	53	<0.001

SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; HbA1C: glycated hemoglobin; TG: triglycerides; CRP: ultrasensitive C-reactive protein.

TABLE 2: CHARACTERISTICS OF PATIENTS ENROLLED IN THE BRASÍLIA HEART STUDY

	LDL-C, mg/dL			
	>100	**70-100**	**<70**	**p**
N (173)	113 (65%)	37(21%)	23 (13%)	
Demographics				
Age, years	62.24±9.9	64.51±11.4	61.48±10.8	0.441
Schooling, years	7.3±4	7.2±5	7.6±5	0.944
Men, %	67.3	64.9	73.9	0.759
BMI, kg/m²	27.7±4	28.2±6	27.4±4	0.726
Medical history				
Previous AMI %	12.4%	27.0%	21.7%	0.093
Smoker, %	69.9	67.6	69.6	0.964
Hypertension, %	29.2	18.9	30.4	0.442
Hemodynamics				
SBP, mmHg	137±30	141±36	128±26	0.317
SBP, mmHg	87±19	86±20	82±19	0.579
HR, bpm	81±17	80±16	72 ±18	0.099
Biochemistry				
Blood glucose, mg/dL	206±87	200±84	191±98	0.741
HbA1c, %	8.1±2.3	8.1±2.0	8.1±1.9	0.983
Cr, mg/dL	1.16±0.35	1.18±0.52	1.15±0.71	0.966
HDL-C, mg/dL	39.0±9.8	37.0±10.4	27.1±10.0	<0.001
LDL-C, mg/dL	140.7±34.9	88.3±8.0	56.7±13.8	<0.001
TG, mg/dL	166.7±73.2	142.2±90.8	175.4±130.1	0.247
PCRus, mg/L	1.39±2.25	2.45±4.04	2.26±4.20	0.120
Treatment				
Simvastatin, %	28.8	27.8	30.4	0.976

SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; HbA1C: glycated hemoglobin; TG: triglycerides; CRP: ultrasensitive C-reactive protein.

trol of dyslipidemia in the general population is lower than 10%.[8] However, it is well known that the Elsa population had an educational background different from that of most of the Brazilian population (52.6% with complete higher education). In the diabetic population, data are even more scarce, which has limited the development of public policies that address this group of patients.

The purpose of this study is to analyze data concerning the dyslipidemia control in diabetic patients using the population from two Brazilian cohorts.

METHOD

The population of this study is composed of individuals from two distinct cohorts, with 173 patients from the BHS (Brasília Heart Study) and 222 patients from the BDS (Brazilian Diabetes Study). There were no patients who participated in both cohorts.

Brasília Heart Study

The patients included in the BHS were consecutively enrolled provided they presented a diagnosis of infarction with ST-segment elevation admitted within 24 hours of the onset of pain at the Basic Hospital of the Federal District, the largest public hospital of the Federal Capital. A total of 173 patients admitted between 2006 and 2017 were included in the analysis. The inclusion criteria were: (i) less than 24 hours from the onset of the AMI symptoms; (ii) ST-segment elevation of at least 1 mm (frontal plane) or 2 mm (horizontal plane) in two contiguous leads; (iii) evidence of myocardial necrosis due to the rise in CK-MB and troponin; (iv) glycated hemoglobin > 6.5% or a previous diagnosis of diabetes with use of anti-diabetic medication; (v) absence of cognitive incompetence that prevents the verbal response to medical questionnaires or the return to medical consultations. During the evaluation, will be carried out: (i) anthropometry; (ii)analysis of dietary composition through a recall questionnaire; (iii) general medical records; (iv) blood samples for biochemical analysis and separation of plasma and DNA for freezing at -80°C. In the plasma biochemical analysis of the first evaluation will be measured: glycated Hemoglobin, insulin, C-peptide, blood glucose, total cholesterol, triglycerides, HDL cholesterol, high-sensitivity C-reactive protein, urea, and creatinine. This study was approved by the ethics committee of the Federal District Secretariat of Health, and all patients enrolled signed an informed consent form authorizing the study.

Brazilian Diabetes Study

The BDS is a cohort formed between 2016 and 2018 with diabetic patients admitted after a campaign in print media, radio, and television. Thus, the population studied is composed of voluntary diabetic individuals assessed in the Clinical Research Center (CPC) of the State University of Campinas (Unicamp). The inclusion criteria were: (i) patients with type 2 diabetes mellitus (DM2), (ii) between 40 and 70 years old, (iii) absence of cognitive incompetence that prevents verbal response to medical questionnaires and the informed consent form. The exclusion criteria were: (i) patients diabetes mellitus phenotypes other than DM2; (ii) insulin-dependent individuals. In the first assessment, the study was explained in detail to the patients, and the informed consent form was presented. Then, the patients' identification and contact information were collected, followed by the medical history and a complete physical examination; the arterial pressure measured while seating and standing, the abdominal circumference, height, and weight were recorded. Finally, the patient's pharmacological characterization was recorded, and a diabetic polyneuropathy assessment conducted, in addition to data concerning the socioeconomic conditions and lifestyle. The research ethics committee of Unicamp approved the study, and all the patients signed the informed consent form in order to be enrolled in the study.

Statistical analysis

The quantitative data were presented as average +/- standard deviation. Comparison between the groups was carried out using the Student t-test for parametric continuous variables, the Mann-Whitey test for non-parametric continuous variables, and the $\chi 2$ test for continuous variables. The statistical analysis was performed using the SPSS version 20.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

In the Brasília Heart Study, there were no statistically significant differences in demographic factors between the groups with the best and worst dyslipidemia control (age, years of formal education, sex, and body mass index). The average of formal education in the cohort was 7.32 of studying. As for the dyslipid-

emia control, 21% of the patients reached LDL-C < 70 (good), 32% between 70-100 (intermediate) and 47% > 100 mg/dL (inadequate).

As for the prevalence of comorbidities between the LDL-C groups, there was also no statistically significant difference between the prevalence of chronic liver disease and hypertension, and the average glycated hemoglobin in the groups with LDL-C < 70, LDL-C = 70-100, and LDL-C > 100 mg/dL was of 8.1 (p = 0.966), which suggests that the adequate control of LDL-Cis independent from the adequate control of blood glucose targets.

In the Brazilian Diabetes Study (BDS), there were also no statistically significant differences between the LDL-C groups in comparison with demographic factors. The is a non-significant tendency of a greater number of men in the LDL-C < 70 (73.9%) group in comparison with the 70-100 and > 100 mg/dL groups (55.6% and 53.8%, respectively; p = 0.058). Although the average formal schooling in this cohort (10.57 years of studying) is higher than in the BHS cohort, the dyslipidemia control was worse, with only 13% of patients reaching DL-C < 70 (good), 21% between 70-100 (intermediate), and 65% > 100 (inadequate).

However, there are noteworthy statistically significant differences concerning comorbidities between the LDL-C groups. Concerning diabetes, there was a higher lack of blood glucose control in the group with a greater lack of dyslipidemia control. The average glycated hemoglobin is of 8.2 in the LDL-C > 100 group in comparison with 7.7 and 7.5 in the 70-100 and < 70 groups, respectively (p = 0.024). There is a higher percentage of hypertensive patients with LDL between 70-100 (63.9%), when comparing the < 70 and > 100 groups (54.3% and 54.9%, respectively; p = 0.005). Diastolic pressure is higher in the group with LDL > 100, with an average of 87 mmHg, in comparison with 82.6 mmHg and 81.9 mmHg in the 70-100 and < 70 groups, respectively (p = 0.019). The group with LDL > 100 has the greatest percentage of smokers (8.7%) in comparison with the groups with LDL between 70-100 and < 70 (5.6% and 4.3%, respectively; p = 0.015). There is also a difference in the previous incidence of coronaropathy. In the group with LDL < 70, 28.3% of patients had already experienced a previous infarction, compared with 11.1% and 10.6% in the 70-100 and > 100 groups, respectively (p < 0.001). Thus, it is reasonable to conclude that chasing more aggressive targets of LDL-C is more frequent for diabetic patients in secondary prevention than to those on primary prevention.

DISCUSSION

Our work was one of the few in Brasil that proposed to evaluate the dyslipidemia control in diabetic populations. The results show that in both populations the control is beneath the desired (prevalence of adequate control of 32% in BHS and 13% in BDS) and that the prevalence of dyslipidemia is high.

A similar study was Elsa, composed of public servants from six institutions of higher education and research in Brasil. The prevalence of diabetes, however, was of only 19.1%, and the control with LDL < 70 was reached in 2.5% patients.[8] Another critical difference in this study is the level of formal education, with a greater prevalence of individuals with completed higher education. This suggests a higher socioeconomic level of the participants, with a likely prevalence of patients who already had medical follow-up by private or insurance medical professionals.

The difference in the prevalence of a previous coronaropathy found in the BDS can be explained by the secondary prevention since patients with a previous ischemic event usually have a more intense and rigorous control of dyslipidemia.[9]

Other findings of the BDS are compatible with previous results in the literature: the patients with worst dyslipidemia control have a higher incidence of smoking[10] in this same population, present a higher diastolic pressure[11-13] and, lastly, which is the purpose of the research, a worse blood glucose control was found in patients with higher levels of LDL[14,15].

In the Elsa study, it was found that the prevalence, awareness, and control of dyslipidemia were higher in men, black, and with less formal education.[8] In the populations studied in our work, however, we found a higher dyslipidemia control in patients with lower levels of formal education. Again, the greater prevalence of patients with a higher socioeconomic level among the group with higher formal education might suggest a follow-up by private or insurance doctors. In this sense, the guidelines by the Brazilian Society of Endocrinology and Metabolism proposes to identify the cardiovascular risk for each patient, considering that each diabetic individual has a unique risk and, thus, rejecting the theory that everyone has a high cardiovascular risk. In contrast, the guidelines by the Brazilian Society of Cardiology (SBC) suggests strict targets for LDL-C, at < 70 for everyone with high cardiovascu-

lar risk - a group that includes diabetic individuals. This recommendation is based on studies that show a decrease in cardiovascular events in diabetic patients undergoing a strict control of lipids[16-18].

As limitations, we can mention the small sample of patients in each of the cohorts used in our work in comparison with the Elsa study, for example. Besides, there were no statistically significant associations between the groups of patients of the BHS. Lastly, the inclusion criteria in the cohorts usually selects patients with specific profiles; thus, the data found are not representative of the general diabetic population in Brasil.

In conclusion, there are few data on the dyslipidemia control in the Brazilian population, especially in people with diabetes. The data in our study shows that the control is inadequate and that there is a tendency of direct association between the lack of blood glucose control and the lack of dyslipidemia control, in addition to the association with other risk factors, such as diastolic hypertension and smoking. The limited control of dyslipidemia might be related to the plateau in the curve of mortality trend for diabetic individuals over the last decade. Inventing in the control of comorbidities, such as dyslipidemia, can potentially prolong survival and translate into improved cardiovascular health for diabetic individuals.

RESUMO

OBJETIVO: *O diabetes é importante causa de mortalidade cardiovascular. Nos últimos anos, a mortalidade diminuiu substancialmente, mais em diabéticos do que em não diabéticos, em grande parte devido ao controle de outros fatores de risco cardiovasculares. Nosso estudo tem como objetivo analisar o controle de dislipidemia em duas coortes de diabéticos.*

MÉTODOS: *Foram estudados pacientes de duas coortes distintas, sendo 173 pacientes do BHS (Brasília Heart Study) e 222 pacientes do BDS (Brazilian Diabetes Study). Os dados sobre controle de dislipidemia foram estudados nas duas populações diferentes. Todos os pacientes eram diabéticos.*

RESULTADOS: *Há diferenças significativas em relação às comorbidades entre os grupos de LDL-C no BDS. A média de hemoglobina glicada é de 8,2 no grupo com LDL-C > 100, comparado com 7,7 e 7,5 nos grupos 70-100 e < 70, respectivamente (p = 0,024). Há maior porcentagem de pacientes hipertensos com LDL entre 70-100 (63,9%), quando comparado aos grupos < 70 e > 100 (54,3% e 54,9%, respectivamente; p = 0,005). A pressão diastólica é mais elevada no grupo com LDL > 100, com média de 87 mmHg, comparado com 82,6 mmHg e 81,9 mmHg nos grupos 70-100 e < 70, respectivamente (p = 0,019). O grupo com LDL > 100 tem maior porcentagem de tabagistas (8,7%) quando comparado aos grupos com LDL entre 70-100 e < 70 (5,6% e 4,3%, respectivamente; p = 0,015). Há, também, diferença na incidência prévia de coronariopatia. No grupo com LDL < 70, 28,3% dos pacientes já apresentaram infarto prévio, comparados com 11,1% e 10,6% nos grupos 70-100 e > 100, respectivamente (p < 0,001).*

CONCLUSÃO: *Os dados do nosso estudo mostram que o controle de dislipidemia em diabéticos é inadequado, e há uma tendência de associação direta entre descontrole glicêmico e descontrole de dislipidemia, além de associação com outros fatores de risco cardiovascular, como hipertensão diastólica e tabagismo. Esse pior controle pode estar relacionado ao platô no descenso da curva de mortalidade, e o investimento nesse quesito pode melhorar a saúde cardiovascular dos diabéticos.*

PALAVRAS-CHAVE: *Dislipidemias. Diabetes mellitus. Fatores de risco. Metas.*

REFERENCES

1. Arnold SV, Kosiborod M, Tang F, Zhao Z, McCollam PL, Birt J, et al. Changes in low-density lipoprotein cholesterol levels after discharge for acute myocardial infarction in a real-world patient population. Am J Epidemiol. 2014; 179(11): 1293-300.
2. Behradmanesh S, Nasri P. Serum cholesterol and LDL-C in association with level of diastolic blood pressure in type 2 diabetic patients. J Renal Inj Prev. 2012;1(1):23-6.
3. Bonaa KH, Thelle DS. Association between blood pressure and serum lipids in a population. The Tromso Study. Circulation. 1991;83(4):1305-14.
4. Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, et al. Ezetimibe added to statin therapy after acute coronary syndromes. N Engl J Med. 2015;372(25):2387-97.
5. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. Lancet. 2017;389(10085):2239-51.
6. Cholesterol Treatment Trialists' (CTT) Collaborators. Kearney PM, Blackwell L, Collins R, Keech A, Simes J, Peto R, et al. Efficacy of cholesterol-lowering therapy in 18,686 people with diabetes in 14 randomised trials of statins: a meta-analysis. Lancet. 2008;371(9607):117-25.
7. Giugliano RP, Cannon CP, Blazing MA, Nicolau JC, Corbalán R, Špinar J, et al. Benefit of adding ezetimibe to statin therapy on cardiovascular outcomes and safety in patients with versus without diabetes mellitus: results from IMPROVE-IT (Improved Reduction of Outcomes: Vytorin Efficacy International Trial). Circulation. 2018;137(15):1571-82.
8. Gossett LK, Johnson HM, Piper ME, Fiore MC, Baker TB, Stein JH. Smoking intensity and lipoprotein abnormalities in active smokers. J Clin Lipidol. 2009;3(6):372-8.
9. Gregg EW, Cheng YJ, Saydah S, Cowie C, Garfield S, Geiss L, et al. Trends in death rates among U.S. adults with and without diabetes between 1997 and 2006. Diabetes Care. 2012;35(6):1252-7.
10. Klisic A, Kavaric N, Jovanovic M, Zvrko E, Skerovic V, Scepanovic A, et al.

Association between unfavorable lipid profile and glycemic control in patients with type 2 diabetes mellitus. J Res Med Sci. 2017;22:122.

11. Lotufo PA, Santos RD, Figueiredo RM, Pereira AC, Mill JG, Alvim SM, et al. Prevalence, awareness, treatment, and control of high low-density lipoprotein cholesterol in Brazil: baseline of the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). J Clin Lipidol. 2016;10(3):568-76.

12. Mullugeta Y, Chawla R, Kebede T, Worku Y. Dyslipidemia associated with poor glycemic control in type 2 diabetes mellitus and the protective effect of metformin supplementation. Indian J Clin Biochem. 2012;27(4):363-9.

13. NCD Risk Factor Collaboration. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. Lancet. 2016;387(10027):1513-30.

14. Faludi AA, Izar MCO, Saraiva JFK, Chacra APM, Bianco HT, Afiune A Neto, et al. Atualização da Diretriz Brasileira de Dislipidemias e Prevenção da Aterosclerose – 2017. Arq Bras Cardiol. 2017;109(2 Supl 1):1-76.

15. Rawshani A, Rawshani A, Franzén S, Eliasson B, Svensson AM, Miftaraj M, et al. Mortality and cardiovascular disease in type 1 and type 2 diabetes. N Engl J Med. 2017;376(15):1407-18.

16. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. N Engl J Med. 2017;376(18):1713-22.

17. Sposito AC. Emerging insights into hypertension and dyslipidemia synergies. Euro Heart J. 2004;G8-G12.

18. World Health Organization. WHO diabetes country profiles 2016. [cited 2018 Jun 11]. Available from: http://www.who.int/diabetes/country-profiles/diabetes_profiles_explanatory_notes.pdf

ORIGINAL ARTICLE

Serum uric acid to HDL-cholesterol ratio is a strong predictor of metabolic syndrome in type 2 diabetes mellitus

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SUMMARY

OBJECTIVE: *Metabolic syndrome (MS) is a clinical entity that associated with increased risk of type 2 Diabetes Mellitus (DM) and cardiovascular diseases. Serum uric acid levels are correlated MS criteria. We hypothesized whether a uric acid to HDL-cholesterol ratio (UHR) could predict diabetic regulation and presence of MS in type 2 diabetic subjects.*

METHODS: *Admissions of the subjects with type 2 DM to outpatient clinics of our institution were retrospectively analyzed. Study population grouped into well-controlled and poorly controlled diabetics according to the HbA1c level (cut off 7%) and further grouped into type 2 DM with and without MS according to the presence of MS. UHR of study groups compared.*

RESULTS: *A hundred diabetic subjects enrolled. Mean UHR was significantly lower in well-controlled diabetics (9.7 ± 3.7%) compared to poorly controlled subjects (14 ± 5.4%) (p<0.001). Median UHR of diabetics with MS (13 (6-29) %) was greater than that of the diabetics without MS (9 (3-16) %) (p<0.001). UHR greater than 11% has 77% sensitivity and 60% specifity in predicting worse diabetic control (AUC: 0.752, p<0.001) and a UHR greater than 10.6% has 83% sensitivity and 71% specifity in predicting MS (AUC: 0.839, p<0.001). Sensitivity and specifity of UHR in predicting MS were better than most of the sensitivities and specifities of the five criteria of MS.*

CONCLUSION: *We suggest utilization of UHR in diagnosis of MS as a novel criteria. Nevertheless, prospective studies with larger population may make a better scientific evidence in that issue.*

KEYWORDS: *Metabolic syndrome. Diabetes mellitus, type 2. Uric acid. HDL-cholesterol.*

INTRODUCTION

The term of metabolic syndrome (MS) first used by Haller and Hanefeld1 and was defined as combination of risk factors attributable to adverse events, such as, type 2 diabetes mellitus and cardiovascular diseases. Synonyms of MS are syndrome X, Reaven syndrome, dysmetabolic syndrome X, CHAOS, plurimetabolic syndrome, the deadly quartet, and insulin resistance syndrome2. Although definition of combined parameters of MS varies, a harmonized definition was accepted by International diabetes Federation (IDF), American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI), the World Heart Federation, the International Atherosclerosis Society, and the International Associa-

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tion for the Study of Obesity in 2009. Combination of three or more of 5 criteria (systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg or treated, fating plasma glucose≥100mg/dl or treated, waist circumference ≥102 cm in men and 88 cm in women, HDL-cholesterol <40 mg/dl in men and <50 mg/dl in women, triglyceride ≥150 mg/dl) make the diagnosis of MS.

Uric acid is synthesized by the enzyme xanthine oxidase during the metabolism of purines. High levels of serum uric acid contribute to development of adverse conditions3. Criteria of MS were found to be correlated with serum uric acid levels4-6. Population with higher uric acid levels are more likely to develop MS7.

Both type 2 diabetes mellitus (DM) and MS are considered as metabolic disorders. Presence of MS and its components are increased by elevated serum uric acid levels in type 2 diabetic subjects8.

According to strong relation between serum uric acid and type 2 DM and MS, we hypothesized whether a uric acid to HDL-cholesterol ratio (UHR) could predict diabetic regulation and presence of MS in type 2 diabetic subjects.

METHOD

Admissions of the subjects with type 2 DM to outpatient clinics of our institution between October 2017 and January 2018 were retrospectively analyzed, after institutional approval obtained. Type 2 diabetics were enrolled to the study. Exclusion criteria were as follows; pregnancy, active malignant disease, treatment with drugs that interfere with serum uric acid levels (thiazides, furosemide, acetyl salicylic acid, etc...) and on medications that may alter serum lipid levels (i.e. statins, fibrates, niacin). Subjects with established hemolytic conditions and end stage renal failure were also excluded.

Age, gender, height, weight, waist circumference, duration of diabetes mellitus, systolic blood pressure and diastolic blood pressure were recorded from the patient files and computerized database of our clinic. Body mass index (BMI) is calculated with division of weight (in kg) by square of height (in meters). Arithmetic mean of blood pressure that measured in consecutive 2 clinic visit in both arms were used as blood pressure measures.

Laboratory parameters, such as, fasting plasma glucose, blood urea, serum creatinine, total-cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride, serum uric acid, glomerular filtration rate (eGFR), glycated hemoglobin (HbA1c) were obtained from the same database and patient files. Plasma non HDL-cholesterol was simply calculated by following formula: total cholesterol- HDL cholesterol. UHR is calculated with division of serum uric acid by HDL cholesterol. Uric acid to non HDL cholesterol ratio is measured by the following formula: uric acid/non HDL cholesterol.

Patients grouped in to two groups according to HbA1c levels. Diabetics with HbA1c lower than 7% were classified as well-controlled type 2 DM group and subjects with HbA1c equal to or greater than 7% were classified as poorly controlled diabetics.

Study population were grouped into another two groups according to the presence of MS. Diagnosis of MS established according to the 2009 harmonized criteria of IDF, AHA/NHLBI, the World Heart Federation, the International Atherosclerosis Society, and the International Association for the Study of Obesity. Presence of three of five criteria (systolic blood pressure ≥130mmHg or diastolic blood pressure ≥85 mmHg or treated, fasting plasma glucose≥100mg/dl or treated, waist circumference ≥102 cm in men and 88 cm in women, HDL-cholesterol <40 mg/dl in men and <50 mg/dl in women, triglyceride ≥150 mg/dl) make the diagnosis of MS.

Data were analyzed by SPSS software. (SPSS 15.0; IBM Inc., Chicago, IL, USA). Distribution of the variables in study groups were conducted by Kolmogorov-Smirnov test. Homogenously distributed variables expressed as mean ± standard deviation and compared with independent samples t test. Non-homogenously distributed variables were expressed as median (minimum-maximum) and compared with Mann Whitney U test. Categorical variables were compared in study groups with Chi-square test. Receiver Operative Characteristics (ROC) analyze used to determine cut-off values of UHR in predicting poorly controlled DM and in predicting MS. A Pearson's analyze was used to find out correlation between uric acid, HDL-cholesterol, triglyceride, BMI, waist circumference, UHR and HbA1c. Statistically significance was set on a p value that lower than 0.05.

RESULTS

A total of 100 type 2 diabetic subjects enrolled to the study. Mean ages of the well-controlled and

poorly controlled diabetics were 58.6 ± 10 years and 59.5 ± 8.4 years, respectively. Age difference was not statistically significant between well-controlled and poorly controlled type 2 DM groups (p=0.61). 22 (55%) of 40 in well-controlled group and 29 (48.3%) of 60 in poorly controlled group were women. Gender was not statistically different among well-controlled and poorly controlled type 2 DM groups (p=0.51).

Height (p=0.97), body weight (p=0.17), waist circumference (p=0.06), BMI (p=0.12), systolic (p=0.28) and diastolic (p=0.73) blood pressures were not significantly different between well-controlled and poorly controlled type 2 DM groups.

Serum uric acid was 4.9 ± 1.4 mg/dl in well-controlled diabetics and 6 ± 1.6 mg/dl in poorly controlled type 2 DM subjects (p=0.001). HDL cholesterol of well-controlled type 2 DM group (50 [33-117] mg/dl) was significantly higher than that of the poorly controlled diabetic group (44 [25-73] mg/dl), (p<0.001). The mean UHRs of well-controlled and poorly controlled type 2 DM groups were 9.7 ± 3.7% and 14 ± 5.4%, respectively. The UHR difference was statistically significant (p<0.001). Comparison of clinical and laboratory data of the well-controlled and poorly controlled diabetic subjects is summarized in table 1.

A ROC analyze performed to determine sensitivity and specifity of UHR in selecting poorly control in type 2 DM. UHR greater than 11% has 77% sensitivity and 60% specifity in predicting worse diabetic control (AUC: 0.752, p<0.001).

Study population further grouped according to the existence of MS. There were 63 subjects with MS and 37 subjects without MS. Mean ages of the subjects with and without MS were 59.6 ± 9.1 years and 58.4 ± 9.2 years, respectively. Age difference was not statistically significant between groups (p=0.54). 29 (46%) of 63 in type 2 DM with MS group and 22 (59.5%) of 37 in type 2 DM without MS group were women. Gender was not significantly different between groups (p=0.20). A total of 36 subjects were on antihypertensive treatment (28 in diabetics with MS and 8 in diabetics without MS groups).

Height (p=0.41), BMI (p=0.07), duration of type 2 DM (p=0.93), blood urea (p=0.92), serum creatinine

TABLE 1: COMPARISON OF CLINICAL AND LABORATORY DATA OF THE WELL-CONTROLLED AND POORLY CONTROLLED TYPE 2 DM GROUPS

		Well-controlled Type 2 DM	Poorly controlled type 2 DM	p
Gender	Men (n (%))	18 (45)	31 (51,7)	0.51
	Women (n (%))	22 (55)	29 (48,3)	
		Mean ± Standard Deviation		
Age (years)		58,6 ± 10	59,5 ± 8,4	0.61
Height (m)		1,62 ± 0,1	1,63 ± 0,1	0.97
Weight (kg)		80 ± 15	84 ± 14	0.17
Total cholesterol (mg/dl)		185 ± 44	214 ± 45	0.003
Non HDL cholesterol (mg/dl)		132 ± 42	169 ± 46	<0.001
LDL cholesterol (mg/dl)		106 ± 33	125 ± 38	0.01
UHR (%)		9,7 ± 3,7	14 ± 5,4	<0.001
Uric acid (mg/dl)		4,9 ± 1,4	6 ± 1,6	0.001
eGFR (mL/min/1.73 m^2)		86 ± 14	81 ± 14	0.08
Urea (mg/dl)		30,4 ± 9,8	34,8 ±12,7	0.07
		Median (Min- Max)		
SBP (mmHg)		130 (100-180)	130 (100-180)	0.28
DBP (mmHg)		80 (70-110)	80 (50-105)	0.73
Waist circumference (cm)		100 (77-128)	105 (81-144)	0.06
Fasting blood glucose (mg/dl)		119 (89-219)	190 (64-466)	<0.001
Triglyceride (mg/dl)		131 (46-369)	174 (59-615)	0.04
HDL-cholesterol (mg/dl)		50 (33-117)	44 (25-73)	<0.001
Uricacid to non-HDL ratio (%)		4 (1-10)	4 (1-8)	0.4
BMI (kg/m^2)		29,4 (22,3-47)	30,4 (21,6-49,4)	0.12
Duration of DM (years)		3,5 (1-20)	8 (1-25)	0.01
HbA1c (%)		6,7 (6,1-6,9)	9,5 (7,1-15,5)	<0.001
Creatinine (mg/dl)		0,81 (0,63-1,2)	0,88 (0,4-1,2)	0.04

(p=0.90), total cholesterol (p=0.06), LDL cholesterol (p=0.08), and eGFR (p=0.24) were not significantly different between type 2 DM with and without MS groups.

Serum uric acid was 6 (2.6-9.6) mg/dl in diabetics with MS and 4.8 (2.1-7.7) mg/dl in diabetics without MS, reaching the difference significance level (p<0.001). HDL cholesterol of diabetics with MS group (44 [25-73] mg/dl) was significantly lower than that of the diabetics without MS group (54 [40-117] mg/dl), (p<0.001). The median UHRs of diabetics with and without MS groups were 13 (6-29) % and 9 (3-16) %, respectively. The UHR difference between groups was statistically significant (p<0.001). Comparison of clinical and laboratory data of the diabetics with and without MS groups is summarized in table 2.

A ROC analyze performed to determine sensitivity and specifity of uric acid, UHR, uric acid to non-HDL cholesterol ratio, SBP (at ≥ 130 mmHg level), DBP (at ≥ 85 mmHg level), triglyceride (at ≥ 150mg/dl level), waist circumference (at levels of ≥102 cm for men and ≥88cm for women) and HDL cholesterol (at < 40 mg/dl for men and <50 mg/dl for women) levels in selecting subjects with MS. UHR greater than 10,6% has 83% sensitivity and 71% specifity in predicting MS (AUC: 0.839, p<0.001). Figure 1 shows the ROC curves of study parameters in predicting MS.

Pearson's correlation analysis showed that UHR was positively correlated with waist circumference (r=0.32, p=0.001), fasting blood glucose (r=0.39, p<0.001), triglyceride (r=0.59, p<0.001) and HbA1c (r=0.54, p<0.001) levels. Neither body weight (r=0.19, p=0.06), nor BMI (r=0.13, p=0.21) were correlated with UHR. Additionally, UHR was not correlated with SBP (r=0.15, p=0.13) and DBP (r=0.06, p=0.58), either.

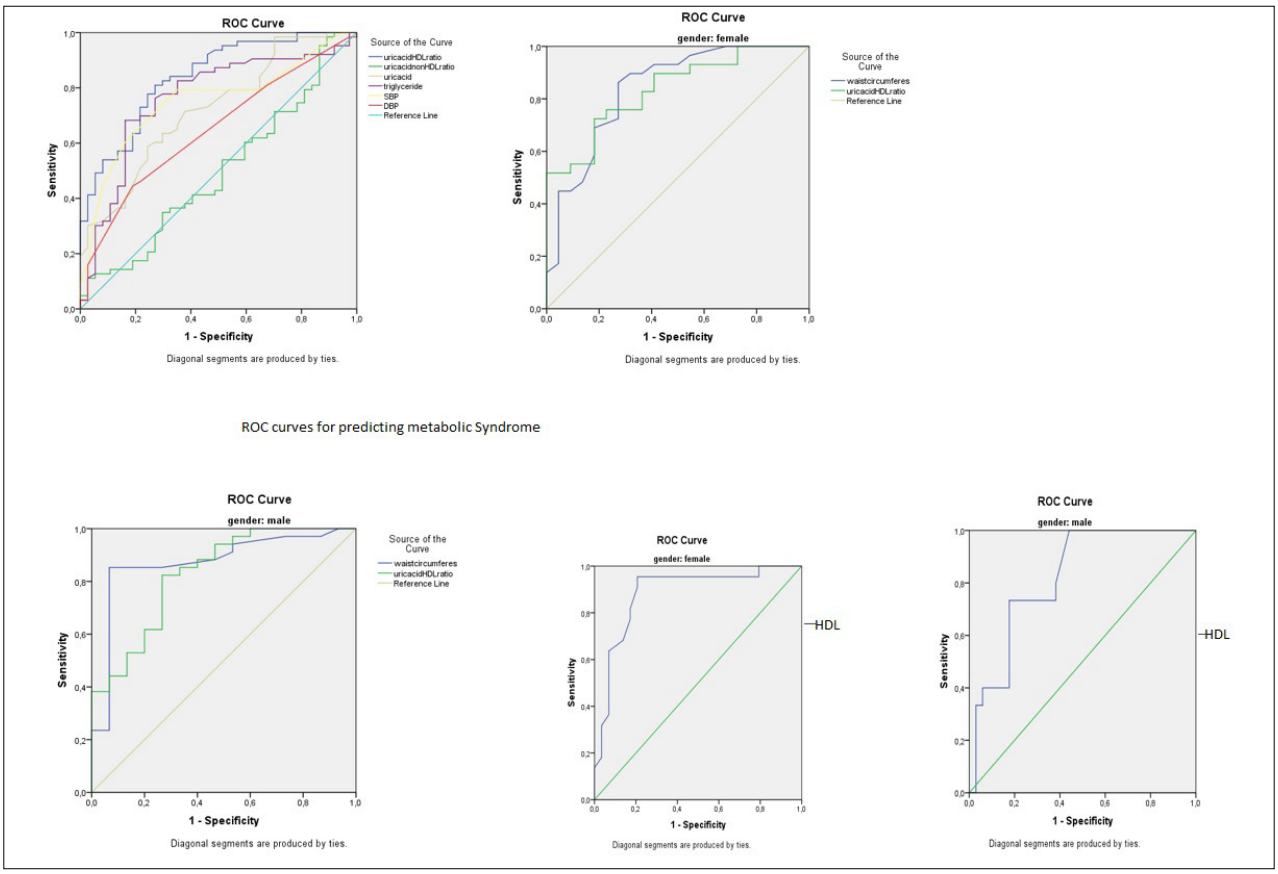
DISCUSSION

In present study, we showed that UHR predicts MS better than all established criteria of this clinical entity. Another important finding of our report

TABLE 2: COMPARISON OF CLINICAL AND LABORATORY DATA OF THE DIABETICS WITH AND WITHOUT METABOLIC SYNDROME

		Type 2 DM with metabolic syndrome	Type 2 DM without metabolic syndrome	p
Gender	Men (n (%))	34 (54)	15 (40,5)	0.20
	Women (n (%))	29 (46)	22 (59,5)	
		Mean ± Standard Deviation		
Age (years)		59,6 ± 9,1	58,4 ± 9,2	0.54
Height (m)		1,63 ± 0,1	1,62 ± 0,1	0.41
Weight (kg)		84.7 ± 14.5	78.2 ± 13.8	0.03
Waist circumference (cm)		109 ± 11	95 ± 10	<0.001
Total cholesterol (mg/dl)		209 ± 48	191 ± 42	0.06
Non HDL cholesterol (mg/dl)		166 ± 49	134 ± 38	0.001
LDL cholesterol (mg/dl)		123 ± 39	109 ± 32	0.08
Creatinine (mg/dl)		0,86 ± 0,17	0,86 ± 0,15	0.90
eGFR (mL/min/1.73 m^2)		81 ± 14	85 ± 14	0.24
		Median (Min- Max)		
SBP (mmHg)		140 (110-180)	125 (100-170)	<0.001
DBP (mmHg)		80 (50-110)	80 (60-105)	0.01
UHR (%)		13 (6-29)	9 (3-16)	<0.001
Fasting blood glucose (mg/dl)		156 (72-466)	129 (62-377)	0.03
Triglyceride (mg/dl)		176 (59-615)	112 (46-456)	<0.001
HDL-cholesterol (mg/dl)		44 (25-73)	54 (40-117)	<0.001
Uricacid to non-HDL ratio (%)		4 (1-10)	4 (1-7)	0.94
BMI (kg/m^2)		31 (23-49)	28 (22-48)	0.07
Duration of DM (years)		6 (1-20)	6 (1-25)	0.93
HbA1c (%)		8,8 (6,2-15,5)	6,8 (6,1-14,2)	0.003
Uric acid (mg/dl)		6 (2,6-9,6)	4,8 (2,1-7,7)	<0.001
Urea (mg/dl)		32 (13-82)	32 (13-58)	0.92

FIGURE 1: ROC CURVES OF THE STUDY PARAMETERS IN PREDICTING MS



is that UHR has high sensitivity and specifity as a marker of diabetic control.

Association between MS and serum uric acid levels is well established in the literature. In 2007, authors identified a substantial association between serum uric acid and MS prevalence[9]. Chang et al.[7] reported that elevated serum uric acid levels were contributed to higher risk of MS. Moreover, elevated serum uric acid has been proposed as a risk factor for MS[10]. In present study, UHR was better than uric acid alone as a marker of MS.

Evidence in literature suggest that serum uric acid levels was in association with each one diagnostic criteria of the MS[10]. Risk of development of type 2 DM is increased in subjects with elevated serum uric acid levels[11,12]. Indeed, increased uric acid was associated with worse diabetic control in type 2 diabetic subjects in a recent study[13]. On the other hand, insulin resistance plays a pivotal role in development of both MS and type 2 DM. Therefore, such an association between high uric acid and development of type 2 DM and MS. Similar to the literature knowledge, since it is a uric acid derived indice, UHR was positively and strongly correlated with fasting plasma glucose and HbA1c levels in present study.

According to the Nakanishi et al.[5]'s study, not only type 2 DM but also hypertension was more common in subjects with higher serum uric acid[14]. In contrary, hypertension was not associated with high uric acid levels in a recent study by Wang et al.[15]. In concordance, there was no significant correlation between blood pressure and UHR levels in our report.

Significant positive correlation between UHR and triglyceride and inverse correlation between UHR and HDL-cholesterol levels in present study, suggest the previous results in literature. Uric acid was found to be positively correlated with serum triglyceride and negatively correlated with HDL-cholesterol levels in Peng et al.[16]'s report. However, increased triglyceride and low HDL levels were not associated with increased risk of hyperuricemia in a Chinese study[15]. Correlation between UHR and triglyceride was more significant and stronger than the correlation between uric acid and triglyceride in our study. Furthermore, inverse correlation between UHR and HDL was more significant and stronger than the correlation between uric acid and HDL.

Obesity is also associated with serum uric acid

levels through increased production pathways[17]. However, literature data about association between uric acid levels and waist circumference, a marker of central obesity, is conflicting. Waist circumference was not associated with hyperuricemia in a recent study[15]. On the other hand, a significant and positive correlation reported between waist circumference and uric acid levels in another Chinese study[18]. We found that correlation between uric acid and waist circumference was weaker than the correlation between UHR and waist circumference in this report.

Uric acid levels increase in chronic kidney disease. However, serum creatinine and GFR values of subjects with and without MS were not different in present report. Thus, elevated UHR in subjects with MS only reflect the presence of this clinical entity.

Results of the present study showed that, in predicting MS, UHR (at a cut of equal to or greater than 10,6%) had greater sensitivity than uric acid level (at a cut off equal to or higher than 5 mg/dl), triglyceride level (at a cut off equal to or higher than 150 mg/dl), SBP (at a cut off equal to or greater than 130 mmHg), DBP (at a cut off equal to or greater than of 85mmHg), waist circumference in men (at a cut off equal to or higher than 102 cm) and HDL in men (at a cut off lower than 40 mg/dl). Only waist circumference in women (at a cut off equal to or higher than 88 cm) and HDL in women (at a cut off lower than 50 mg/dl) have higher sensitivity than UHR in predicting MS. Specifity of UHR (at a cut of equal to or greater than 10,6%) in selecting MS was higher than uric acid level (at a cut off equal to or higher than 5mg/dl), SBP (at a cut off equal to or greater than 130 mmHg), HDL in men (at a cut off lower than 40mg/dl) and waist circumference in women (at a cut off equal to or higher than 88cm). Triglyceride level (at a cut off equal to or higher than 150 mg/dl), DBP (at a cut off equal to or greater than of 85 mmHg), HDL in women (at a cut off lower than 50 mg/dl) and waist circumference in men (at a cut off equal to or higher than 102 cm) have higher specifity than UHR in selecting subjects with MS. Unlike waist circumference and HDL, it was not affected by gender, therefore, we are proud to introduce UHR as a novel and useful criteria in the diagnosis of MS.

Limitations of present study are retrospective design which could cause selection bias, and relatively small size of study cohort. However, to our knowledge, it is the first study in literature that reported UHR as a predictor of MS and worse diabetic control in type 2 DM.

CONCLUSION

Present study suggest that utilization of UHR is a useful tool in diagnosis of MS as a novel criteria. Nevertheless, prospective studies with larger population may make a better scientific evidence in that issue.

Conflict of interest: None
Sources of Funding: None

RESUMO

CONTEXTO E OBJETIVO: *A síndrome metabólica (SM) é uma entidade clínica associada ao aumento do risco de diabetes mellitus tipo 2 (DM) e doenças cardiovasculares. Os níveis séricos de ácido úrico estão correlacionados com os critérios estabelecidos de EM. Uma vez que DM tipo 2 e MS são distúrbios metabólicos, nós hipotetizamos se uma relação ácido úrico para HDL-colesterol (UHR) poderia predizer a regulação diabética e a presença de MS em diabéticos tipo 2.*

MÉTODOS: *As admissões dos sujeitos com DM tipo 2 aos ambulatórios de nossa instituição foram analisadas retrospectivamente. A população do estudo agrupou-se em diabéticos bem controlados e mal controlados, de acordo com o nível de HbA1c (corte de 7%) e posteriormente agrupados em DM tipo 2 com e sem EM de acordo com a presença de EM. UHR dos grupos de estudo comparados.*

RESULTADOS: *Um total de 100 indivíduos diabéticos tipo 2 inscritos no estudo. A média UHR foi significativamente menor em diabéticos bem controlados (9,7 ± 3,7%) em comparação com indivíduos com DM tipo 2 mal controlada (14 ± 5,4%) (p < 0,001). A mediana da UAR de diabéticos com EM (13 (6-29)%) foi maior que a dos diabéticos sem SM (9 (3-16)%) (p < 0,001). Um UHR maior que 11% tem 77% de sensibilidade e 60% de especificidade em predizer um pior controle diabético (AUC: 0,762, p < 0,001) e um UHR maior que 10,6% tem 83% de sensibilidade e 71% de especificidade em prever MS (AUC : 0,839, p < 0,001). A sensibilidade e especificidade de UHR em predizer MS foram melhores do que a maioria das sensibilidades e especificidades dos cinco critérios de MS.*

CONCLUSÃO: *Sugerimos a utilização da UHR no diagnóstico da SM como um novo critério. No entanto, estudos prospectivos com maior população podem fazer uma melhor evidência científica nessa questão.*

PALAVRAS-CHAVE: *Síndrome metabólica. Diabetes mellitus tipo 2. Ácido úrico. HDL-colesterol.*

REFERENCES

1. Haller H, Hanefeld M. Synoptische betrachtung metabolischer risikofaktoren. Lipidstoffwechselstörungen. 1975;254-64.
2. Samson SL, Garber AJ. Metabolic syndrome. Endocrinol Metab Clin North Am. 2014;43(1):1-23.
3. Nejatinamini S, Ataie-Jafari A, Qorbani M, Nikoohemat S, Kelishadi R, Asayesh H, et al. Association between serum uric acid level and metabolic syndrome components. J Diabetes Metab Disord. 2015;14(1):1-7.
4. Sundström J, Sullivan L, D'Agostino RB, Levy D, Kannel WB, Vasan RS. Relations of serum uric acid to longitudinal blood pressure tracking and hypertension incidence. Hypertension. 2005;45(1):28-33.
5. Nakanishi N, Okamoto M, Yoshida H, Matsuo Y, Suzuki K, Tatara K. Serum uric acid and risk for development of hypertension and impaired fasting glucose or type II diabetes in Japanese male office workers. Eur J Epidemiol. 2003;18(6):523-30.
6. Shih M-H, Lazo M, Liu S-H, Bonekamp S, Hernaez R, Clark JM. Association between serum uric acid and nonalcoholic fatty liver disease in the US population. J Formos Med Assoc. 2015;114(4):314-20.
7. Chang JB, Chen YL, Hung YJ, Hsieh CH, Lee CH, Pei D, et al. The role of uric acid for predicting future metabolic syndrome and type 2 diabetes in older people. J Nutr Health Aging. 2017;21(3):329-35.
8. Bonakdaran S, Kharaqani B. Association of serum uric acid and metabolic syndrome in type 2 diabetes. Curr Diabetes Rev. 2014;10(2):113-7.
9. Choi HK, Ford ES. Prevalence of the metabolic syndrome in individuals with hyperuricemia. Am J Med. 2007;120(5):442-7.
10. Chen YY, Kao TW, Yang HF, Chou CW, Wu CJ, Lai CH, et al. The association of uric acid with the risk of metabolic syndrome, arterial hypertension or diabetes in young subjects: an observational study. Clin Chim Acta. 2018;478:68-73.
11. Krishnan E, Pandya BJ, Chung L, Hariri A, Dabbous O. Hyperuricemia in young adults and risk of insulin resistance, prediabetes, and diabetes: a 15-year follow-up study. Am J Epidemiol. 2012;176(2):108-16.
12. Woyesa SB, Hirigo AT, Wube TB. Hyperuricemia and metabolic syndrome in type 2 diabetes mellitus patients at Hawassa university comprehensive specialized hospital, South West Ethiopia. BMC Endocr Disord. 2017;17(1):76.
13. Duman TT, Kocak MZ, Atak BM, Erkus E. Serum uric acid is correlated with HbA1c levels in type 2 diabetes mellitus. Exp Biomed Res. 2018;1(1):6-9.
14. Kelliny C, William J, Riesen W, Paccaud F, Bovet P. Metabolic syndrome according to different definitions in a rapidly developing country of the African region. Cardiovasc Diabetol. 2008;7(1):1-11.
15. Wang J, Chen RP, Lei L, Song QQ, Zhang RY, Li YB, et al. Prevalence and determinants of hyperuricemia in type 2 diabetes mellitus patients with central obesity in Guangdong Province in China. Asia Pac J Clin Nutr. 2013;22(4):590-8.
16. Peng TC, Wang CC, Kao TW, Chan JY, Yang YH, Chang YW, et al. Relationship between hyperuricemia and lipid profiles in US adults. Biomed Res Int. 2015;2015(2015):127596.
17. Hikita M, Ohno I, Mori Y, Ichida K, Yokose T, Hosoya T. Relationship between hyperuricemia and body fat distribution. Intern Med. 2007;46(17):1353-8.
18. Chen LY, Zhu WH, Chen ZW, Dai HL, Ren JJ, Chen JH, et al. Relationship between hyperuricemia and metabolic syndrome. J Zhejiang University Science B. 2007;8(8):593-8.

ORIGINAL ARTICLE

Clinical registry of cardiovascular surgeries in a university hospital

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SUMMARY

OBJECTIVES: *To create and implement a computerized clinical registry to verify in the short-, medium- and long-term the mortality and the incidence of significant surgical outcomes in adult patients submitted to cardiovascular surgeries.*

METHODS: *This is a prospective, observational registry-based study aimed at documenting the characteristics of patients undergoing cardiovascular surgery.*

RESULTS: *Variables were standardized according to international references from the Society of Thoracic Surgeons (STS), American College of Cardiology (ACC), Michigan Society of Thoracic and Cardiovascular Surgeons (MSTCVS) and the Department of Informatics of SUS (DATASUS). The standardization was performed in English with an interface in Portuguese to make the data collection easier in the institution. Quality of care indicators, surgical procedure characteristics, in addition to significant cardiovascular outcomes will be measured. Data were collected during the hospitalization until hospital discharge or at the seventh day, in thirty days, six months, twelve months and annually until completing five years.*

CONCLUSION: *The importance of a database maintenance with international standards that can be reproducible was evidenced, allowing the evaluation of techniques and assistance and the integration of data among health institutions.*

KEYWORDS: *Cardiovascular surgical procedures. Database. Electronic health records.*

INTRODUCTION

Cardiovascular diseases (CVD) are the leading causes of mortality in Brasil and in the world. In 2003, 32% of 69% of the well-defined deaths in Brasil were due to CVD[1]. According to the World Health Organization (WHO)[2], in 2030, 23.6 million people will die from CVD. Factors related to CVD can be of biological and/or behavioral nature[3].

Surgery is one of the therapeutic options. In Brasil, 63,529 heart surgeries were performed between 2005 and 2007; while in 2011, they went up to 100,000, showing a significant increase in the number of these procedures. There are 170 centers all over Brasil, with more than 1,000 surgeons associated with the Brazilian Society of Cardiovascular Surgery[4,5].

Despite the high direct and indirect costs, there are few sources of information with high-quality data collected systematically to monitor the procedures and outcomes of these patients. There is no system to monitor adverse events of cardiovascular surger-

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ies following international quality standards yet. The registries started to be used in the clinical practice due to the need of using clinical and evolution history of these patients[6]. A high-quality medical record helps to monitor and improve the patients' health, describing the epidemiological and clinical characteristics of the diseases and the assessment of the interventions[7].

Electronic technology improved the registry and facilitated the communication between doctors and patients, among health providers, and the access to medical information[8]. Barriers like costs, inappropriate medical information, lack of standards, patient privacy, knowledge, and data management still need to be surpassed[9,10].

Electronic databases are not adopted in several medical and academic centers yet[11]. The use of validated data collection tools and forms based on standards make it easier to visualize and share these data, as well to participate in multicentric studies[12].

REDCap (Research Electronic Data Capture) is a web-based software solution with tools that reliably create online forms to capture, manage and analyze data during the process of investigation[13]. REDCap allows data to be collected in an offline environment and synchronized in the REDCap server when it is connected to the Internet[14,15]. Our aim was to create and implement a computerized clinical record.

METHODS

Study design

A prospective, observational registry-based study, aimed to record cardiovascular variables, surgical procedures and extracorporeal circulation (ECC), as well as the clinical evolution. The longitudinal follow-up of the patients will happen from the moment of the procedure until hospital discharge, at thirty days, six months, twelve months and annually until completing five years.

Sample/target population

Adult patients submitted to cardiovascular surgery in Santa Maria University Hospital (SMUH).

Inclusion criteria

Patients who are 18-years-old or older from both sexes, suffering from cardiovascular disease and submitted to the following surgeries will be included:

- Myocardial revascularization surgery
- Valve surgery
- Aorta surgery
- Surgical correction of congenital disease in the adult

Outcomes of interest

Mortality from all causes and cardiovascular events (reinfarction, stroke, fatal and non-fatal cardiac arrest, and mortality for cardiovascular cause) during the hospitalization and up to thirty days, six months, twelve months and annually until completing five years.

Ethical aspects

The clinical study described in this paper was conducted under the principles of the current revision of the Declaration of Helsinki and the most recent version of the Guideline for Good Clinical Practice (ICH-GCP), as well as the Resolution 466/12. The study was developed in compliance with Brazilian legal and regulatory requirements.

The names of all participants were kept as confidential. They were identified in the documentation and during the evaluation by the number assigned to each one in the study. It was guaranteed to the patients that all the findings were stored in computers and handled according to the strictest rules of confidentiality. All the data collected and stored in the registry were screened. This screening will have the function of guaranteeing that no patient identification data will be shown in the final version of the registry, following international requirements for privacy of clinical data. The de-identification of the database is made through the transformation of all the dates in intervals with reference to a random date randomly selected and stored by the individual responsible for the data management. Geolocalization data were modified to respect the national and international data safety rules. For storage in Research Ethics Committees (REC), only the three initial digits will be kept, following HIPAA (*Health Insurance Portability and Accountability Act*) rules. However, as the Brazilian rules do not require the storage of REC 3 digits only, a secondary bank with five digits was kept in our server, protected for the exclusive use of computers with a national internet protocol (IP) address.

The study was submitted and approved by the Education and Research Department of SMUH under

the number 046/2016 and by the REC of the Institute of Cardiology/University Foundation of Cardiology (ICUFC) under the UP number 5017/14. All the patients who will participate in the study will sign the Consent Form (CF).

Risks and benefits

There will be no any additional risks to the patients neither to the institution due to inclusion in this study, considering that this is an observational registry-based study. Therefore, it does not submit the patients to any intervention that is not correctly

indicated and implemented by the assistant doctor, in his/her usual clinical practice procedures. There will be no additional costs or discomfort to the patients.

RESULTS

The outcomes of the development and implementation of the CardioCEC clinical registry will be shown in steps (Figure 1).

Creation of the CardioCEC clinical registry

Given the need to monitor the high number of high-cost procedures and to evaluate the outcomes of these patients in SMUH, we decided to create and develop an information source with data collected in a systematized and high-quality way.

Software

We used the REDCap (Research Electronic Data Capture) software for collection and storage of the research data. Data were physically stored in a server located in ICUFC, in the department of informatics of the institution and protected by the firewall of the ICUFC network and the REDCap access permission system itself (Figure 2). The infrastructure requirements, such as a web server that offers support to PHP, a MySQL database server and Secure Sockets Layer (SSL) connections, need to be present (http://project-redcap.org/).

Variables standardization

Initially, the variables and the standardization of the nomenclatures were defined in order to guarantee that the data input into the registry was compatible with international databases. The variable standardization was followed international references from the American College of Cardiology Foundation (ACCF), the American Heart Association (AHA), the Society of Thoracic Surgeon (STS), The Michigan Society of Thoracic and Cardiovascular Surgeons (MSTCVS) and the SUS Department of Informatics - DATASUS. International scope variables that are usually used for the connection with vast repositories of global databases were included. The standardization was made in English, and an interface in Portuguese was implemented to make the data collection easier in the institution.

Inclusion of variables

The CardioCEC database has 553 variables distributed in 12 data collection tools, standardized according to international references, allowing for the participation in multicentric studies (Table 1).

Data collection logistics

The data collection of the patients will be made by a skilled team engaged in the procedure (CRF, case report forms). The CardioCEC Clinical Registry will be accomplished through internet and intranet access through any computer of the hospitalization units, or the thoracic surgery room, or through the REDCap app, and all the information collected will be input into the system.

All the patients who meet the eligibility criteria will have their data collected following the CF signature. The data of the patients will be filled out in the clinical chart preferably in the day previous to the surgery (demographic data and admission, preoperative). The surgical procedure (intraoperative) will be informed in the period following the discharge from the intensive care unit (ICU discharge). The prospective data will be informed until the hospital discharge or up to seven days. The clinical follow-up chart of 30 days, six months and 12 months, as well as the annual follow-up, will always be filled out within seven days. The hard outcomes will be obtained through the medical record or a telephone call to the patient, family member or third party.

The team may collect information on the patient's mortality and major cardiovascular events through a telephone call to the patient, a family member or a third party, or from the patient's medical record.

Bias prevention

It is essential to have a mechanism to reduce the possibility of data entry errors. With REDCap, we restricted the format/type of data and established inter-

FIGURE 1: CONSTRUCTION AND DEVELOPMENT OF THE CARDIOCEC REGISTRY FOR THE SMUH (ACTIVITIES DIAGRAM)

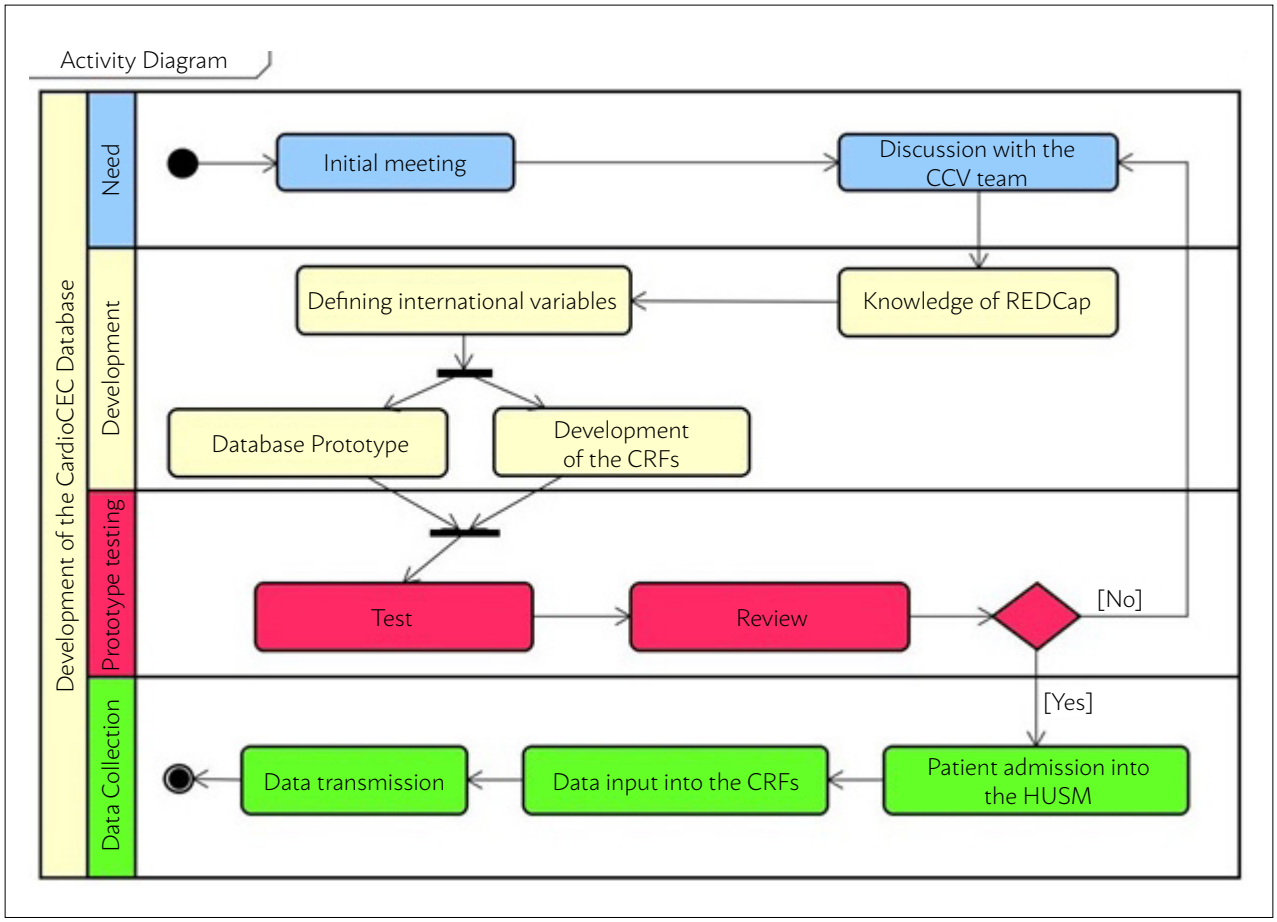

FIGURE 2: REDCAP SOFTWARE FOR THE CONSTRUCTION OF THE CARDIOCEC CLINICAL REGISTRY.

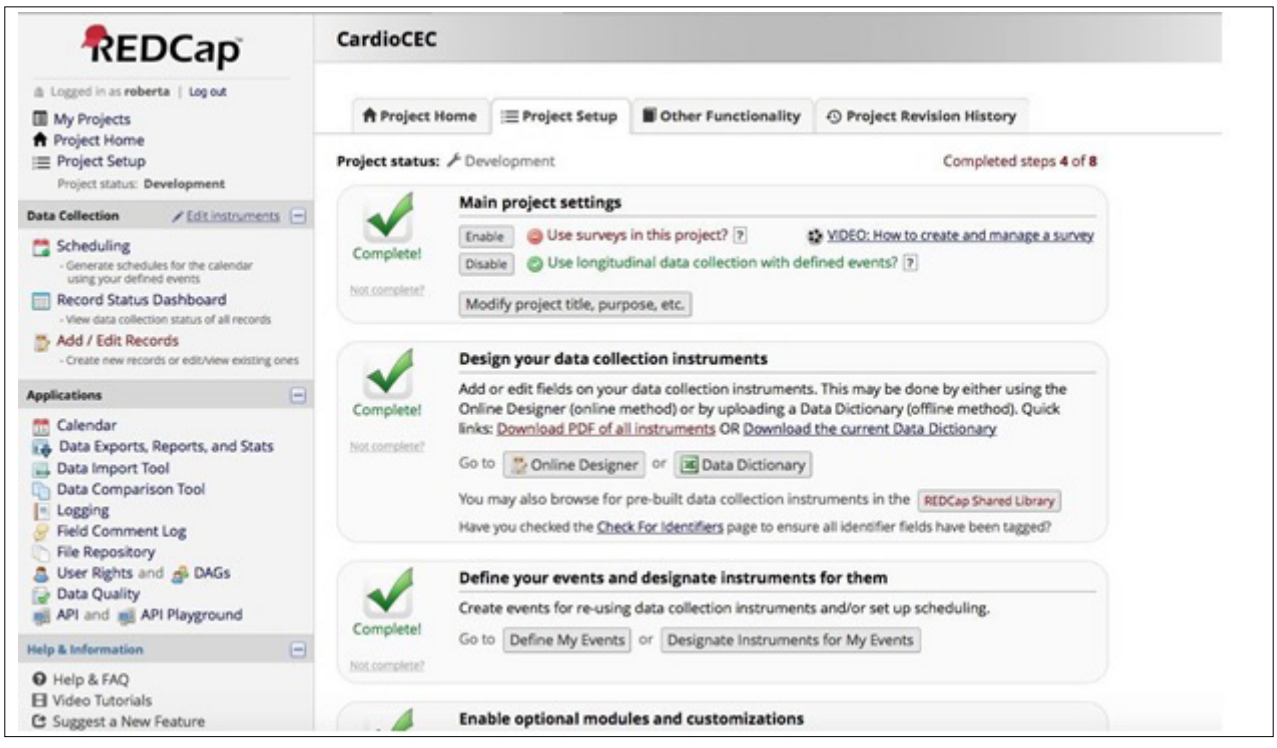

TABLE 1: VARIABLE STANDARDIZATION

Name of the instrument	Variables	Source of standardization
Demographic data - Admission	Record ID, Date of admission, Form of admission, Last name, Name, Middle name, Number of the National Health Card (CNS), SAME, Date of birth, Age, Race, Gender, Address, City	DATASUS, IBGE, STS
Preoperative risk factors, Preoperative heart status, Preoperative drugs	Record ID, Weight, Height, BMI, Smoker, Current smoker, Diabetes, Diabetes control, Dyslipidemia, Dyslipidemia control, Kidney failure, Dialysis, Hypertension, Stroke, Time since stroke, Infectious endocarditis, Type of endocarditis, Pulmonary disease, Immunosuppressing therapy, Peripheral vascular disease, Cerebrovascular disease, AMI, Time AMI, Heart failure last 2 weeks, CHF, Heart condition at admission, Cardiogenic shock, Cardiac resuscitation, Arrhythmia, Type of arrhythmia, NYHA, Beta blockers, Use of BB 2 weeks, ACE-inhibitor, Nitrates, Anticoagulation, Name anticoagulant, Warfarin, IV Inotropes, Corticoids, *Aspirin, Adenosine diphosphate* (ADP) *receptor inhibitors*, *Adenosine diphosphate (ADP) receptor inhibitors 24 hours before*, Glycoprotein IIb/IIIa, Number of compromised vases, Percentage main stenosis left side vase, EF measure, EF, Presence of valvopathy, Aortic failure, Aortic stenosis, Mitral failure, Mitral stenosis, Tricuspid failure, Pulmonary failure, Incidence of surgery, Status, IABP, Ht, Hb, Na, K, Urea, Creatinine, Glucose, Platelets, TP, TTPA	ACCF, NCDR, STS
Intraoperative	Record ID, Name of surgeon, Surgery category, Use of robotic technology, Minimally invasive procedure, Conversion to standard procedure, Use of ECC, Use of arterial filter, Strategy of acid-base management, Bioactive coating area, Type of system, Assistance in venous draining, Type of arterial pump, Use of autotransfusion Pulsatile Perfusion device, Saline solution filling volume, Ringer lactate filling volume, Plasma-Lyte filling volume, Albumin 5% filling volume, Albumin 25% filling volume, Starch solution filling volume, Mannitol filling volume, Sodium bicarbonate filling volume, Red cells concentrates in priming, Plasma in priming, Heparin dose, Total volume of priming, Time of perfusion, Type of aorta clamping, Site of aorta clamping, Time of aorta occlusion or clamping, Circulatory arrest (CA), Time of CA, Selective cerebral perfusion (SCP), Time of SCP, Time of reperfusion, Return to ECC, Reasons for returning to ECC, Cardioplegia, Type of cardioplegia, Regimen of cardioplegia, Temperature of cardioplegia induction, Direction of cardioplegia induction flow, Temperature of cardioplegia maintenance, Direction of cardioplegia maintenance flow, Total volume of cardioplegia, Assessment of the presence of preexistent calcifications in the site of aorta handling, Aorta classification, Site of temperature measurement, Lower temperature reached in the nasopharyngeal path, Lower temperature reached in the esophageal path, Lower temperature reached in the jugular bulb region	STS, PERForm
	Lower temperature reached in the rectal path, Lower tympanic temperature reached, Lower temperature reached in other paths, Higher temperature reached in the nasopharyngeal path, Higher temperature reached in the esophageal path, Higher temperature reached in the jugular bulb region, Higher temperature reached in the rectal path, Higher tympanic temperature reached, Higher temperature reached in other paths, Temperature following ECC, Initial Hematocrit (Ht) in ECC, Initial hemoglobin (Hb) in ECC, Hematocrit (Ht) when leaving the surgery room, Hemoglobin (Hb) when leaving the surgery room, Intra-Aortic Balloon Pump (IABP) in the surgery room, Period of insertion of IABP in the surgery room, Indication of IABP in the surgery room, Use of hemoderivatives, Use of red cells concentrate, Use of fresh frozen plasma, Use of platelets, Use of cryoprecipitate, Fibrinogen concentrate was administered, *Prothrombin complex concentrate was administered*, Administered dose of fibrinogen concentrate, Administered dose of *prothrombin complex concentrate*, Volume of SS 0.9% used in ECC, Volume of Ringer lactate used in ECC, Volume of Plasma-Lyte used in ECC, Volume of Albumin 5% used in ECC, Volume of Albumin 25% used in ECC, Volume of Starch Solution used in ECC, Use of UF, Total UF volume, Volume of residual blood that returned to the patient, Volume of urine in the intraoperative period Presence of hemoglobin, Inotropic in use in the transfer to the ICU, Myocardial revascularization surgery Number of distal anastomoses with arterial graft, Number of distal anastomoses with venous graft, Number of distal anastomoses with internal mammary artery graft, Number of distal anastomoses with radial artery graft, Number of distal anastomoses with *Gastroepiploic artery* graft, Number of anastomoses with other arterial grafts, Procedure in the aortic valve, Procedure in the mitral valve, Procedure in the tricuspid valve, Procedure in the pulmonary valve, Correction of aorta aneurysm, surgery of congenital cardiopathy, Surgery of aorta dissection correction, Other cardiovascular surgeries, Extubation in the surgery room	
ICU discharge	Record ID Date and time of entrance at ICU, Date and time of discharge from ICU, Reintubation, Additional hours in MV, Total hours in MV, creatinine, hemoderivatives, units of red blood concentrate, Units of fresh frozen plasma, units of platelets, units of cryoprecipitate, Hematocrit at discharge, Hemoglobin at discharge, Echocardiogram, Presence of aortic valve failure, Presence of mitral valve failure, Presence of tricuspid valve failure, Presence of pulmonary valve failure, EF, Cardiac enzymes, Higher CKMB value measured, Higher Troponin value measured, Higher Troponin T value measured, ECG result, surgical intervention, Reintervention for bleeding, Date of intervention for bleeding, Intervention for valve dysfunction, Reintervention for coronary occlusion, Reintervention for other cardiac reasons, Reintervention for other non-cardiac reasons, complication in PO, Deep infection of the sternum, Infection of thoracotomy, Infection of OW in the site of grafts removal, Sepsis, Stroke, TIS, *Prolonged mechanical ventilation* (MV), Pneumonia, Infection in the urinary tract, Kidney failure, Dialysis, *Deep vein thrombosis* (DVT), Pulmonary thromboembolism	ACCF, NCDR, STS, PERForm
	Limb ischemia, Myocardial *infarction*, Rhythm dysfunction requiring device, PCR, Events related to anticoagulant therapy, GI, Dysfunction of multiple organs, Atrial fibrillation / flutter, Injury of the laryngeal nerve, Injury of the phrenic nerve, Other events, Identification of the event, ACE-inhibitor, Beta blockers, Nitrates, Anticoagulants, Name of anticoagulant, Warfarin, IV Inotropes, Corticoids, *Acetylsalicylic acid, Adenosine diphosphate* (ADP) *receptor inhibitors*, Glycoprotein IIb/IIIa	

Name of the instrument	Variables	Source of standardization
Hospital discharge	Patient status, Date and time of the hospital discharge, Date and time of death, Cause of death, Units of red cell concentrate, Units of fresh frozen plasma, Units of platelets, Units of Cryoprecipitate, Echocardiogram, Presence of aortic valve failure, Presence of mitral valve failure, Presence of tricuspid valve failure, Presence of pulmonary valve failure, EF, PO complications, Deep infection of the sternum, Infection of the thoracotomy, Infection of the OW in the site of the grafts removal, Sepsis, stroke, TIS, Prolonged mechanical ventilation (MV), Pneumonia, Infection in the urinary tract, Kidney failure, Dialysis, *Deep vein thrombosis* (DVT), Pulmonary thromboembolism, Limb ischemia, myocardial *infarction*, Rhythm dysfunction requiring device, PCR, Events related to anticoagulant therapy, GI, Dysfunction of multiple organs, Atrial fibrillation / flutter, Injury of the laryngeal nerve, Injury of the phrenic nerve, Other events, Identification of the event, ACE-inhibitor, Beta blockers, Nitrates, Anticoagulants, Name of the anticoagulant, Warfarin, IV Inotropes, Corticoids, *Acetylsalicylic acid, Adenosine diphosphate* (ADP) *receptor inhibitors*, Glycoprotein IIb/IIIa	ACCF, NCDR, STS, PERForm
Follow-up One month Six months One year Two years Three years Four years Five years	Patient status, Date of hospital discharge, Hospitalization, Still hospitalized, Days of permanence in the ICU, Date and time of death, Cause of death, Complications, Deep infection of the sternum, Infection of the thoracotomy, Infection of the OW in the site of the grafts removal, Sepsis, stroke, TIS, Prolonged mechanical ventilation (MV), Pneumonia, Infection in the urinary tract, Kidney failure, Dialysis, *Deep vein thrombosis* (DVT), Pulmonary thromboembolism, Limb ischemia, myocardial *infarction*, Rhythm dysfunction requiring device, PCR, Events related to anticoagulant therapy, GI, Dysfunction of multiple organs, Atrial fibrillation / flutter, Injury of the laryngeal nerve, Injury of the phrenic nerve, Other events, Identification of the event, ACE-inhibitor, Beta blockers, Nitrates, Anticoagulants, Name of the anticoagulant, Warfarin, IV Inotropes, Corticoids, *Acetylsalicylic acid, Adenosine diphosphate* (ADP) *receptor inhibitors*, Glycoprotein IIb/IIIa	ACCF, NCDR, STS, PERForm

vals for numerical and date fields, thus allowing data validation. Problems with data consistency, like the wrong types of data, out of reach values and outliers for numerical fields can be reported using the module of quality of data. Besides, we apply predefined rules that make it easier to identify discrepant data value, which is quite important because the project has several variables. Several procedures guarantee the appropriate control of systematic errors (biases) and assure the methodological quality of the registry, amongst them:

- Successive sampling;
- Blinded evaluation of outcomes;
- Prevention of losses during the follow-up;
- Use of partial reports of quality of data;
- Use of electronic signature.

Data management

The Santa Maria University Hospital (SMUH) is responsible for the management of the data registry, whose information will be collected through electronic forms.

System of data entry

The data management will be accomplished using the REDCap (Research Electronic Data Capture) system. The case report forms (CRFs) will be transcribed through web medical records and will be included in a validation database.

Case report form

The study's CRFs will be filled out and sent through internet/intranet or web; the electronic signature is available through the access with a personal and non-transferable password.

Data quality control

For the quality control of the data, the following strategies are used:

- Training for data collection;
- Standard operational procedures handbook for each step of the protocol;
- Electronic CRF: aiming to prevent the occurrence of incomplete (mandatory data) or inconsistent data, or containing non-plausible values from the biological point of view;
- Central checking of data through statistical analysis to check possible inconsistencies;
- Quarterly reports of screening, recruiting, data quality, compliance to the protocol, consistency and filling out of collection forms.

DISCUSSION

The development and the implementation of the CardioCEC Registry were due to the need for identification of the outcomes from different techniques and equipment/materials used for cardiovascular surgical procedures. The planning of the database used variables recommended by interna-

tional organizations, thus allowing the interoperability of the data[12].

The involvement of the cardiovascular surgery multidisciplinary team through meetings and training favors the success of the registry's implementation. The assignment of distinct levels of access to each one of the members of the research team brings safety to the appropriate use of the database. The possibility of data tabulation in an offline environment in the REDCap mobile app favors the compliance[16] to the filling out of the CardioCEC registry.

Mechanisms used to prevent systematic errors will be extensively studied. The branching logic used rendered more coherent and rational questionnaires[14].

The automatized reports of data quality data give the researcher absolute control over the quality of the records made[17].

CONCLUSION

The CardioCEC Registry is the first Brazilian electronic registry with several variables related to ECC, which are recommended by international repositories, allowing the interoperability between major national and international institutions, presenting relevant data in the conduction of the ECC in cardiovascular surgeries, as well as in the evaluation of the primary outcomes in this group of patients.

The registry has excellent potential for research in the field and allows for a significant contribution to the development of new technologies and innovations in the field of and cardiovascular surgery.

Acknowledgment

We thank the Registry Clinics Unit of the Institute of Cardiology / University Foundation of Cardiology of Rio Grande do Sul and the Santa Maria University Hospital.

RESUMO

OBJETIVO: *Criar e implantar um registro clínico informatizado para verificar mortalidade e incidência de desfechos cirúrgicos maiores em pacientes adultos submetidos a cirurgias cardiovasculares a curto, médio e longo prazo.*

METODOLOGIA: *Trata-se de um estudo observacional do tipo Registro, prospectivo, que visa documentar as características dos pacientes submetidos à cirurgia cardiovascular.*

RESULTADOS: *As variáveis foram padronizadas de acordo com referências internacionais padronizadas pela The Society of Thoracic Surgeons (STS), American College of Cardiology (ACC), The Michigan Society of Thoracic and Cardiovascular Surgeons (MSTCVS) e o Departamento de Informática do SUS (Datasus). A padronização foi realizada na língua inglesa com uma interface em português para facilitar a coleta de dados na instituição. Serão mensurados indicadores de qualidade de atendimento, características do procedimento cirúrgico, além dos principais desfechos cardiovasculares. Os dados serão coletados durante a internação até a alta hospitalar ou até o sétimo dia, em 30 dias, seis meses, 12 meses e anualmente até completar cinco anos.*

CONCLUSÃO: *Evidenciou-se a importância da manutenção de banco de dados com padrões internacionais que podem ser reprodutíveis, possibilitando a avaliação de técnicas e assistência integrando os dados entre instituições de saúde.*

PALAVRAS-CHAVE: *Procedimentos cirúrgicos cardiovasculares. Base de dados. Registros eletrônicos de saúde.*

REFERENCES

1. Ministério da Saúde, Departamento de Ciência e Tecnologia, Secretaria de Ciência, Tecnologia e Insumos Estratégicos. ELSA Brasil: maior estudo epidemiológico da América Latina. Rev Saúde Pública. 2009;43(1):1-2.
2. World Health Organization. Cardiovascular diseases (CVDs). [cited 2017 Jun 18]. Available from: http://www.who.int/en
3. Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren WM, et al. European guidelines on cardiovascular disease prevention in clinical practice (version 2012): the fifth joint task force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). Int J Behav Med. 2012;19(4):403-88.
4. Piegas LS, Bittar OJNV, Haddad N. Cirurgia de revascularização miocárdica: resultados do Sistema Único de Saúde. Arq Bras Cardiol. 2009;93(5):555-60.
5. Braile DM, Godoy MF. História da cirurgia cardíaca no mundo. Rev Bras Cir Cardiovasc. 2012;27(1):125-34.
6. Cavalcante RB, Pinheiro MMK, Watanabe YJÁ, Silva CJ. Grupo Técnico de Informação em Saúde e Populações: contribuições para a Política Nacional de Informação e Informática em Saúde. Perspectivas em Ciência da Informação. 2015;20(1):92-119.
7. Pang X, Kozlowski N, Wu S, Jiang M, Huang Y, Mao P, et al. Construction and management of ARDS/sepsis registry with REDCap. J Thorac Dis. 2014;6(9):1293-9.
8. Bodenheimer T, Grumbach K. Electronic technology: a spark to revitalize primary care? JAMA. 2003;290(2):259-64.
9. Majeed A. Ten ways to improve information technology in the NHS. BMJ. 2003;326(7382):202-6.
10. Snyder DC, Epps S, Beresford HF, Ennis C, Levens JS, Woody SK, et al. Research management team (RMT): a model for research support services at Duke University. Clin Transl Sci. 2012;5(6):464-9.
11. Obeid JS, Johnson LM, Stallings S, Eichmann D. Research Networking Systems: the state of adoption at institutions aiming to augment translational research infrastructure. J Transl Med Epidemiol. 2014;2(2):1026.
12. Anderson HV, Weintraub WS, Radford MJ, Kremers MS, Roe MT, Shaw RE, et al. Standardized cardiovascular data for clinical research, registries, and patient care: a report from the Data Standards Workgroup of the Na-

tional Cardiovascular Research Infrastructure project. J Am Coll Cardiol. 2013;61(18):1835-46.

13. REDCap [Internet]. [cited 2017 Jun 18]. Available from: http://project-redcap.org/

14. REDCap [Internet]. [cited 2017 Jun 18]. Available from: https://projectredcap.org/wp-content/uploads/2016/08/CFRI-DM-REDCap-Mobile-App-Manual.pdf

15. REDCap [Internet]. [cited 2017 Jun 18]. Available from: https://projectredcap.org

16. Dayer L, Heldenbrand S, Anderson P, Gubbins PO, Martin BC. Smartphone medication adherence apps: potential benefits to patients and providers. J Am Pharm Assoc (2003). 2013;53(2):172-81.

17. Lyon JA, Garcia-Milian R, Norton HF, Tennant MR. The use of Research Electronic Data Capture (REDCap) software to create a database of librarian-mediated searches. Med Ref Serv Q. 2014;33(3):241-52.

ORIGINAL ARTICLE

Intensive treatment of hyperglycemia in the acute phase of myocardial infarction: the tenuous balance between effectiveness and safety – a systematic review and meta-analysis of randomized clinical trials

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SUMMARY

INTRODUCTION: *In acute myocardial infarction (AMI), each 18 mg/dl (1 mmol/L) increment is associated with a 3% increase in mortality rates. All strategies applied for reducing blood glucose to this date, however, have not presented encouraging results.*

METHODOLOGY: *We searched the Medline (PubMed) and Cochrane Library databases for randomized clinical trials (RCTs) from 1995 to 2017 that used the intensive strategy or GIK therapy for blood glucose control during the acute stage of the AMI. We included eight studies. In order to identify the effects of GIK or insulin therapy, we calculated a overall risk ratio (RR) with meta-analysis of fixed and random effects models. A two-tail p-value of < 0.05 was considered statistically significant.*

RESULTS: *A total of 28,151 patients were included: 1,379 intensively treated with insulin, 13,031 in GIK group, and 13,741 in the control group. The total mortality was 10.5% (n=2,961) and the RR of 1.03 [95%CI 0.96–1.10]; I2 = 31%; p = 0.41 for the combined intensive insulin plus GIK groups in comparison with the control group. In meta-regression analyses, intense reductions in blood glucose (> 36 mg/dL) in relation to the estimated average blood glucose (estimated by HbA1c) were associated with higher mortality, whereas lower reductions in blood glucose (< 36 mg/dL) were not associated with mortality. The lowering of blood glucose in the acute phase of MI compared with the average blood glucose was more effective around 18 mg/dL.*

CONCLUSION: *This meta-analysis suggests that there may be a tenuous line between the effectiveness and safety of reducing blood glucose in the acute phase of MI. The targets must not exceed a reduction greater than 36 mg/dL in relation to estimated average blood glucose.*

KEYWORDS: *Hyperglycemia. Myocardial infarction. Meta-analysis as the subject. Effectiveness. Safety. Critical care.*

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INTRODUCTION

Stress hyperglycemia is a powerful prognostic marker for acute myocardial infarction (AMI)[1,2]. It represents not only a consequence of inflammatory activation, adrenergic and endocrine but also contributes to the continuation of these phenomena. In AMI patients, each 18 mg/dl (1 mmol/L) increment is associated with a 3% increase in mortality due to all causes[1]. In a different study, each increment of capillary blood glucose over 140 mg/dl was translated as a directly proportional increase in the mortality of these patients over five years[2].

Overall, however, all strategies applied for reducing blood glucose to this date have not presented encouraging results. As the first strategy for the intensive control of blood glucose, the Digami-1[3] presented an aggressive reduction in the blood glucose of diabetic patients with AMI was associated with a decrease in mortality. However, these findings could not be reproduced by subsequent studies – both Digami-2[4] and HI-5[5] failed to demonstrate the benefits of using the intensive treatment for blood glucose during an acute coronary event. Randomized studies with the same purpose of comparing a more rigorous treatment for stress hyperglycemia with more conservative methods, in the context of other critical conditions, also failed, like the multicenter Nice-Sugar[6]. This study suggested a 14% increase in patient mortality with the intensive control of hyperglycemia, overall, due to an increase in the risk of hypoglycemia.

The concept of metabolic modulation in the acute stage of AMI with the purpose of promoting electrical stability in the myocardium after an ischemic event started in 1960 with the glucose-insulin-potassium therapy (GIK)[7]. However, there is an extremely tenuous balance between the plasma glucose and insulin resistance in the ischemic myocardium, in which, on the one hand, there is a necessity for high blood glucose levels in order to defeat the cellular mechanisms for insulin resistance and, on the other hand, a relative insulin deficiency in the acute stage. The mere provision of glucose and insulin could facilitate the glucose uptake by the myocardium, provided that in balance with the glucose consumption rate. If that balance is broken, the deleterious effect of hypoglycemia or hyperglycemia will prevail.

In studies such as Digami I and II, Gips I[8] and II[9], Create-Ecla[10], and other trials, the diametrically opposite impacts on cardiovascular mortality due to all causes were not revealed. Although the proportion of hypoglycemia partially explains these findings, there is no clear explanation for such divergent results. In order to answer that question, we propose a meta-analysis followed by meta-regression using data published from trials on the intensive control of blood glucose in the acute stage of AMI.

METHODS

A detailed description of all the procedures is included in the Supplementary Data (from the Data Source and Research section to the Studies Included and Excluded). In short, the Medline (PubMed), Cochrane Library and ClinicalTrials.gov databases were searched for original articles from 1995 to 2017 in order to identify all randomized clinical trials (RCTs) that used an intensive strategy or GIK therapy for the control of blood glucose levels during the acute stage of AMI. Original essays were considered eligible for the present meta-analysis if they met the following criteria: 1) Phase 2 or 3 RCT; 2) participants of the AMI acute event; 3) the participants of the treatment group used insulin or GIK therapy during the study. We excluded the studies that, despite being related to this subject, did not present mortality data or a comparison between a more intensive strategy for the control of stress hyperglycemia with more conservative strategies, or that presented biases in the randomization or data analysis. Two researchers analyzed the data using pre-set forms and independently assessed the precision of the analysis, resolving any discrepancies through a consensus after a discussion with the third researcher. The baseline data were obtained through weighted calculation. In order to identify the potential effects of GIK or insulin therapy in the AMI, we calculated an overall risk ratio (RR) with meta-analysis of fixed and random effects models. Odds Ratios and risk ratios were universally identical during the data analysis. More details on the data analysis can be found in Annex 1 (Data Analysis and Synthesis). For the estimate of the effects of the resumed treatment, a two-tail p-value of < 0.05 was considered statistically significant. We used Stata 13 to analyze the data.

RESULTS

In total, we identified over 20 studies on the control of hyperglycemia in events of acute myocardial

infarction, out of which nine were included in our study (Table 1). All other studies were not included in our meta-analysis because they did not present assessments of the polarity of more intensive or conservative approaches.

With that in mind, the studies analyzed were conducted between the years of 1995 and 2007 and all were clinical trials with a multicenter and randomized design. The average of follow-up for all the studies was of 11.5 months; Digami-1 was the one with the longest patient follow-up time (40.8 months), while all the others (except for Digami-2, which followed-up with the patients for 36 months) had a follow-up time between one and six months. In total, there were 28,151 patients included; of these, 1,379 were included in the group for intensive capillary blood glucose treatment, while 13,031 were in the group for GIK treatment, and 13,741 in the control group (734 in the studies that were compared with the intensive strategy, and 13,007 in the studies that assessed the use of GIK). In total, about 34.7% of patients were female, with an average age of 62.6 years.

After dividing the sample between the studies that sought blood glucose levels with an emphasis on a more intensive regimen of insulin in the acute stage of infarction, we had 2,113 patients in the intensive treatment group, and 734 in the control group, with 39% of patients being female, with an average age of 66.1 years. In this subgroup, the average pre-randomized blood glucose of patients was of 234.72 mg/dl, with a 7.45% mean value for glycated hemoglobin (HbA1C) for these patients. After separating the patients into the intensive and conservative treatment groups, for the first group, the average blood glucose value was of 148.68 mg/dl, while on the latter it was of 159.48 mg/dl, with post-follow-up HbA1C values of 7.06% and 7.1 %, respectively.

As for the studies that assessed the use of the glucose-insulin-potassium therapy solution, there was a total of 26,038 patients, with 13,031 in the GIK group and 13,007 in the conservative treatment group. There was an average of 32.6% female patients, with an average age of 60.9 years. The average pre-randomized blood glucose was of 150.48 mg/dl between the sub-

TABLE 1 – DESCRIPTIVE ANALYSIS OF THE STUDIES ANALYZED

Trial	Digami	Digami 2	HI-5	Gips I	Oasis 6	Cre-ate-Ecla *trial*	Pache et al	Gips II	Pol-GIK
Year	1995	2005	2006	2003	2007	2005	2004	2006	1999
Design	RCT	RCT	RCT	RCT	RCT	RCT	RCT	RCT	RCT
Follow-up (months)	40.8	36	6	1	6	1	6	1	6
Female (%)	62.5	33	21.6	60.1	27.6	22.4	27.9	26.4	31.4
Average pre-randomized age (years)	67.5	68.3	62.6	60.3	61.8	58.6	62.4	61.5	61.0
Pre-randomized DM II (%)	100	100	48.3		14.5	17.7	23.1	9.5	6.3
DM II in insulin use (%)	34.6	31.1	7.9					2.5	1.9
Heart failure (%)	21.9	23.9		8.2	13.5	1.65			7.7
Undiagnosed DM II (%)	12.5								
Pre-randomized HbA1C (%)	8.1	7.26	7.0						
Pre-randomized blood glucose (mg/dl)	279	229.1	196.2	153	160.2	162		153	124.2
Intensive treatment sample	306	947	126						
Conservative treatment sample	314	306	114	464	1,374	10,107	157	445	460
GIK sample				476	1,374	10,088	155	444	494
Post-intensive treatment blood glucose (mg/dl)	210.6	163.8	144						
Post-conservative treatment blood glucose (mg/dl)	172.8	180	145.8	145.8	133.2	135			111.6
Post-GIK blood glucose (mg/dl)				138.6	151.2	154.8			106.2
Post-intensive treatment HbA1C (%)	7.0	6.8	7.4						
Post-conservative treatment HbA1C (%)	7.5	6.8	7.0						

RCT – Randomized Clinical Trial; DM II – Type II Diabetes Mellitus; GIK – glucose-insulin-potassium solution; HbA1C – Glycated Hemoglobin.

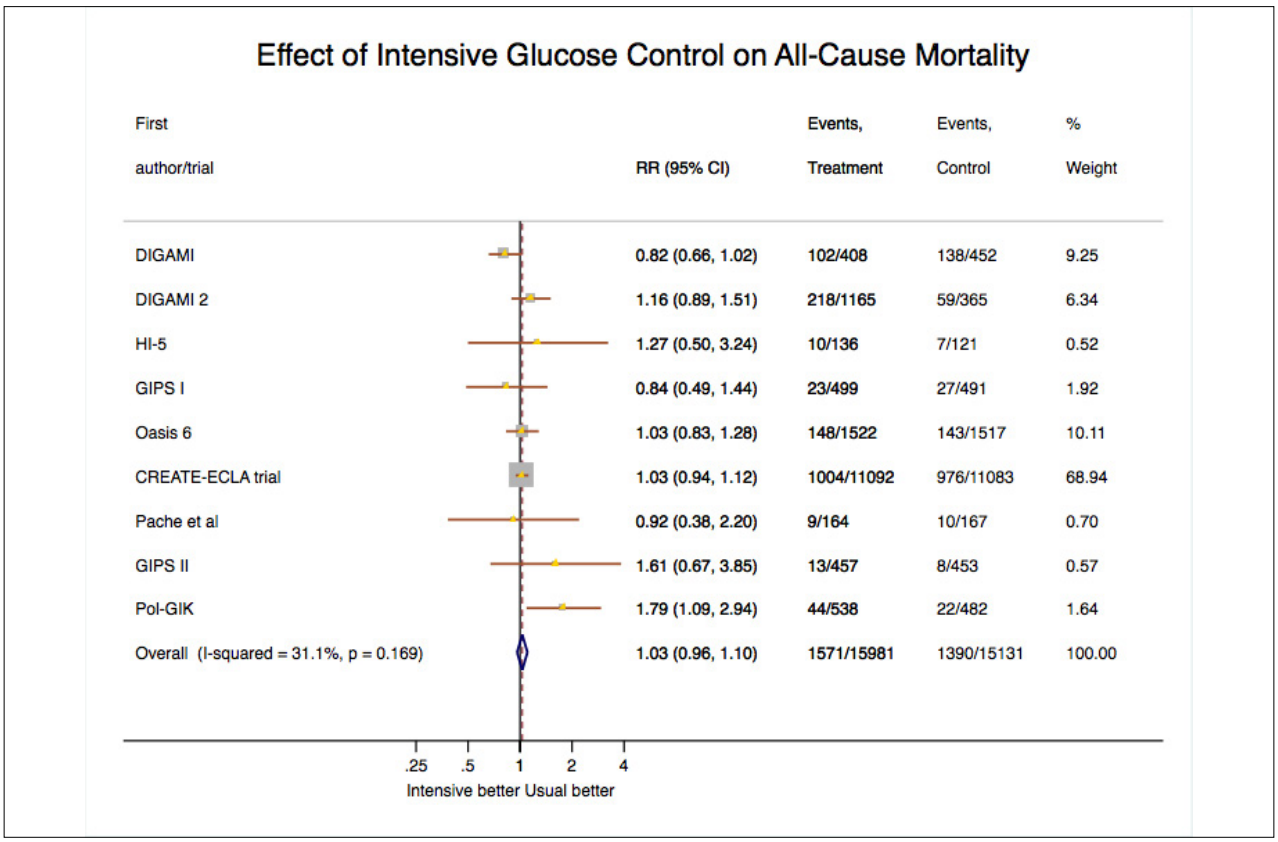

FIGURE 1

groups, with 131.4 mg/dl for patients of the conservative treatment and 137.7 mg/dl in the GIK group.

The mortality rates for all causes are described in Figure 1. The grouped data show that the total mortality of the studies included was of 2,961 (10.5%). When once again divided into subgroups, there were 330 in the group for intensive control of capillary blood glucose, against 204 in the group for the conservative treatment (15.6% against 9.6%, respectively). Among the patients assessed for the GIK treatment, the total mortality was of 1,186 individuals (4.7%) for patients who used this strategy, against 1,186 patients of the conservative treatment (4.5%). Thus, the risk ratio for mortality due to all causes was of 1.03 [95% CI 0.96–1.10]; $I^2 = 31\%$; p = 0.41). As shown in Supplementary Figure 1, there was no significant publication bias in the funnel charts and no significant bias concerning small studies, according to the Egger tests (p = 0.57).

Finally, with the purpose of finding possible explanations for the discrepant findings between the trials, we conducted a series of meta-regressions (Supplementary Figures 2 and 3). As shown in figure 2, we can see that abrupt and intense reductions in blood glucose in relation to the average estimated blood glucose (> 2 mmol/L or > 35 mg/dL) were associated with a higher risk of death, while reductions with low intensity (around zero) were not significantly associated with an increment or reduction in mortality due to all causes. Contrastingly, the lowering of blood glucose in the acute stage compared with the average blood glucose was more effective around 1 mmol/L 18 mg/dL (p = 0.008 for the tendency in the restricted cubic *spline*).

DISCUSSION

The present systematic review assessed the impact of the intensive control of blood glucose levels in the acute stage of AMI, following different strategies, over the mortality due to all causes. A raw analysis of the impact of intensive control suggests there is no difference in comparison with the usual control. However, we have shown there is a fine limit between the magnitude of the fasting glucose reduction in relation with the average blood glucose levels before the AMI (based on the glycated hemoglobin) and the results of the intensive blood glucose control approach. In this context, intense and abrupt reductions of blood glucose (> 2 mmol/L or > 35 mg/dL) seem to lead to a higher risk of death, while re-

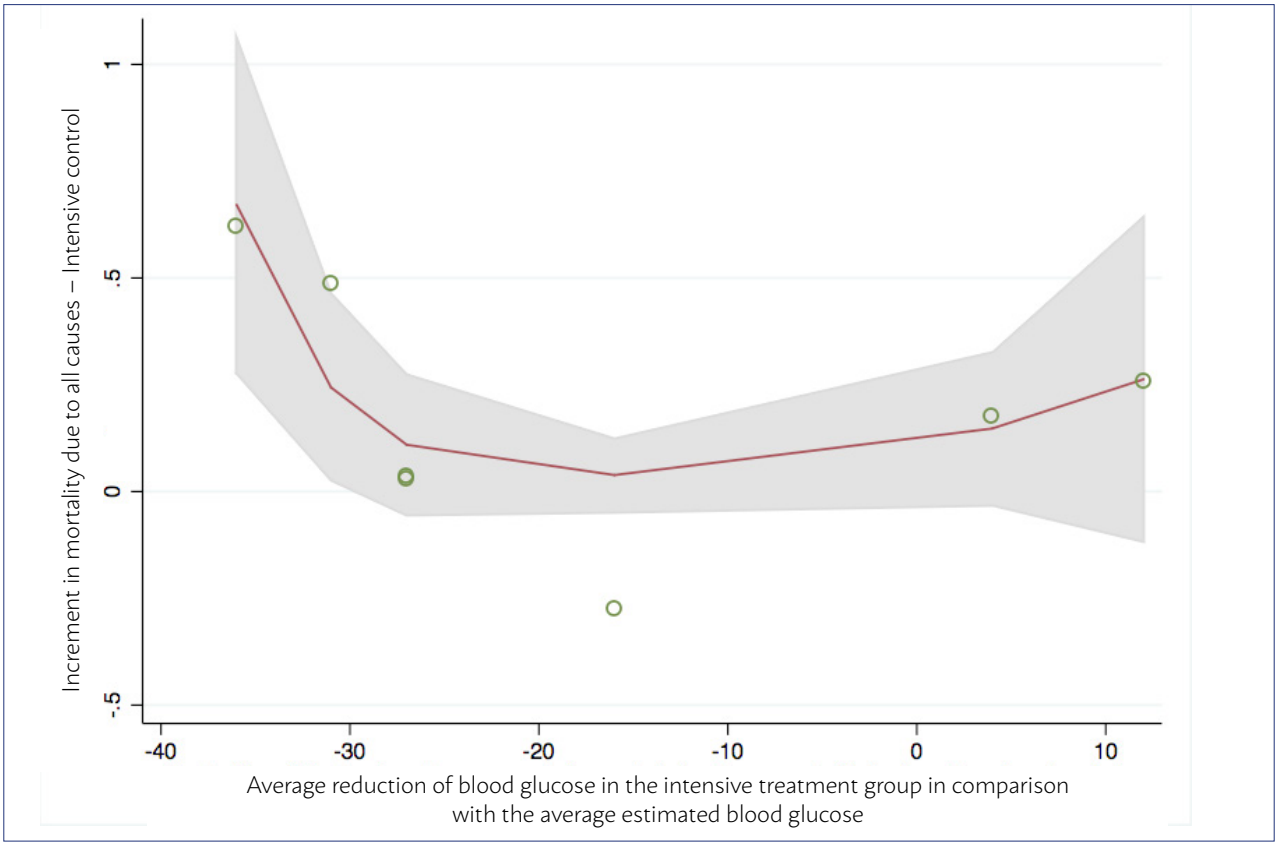

FIGURE 2

ductions with low intensity (around zero) seem not to influence death risk. On the other hand, a reduction of around 1 mmol/L (or 18 mg/dL) of the blood glucose during the acute stage compared with the estimated average seems to be the most secure and effective strategy to reduce the risk of death due to all causes in the context of AMI.

Stress hyperglycemia is common in the context of critically ill patients[6] and is not different from the acute stage of the acute myocardial infarction[1,2,11,12]. It is a powerful risk marker since it reflects the systemic and sympathetic activity and plasma levels of catecholamines, cortisol, and glucagon after the ischemic event[13]. When it is present at the AMI patient's admission into hospital, it is related with higher mortality rates, worse outcomes in primary angioplasty and an increase in the number of Timi 0 flows in coronary angiographies, as well as a more significant development of heart failure and cardiogenic shock. The pathophysiology is still little known; however, it is known that the hyperglycemia is associated with endothelial dysfunction in the context of acute AMI[15].

The cells of the vascular endothelium play an essential part in cardiovascular homeostasis, secreting a series of mediators that regulate the platelet aggregation, coagulation, and vascular tonus[16]. The term "endothelial dysfunction" refers to a condition in which the endothelium loses its physiological properties, with a tendency of promoting arteriolar vasodilation, fibrinolysis, and anti-aggregation. The mediators secreted by these cells can perform both vasoconstriction, through endothelin-1 and thromboxane A2, and vasodilation through nitric oxide and prostacyclin. In patients with hyperglycemia and diabetes, there is a deficiency in the production of nitric oxide, which causes a vascular imbalance towards vasoconstriction[16].

It is also known that., in physiological conditions, the myocardium metabolizes, preferably, free fatty acids; however, in ischemic conditions, it uses glucose in a relatively higher proportion. Nevertheless, the insulin resistance developed during the AMI acute stage generates an immediate limiting factor to the absorption of the energy substrate, which can contribute to the worsening of the myocardium injury. Thus, both endothelial dysfunction caused by hyperglycemia and insulin resistance can explain the adverse outcomes of exacerbated hyperglycemia in the second acute stage of AMI[1,11].

After these conclusions, the question was what

would be the ideal blood glucose target after the AMI, since, for the reasons previously mentioned, even patients who did not have diabetes were hyperglycemic upon the admission of the ischemic event. Thus, was born the 1995 Digami[3] study, which set out to study 620 patients grouped into a more permissive hyperglycemic approach and a more intensive approach for the control of blood glucose levels through the use of a glucose-insulin solution. They were, initially, encouraging of an intensive strategy, since after a 40.8 months follow-up there was a reduction of 11% of mortality in the intensive group, which was more evident in the low-risk groups who were not previously treated with insulin therapy.

The following studies then – Digami-2[4] e HI-5[5] – tried to reproduce a similar design to the Digami but were not very successful. In the first one, there was no difference in mortality between the groups, possibly due to the excellent blood glucose levels on both. In the HI-5, the absence of significant differences in mortality could be explained by the reduced number of patients in a study that, perhaps, was not adequately blinded.

When we analyze the results of these three studies (Digami, Digami-2 e HI-5), we can see that the post-intensive treatment blood glucose level was of 172.8 mg/dl, 163.8 mg/dl, and 144 mg/dl, respectively, while in the groups randomized for the conservative treatment it was 210.6 mg/dl, 180 mg/dl, and 145.8 mg/dl; the average for HbA1c was 8.1%, 7.23%, and 7%, also respectively. The Digami data show that this study had the highest values for blood glucose in both groups in comparison with the other two studies, while the HI-5 was the one with the lowest values. This analysis suggests that higher blood glucose values might be related to better outcomes when compared with lower values in more intensive approaches. Accordingly, the meta-regression results suggested there is a tenuous line between effectiveness and safety for the reduction of blood glucose in the acute stage.

The studies on GIK therapy were motivated by the theoretical benefits of the solution in increasing the availability of glucose and potassium to the myocardium associated with the insulin effect that facilitates the glucose oxidation, reduces circulating fatty acids, thus improving the parameters for coagulation and the anti-inflammatory effects, and reducing arrhythmias and changes in contractility. The studies included in this meta-analysis assessed 13,031 patients who underwent GIK therapy and 13,007 who underwent the conventional treatment; Create-Ecla[10] had the largest sample, with 20,201 patients.

The Gips-I[8] study compared the GIK therapy infusion or placebo in 940 AMI patients eligible for primary angioplasty in 8-12 hours, showing clinical benefits for the patients, with no signs of heart failure; however, the following study, Gips-II[9], could not confirm this finding.

Similarly, in the most recent Create-Ecla, a study that assessed the effects of a high dose of GIK did not present a significant difference in mortality. The Oasis-6[17] study was prematurely interrupted after 2,748 patients were enrolled after the publication of the Create-Ecla; the Oasis-6 showed no benefits to the GIK treatment.

Despite that, a recent analysis of the Oasis-6 and Create-Ecla studies combined the populations of both, showing an increase in mortality for the treatment with GIK, especially over the first three days.

In a meta-analysis published in 1997 by Fath-Ordoubadi & Beatt[18] and reviewed by Mamas et al.[19] in 2010, it was demonstrated that the GIK therapy in the studies analyzed conducted during a pre-revascularization/fibrinolysis period presented benefits for the clinical outcome. Upon comparing these data with more recent studies, it is possible that the GIK therapy benefits only patients in whom the reperfusion has not been reached.

When analyzing the studies, it is evident that the interventions by the GIK studies, overall, provided better blood glucose control for patients in both groups. For those submitted to GIK therapy, there was a discrete increase in blood glucose in comparison with the placebo group, whose average pre-randomization blood glucose was of 150.48. After the intervention, the blood glucose average in the treatment group was of 137.7, and the average in the placebo group was 131.4. Both results were very similar, which might justify the absence of benefits for the reduction of mortality when both groups were compared.

CONCLUSION

Few reported studies assess blood glucose control guided by goals in an intensive environment and that included acute coronary syndrome patients. The present meta-analysis demonstrated that the data on the interval between blood glucose levels that

should be sought during the acute stage of the AMI is inconsistent and that GIK therapy did not present any benefits for this same group of patients. On the other hand, our study supports the hypothesis that there must be a fine limit between effectiveness and safety for blood glucose reduction in the acute stage. Thus, the reasonable blood glucose targets should be guided, preferably, by the average estimated blood glucose and should not exceed a reduction of 36 mg/dL of blood glucose. Additional studies are still necessary to confirm the ideal targets and dissect the differences between diabetic and non-diabetic patients.

RESUMO

INTRODUÇÃO: *No infarto agudo do miocárdio (IAM), cada incremento de 18 mg/dl (1 mmol/L) se associa a um aumento de 3% na mortalidade. As estratégias de redução da glicemia tentadas até o momento, entretanto, não trouxeram resultados animadores.*

METODOLOGIA: *Foram pesquisadas nas bases de dados Medline (PubMed) e Cochrane Library os ensaios clínicos randomizados (ECRs) de 1995 a 2017 que utilizaram estratégia intensiva ou a terapia GIK no controle glicêmico durante a fase aguda do IAM. Foram incluídos oito estudos. Para identificar os efeitos da insulinoterapia ou da terapia GIK, calculamos um risco relativo geral (RR) com meta-análises de modelos de efeitos fixos e aleatórios. Um valor de p-bicaudal < 0,05 foi considerado estatisticamente significativo.*

RESULTADOS: *Foram incluídos 28.151 pacientes, sendo 1.379 no grupo de tratamento intensivo da glicemia, 13.031 no GIK e 13.741 no controle. A mortalidade total foi de 2.961 (10,5%), computando um risco relativo de 1,03 [95%CI 0,96–1,10]; I^2 = 31%; p = 0,41 para o grupo intensivo ou GIK contra o grupo conservador. Reduções intensas (> 36 mg/dL) em relação à glicemia estimada média se associaram à maior mortalidade, enquanto reduções menores não se associaram com seu incremento ou redução. A redução glicêmica na fase aguda em relação à glicemia estimada média foi mais efetiva e segura na faixa em torno de 18 mg/dL.*

CONCLUSÃO: *Esta meta-análise levanta a hipótese de haver um limite tênue entre efetividade e segurança para a redução glicêmica na fase aguda, sendo que os alvos não devem exceder uma redução maior do que 36 mg/dL de glicemia.*

PALAVRAS-CHAVE: *Hiperglicemia. Infarto do miocárdio. Meta-análise como assunto. Efetividade. Segurança. Cuidados críticos.*

REFERENCES

1. Stranders I, Diamant M, van Gelder RE, Spruijt HJ, Twisk JW, Heine RJ, et al. Admission blood glucose level as risk indicator of death after myocardial infarction in patients with and without diabetes mellitus. Arch Intern Med. 2004;164(9):982-8.
2. Mulder M, Cornel JH, van der Ploeg T, Boersma E, Umans VA. Elevated admission glucose is associated with increased long-term mortality in myocardial infarction patients, irrespective of the initially applied reperfusion strategy. Am Heart J. 2010;160(3):412-9.
3. Malmberg K, Rydén L, Efendic S, Herlitz J, Nicol P, Waldenström A, et al. Randomized trial of insulin-glucose infusion followed by subcutaneous insulin treatment in diabetic patients with acute myocardial infarction (DIGAMI study): effects on mortality at 1 year. J Am Coll Cardiol. 1995;26(1):57-65.
4. Malmberg K, Rydén L, Wedel H, Birkeland K, Bootsma A, Dickstein K, et al. DIGAMI 2 Investigators. Intense metabolic control by means of insulin in patients with diabetes mellitus and acute myocardial infarction (DIGAMI 2): effects on mortality and morbidity. Eur Heart J. 2005;26(7):650-61.
5. Cheung NW, Wong VW, McLean M. The hyperglycemia: intensive insulin infusion in infarction (HI-5) study: a randomized controlled trial of insulin infusion therapy for myocardial infarction. Diabetes Care. 2006;29(4):765-70.
6. Inzucchi SE, Siegel MD. Glucose control in the ICU: how tight is too tight? N Engl J Med. 2009;360(13):1346-9.
7. Sodi-Pallares D, Testelli MR, Fishleder BL, Bisteni A, Medrano GA, Friedland C, et al. Effects of an intravenous infusion of a potassium-glucose-insulin solution on the electrocardiographic signs of myocardial infarction. A preliminary clinical report. Am J Cardiol. 1962;9:166-81.
8. Van der Horst IC, Zijlstra F, van't Hof AW, Doggen CJ, de Boer MJ, Suryapranata H, et al.; Zwolle Infarct Study Group. Glucose-insulin-potassium infusion in patients treated with primary angioplasty for acute myocardial infarction: the glucose-insulin-potassium study: a randomized trial. J Am Coll Cardiol. 2003;42(5):784-91.
9. Timmer JR, Svilaas T, Ottervanger JP, Henriques JP, Dambrink JH, van den Broek SA, et al. Glucose-insulin-potassium infusion in patients with acute myocardial infarction without signs of heart failure: the Glucose-Insulin-Potassium Study (GIPS)-II. J Am Coll Cardiol. 2006;47(8):1730-1.
10. Mehta SR, Yusuf S, Diaz R, Zhu J, Pais P, Xavier D, et al.; CREATE-ECLA Trial Group Investigators. Effect of glucose-insulin-potassium infusion on mortality in patients with acute ST-segment elevation myocardial infarction: the CREATE-ECLA randomized controlled trial. JAMA. 2005;293(4):437-46.
11. Kosiborod M, Rathore SS, Inzucchi SE, Masoudi FA, Wang Y, Havranek EP, et al. Admission glucose and mortality in elderly patients hospitalized with acute myocardial infarction: implications for patients with and without recognized diabetes. Circulation. 2005;111(23):3078-86.
12. Goyal A, Mahaffey KW, Garg J, Nicolau JC, Hochman JS, Weaver WD, et al. Prognostic significance of the change in glucose level in the first 24 h after acute myocardial infarction: results from the CARDINAL study. Eur Heart J. 2006;27(11):1289-97.
13. Oswald GA, Smith CC, Betteridge DJ, Yudkin JS. Determinants and importance of stress hyperglycaemia in non-diabetic patients with myocardial infarction. Br Med J. 1986;293(6552):917-22.
14. Capes SE, Hunt D, Malmberg K, Gerstein HC. Stress hyperglycaemia and increased risk of death after myocardial infarction in patients with and without diabetes: a systematic overview. Lancet. 2000; 355(9206):773-8.
15. Chen SM, Tsai TH, Hang CL, Yip HK, Fang CY, Wu CJ, et al. Endothelial dysfunction in young patients with acute ST-elevation myocardial infarction. Heart Vessels. 2011;26(1):2-9.
16. Fadini GP, Sartore S, Agostini C, Avogaro A. Significance of endothelial progenitor cells in subjects with diabetes. Diabetes Care. 2007;30(5):1305-13.
17. Díaz R, Goyal A, Mehta SR, Afzal R, Xavier D, Pais P, et al. Glucose-insulin-potassium therapy in patients with ST-segment elevation myocardial infarction. JAMA. 2007;298(20):2399-405.
18. Fath-Ordoubadi F, Beatt KJ. Glucose-insulin-potassium therapy for treatment of acute myocardial infarction: an overview of randomized placebo-controlled trials. Circulation. 1997;96(4):1152-6.
19. Mamas MA, Neyses L, Fath-Ordoubadi F. A meta-analysis of glucose-insulin-potassium therapy for treatment of acute myocardial infarction. Exp Clin Cardiol. 2010;15(2):e20-4.

ANNEX 1
Data Source and Research

The following terms were used in our search: "acute myocardial infarction", "diabetes mellitus" and "stress hyperglycemia" and "stress hyperglycemia in acute myocardial infarction" and (ensaio clínico aleatório controlado OU ensaio clínico controlado OU randomizado OU placebo OU terapia medicamentosa OU aleatoriamente OU julgamento ou grupos NÃO animais) and (ensaio clínico randomizado controlado e humanos) and (ensaio clínico controlado aleatório e humanos).

During the search strategy, we selected the following languages: English, Spanish, and Portuguese. However, all relevant articles were published in English, conducted in human beings, and classified as RCTs.

– Definitions

Hyperglycemia in an acute myocardial infarction event was characterized according to the definition of each researcher. Usually, these definitions included the following terms from the MedDRA terminology: "hyperglycemia after acute myocardial infarction" and "hyperglycemia and mortality in acute myocardial infarction."

– Data Extraction and Quality Assessment

Two researchers who were not involved in any of the studies selected collected the data using a pre-set table and assessed, independently, the precision of the data, solving any discrepancies through consensus after a discussion with a third researcher. The following items were extracted from the studies included: name of the first author, year of publication, study design, characteristics of the patients, sample size, duration of the intervention, type of dose control, clinical outcomes, and adverse events. If a study was published more than once, we included the most recent report. If the patients were recruited for more than one study, they were not counted twice. The Cochrane Collaboration[1] tool to assess the risk of bias was used to assess the different types of bias within the studies included in our meta-analysis, and the quality of the study was assessed using the Grade[2] system. Two unblinded researchers independently assessed the potential risk of bias in the RCTs using the methods described in the Cochrane Collaboration guidelines. Our co-primary outcomes were: 1) Blood glucose levels after one of the approaches had been applied, and 2) mortality for each of the approaches.

– Studies Included and Excluded

Using the Medline/PubMed, Cochrane Library and ClinicalTrials.gov databases, we identified 36 citations that used the search terms previously defined. After implementing our inclusion/exclusion criteria, we excluded 25 studies that did not present data on mortality or a comparison between a more intensive approach for the control of stress hyper-

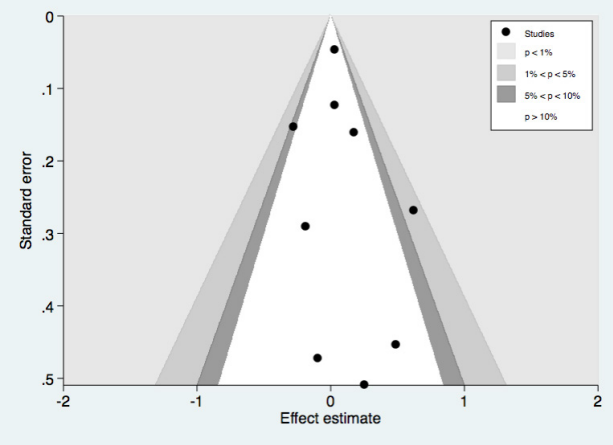

FIGURE 1

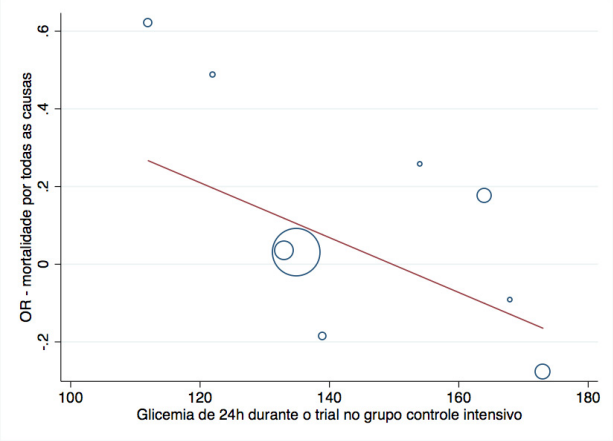

FIGURE 2

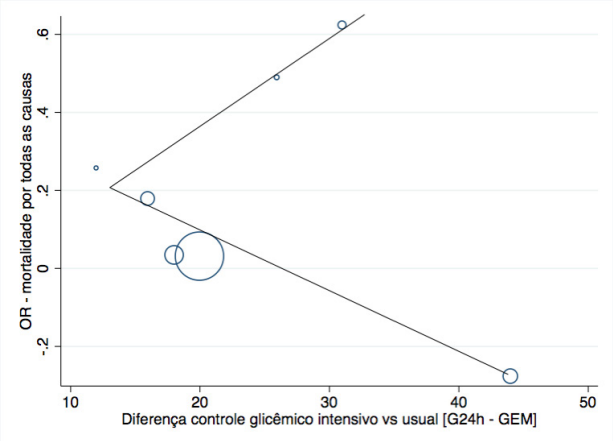

FIGURE 3

glycemia and more conservative approaches, or that presented randomization or data analysis (n=2) biases, or were previous meta-analysis, which then resulted in 11 studies considered relevant for this meta-analysis.

Data Handling and Analysis

Dichotomous variables are reported as percentages, while continuous variables are reported as average ± SD or median (interquartile range). The baseline data were obtained through weighted calculation. To identify the potential effects of the intensive strategy for blood glucose control, we calculated an overall risk ratio (RR) with meta-analyses of fixed and random effects models. Probability indexes and risk ratios were universally identical during the data analysis. We assessed the statistical heterogeneity between the trials using I^2 statistics (with 95% CI)[3], which provides a measure of the proportion of overall variation that can be attributed to heterogeneity between trials. We used risk ratios obtained through a fixed and random effects meta-analysis because they can be used as a sensitivity analysis. We used meta-regression analyses to investigate the possible sources of heterogeneity among the trials.

REFERENCES

1. Higgins JP, Altman DG. Assessing risk of bias in included studies. In: Cochrane handbook for systematic reviews of interventions. Chichester: John Wiley & Sons; 2008. p.187-241.
2. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ. 2008;336(7650):924-6.
3. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327(7414):557-60.

ORIGINAL ARTICLE

The efficacy of saxagliptin in T2DM patients with non-alcoholic fatty liver disease: preliminary data

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SUMMARY

OBJECTIVE: *To investigate the clinical efficacy and the possible mechanisms of saxagliptin in the treatment of type 2 diabetes mellitus (T2DM) combined with non-alcoholic fatty liver disease (NAFLD).*

METHODS: *A total of 95 T2DM and NAFLD patients were randomly divided into group A (saxagliptin group), group B (glimepiride group), and group C (glimepiride combined with polyene phosphatidylcholine group).*

RESULTS: *After intervention treatment for 24 w, body mass index (BMI), waist-to-hip ratio (WHR), glycated haemoglobin (HbA1c), fasting plasma glucose (FPG), fasting insulin (FINS), homeostatic model assessment of insulin resistance (HOMA-IR), interleukin-6 (IL-6), triglyceride (TG), total cholesterol (TC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (γ-GT), and quantitative detection of liver steatosis of study subjects were observed, the action of liver steatosis in subjects of groups A and C were significantly different from those of group B; however, there were no differences between groups A and C. The FINS, HOMA-IR, and IL-6 of subjects in group A was lower than those in groups B and C; however, there were no significant differences between the latter two groups.*

CONCLUSION: *For T2DM combined with NAFLD patients, the saxagliptin treatment could not only effectively control blood glucose but also attenuate insulin resistance and inflammatory injury of the liver to improve fatty liver further.*

KEYWORDS: *Dipeptidyl-peptidase IV inhibitors/therapeutic use. Diabetes mellitus, type 2. Interleukin-6. Fatty liver. Non-alcoholic fatty liver disease.*

INTRODUCTION

Non-alcoholic fatty acid disease (NAFLD) refers to one type of metabolic stress-induced liver injury syndrome that is closely associated with insulin resistance and genetic susceptibility resulting from factors other than alcohol and other clear liver injury factors[1]. Epidemiological survey results have shown that the prevalence of NAFLD in the world reaches 30%, the prevalence of combined NAFLDs among type 2 diabetes mellitus (T2DM) patients reaches 34%-74%, and NAFLD is present in almost all T2DM combined with obesity patients[2]. Insulin resistance is even more evident in T2DM combined with NAFLD

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TABLE 1. COMPARISON OF BASELINE CLINICAL INFORMATION OF SUBJECTS AMONG ALL GROUPS

Group	Number of cases (male/female)	Age (year)	Disease history (month)	BMI (kg/m2)	WHR (cm/cm)	HbA1c (%)	FPG (mmol/L)	FINS (μIU/ml)	HOMA-IR	IL-6 (pg/ml)
Group A	31 (15/16)	46.58±8.15	11.32±6.64	27.20±4.06	0.89±0.06	7.79±0.52	7.86±1.25	10.63±2.25	3.72±0.98	10.60±8.20
Group B	33 (17/16)	47.36±9.40	10.15±7.55	26.46±3.23	0.89±0.05	7.82±0.61	7.57±1.24	9.97±2.45	3.37±0.99	10.05±7.57
Group C	31 (16/15)	49.26±8.94	9.55±5.37	26.02±2.91	0.88±0.05	7.85±0.57	7.54±0.98	10.57±2.61	3.55±1.02	11.36±9.10
F	0.084	0.908	2.448	2.544	0.218	1.659	0.230	0.639	0.148	0.270
p	0.959	0.407	0.092	0.084	0.805	0.196	0.795	0.530	0.863	0.764

Group	Number of cases (male/female)	Age (year)	Disease history (month)	TC (mmol/L)	TG (mmol/L)	ALT (U/L)	AST (U/L)	γ-GT (U/L)	Quantitative detection of liver steatosis (%)
Group A	31 (15/16)	46.58±8.15	11.32±6.64	5.35±0.94	1.98±1.08	31.74±18.99	32.48±20.33	51.19±27.01	20.50±8.96
Group B	33 (17/16)	47.36±9.40	10.15±7.55	5.55±0.79	2.13±0.84	28.36±14.55	28.88±14.08	46.61±20.26	21.11±8.85
Group C	31 (16/15)	49.26±8.94	9.55±5.37	5.57±1.20	2.06±1.02	30.26±13.05	27.13±12.17	45.00±21.37	22.03±9.19
F	0.084	0.908	2.448	1.816	0.164	1.643	2.314	2.436	0.229
p	0.959	0.407	0.092	0.169	0.849	0.199	0.105	0.093	0.796

patients, and the risk of cardio-cerebrovascular diseases increases in these patients. Therefore, during the control of blood glucose compliance, it would be more beneficial also to improve fatty liver at the same time. Studies in recent years have suggested that the development and progression of various chronic liver diseases are associated with dipeptidyl peptidase-4 (DPP-4). NAFLD patients have higher levels of serum DPP-4, and administration of DPP-4 inhibitor treatment can improve liver functions and hepatocyte degeneration levels in these patients[3,4]; however, its possible mechanisms are still not completely elucidated. This study aimed to observe the efficacy of saxagliptin in the reduction of glucose and the improvement of fatty liver in newly diagnosed T2DM patients combined with NAFLD and to investigate the possible mechanisms underlying the improvement of fatty liver.

INVESTIGATIONS AND RESULTS

A total of 95 subjects were enrolled in this study. Group A had 31 cases, and 1 case was lost during follow-up; group B had 33 cases, and no case was lost during follow-up, and group C had 31 cases, and 2 cases were lost during follow-up. A total of 92 subjects completed this study.

Comparison of baseline clinical information among all groups

Age, gender, DM history, BMI, WHR, HbA1c, FPG, FINS, IL-6, TG, TC, ALT, AST, γ-GT, and quantitative detection of liver steatosis of subjects among the three groups before enrolment were not significantly different ($p>0.05$) (Table 1) and had comparability.

Comparison of the control of BMI, WHR, blood glucose, and blood lipids after 24 w of treatment among all groups

BMI, WHR, HbA1c, FPG, TC, and TG of subjects among the three groups were not significantly different at the observation endpoint ($p>0.05$) (Table 2), suggesting that the hypoglycemic efficacy in the saxagliptin treatment group was equivalent to that in the glimepiride group.

Comparison of liver functions, quantitative detection of liver steatosis, FINS, HOMA-IR, and IL-6 among all groups after 24 w of treatment

ALT, AST, γ-GT, and quantitative detection of liver steatosis of subjects in groups A and C were all lower than that in group B, and the differences were significant ($p<0.05$); in contrast, there were no differences between groups A and C. FINS, HOMA-IR, and IL-6 of subjects in group A was lower than those in groups B and C ($p<0.05$); however, there was no significant difference between the latter 2 groups (Table 3).

DISCUSSION

Dipeptidyl peptidase-4 (DPP-4) inhibitors can stimulate pancreatic islet β cells to secrete insulin and

can also inhibit abnormal secretion of glucagon by α cells, functioning on the α,β-double-channel glucose-dependent blood glucose regulation mechanism[5]. Based on metformin administration, the comparison of blood glucose control compliance rates between combined saxagliptin treatment and combined glimepiride treatment did not have a significant difference in elderly T2DM patients[6]. This study observed that the treatment of newly diagnosed DM patients using saxagliptin or glimepiride alone did not have a significant difference in the control of FPG and HbA1c, suggesting that the blood glucose control effects between these two treatments were equivalent. In addition, the results in this study showed that the HOMA-IR indicator in the saxagliptin treatment group was lower than that in the glimepiride group, suggesting that saxagliptin could improve insulin resistance in newly diagnosed T2DM combined with NAFLD patients. Previous study results mainly suggested that saxagliptin could stimulate insulin secretion and improve β cell functions but did not have apparent functions on insulin resistance[7,8]. Some studies have suggested that saxagliptin could also improve insulin resistance in DM patients[9]. The differences in the study results might be associated with characteristics of the populations enrolled in the different studies. Therefore, large-scale clinical trials are still needed for further studies and observations.

NAFLD is characterized by hepatocyte steatosis, and fat deposition includes simple fatty liver, steatohepatitis, and fatty liver fibrosis and cirrhosis. Currently, its pathogenetic mechanisms have not been completely elucidated. The "second hit" theory is currently the most recognized viewpoint by most scholars. In the first hit, fat accumulation in the liver causes hepatocyte apoptosis and induces insulin resistance. Insulin resistance, directly and indirectly, participates in the second hit to cause inflammatory responses, hepatocyte injury, and fibrosis[10]. Studies on NAFLD-associated chronic inflammation have received extensive attention in recent years, and cytokines and inflammatory factors have become research hot spots[11,12]. IL-6 is mainly produced by immune cells, including macrophages, T cells, and B cells; it is an important pro-inflammatory cytokine, and the deregulation of its expression is closely associated with various diseases. The functions of IL-6 in hepatocyte steatosis are very complicated. It has been shown that IL-6 plays a role in liver protection through the inhibition of oxidative stress and the prevention of mitochondrial dysfunction in the early stage of fatty liver. However, during the pathological changes in the late stage of fatty liver, IL-6 can induce hepatocyte apoptosis, produce insulin resistance, participate in NAFLD development and progression, and cause hepatocyte injury[13,14]. This study showed that the blood glucose control, BMI, and WHR of subjects in the saxagliptin group were not significantly different compared to the glimepiride group. However, the IL-6 and HOMA-IR levels were both lower than those in the glimepiride group and the glimepiride combined with polyene phosphatidylcholine group. In addition, the level of liver steatosis quantitation of subjects in the saxagliptin group was significantly lower than that in the glimepiride group and was equivalent to that in the glimepiride combined with polyene phosphatidylcholine group. These results suggested that based on the same levels of blood glucose control and body weight management, the administration of saxagliptin treatment could improve insulin resistance and reduce the levels of inflammatory factors such as IL-6 to improve hepatocyte steatosis and protect hepatocytes in newly diagnosed patients with T2DM combined with NAFLD.

TABLE 2. COMPARISON OF THE CONTROL CONDITIONS OF BMI, WHR, HBA1C, FBG, TC, AND TG AMONG ALL GROUPS

Group	Number of cases (male/female)	BMI (kg/m2)	WHR (cm/cm)	HbA1c (%)	FPG (mmol/L)	TC (mmol/L)	TG (mmol/L)
Group A	30 (14/16)	26.69±4.16	0.88±0.06	6.91±0.48	6.46±0.44	5.12±0.78	1.58±0.85
Group B	33 (17/16)	25.65±3.03	0.88±0.05	6.92±0.58	6.42±0.57	5.40±0.62	1.65±0.77
Group C	29 (14/15)	25.52±2.79	0.87±0.05	6.85±0.47	6.42±0.48	5.28±1.08	1.74±1.12
F	0.155	1.132	0.477	0.197	0.080	0.898	0.245
p	0.926	0.327	0.622	0.821	0.923	0.411	0.784

In summary, the administration of saxagliptin treatment could effectively control blood glucose in patients newly diagnosed with T2DM combined with NAFLD, and its efficacy was no worse than that of glimepiride. In addition, saxagliptin treatment could improve insulin resistance, reduce IL-6 levels, and attenuate inflammatory responses to improve hepatocyte steatosis, protect hepatocytes, and obtain extra benefits other than the hypoglycemic effect in NAFLD patients. However, whether the treatment effect of saxagliptin on the fatty liver is independent of the hypoglycemic effect still requires further studies for investigation.

EXPERIMENTAL

Patients

A total of 95 T2DM patients (48 male and 47 female) who were treated in the Department of Endocrinology of our hospital between July 2014 and December 2016 were selected. All subjects signed an informed consent form. This study was approved by the Research Ethics Committee of Qilu Hospital of Shandong University.

Case inclusion and exclusion criteria

Inclusion criteria: (1) patients who met the World Health Organisation (WHO) 1999 T2DM diagnostic criteria; (2) patients who were newly diagnosed with T2DM or had a disease history of less than 2 years and did not receive hypoglycaemic drug treatment; (3) patients with ages between 30-60 years, body mass index (BMI) between 23-30 kg/m^2 and glycated haemoglobin (HbA1c) between 7%-9%; and (4) patients who met the relevant diagnostic criteria of the *Guidelines for the Management of Non-alcoholic Fatty Liver Disease (2010 revised edition)* by the Chinese Society of Hepatology, Chinese Medical Association and did not receive drug treatment for liver protection. Exclusion criteria: (1) patients with acute complications and severe chronic complications of DM; (2) patients with viral hepatitis, drug hepatitis, autoimmune liver disease, other liver diseases caused by clear damage factors, hepatolenticular degeneration, and total parenteral nutrition; and (3) patients with liver cirrhosis, severe liver and kidney insufficiency, cardio-cerebrovascular diseases, acute infection, and genetic diseases.

Patient grouping and treatment

Subjects were randomly divided into groups A, B, and C according to a computer-generated random number table. Group A received oral saxagliptin at 5 mg once a day (QD), group B received oral glimepiride at 2 mg QD, and group C received oral glimepiride at 2 mg QD and polyene phosphatidylcholine at 456 mg orally three times a day (PO TID) based on providing diet and exercise therapy guidance. The doses of glimepiride for subjects in groups B and C were adjusted based on blood glucose. Patients were observed for 24 w.

Observation indicators

Body weight, height, waist circumference, hip circumference, HbA1c, fasting plasma glucose (FPG), fasting insulin (FINS), interleukin-6 (IL-6), triglyceride (TG), total cholesterol (TC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (γ-GT), and quantitative detection of liver steatosis of study subjects were observed before enrolment and after 12 and 24 w of treatment. In addition, BMI, waist-to-hip ratio (WHR), and homeostatic model assessment of insulin resistance (HOMA-IR) were calculated. Fingertip FPG and two h postprandial blood glucose were measured every two w. Patients with liver fat contents between 5%-10% were considered to have

TABLE 3. COMPARISON OF FINS, HOMA-IR, IL-6, ALT, AST, Γ-GT, AND QUANTITATIVE DETECTION OF LIVER STEATOSIS AMONG ALL GROUPS

Group	Number of cases (male/female)	FINS (μIU/ml)	HOMA-IR	IL-6 (pg/ml)	ALT (U/L)	AST (U/L)	γ-GT (U/L)	Quantitative detection of liver steatosis (%)
Group A	30 (14/16)	7.82±2.14*#	2.25±0.63*#	6.04±4.01*#	22.10±9.25*	16.94±6.43*	30.84±21.30*	14.57±7.78*
Group B	33 (17/16)	9.75±2.34	2.79±0.75	9.84±6.81	29.00±14.62	22.03±10.27	44.21±12.42	20.13±8.18
Group C	29 (14/15)	10.29±2.40	2.94±0.76	10.46±7.56	19.32±9.50*	17.68±5.60*	31.48±28.12*	15.09±9.09*
F	0.155	9.940	8.229	4.470	6.072	3.600	3.999	4.733
p	0.926	0.000	0.001	0.014	0.003	0.031	0.022	0.011

Note: * compared with group B, p<0.05; # compared with group C, p<0.05.

mildly fatty livers, between 11%-30%, were considered to have moderately fatty livers, and above 30% were considered to have severely fatty livers.

STATISTICAL ANALYSES

Analyses were performed using SPSS 21.0 software. Continuous variables conformed to the normal distribution and were expressed as $\bar{x}\pm s$. Based on the distribution features of clinical information among all groups, the comparison of measurement data that conformed to the normal distribution and had homogenous variances was performed using the analysis of variance (ANOVA). The comparison inside a group was performed using the least significant difference (LSD) method. $P<0.05$ indicated that the difference had statistical significance.

Declaration of conflict of interest

None.

RESUMO

OBJETIVO: *Investigar a eficácia clínica e os possíveis mecanismos da saxagliptina no tratamento do diabetes mellitus tipo 2 (DM2) associado à doença hepática gordurosa não alcoólica (DHGNA).*

MÉTODOS: *Um total de 95 DM2 combinados com pacientes com DHGNA foram aleatoriamente divididos em grupo A (grupo saxagliptina), grupo B (grupo glimepirida) e grupo C (glimepirida combinado com grupo fosfatidilcolina polienizada).*

RESULTADOS: *Após a intervenção tratamento por 24 w, índice de massa corporal (IMC), relação cintura-quadril (RCQ), hemoglobina glicada (HbA1c), glicemia de jejum (FPG), insulina de jejum (Fins), avaliação do modelo homeostático de insulina resistência (Homa-IR), interleucina-6 (IL-6), triglicérides (TG), colesterol total (CT), alanina aminotransferase (ALT), aspartato aminotransferase (AST), γ-glutamiltransferase (γ-GT) e detecção de esteatose hepática dos sujeitos do estudo foram observados. Ação de esteatose hepática de indivíduos nos grupos A e C foram significativamente diferentes do grupo B; no entanto, não houve diferenças entre os grupos A e C. Os grupos Fins, Homa-IR e IL-6 dos participantes do grupo A foram menores que os dos grupos B e C; no entanto, não houve diferenças significativas entre os dois últimos grupos.*

CONCLUSÃO: *Para o DM2 combinado com pacientes com DHGNA, o tratamento com saxagliptina pode não apenas controlar efetivamente a glicemia, mas também atenuar a resistência à insulina e a lesão inflamatória do fígado para melhorar ainda mais o fígado gorduroso.*

PALAVRAS-CHAVE: *Inibidores da dipeptidil peptidase IV/uso terapêutico. Diabetes mellitus tipo 2. Interleucina-6. Fígado gorduroso. Hepatopatia gordurosa não alcoólica.*

REFERENCES

1. Gastaldelli A. Insulin resistance and reduced metabolic flexibility: cause or consequence of NAFLD? Clin Sci. 2017;131(22):2701-4.
2. Dai W, Ye L, Liu A, Wen SW, Deng J, Wu X, et al. Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: a meta-analysis. Medicine (Baltimore). 2017;96(39): e8179.
3. Dixon LJ, Berk M, Thapaliya S, Papouchado BG, Feldstein AE. Caspase-1-mediated regulation of fibrogenesis in diet-induced steatohepatitis. Lab Invest. 2012;92(5):713-23.
4. Sugino H, Kumagai N, Watanabe S, Toda K, Takeuchi O, Tsunematsu S, et al. Polaprezinc attenuates liver fibrosis in a mouse model of non-alcoholic steatohepatitis. J Gastroenterol Hepatol. 2008;23(12):1909-16.
5. Irons BK, Weis JM, Stapleton MR, Edwards KL. An update in incretin-based therapy: a focus on dipeptidyl peptidase: 4 inhibitors. Curr Diabetes Rev. 2012;8(3):169-82.
6. Schernthaner G, Durán-Garcia S, Hanefeld M, Langslet G, Niskanen L, Östgren CJ, et al. Efficacy and tolerability of saxagliptin compared with glimepiride in elderly patients with type 2 diabetes: a randomized, controlled study (GENERATION). Diabetes Obes Metab. 2015;17(7):630-8.
7. Bugliani M, Syed F, Paula FMM, Omar BA, Suleiman M, Mossuto S, et al. DPP-4 is expressed in human pancreatic beta cells, and its direct inhibition improves beta cell function and survival in type 2 diabetes. Mol Cell Endocrinol. 2018;473:186-93.
8. Henry RR, Smith SR, Schwartz SL, Mudaliar SR, Deacon CF, Holst JJ, et al. Effects of saxagliptin on beta-cell stimulation and insulin secretion in patients with type 2 diabetes. Diabetes Obes Metab. 2011;13(9):850-8.
9. Kenawy S, Hegazy R, Hassan A, El-Shenawy S, Gomaa N, Zaki H, et al. Involvement of insulin resistance in D-galactose-induced age-related dementia in rats: protective role of metformin and saxagliptin. PLoS One. 2017;12(8):e0183565.
10. Hajighasem A, Farzanegi P, Mazaheri Z. Effects of combined therapy with resveratrol, continuous and interval exercises on apoptosis, oxidative stress, and inflammatory biomarkers in the liver of old rats with non-alcoholic fatty liver disease. Arch Physiol Biochem. 2018;1-8.
11. Qu HJ, Wang L, Jin PH, Gao Q, Zhou JX, Song Y, et al. Value of hydrogen proton magnetic resonance spectroscopy in the determination of liver triglyceride in patients with fatty liver disease and its influencing factors. Zhonghua Gan Zang Bing Za Zhi. 2017;25(11):858-63.
12. Younossi ZM, Stepanova M, Rafiq N, Henry L, Loomba R, Makhlouf H, et al. Nonalcoholic steatofibrosis independently predicts mortality in nonalcoholic fatty liver disease. Hepatol Commun. 2017;1(5):421-8.
13. Dembek A, Laggai S, Kessler SM, Czepukojc B, Simon Y, Kiemer AK, et al. Hepatic interleukin-6 production is maintained during endotoxin tolerance and facilitates lipid accumulation. Immunobiology. 2017;222(6):786-96.
14. Mohammadi S, Karimzadeh Bardei L, Hojati V, Ghorbani AG, Nabiuni M. Anti-inflammatory effects of curcumin on insulin resistance index, levels of interleukin-6, C-reactive protein, and liver histology in polycystic ovary syndrome-induced rats. Cell J. 2017;19(3):425-33.

ORIGINAL ARTICLE

Diabetes control could through platelet-to-lymphocyte ratio in hemograms

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SUMMARY

OBJECTIVE: *Association between type 2 diabetes mellitus and inflammation is well-established. We aimed to study platelet-to-lymphocyte ratio (PLR), a novel inflammatory index derived from hemogram, in diabetic patients in comparison to those in healthy volunteers.*

METHODS: *Medical data of type 2 diabetics that showed up in general outpatient medical clinics of our institution between February 2017 and August 2017 were recorded and analyzed.*

RESULTS: *Median PLR of type 2 diabetic patients was significantly higher than the PLR of healthy controls (p=0.001). Moreover, PLR was significantly and positively correlated with HbA1c (p<0.001, r=0.58), fasting plasma glucose (p<0.001, r=0.49), and c-reactive protein (p=0.003, r=0.30) levels. Type 2 diabetic subjects with proteinuria had significantly higher PLR levels than that of diabetic subjects without proteinuria.*

CONCLUSION: *As an inexpensive and easy to use index, PLR may be useful in predicting the development and control levels of type 2 diabetes mellitus. However, its correlation with HbA1c needs to be validated by larger prospective studies.*

KEYWORDS: *Diabetes mellitus, type 2. Inflammation. Glycated hemoglobin A. Lymphocyte count. Platelet count.*

INTRODUCTION

Type 2 diabetes mellitus (DM) has reached nearly epidemic levels worldwide. Clinical and experimental studies focus on a better understanding of the pathophysiology in order to develop more effective treatment options. Type 2 DM is associated with low-grade inflammation. Moreover, diabetes control levels, such as glycated hemoglobin (HbA1c) levels, can be predicted by circulating inflammatory biomarkers. It is reported in the literature that inflammatory indexes in serum are correlated with HbA1c levels in type 2 diabetic subjects.[1]

Hemogram parameters have attracted great attention from researchers as novel inflammatory parameters. One of them is the platelet-to-lymphocyte ratio, which is derivated through the simple division of the platelet count by the lymphocyte count in a hemogram test. Platelet-to-lymphocyte ratio (PLR) has been proposed as a novel inflammatory marker for

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several cardiac, rheumatologic, and neoplastic conditions.[2-4] Therefore, we hypothesized that PLR could be associated with type 2 DM as well.

The aim of the present study was to compare PLR levels of type 2 diabetic patients to those of healthy controls. We also aimed to research the possible correlation between HbA1c and PLR in patients with type 2 DM.

METHODS

After the institutional board approved the study, we retrospectively analyzed the data of patients with type 2 DM whose admissions to our hospital occurred between February and August 2017. Control subjects enrolled were healthy subjects that visited the clinic for routine check-ups. Age, gender, height, weight, waist circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), diabetes complications (if any), comorbidities, use of anti-diabetic drugs, HbA1c level, hemogram parameters – such as white blood cell count (WBC), lymphocyte count (lym), hemoglobin (Hb), hematocrit (Htc), and platelet count (PLT) – were recorded. Fasting glucose, total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, c- reactive protein (CRP), uric acid levels were also obtained from a biochemical laboratory database of test results. The body mass index (BMI) was simply calculated by dividing the weight in kilograms by the squared height in meters. PLR was measured as a derived parameter by dividing the PLT by lym. Active inflammatory diseases, active infection, malign conditions, pregnancy, age below 18 years, and use of medications that may interfere with platelet count were set as exclusion criteria.

Commercial SPSS software (SPSS 15.0; IBM Inc., Chicago, IL, USA) was used for statistical analysis. Comparison of homogenously distributed variables in study and control groups was conducted by independent samples t-test and values were expressed as a mean ± standard deviation. On the other hand, comparison of non-homogenously distributed variables in study and control groups was made by Mann-Whitney U test and variables expressed as median (minimum-maximum). The comparison of categorical variables in study groups was conducted with the Chi-square test. Pearson's correlation analysis was used for the correlation between study parameters. A p value of < 0.05 was considered as statistically significant.

RESULTS

A total of 93 individuals, 63 diabetic and 30 control subjects, were enrolled in the study. The mean age of the study and control groups were 56 ± 9 years and 45 ± 12 years, respectively. Diabetic subjects were significantly older than healthy controls ($p<0.001$). A total of 33 of 63 diabetic subjects and 21 of 30 healthy controls were women. The sex difference between study and control groups was not statistically significant ($p=0.11$).

Although weight was significantly different between study and control groups ($p<0.001$), height was similar between them ($p=0.08$). Both body mass index and waist circumference were significantly higher in the study group compared to the control group ($p<0.001$ for both). Systolic and diastolic blood pressures were significantly higher in diabetic subjects compared to healthy ones ($p=0.001$ for SBP and $p=0.048$ for DBP). General characteristics of the study and control groups were summarized in table 1.

While there was no significant difference between study and control groups in terms of LDL cholesterol ($p=0.12$), serum creatinine ($p=0.06$), Hb (0.92) and Htc (0.72) levels, total cholesterol ($p=0.02$), HDL cholesterol ($p<0.001$), triglyceride ($p<0.001$), fasting plasma glucose ($p<0.001$), uric acid ($p<0.001$), CRP ($p<0.001$), WBC ($p=0.001$) and PLT ($p<0.001$), values were significantly different between diabetic subjects and healthy controls.

Calculated PLR in type 2 diabetic and control groups were 122 (44-472) and 94 (48-170), respectively. The difference between study and control groups was statistically significant ($p=0.001$). The laboratory data of the study and control subjects were summarized in table 2.

PLR was significantly and positively correlated with HbA1c ($p<0.001$, $r=0.58$), fasting plasma glucose ($p<0.001$, $r=0.49$), and CRP ($p=0.003$, $r=0.30$) levels in Pearson's correlation analysis test. However, PLR was not correlated with BMI, nor with waist circumference. Figure 1 shows the relationship between HbA1c and PLR.

In the subgroup analysis of 63 diabetic patients, 26 had proteinuria of various levels, and 37 had not. The PLR of type 2 diabetics with proteinuria was significantly higher than that of the diabetic patients without it ($p=0.04$). PLR of diabetic subjects with and without retinopathy was similar ($p=0.24$). PLR of diabetic subjects with confirmed diabetic neuropathy

TABLE 1. GENERAL CHARACTERISTICS OF THE STUDY POPULATION

		Study Group	Control Group	p
Gender	Men (n)	30	9	0.11
	Women (n)	33	21	
Mean ± standard deviation				
Age (years)		56 ± 9	45 ± 12	<0.001
Height (m)		1.62 ± 0.9	1.66 ± 0.8	0.08
Weight (kg)		82 ± 13	70 ± 8	<0.001
Body mass index (kg/m²)		31.4 ± 5.4	25.6 ± 4.2	<0.001
Waist circumference (cm)		106 ± 12	89 ±10	<0.001
Median (min-max)				
Systolic blood pressure (mmHg)		120 (90-180)	110 (100-130)	0.001
Diastolic blood pressure (mmHg)		80 (50-110)	70 (50-80)	0.04

TABLE 2. LABORATORY DATA OF THE STUDY POPULATION

	Study group	Control group	p
Median (min-max)			
Fasting plasma glucose (mg/dl)	162 (86-565)	95 (79-107)	<0.001
Serum creatinine (mg/dl)	0.82 (0.63-1.5)	0.75 (0.56-1.05)	0.06
Uric acid (mg/dl)	5.6 (3.2-8.6)	4.5 (2.1-10.4)	<0.001
CRP (U/l)	5 (0.1-45)	2.2 (0.1-11.9)	<0.001
Total cholesterol (mg/dl)	204 (94-325)	168 (114-248)	0.02
LDL- cholesterol (mg/dl)	123 (42-244)	111 (53-162)	0.12
HDL-cholesterol (mg/dl)	45 (25-86)	55 (39-77)	<0.001
Triglyceride (mg/dl)	166 (72-1050)	106 (52-297)	<0.001
WBC (u/mm³)	7.5 (4.4-13.7)	6.3 (3.4-9.7)	0.001
PLT (u/mm³)	245 (141-482)	208 (148-263)	<0.001
PLR	122 (44-472)	94 (48-170)	0.001
Mean ± Standard Deviation			
Hb (g/dl)	13.6 ± 1.6	13.6 ±1.9	0.92
Htc (%)	40 ± 4.6	40.3 ± 2.2	0.72

was not significantly different from that of diabetics without diabetic neuropathy (p=0.29).

DISCUSSION

The present study showed that PLR was significantly higher in type 2 diabetic patients compared to healthy subjects. Another interesting finding was the positive correlation between HbA1c and PLR in diabetic subjects.

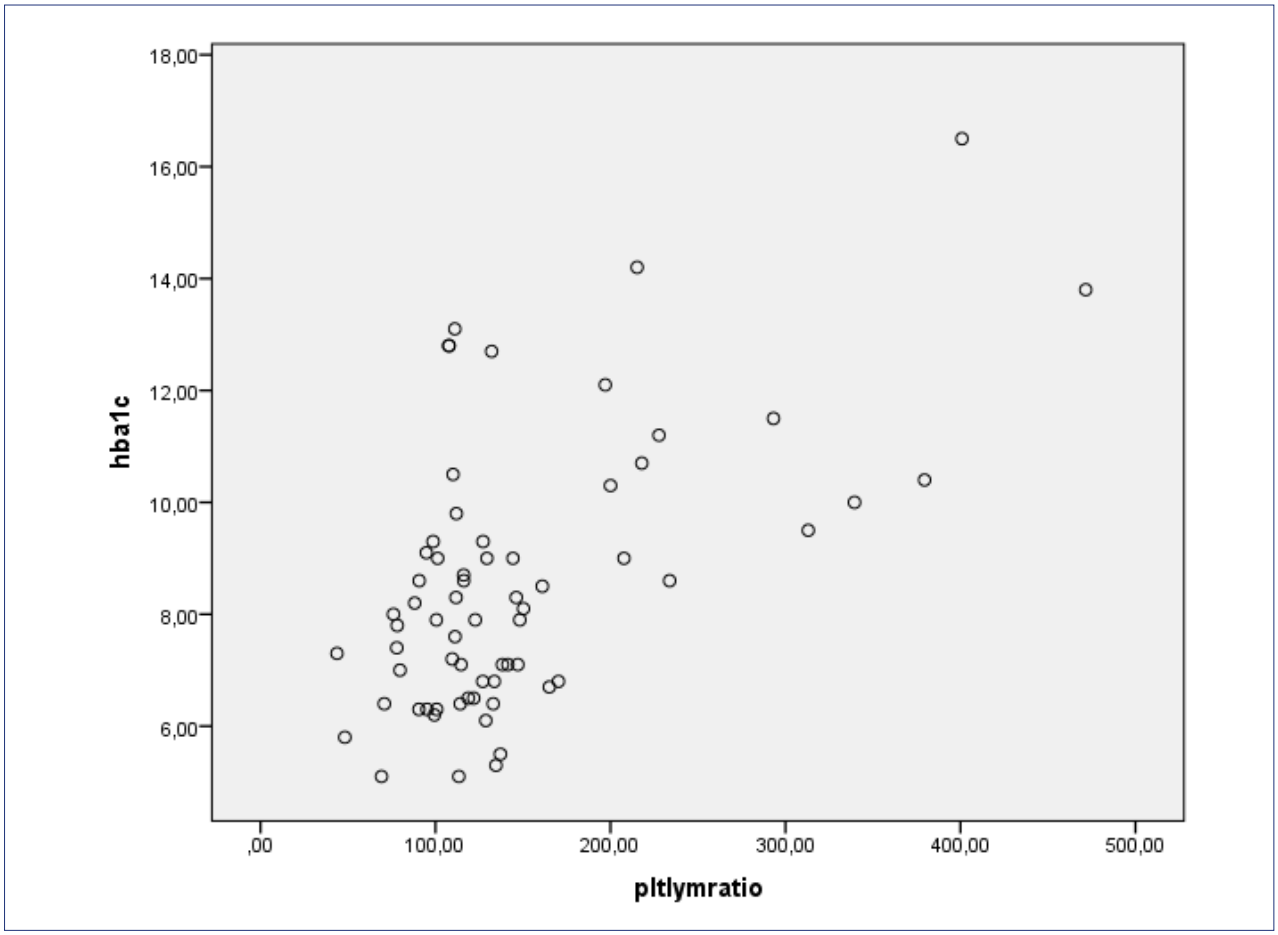
Elevated PLR in patients with type 2 diabetes mellitus may be a reflection of the underlying inflammatory burden of the disease. As HbA1c worsens due to poor diabetes control, underlying chronic low-grade inflammatory status intensifies and thus, inflammatory markers, including PLR, increase.

Pathogenesis of type 2 diabetes mellitus is an inflammatory process.[5] There are many studies in the literature that point to an association between diabetes mellitus and inflammation. For example, Pradhan et al.[6] suggested that an increase in inflammatory markers such as CRP and interleukin-6 may predict the development of diabetes mellitus. In another study, authors concluded that chronic inflammation in type 2 DM was associated with mortality risk.[7] Chronic complications from diabetes, in particular, have been linked to the inflammatory status of the patients.[8,9] Navarro et al.[10] showed that albuminuria was closely associated with inflammatory markers, including high sensitive CRP and tumor necrosis factor- alpha. Similarly, we found that PLR was higher in diabetic patients with proteinuria compared to those without it. However, this report did not show such a relation in diabetic neuropathy and retinopathy, which may be due to the small study population. Therefore, as an inflammatory index, one can expect increased PLR in diabetic subjects.

PLR has been studied in several clinical conditions as a marker of inflammation. Akboga et al.[11] studied PLR in coronary artery disease and concluded that it was cheap and easy to assess inflammatory marker, which was helpful in predicting the severity of coronary artery disease. Moreover, PLR has been suggested as an inflammatory indicator in psoriasis,[12] and rheumatoid arthritis.[13] Beside diseases with a high inflammatory burden, PLR has also been found to be associated with low-grade inflammatory states, such as cancers. Authors claimed that high PLR has predicted poor prognosis in patients with breast cancer.[14] Kokcu et al.[15] reported that PLR was positively correlated with the stage of ovarian cancer. Furthermore, the prognosis of epithelial ovarian cancer could be predicted by an increase in PLR.[16] These data in the literature suggest that PLR is a strong inflammatory index and type 2 DM, as a low-grade inflammatory condition may be associated with elevated PLR.

The inflammatory burden of type 2 diabetes mellitus might be aggravated by poor diabetes control. The positive correlation between PLR and HbA1c reported in the present study suggests this.

FIGURE 1. ASSOCIATION BETWEEN HBA1C AND PLR



The reason for increased PLR in type 2 diabetes mellitus should be explained by an increased platelet count in diabetic patients.[17] Limitations of the present report are its retrospective design and the relatively small study population. However, to the best of our knowledge, this is the first study that found an association between PLR and type 2 diabetes mellitus.

CONCLUSION

The PLR is an inexpensive and simple assessment marker, which can be useful in predicting the development and control level of type 2 diabetes mellitus. However, we believe that its correlation with HbA1c needs to be validated by larger prospective studies.

Conflict of Interest

None

RESUMO

O controle diabético poderia ser predicado por Platelet para a relação de linfócitos no hemograma

OBJETIVO: *A associação entre diabetes mellitus tipo 2 e inflamação está bem estabelecida. Pretendemos estudar a relação plaquetária com linfócitos (PLR), um novo índice inflamatório derivado do hemograma, em pacientes diabéticos e comparar com aqueles em voluntários saudáveis.*

MÉTODOS: *Foram registrados e analisados dados médicos de diabéticos de tipo 2 que apareceram em clínicas ambulatoriais de medicina geral de nossa instituição entre fevereiro de 2017 e agosto de 2017.*

RESULTADOS: *A PLR mediana dos pacientes com diabetes tipo 2 foi significativamente maior que a PLR de controles saudáveis (p=0,001). Além disso, a PLR foi correlacionada de forma significativa e positiva com os níveis de glicemia de jejum (p<0,001, r=0,49) e níveis de proteína c-reativa (p=0,003, r=0,30) com HbA1c (p<0,001, r=0,58). Os indivíduos diabéticos de tipo 2 com proteinúria aumentaram significativamente os níveis de PLR do que os indivíduos diabéticos sem proteinúria.*

CONCLUSÃO: *Como um índice barato e fácil de usar, a PLR pode ser útil para prever o desenvolvimento e controle do nível de diabetes mellitus tipo 2. No entanto, sua correlação com HbA1c precisa ser validada por estudos prospectivos maiores.*

PALAVRAS-CHAVE: *Diabetes mellitus tipo 2. Inflamação. Hemoglobina A glicada. Contagem de linfócitos. Contagem de plaquetas.*

REFERENCES

1. Gustavsson CG, Agardh CD. Inflammatory activity increases with haemoglobin A1c in patients with acute coronary syndrome. Scand Cardiovasc J. 2009;43(6):380-5.
2. Ayça B, Akin F, Çelik Ö, Yüksel Y, Öztürk D, Tekiner F, et al. Platelet to lymphocyte ratio as a prognostic marker in primary percutaneous coronary intervention. Platelets. 2015;26(7):638-44.
3. Balta S, Ozturk C. The platelet-lymphocyte ratio: a simple, inexpensive and rapid prognostic marker for cardiovascular events. Platelets. 2015;26(7):680-1.
4. Peng W, Li C, Zhu WJ, Wen TF, Yan LN, Li B, et al. Prognostic value of the platelet to lymphocyte ratio change in liver cancer. J Surg Res. 2015;194(2):464-70.
5. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nat Rev Immunol. 2011;11(2):98-107.
6. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. Jama. 2001;286(3):327-34.
7. Stehouwer CD, Gall MA, Twisk JW, Knudsen E, Emeis JJ, Parving HH. Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes: progressive, interrelated, and independently associated with risk of death. Diabetes. 2002;51(4):1157-65.
8. Jialal I, Devaraj S, Venugopal SK. Oxidative stress, inflammation, and diabetic vasculopathies: the role of alpha tocopherol therapy. Free Radic Res. 2002;36(12):1331-6.
9. Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care. 2004;27(3):813-23.
10. Navarro JF, Mora C, Maca M, Garca J. Inflammatory parameters are independently associated with urinary albumin in type 2 diabetes mellitus. Am J Kidney Dis. 2003;42(1):53-61.
11. Akboga MK, Canpolat U, Yayla C, Ozcan F, Ozeke O, Topaloglu S, et al. Association of platelet to lymphocyte ratio with inflammation and severity of coronary atherosclerosis in patients with stable coronary artery disease. Angiology. 2016;67(1):89-95.
12. Kim DS, Shin D, Lee MS, Kim HJ, Kim DY, Kim SM, et al. Assessments of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in Korean patients with psoriasis vulgaris and psoriatic arthritis. Dermatol. 2016;43(3):305-10.
13. Fu H, Qin B, Hu Z, Ma N, Yang M, Wei T, et al. Neutrophil-and platelet-to-lymphocyte ratios are correlated with disease activity in rheumatoid arthritis. Clin Lab. 2015;61(3-4):269-73.
14. Krenn-Pilko S, Langsenlehner U, Thurner EM, Stojakovic T, Pichler M, Gerger A, et al. The elevated preoperative platelet-to-lymphocyte ratio predicts poor prognosis in breast cancer patients. Br J Cancer. 2014;110(10):2524-30.
15. Kokcu A, Kurtoglu E, Celik H, Tosun M, Malatyalioglu E, Ozdemir AZ. May the platelet to lymphocyte ratio be a prognostic factor for epithelial ovarian cancer? Asian Pac J Cancer Prev. 2014;15(22):9781-4.
16. Raungkaewmanee S, Tangjitgamol S, Manusirivithaya S, Srijaipracharoen S, Thavaramara T. Platelet to lymphocyte ratio as a prognostic factor for epithelial ovarian cancer. J Gynecol Oncol. 2012;23(4):265-73.
17. Akinsegun A, Akinola Olusola D, Sarah JO, Olajumoke O, Adewumi A, Majeed O, et al. Mean platelet volume and platelet counts in type 2 diabetes: mellitus on treatment and non-diabetic mellitus controls in Lagos, Nigeria. Pan Afr Med J. 2014;18:42.

ORIGINAL ARTICLE

Consumption of animal-based and processed food associated with cardiovascular risk factors and subclinical atherosclerosis biomarkers in men

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SUMMARY

OBJECTIVE: *To evaluate the frequency of food consumption in apparently healthy men and their association with cardiovascular risk factors and biomarkers of subclinical atherosclerosis.*

METHODS: *In this observational study, 88 men had their food standard obtained through the food frequency questionnaire (FFQ). Associations of dietary patterns with cardiovascular risk factors, such as anthropometric data, laboratory and clinical evaluations, carotid-femoral arterial stiffness (IMT) and pulse wave velocity were evaluated.*

RESULTS: *The highest values were observed, for most of the risk factors evaluated, with the highest frequency of weekly consumption of dairy products, meats, sweets, fats, cold meats, sodas, milk and white chocolate; and lower frequency of weekly consumption of fruits, cereals, vegetables, legumes, oilseeds, and soy. There was no significant difference for coffee and dark chocolate*

CONCLUSIONS: *A diet with high consumption of animal products has a higher correlation with cardiovascular risk factors; the opposite is true for the consumption of plant-based food, associated with the profile of more favorable biomarkers for cardiovascular health and better biochemical and structural parameters.*

KEYWORDS: *Atherosclerosis. Food Consumption. Pulse Wave Analysis. Vascular Stiffness.*

INTRODUCTION

Chronic non-communicable diseases (CNCD) are the leading cause of disability. Among them are included cardiovascular disease, responsible for 30% of deaths worldwide. Epidemiological data suggest that individuals who follow diets rich in fruits and vegetables have a lower risk of CNCD and mortality than those who follow diets poor in vegetables[1]. Several studies have shown a connection between the consumption of meat and hypertension, risk of heart disease, metabolic disorders, and mortality[2-9]. The relationship between food and diseases in groups who follow specific diets, such as vegetarians, have required the attention of scholars. In the past, the fo-

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cus was on evidencing the problems caused by the deficiency of certain nutrients; now, it is evident a tendency to study the possible health benefits of a diet completely or partially plant-based, such as the ovo-lacto vegetarian diet[10].

Recent studies show the link between diet and nutritional state with the occurrence of CNCD[10,11]. Despite vast clinical and epidemiological evidence that diet has a direct effect on the onset of chronic diseases, the mechanisms of action are not yet clearly understood, especially regarding cardiovascular risk[12,13]. Furthermore, some studies have investigated the relationship between the consumption of certain nutrients or food and the intima-media thickness[14,15] and the pulse wave velocity[16].

Due to the scientific interest in food consumption, healthy diet, cardiovascular health, and the scarcity of studies that carry out that analysis, the objective of this study was to assess the frequency of food consumption in men who were apparently healthy and its link with cardiovascular risk.

METHODS

Study population

In this cross-sectional observational study, 745 adult volunteers were recruited in the city of São Paulo, Brazil, through social activities and internet publicity, initially between October 2012 and June 2013. The participants filled out questionnaires on family and personal medical history, diet preferences, physical activity, formal education, and personal data. The exclusion criteria were: 1) female; 2) under 35 years old; 3) history of diabetes; 4) history of dyslipidemia; 5) history of cardiovascular or cerebrovascular diseases; 6) history of hypertension or of use of antihypertensive medication; and 7) smoking. All individuals that declared to be smokers or "occasional smokers," or who had quit smoking one month before the interview were considered smokers. The sample consisted of men who were apparently healthy in order to prevent confounding risk factors for subclinical atherosclerosis.

Healthy participants who were ≥35 years old were allocated into two groups – VEG and OMNI –, according to their diets. VEG men were defined as having had a vegetarian diet free of meat, fish, and poultry for at least four years; these men could be ovo-lacto vegetarian (consumption of eggs, milk and dairy), lacto-vegetarians (consumption of milk and dairy), or vegan (no consumption of eggs, milk, or dairy). OMNI men were defined as those who consume any type of meat, at least five portions or more per week. During the period from July 2013 to January 2014, after the inclusion and exclusion criteria were applied, 88 men, ≥35 years old, apparently healthy were included in the study (44 vegetarians and 44 omnivores). The original study design had the intention of studying the link between a vegetarian and omnivore diet on cardiovascular risk factors; however, in this study, our strategy was to consider both groups as a whole. The research committee of the Heart Institute (InCor) of the Hospital of the Faculty of Medicine of the University of São Paulo approved the protocol of the study, and all participants signed an Informed Consent Form to participate in the study.

Laboratory and clinical analysis, ultrasonography of the carotid, pulse wave velocity (PWV)

The methodology of these variables is described in the previous publication of the CARVOS Study[17].

Anthropometric assessment

For the anthropometric assessment, we used the Body Mass Index (BMI) and the bioelectrical impedance analysis (BIA). To assess the level of physical activity we used the International Physical Activity Questionnaire-Short Form (Ipaq). The methodology is described in the CARVOS[18] study on body composition.

The assessment of the frequency of food consumption

The individuals were interviewed, and a food frequency questionnaire (FFQ) was applied to assess the pattern of consumption of food groups.

Each individual was asked: Over the last seven days, in how many days did you consume the following food or beverages? The following alternatives were possible: Did not eat over the last seven days; 1 day over the last seven days; 2 days over the last seven days; 3 days over the last seven days; 4 days over the last seven days; 5 days over the last seven days; 6 days over the last seven days; and all seven days.

The food groups assessed were: 1. Grains, tubers, and roots (rice, corn, bread, cookies, pasta, pastry, potato, manioc); 2. Leafy greens (chard, lettuce, broccoli, collard greens, kale, spinach, cabbage, arugula, etc.); 3. Vegetables (pumpkins, beet, carrot, chayote, cucumbers, tomato, etc.); 4. Fresh fruits (pineap-

ple, banana, apple, papaya, strawberry, grape, fruit salad, etc.); 5. Legumes (beans, peas, lentils, chick-peas, soy); 6. Oilseeds (nuts, almonds, pistachios, linseed, sunflower seeds); 7. Dairy products (milk, yogurt, and cheese); 8. Meat (poultry, beef, pork, game – such as rabbit and pheasant, etc.); 9. Fish and seafood (shrimp, crab, lobster, etc.); 10. Eggs (fried, boiled, poached, etc.). 11. Cold meats (sausage, salami, hamburger, bologna, ham, turkey breast, etc.); 12. Soy-based food and drinks (soy-based drinks, textured vegetable protein, etc.); 13. Fats (fried food, such as potato or manioc, croquettes, hand pies); 14. Sweets (candy, gum, candied fruit, etc.); 15. Soda (not including diet or light ones); 16. Milk Chocolate; 17. Dark chocolate; 18. White chocolate; and 19. Coffee.

The instrument of food consumption frequency used was based on Sisvan, which according to the Ministry of Health[19] is similar to versions used in research for monitoring practices that pose a risk to health, such as the North-American Youth Risk Behavior Survey - YRBS, carried out by the Center for Disease Control and Prevention - CDC. Moreover, we considered the questionnaire used in Vigitel (Vigilance of Chronic Diseases through Phone Survey), conducted by the Department of Health Vigilance of the Ministry of Health in Brazil.

In this study, the frequency of food consumption was categorized in 0-3 times per week and 4-7 times per week; and the tables and images were divided in plant-based, animal-based, and processed/homemade food.

Statistical analysis

The variables analyzed in this study were: systolic blood pressure (SBP), diastolic blood pressure (DBP), thickness of the intima-media thickness (IMT), pulse wave velocity (PWV), Framingham risk score, percentage of lean body mass (%LBM), percentage of body fat (%BF), percentage of total body water (%TBW), total cholesterol (TC), low-density lipoprotein (LDL), TC/HDL ratio, triglycerides (TG), glucose (GL), measurement of glycated hemoglobin (Glyc-Hb), apolipoprotein B (ApoB), C-reactive protein (CRP).

The continuous variables are presented as Standard Deviation (SD) ± Means. T-tests and chi-square tests were used to test differences in numeric and nominal variables.

All the calculations were made using the Stata software, version 10.0.

TABLE 1. Mean values of cardiovascular risk factors, according to the frequency of consumption of products of plant origin.

Frequency of consumption			
Variable	0 to 3x/week	4 to 7x/week	P value
Fruits			
	n=18	n=70	
Diastolic blood pressure	85.5 (±11.49)	78.40 (±9.63)	0.004
Glycaemia	102.94 (±20.33)	97.90 (±7.56)	0.049
PCR	4.56 (±9.55)	1.74 (±2.72)	0.016
Cereals			
	n=3	n=85	
Total cholesterol	243.67 (±37.81)	189.60 (±38.42)	0.009
LDL	163.00 (±29.82)	117.73 (± 33.14)	0.011
Percentage of lean mass	71.80 (±7.92)	78.94 (±5.73)	0.019
Percentage of fat mass	28.20 (±7.92)	21.12 (±5.97)	0.024
Percentage of water	51.88 (±5.99)	57.42 (±4.65)	0.024
Framingham score	8.33 (±6.81)	3.74 (±4.45)	0.043
Vegetables			
	n=22	n=66	
Diastolic blood pressure	83.41 (±11.75)	78.67 (±9.68)	0.031
Total cholesterol/HDL	4.83 (±1.45)	4.17 (±1.28)	0.021
Triglycerides	160.77 (±77.79)	117.15 (±63.73)	0.005
Glycated haemoglobin	5,57 (±0.48)	5.39 (±0.34)	0.029
Percentage of lean mass	76.76 (±6.26)	79.32 (±5.69)	0.041
Greens			
	n=20	n=68	
Diastolic blood pressure	83.30 (±11.79)	78.84 (±9.79)	0.045
Total cholesterol/HDL	4.88 (±1.42)	4.17 (±1.29)	0.019
LDL	43.05 (±10.47)	47.54 (±10.37)	0.046
Triglycerides	157.70 (±77.39)	119.34 (±65.33)	0.014
Glycaemia	103.10 (±18.67)	97.63 (±7.61)	0.028
Glycated haemoglobin	5.60 (±0.51)	5.39 (±0.33)	0.013
Percentage of fat mass	23.85 (±6.64)	20.66 (±5.84)	0.022
Percentage of water	55.48 (±3.48)	57.73 (±5.00)	0.035
Legumes			
	n=19	n=69	
Total cholesterol/HDL	4.79 (±1.54)	4.21 (±1.27)	0.046
LDL	131.32 (±28.57)	115.96 (±34.68)	0.04
Oilseeds			
	n=54	n=34	
Systolic blood pressure	126.37 (±15.12)	121.15 (±10.87)	0.042
Diastolic blood pressure	81.57 (±10.57)	77.12 (±9.57)	0.024
Total cholesterol	197.54 (±35.94)	181.76 (±43.23)	0.033
Total cholesterol/HDL	4.58 (±1.24)	3.94 (±1.42)	0.013
LDL	124.11 (±29.73)	110.79 (±38.58)	0.031
Triglycerides	138.67 (±65.43)	111.21 (±73.82)	0.035
Glycated haemoglobin	5.50 (±0.43)	5.33 (±0.28)	0.021
IMT	662.04 (±114.88)	572.35 (±98.35)	0
VOP	7.62 (±0.85)	7.04 (±0.75)	0
Framingham score	4.68 (±5.34)	2.65 (±2.58)	0.02
Soy and derivatives			
	n=73	n=15	
Total cholesterol/HDL	4.48 (±1.35)	3.64 (±1.13)	0.013
LDL	122.04 (3±3.24)	105.80 (±34.97)	0.045

RESULTS

In the present study, we analyzed 88 individuals, with a mean age of 46.1 (±8.68). According the body mass index, 54.5% (n=48) had adequate weight and 29.5% (n=26) presented risk of obesity. Regarding the level of formal education, 47.7% (n=42) had higher education, and 33% secondary education. Most individuals, 69.3% (n=61) had a high level of physical activity.

In Table 1, which shows the variables with plant-based food, we can see that those who eat fruits 4-7 times a week have lower glucose, CRP, and DBP values. For grains, those who consume it more times per week have lower values for LDL, TC, and Framingham risk score. Individuals who eat vegetables more times per week have lower TG, glyc-Hb, TC/HDL, DBP. Those who eat leafy greens more times per week have lower values for DBP, TC/HDL, TG, GL, glyc-Hb, and higher HDL for legumes, those who consume them more times per week have lower TC/HDL and LDL. As for oilseeds, those who consume them more times per week have lower SBP, DBP, TC, TC/HDL, LDL, TG, Glyc-Hb, IMT, PWV, and Framingham score. For soy, lower TC/HDL and LDL.

TABLE 2. Mean values of cardiovascular risk factors, according to frequency of consumption of products of animal origin.

Frequency of consumption			
Variable	0 to 3x/week	4 to 7x/week	P value
	Dairy		
	n=36	n=52	
HDL	49.19 (±11.35)	44.67 (±9.56)	0.023
Triglycerides	109.86 (±63.41)	140.65 (±71.62)	0.02
Glycated haemoglobin	5.34 (±0.33)	5.51 (±0.41)	0.02
Haemoglobin	15.89 (0.96)	15.45 (±0.95)	0.022
Framingham score	2.92 (±3.89)	4.58 (±4.90)	0.046
	Meats		
	n=50	n=38	
Total Cholesterol/HDL	3.98 (±1.27)	4.80 (±1.31)	0.002
Triglycerides	114.66 (±71.06)	145.68 (±64.57)	0.018
Glycaemia	95.71 (±7.36)	102.97 (±13.92)	0.001
Glycated haemoglobin	5.34 (±0.28)	5.57 (±0.47)	0.002
Systolic blood pressure	120.28 (±11.56)	129.71 (±14.81)	0
Diastolic blood pressure	76.20 (±8.43)	84.66 (±10.83)	0
Percent of fat mass	19.73 (±4.74)	23.61 (±7.11)	0.001
Water percentage	58.17 (±4.75)	55.95 (±4.57)	0.017
IMT	596.10 (±97.10)	668.55 (±128.52)	0.001
VOP	7.21 (±0.85)	7.64 (±0.83)	0.009
	Eggs		
	n=74	n=11	
Percentage of fat mass	20.91 (±5.76)	24.44 (±7.84)	0.037

The group of individuals that indicated the highest frequency of consumption of dairy products presented lower values for HDL, and HB; and higher values for TG, Glyc-Hb, and Framingham score. In the category of greater frequency of consumption of meats, we found higher values for TC/HDL, TG, GL, Glyc-Hb, SBP, DBP, PWV, and IMT. It was not possible to carry out the analysis of fish consumption for only two men in the sample indicated a regular consumption of the item from 4-7 times per week (Table 2).

For the consumption of sweets, higher TC/HDL, TG, ApoB; and lower HDL. For fat, higher DBP, SBP, TC, TC/HDL, TG, GL, Glyc-Hb, HB, Apo B, CRP. For cold meats, higher GL and CRP. For sodas, higher DBP, TC/HDL, LDL, TG, GL, Apo B, CRP, IMT. For milk chocolate, higher SBP and Glyc-Hb. For white chocolate, higher Glyc-Hb.

The pattern observed in most risk factors assessed was of higher values for higher weekly consumption of sweets, fats/fried foods, cold meats, sodas, white and milk chocolate. There was no significant difference for coffee and dark chocolate (Table 3).

Regarding body composition, we found that those who consume more grains more times per week have lower %BF and higher %LBM and water; those who eat vegetables more often have higher %LBM; and those who consume more leafy greens have lower %BF and higher %water (Figure 1). As for animal-based products, for a more often consumption of meat we found a higher %BF and lower water; for eggs, higher %BF (Figure 2). The group of individuals the indicated a higher frequency for the consumption of sweets presented higher %BF; for fats/fried food, sodas, milk, and white chocolate, we found higher %BF, and lower %LBM and water (Figure 3).

DISCUSSION/CONCLUSION

There are many clinical and epidemiological studies on the influence of diet energy and nutrients over the health state and incidence of diseases. However, individuals do not consume nutrients in isolation but combined in the food that makes up their diet patterns[19]. In the combination of macro and micronutrients, antioxidants and bioactive substances present in food can have a synergy effect in the organism, with a different effect from isolated nutrients. In this context, the present study on the influence of the

TABLE 3. Mean values of cardiovascular risk factors, according to frequency of consumption of sweets, fats and ultraprocessed foods.

	Frequency of consumption		
Variable	0 to 3x/weeks	4 to 7x/week	P Value
	Sweets		
	n=60	n=28	
Total Cholesterol/HDL	4.13 (±1.20)	4.77 (±1.54)	0.018
HDL	48.27 (±10.76)	42.79 (±9.04)	0.01
Triglycerides	119.17 (±66.36)	147.11 (±73.98)	0.039
Apob	0.91 (±0.27)	1.03 (±0.29)	0.028
Percentage of fat mass	20.62 (±5.96)	23.00 (±6.28)	0.047
	Fats/Fried		
	n=74	n=14	
Systolic blood pressure	123.09 (±13.39)	131. 00 (±14.56)	0.024
Diastolic blood pressure	78.05 (±8.55)	89.36 (±13.95)	0
Total cholesterol	187.66 (±39.93)	211. 43 (±30.74)	0.019
Total Cholesterol/HDL	4.18 (±1.27)	5.13 (±1.47)	0.007
Triglycerides	119.45 (±64.25)	173. 57 (±81.62)	0.003
Glycaemia	97. 85 (±7.51)	104. 29 (±22.10)	0.024
Glycated haemoglobin	5.40 (±0.34)	5.61 (±0.57)	0.031
Haemoglobin	15.53 (±0.97)	16.27 (±0.77)	0.01
Apob	0.92 (±0.28)	1.09 (±0.19)	0.019
PCR	1.87 (±2.79)	4.65 (±10.81)	0.028
Percentage of lean mass	79.16 (±5. 84)	75. 82 (±5.74)	0.034
Percentage of fat mass	20.91 (±6.11)	24.18 (±5.74)	0.043
Percentage of water	57.65 (4.82)	54. 62 (±3.69)	0.02
	Sausages		
	n=76	n=11	
Glycaemia	98. 05 (±7.70)	104. 64 (±24.58)	0.034
PCR	1.93 (±2.79)	5.05 (±12.21)	0.026
	Soft Drinks		
	n=79	n=9	
Diastolic blood pressure	78.78 (±9.66)	89.22 (±12.30)	0.001
Total cholesterol/HDL	4.23 (±1.32)	5.24 (±1.27)	0.016
LDL	116.77 (±33.43)	141.22 (±31.55)	0.019
Triglycerides	121. 20 (±66.14)	188.22 (±75.01)	0.002
Glycaemia	98.15 (±7.57)	105. 22 (±27.59)	0.037
Apob	0.92 (±0.27)	1.20 (±0.21)	0.001
PCR	1.84 (±2.74)	6.50 (±13.31)	0.003
Percentage of lean mass	79.14 (±5.71)	74. 32 (±6.33)	0.013
Percentage of fat mass	20.93 (±5.98)	25.67 (±6.33)	0.018
Percentage of water	57.57 (±4.72)	53.86 (±4.17)	0.017
IMT	618.26 (±114.05)	707.50 (±115. 97)	0.014
	Milk Chocolate		
	n=79	n=9	
Systolic blood pressure	123.53 (±13.57)	131.56 (±14.61)	0.049
Glycated haemoglobin	5.4 (0.37)	5.72 (0.40)	0.009
Percentage of lean mass	79.20 (±5.68)	74.38 (±6.40)	0.009
Percentage of fat mass	20.87 (±5.95)	25.61 (±6.39)	0.013
Percentage of water	57.62 (±4.70)	53.90 (±4.29)	0.013
	White Chocolate		
	n=83	n=2	
Glycated haemoglobin	5.42 (±0.38)	5.95 (±0.21)	0.029
Percentage of lean mass	78. 92 (±5.79)	69.35 (±2.33)	0.011
Percentage of fat mass	21.15 (±6.03)	30.60 (±2.40)	0.015
Percentage of water	57.38 (±4.72)	50.69 (±0.37)	0.024

consumption of food groups over cardiovascular risk factors can be considered promising.

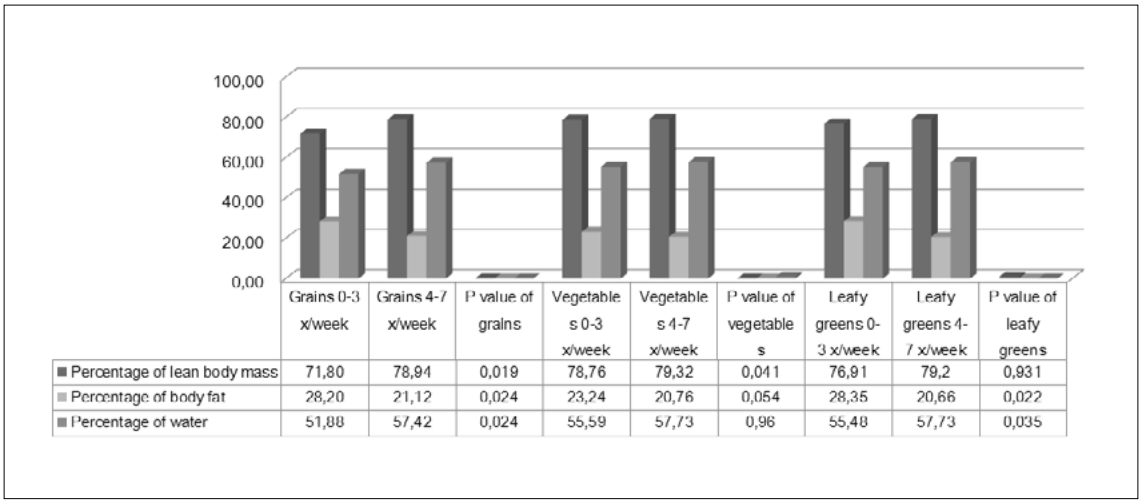
This way, some studies have verified an association between diet patterns and chronic diseases, such as obesity[20], endothelial function, and inflammation[21], and some types of cancer, amongst them oral[22], gastric[23], and cerebral[24].

In the present study, we found significant differences in the cardiovascular risk factors assessed according to the frequency of consumption of specific food items, with the exception of fish, coffee, and dark chocolate. Some studies have investigated food consumption and cardiovascular risk factors. Kerver et al.[25] found that the food pattern named "Western," characterized by the high consumption of processed meats, eggs, red meat, and dairy products with high fat levels, is positively associated with serum levels of insulin, C-peptide, and glycated hemoglobin. As for the pattern know as "Healthy American" (high amounts of leafy green vegetables, salad dressing, tomatoes, and other vegetables, and tea), there was no correlation found with the biomarkers analyzed.

In the study by Centritto et al.[26], three patterns of diet were categorized. The "Olive Oil and Vegetables" pattern, characterized by the high intake of olive oil, vegetables, soup, fruit, and fish, was associated with relatively low values of glucose, fats, CRP, arterial pressure, and individual cardiovascular risk scores. The "Meats and Pasta" pattern, characterized by a high intake of pasta, tomato sauce, red meat, animal fat, and alcohol, was positively associated with blood glucose, fats, CRP, and cardiovascular risk scores. The "Eggs and Sweets" pattern, characterized by positive loads of eggs, processed meats, margarine, butter, sugar, and sweets, was associated with high CRP values.

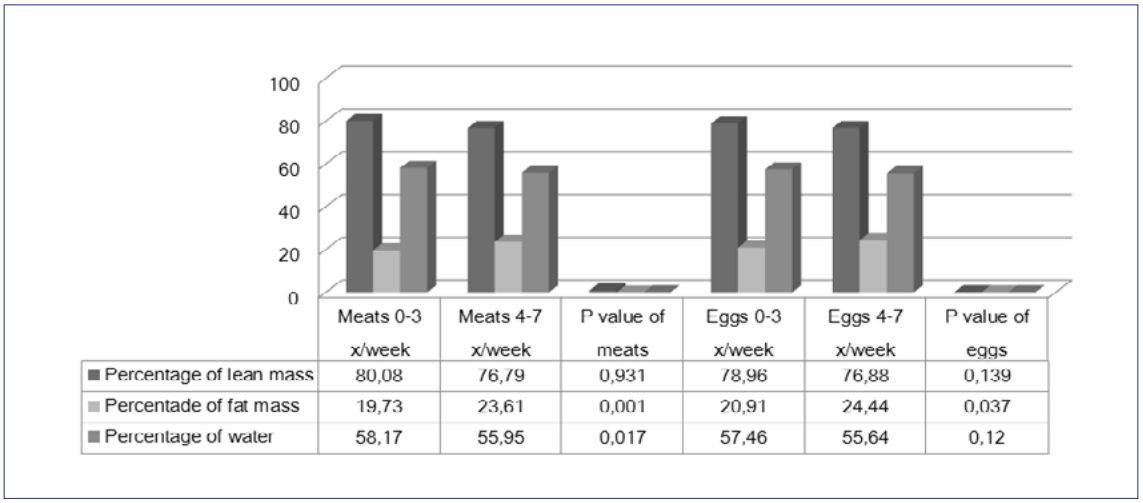
There was also a study conducted with the Brazilian population. Olinto et al.[27] classified the food consumption into two patterns: the first one, the Average Brazilian, characterized by sugar, white bread, coffee, margarine/butter, rice, and black beans. The second, the Processed Food, was characterized by red and processed meats, salty snacks, french fries, beer, soda, and other processed food. The Average Brazilian was inversely associated with values of LDL, HDL, and total cholesterol in men. Amongst women, they found tendencies of an inverse association with SBP, DBP, LDL, HDL, and TC. The patter of Processed Food was positively associated with values of LDL, HDL, and TC amongst men. Amongst wom-

FIGURE 1. MEAN VALUES OF BODY COMPOSITION ACCORDING TO THE FREQUENCY OF CONSUMPTION OF PLANT-BASED FOOD.



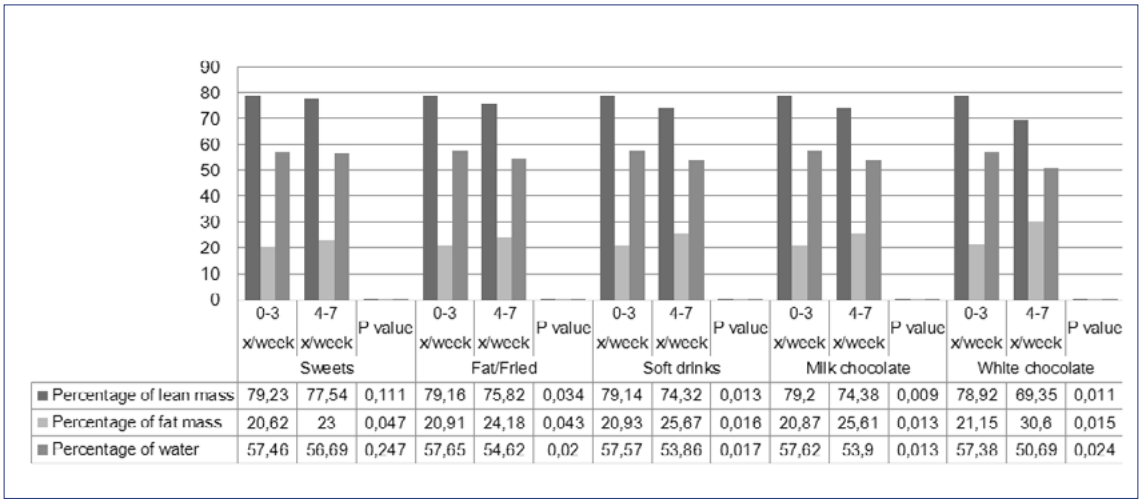
	Grains 0-3 x/week	Grains 4-7 x/week	P value of grains	Vegetables 0-3 x/week	Vegetables 4-7 x/week	P value of vegetables	Leafy greens 0-3 x/week	Leafy greens 4-7 x/week	P value of leafy greens
Percentage of lean body mass	71,80	78,94	0,019	78,76	79,32	0,041	76,91	79,2	0,931
Percentage of body fat	28,20	21,12	0,024	23,24	20,76	0,054	28,35	20,66	0,022
Percentage of water	51,88	57,42	0,024	55,59	57,73	0,96	55,48	57,73	0,035

FIGURE 2. MEAN VALUES OF BODY COMPOSITION, ACCORDING TO FREQUENCY OF CONSUMPTION OF PRODUCTS OF ANIMAL ORIGIN.



	Meats 0-3 x/week	Meats 4-7 x/week	P value of meats	Eggs 0-3 x/week	Eggs 4-7 x/week	P value of eggs
Percentage of lean mass	80,08	76,79	0,931	78,96	76,88	0,139
Percentade of fat mass	19,73	23,61	0,001	20,91	24,44	0,037
Percentage of water	58,17	55,95	0,017	57,46	55,64	0,12

FIGURE 3. MEAN VALUES OF BODY COMPOSITION, ACCORTDING TO FREQUENCY OF ULTRAPROCESSED FOOD.



	Sweets			Fat/Fried			Soft drinks			Milk chocolate			White chocolate		
	0-3 x/week	4-7 x/week	P value	0-3 x/week	4-7 x/week	P value	0-3 x/week	4-7 x/week	P value	0-3 x/week	4-7 x/week	P value	0-3 x/week	4-7 x/week	P value
Percentage of lean mass	79,23	77,54	0,111	79,16	75,82	0,034	79,14	74,32	0,013	79,2	74,38	0,009	78,92	69,35	0,011
Percentage of fat mass	20,62	23	0,047	20,91	24,18	0,043	20,93	25,67	0,016	20,87	25,61	0,013	21,15	30,6	0,015
Percentage of water	57,46	56,69	0,247	57,65	54,62	0,02	57,57	53,86	0,017	57,62	53,9	0,013	57,38	50,69	0,024

en, the Processed Food pattern was not significantly associated with cardiovascular risk factors.

In the clinical practice, the assessment of food consumption has the purpose of helping with the development and implementation of nutritional planning[28]. There are several methods for assessing food intake and nutrient consumption, and it is important to choose the one that aims at promoting health, preventing injuries, and adjusting the nutritional state of the patient. In this sense, the method of food consumption or food groups is the food frequency questionnaire (FFQ)[28].

The FFQ is considered to be the most practical and informative method for assessment in studies that investigate the connection between diet and clinical outcomes, usually associated with CNCD[28].

The fact that this is a cross-sectional study compromises its causality proof. In addition, there is a restricted number of individuals. However, the sample is highly homogenized in sex and age, and reproducible methods were used.

The present study was conducted with a sample of apparently healthy men who make up a large portion of the population and which is of great interest in terms of prevention of chronic diseases since these are associated with inadequate food consumption parameters.

CONCLUSION

In the present study, higher consumption of plant-based food was less associated with cardiovascular risk factors; the contrary was found for animal-based food, sweets, fats, and ultra-processed food, with a higher association to cardiovascular risk factors, the higher the weekly frequency of their consumption.

RESUMO

OBJETIVO: *Avaliar a frequência do consumo alimentar de indivíduos homens aparentemente saudáveis e a associação desta com fatores de risco cardiovascular e biomarcadores de aterosclerose subclínica.*

MÉTODOS: *Neste estudo observacional, 88 homens tiveram o padrão alimentar obtido por meio do questionário de frequência alimentar (QFA). Foram avaliadas as associações dos padrões alimentares com os fatores de risco cardiovascular, como dados antropométricos, avaliações laboratoriais e clínica, rigidez arterial determinada pela carótida-femoral (IMT) e velocidade da onda de pulso (VOP).*

RESULTADOS: *O padrão observado para a maioria dos fatores de risco avaliados foi de valores mais altos, segundo maior frequência de consumo semanal de lácteos, carnes, doces, gorduras/frituras, embutidos, refrigerantes, chocolates ao leite e branco; e de menor frequência de consumo semanal de frutas, cereais, legumes, verduras, leguminosas, oleaginosas e soja. Não houve diferença significativa para café e chocolate amargo.*

CONCLUSÕES: *Uma dieta com alto consumo de produtos animais apresenta maior correlação com fatores de risco cardiovascular, sendo o oposto para o consumo de alimentos de origem vegetal, associado ao perfil de biomarcadores de saúde cardiovascular mais favorável e melhores parâmetros bioquímicos e estruturais.*

PALAVRAS-CHAVE: *Aterosclerose. Consumo de alimentos. Análise de onda de pulso. Rigidez vascular.*

REFERENCES

1. Silva LB, Silva SS, Marcílio AG, Pierin AM. Prevalence of arterial hypertension among Seventh-Day Adventists of the São Paulo state capital and inner area. Arq Bras Cardiol. 2012;98(4):329-37.
2. Acosta-Navarro JC, Prado SMC, Sanchez DE, Ayala CC, Cabezas JT, Mejia ZP, et al. Pressão sanguínea, perfil lipídico e outros parâmetros bioquímicos entre peruanos vegetarianos, semi-vegetarianos e omnívoros. O estudo Lima. An Paul Med Cir. 1998;125(3):87-101.
3. Acosta-Navarro JC, Prado SC, Guimarães G, Martins M, Caramelli B. Vegetarians and semi-vegetarians are less exposed to cardiovascular risk factors. Int J Atheroscler. 2006;1(1):48-54.
4. Navarro JA, Caramelli B. Vegetarians from Latin America. Am J Cardiol. 2010;105(6):902.
5. Craig WJ. Nutrition concerns and health effects of vegetarian diets. Nutr Clin Pract. 2010;25(6):613-20.
6. Sticher MA, Smith CB, Davidson S. Reducing heart disease through the vegetarian diet using primary prevention. J Am Acad Nurse Pract. 2010;22(3):134-9.
7. Huang T, Yang B, Zheng J, Li G, Wahlqvist ML, Li D. Cardiovascular disease mortality and cancer incidence in vegetarians: a meta-analysis and systematic review. Ann Nutr Metab. 2012;60(4):233-40.
8. Pettersen BJ, Anousheh R, Fan J, Jaceldo-Siegl K, Fraser GE. Vegetarian diets and blood pressure among white subjects: results from the Adventist Health Study-2 (AHS-2). Public Health Nutr. 2012;15(10):1909-16.
9. Navarro JC, Prado SM, Cárdenas PA, Santos RD, Caramelli B. Pre-historic eating patterns in Latin America and protective effects of plant-based diets on cardiovascular risk factors. Clinics (Sao Paulo). 2010;65(10):1049-54.
10. Brasil. Ministério da Saúde. Departamento de Atenção Básica. Guia alimentar para a população brasileira. 2ª ed. Brasília: Ministério da Saúde; 2014.
11. Casado L, Vianna LM, Thuler LCS. Fatores de risco para doenças crônicas não transmissíveis no Brasil: uma revisão sistemática. Rev Bras Cancerol. 2009;55(4):379-88.
12. Dourado KF, Campos FACS, Rojas HF, Simões SKS, Siqueira LP. Estado nutricional, estilo de vida e risco cardiovascular de ovolactovegetarianos e onívoros. ALAN. 2010;60(3):220-6.

13. Teixeira RCMA, Molina MCB, Zandonade E, Mill JG. Cardiovascular risk in vegetarians and omnivores: a comparative study. Arq Bras Cardiol. 2007;89(4):237-44.

14. Sala-Vila A, Romero-Mamani ES, Gilabert R, Núñez I, de la Torre R, Corella D, et al. Changes in ultrasound-assessed carotid intima-media thickness and plaque with a Mediterranean diet: a substudy of the PREDIMED trial. Arterioscler Thromb Vasc Biol. 2012;34(2):439-45.

15. Cai Y, Guo K, Chen C, Wang P, Zhang B, Zhou Q, et al. Soya isoflavone consumption in relation to carotid intima-media thickness in Chinese equol excretors aged 40–65 years. Br J Nutr. 2012;108(9):1698-704.

16. Chan HT, Chan YH, Yiu KH, Li SW, Tam S, Lau CP, et al. Worsened arterial stiffness in high-risk cardiovascular patients with high habitual carbohydrate intake: a cross-sectional vascular function study. BMC Cardiovasc Disord. 2014;14:24.

17. Acosta-Navarro J, Antoniazzi L, Oki AM, Bonfim MC, Hong V, Acosta-Cardenas P, et al. Reduced subclinical carotid vascular disease and arterial stiffness in vegetarian men: The CARVOS Study. Int J Cardiol. 2017;230:562-6.

18. Acosta-Navarro JC, Oki AM, Gouveia LAG, Hong V, Bonfim MC, Acosta-Cardenas P, et al. Healthier body composition in vegetarian men compared to omnivorous men. J Nutr Food Sci. 2016;6:529.

19. Brasil. Ministério da Saúde. Secretaria de Atenção à Saúde. Departamento de Atenção Básica. Protocolos do Sistema de Vigilância Alimentar e Nutricional – SISVAN na assistência à saúde. Brasília: Ministério da Saúde; 2008.

20. Sichieri R. Dietary patterns and their associations with obesity in the Brazilian city of Rio de Janeiro. Obes Res. 2002;10(1):42-8.

21. Thomazella MCD. Efeito da dieta tipo Mediterrânea na função endotelial e inflamação da aterosclerose: estudo comparativo com a dieta TLC (Therapeutic Lifestyle Changes, no NCEP–ATPIII) [Tese de Doutorado]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2010. 144p.

22. Marchioni DM, Fisberg RM, Góis Filho JF, Kowalski LP, Carvalho MB, Abrahão M, et al. Dietary patterns and risk of oral cancer: a case-control study in São Paulo, Brazil. Rev Saude Publica. 2007;41(1):19-26.

23. Resende ALS, Mattos IE, Koifman S. Dieta e câncer gástrico: aspectos históricos associados ao padrão de consumo alimentar no estado do Pará. Rev Nutr. 2006;19(4):511-9.

24. Albuquerque RCR, Monteiro GTR, Pereira RA, Koifman S. Padrões dietéticos e tumores cerebrais: estudo caso-controle no Rio de Janeiro. Cad Saúde Colet. 2012;20(3):271-9.

25. Kerver JM, Yang EJ, Bianchi L, Song WO. Dietary patterns associated with risk factors for cardiovascular disease in healthy US adults. Am J Clin Nutr. 2003;78(6):1103-10.

26. Centritto F, Iacoviello L, di Giuseppe R, De Curtis A, Costanzo S, Zito F, et al; Moli-sani Investigators. Dietary patterns, cardiovascular risk factors and C-reactive protein in a healthy Italian population. Nutr Metab Cardiovasc Dis. 2009;19(10):697-706.

27. Olinto MT, Gigante DP, Horta B, Silveira V, Oliveira I, Willett W. Major dietary patterns and cardiovascular risk factors among young Brazilian adults. Eur J Nutr. 2012;51(3):281-91.

28. Fisberg RM, Marchioni DML, Colucci ACA. Avaliação do consumo alimentar e da ingestão de nutrientes na prática clínica. Arq Bras Endocrinol Metab. 2009;53(5):617-24.

ORIGINAL ARTICLE

Diabetic regulation of subjects with type 2 diabetes mellitus is associated with serum vitamin D levels

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SUMMARY

OBJECTIVE: *Vitamin D deficiency is not only associated with bone metabolism but also with diabetes mellitus. We aimed to study the possible association between serum vitamin D concentration and HbA1c level in patients with type 2 diabetes mellitus (T2DM) in this retrospective report.*

METHODS: *Patients with T2DM were enrolled to the study either in regulated or non-regulated T2DM groups, according to HbA1c levels. An HbA1c level of <8% was considered as relatively controlled and others were considered as poorly controlled T2DM.*

RESULTS: *Serum vitamin D levels in poorly controlled T2DM subjects (9.4 (4.9-34) ng/ml) were significantly lower than that of the relatively well regulated T2DM patients (13.5 (3.4-36) ng/ml) (p=0.03). Vitamin D was strongly and inversely correlated with HbA1c levels (r= -0.295, p=0.005).*

CONCLUSION: *Whatever the cause or result of the diabetes mellitus, it is clear that lower vitamin D is strongly associated with worse diabetic regulation in T2DM subjects. Randomized controlled larger studies, which research the relation between diabetic regulation and vitamin D status, are needed to claim whether it could be a therapeutic target in future in diabetic subjects.*

KEY WORDS: *Vitamin D. Type 2 diabetes mellitus. Glycated hemoglobin A.*

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder, which is caused by deterioration in secretion or action of insulin. Prevalence of T2DM has been reached nearly pandemic levels worldwide, in parallel to the increase in obesity.

The role of vitamin D in many bodily functions has been researched after discovery of vitamin D receptors in pancreas and immune cells[1]. It has been reported that subjects with insufficient vitamin D were prone to develop type 2 diabetes mellitus[2]. Moreover, impaired glucose tolerance has been shown to be meliorated in vitamin D deficient subjects after vitamin D replacement [3].

Few studies analyzed the possible association between vitamin D serum levels and glycated hemoglobin (HbA1c) in patients with T2DM. Several

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of these reports found an association and others did not. Indeed, vitamin D deficiency could be associated with development of T2DM or diabetic control level. Therefore, we planned a present retrospective study.

OBJECTIVE

In the present retrospective analysis, we aimed to study the possible association between serum vitamin D concentration and HbA1c level in patients with type 2 diabetes mellitus.

METHODS

Patients' admissions to outpatient clinics of our institution who have T2DM retrospectively investigated from patient files and computerized database between November 2016 and June 2017. Subjects grouped either into regulated T2DM or into non-regulated T2DM groups, according to HbA1c levels. An HbA1c level of <8% was considered as relatively controlled and others were considered as uncontrolled T2DM. Patients with active inflammation, infection, malignancy or chronic kidney disease were excluded from the study. Patients receiving vitamin D supplements were also excluded. Age, gender and other general characteristics, such as height, weight, duration of T2DM and waist circumference were recorded. A body mass index (BMI) was calculated by dividing of the weight in kilograms to the square of height in meters.

Vitamin D levels, serum urea, creatinine, fasting plasma glucose, total, HDL and LDL cholesterol, triglyceride and albumin were obtained from the same database and recorded. HbA1c levels were also obtained and recorded from patient file system. Vitamin D levels were detected by measuring serum 25-hydroxyvitamin D.

Data were analyzed by SPSS software. (SPSS 15.0; IBM Inc., Chicago, IL, USA). Results expressed as mean ± SD or median (minimum - maximum). Variables are conducted with independent samples t test or Mann-Whitney U test. Chi square test used in comparison of categorical variables between study groups. A p value of < 0.05 is considered as statistically significant. Pearson's correlation analysis test used to reveal possible correlation between vitamin D and HbA1c levels.

RESULTS

The study population was consisted of 89 diabetic subjects; 47 in poorly controlled and 42 in relatively

TABLE 1. GENERAL CHARACTERISTICS AND LABORATORY DATA OF STUDY GROUPS

		Poorly controlled T2DM	Well-controlled T2DM	p
Gender	Women (n)	30	20	0.12
	Men (n)	17	22	
		Median (Min.-Max.)		
Age (years)		61 (40-80)	56.5 (29-86)	0.49
Duration of T2DM (years)		10 (1-30)	4.5 (1-20)	0.02
Height (m)		1.62 (1.54-1.83)	1.65 (1.45-1.82)	0.40
Weight (kg)		82 (60-137)	80 (56-120)	0.40
Waist circumference (cm)		104 (80-141)	100 (75-141)	0.33
Body mass index (kg/m^2)		31.1 (22.6-52.2)	29.7 (20.6-46.9)	0.22
Fasting plasma glucose (mg/dl)		209 (89-422)	126 (78-294)	<0.001
HbA1c (%)		9.7 (8-16.6)	6.9 (5.4-7.7)	<0.001
Vitamin D (ng/ml)		9.4 (4.9-34)	13.5 (3.4-36)	0.03
Blood urea (mg/dl)		30 (14-79)	30 (17-62)	0.62
Plasma creatinine (mg/dl)		0.83 (0.6-1.1)	0.79 (0.56-1.1)	0.26
Na (mmol/l)		138 (134-145)	139 (129-145)	0.24
K (mmol/l)		4.5 (3.6-5.3)	4.4 (3-5.4)	0.12
Triglyceride (mg/dl)		182 (65-841)	149 (69-850)	0.03
Serum albumin (g/dl)		4.2 (2.5-4.8)	4.3 (3.5-4.8)	0.17
		Mean ± Standard Deviation		
Total cholesterol (mg/dl)		194 ± 51	188 ± 46	0.53
HDL cholesterol (mg/dl)		46 ± 10	45 ± 10	0.53
LDL cholesterol (mg/dl)		127 ± 31	112 ± 36	0.04

well-controlled diabetes mellitus groups. Median age of poorly and well-regulated T2DM group was 61 (40-80) years and 56.5 (29-86) years, respectively. Age difference was not statistically significant (p=0.49).

Twenty of 42 subjects in relatively well-controlled T2DM group and 30 of 47 in poorly controlled T2DM group were women. Gender was not statistically different between study groups (p=0.12).

Fasting plasma glucose levels of poorly controlled and well-controlled diabetic subjects were 209 (89-422) mg/dl and 126 (78-294) mg/dl, respectively. The difference was statistically significant, as expected (p<0.001). Similarly, HbA1c of poorly controlled diabetics (9.7 [8-16.6] %) was significantly higher than that of the relatively well-controlled diabetic subjects (6.9 [5.4-7.7] %), again expectedly (p<0.001). General characteristics and laboratory data of study population were expressed in table 1.

Body mass index (p=0.22), waist circumference (p=0.33), blood urea (p=0.62) and creatinine (p=0.26), total (p=0.53) and HDL (p=0.53) cholesterol, plasma sodium (p=0.24) and potassium (p=0.12) concentrations, and serum albumin levels (p=0.17) were not significantly different between study groups.

Serum LDL (p=0.04) and triglyceride (p=0.03) were significantly higher in poorly controlled compared to well-controlled diabetic subjects. Duration of T2DM was significantly longer in poorly regulated compared to relatively well-regulated T2DM group (p=0.02).

Vitamin D serum levels in poorly and well-regulated T2DM groups were 9.4 (4.9-34) ng/ml and 13.5 (3.4-36) ng/ml, respectively. The difference between study groups was statistically significant (p=0.03).

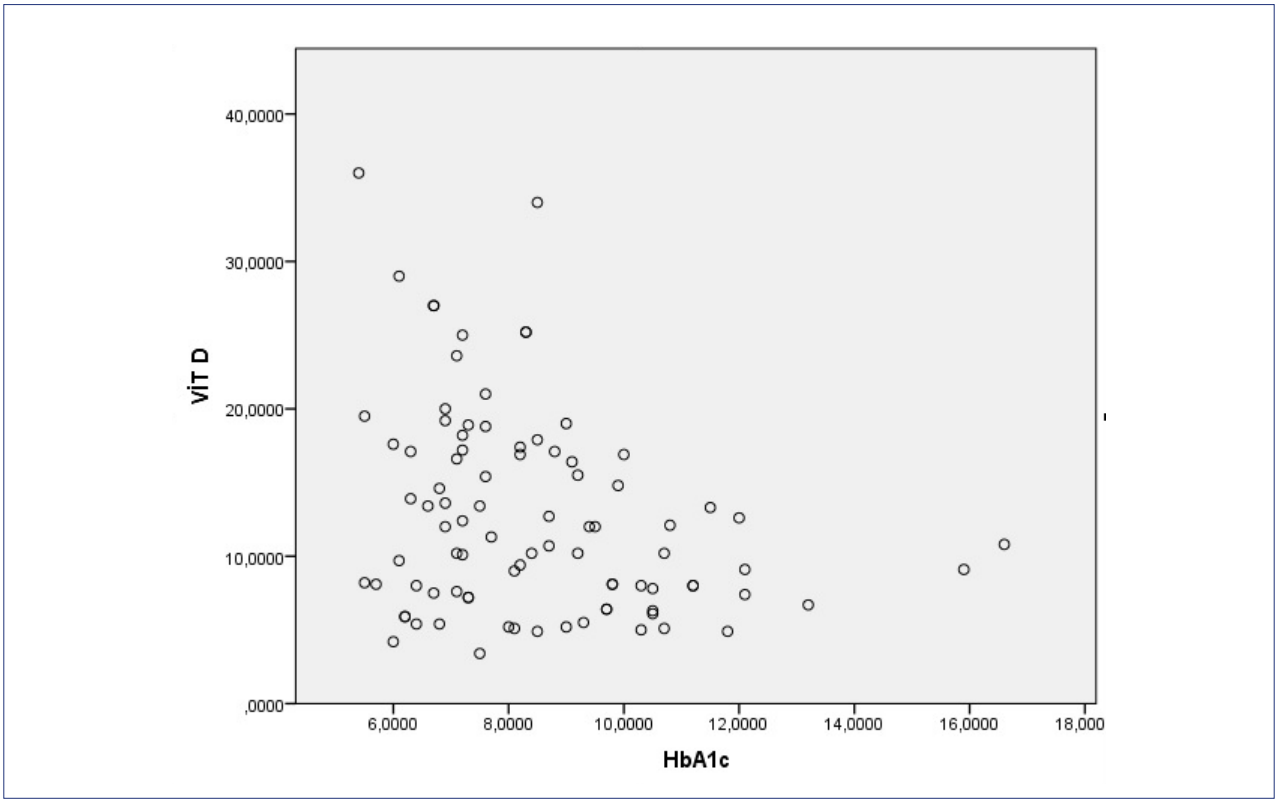
Pearson correlation analysis revealed that serum vitamin D was strongly and inversely correlated with HbA1c levels (r= -0.295, p=0.005). Figure 1 shows the correlation between vitamin D and HbA1c (figure 1).

DISCUSSION

The main finding of the present study is that vitamin D levels were negatively and strongly correlated with HbA1c levels in patients with T2DM. HbA1c reflects blood glucose of the last 3 months and is considered as a marker of diabetic regulation. This negative correlation could be the consequence of vitamin D being a metabolic biomarker besides its action in bone and calcium metabolism.

Hypovitaminosis of vitamin D have negative effects on both calcium and bone metabolism and diabetes mellitus[4]. Besides its crucial role in calcium and bone metabolism, recent discoveries showed

FIGURE 1. CORRELATION BETWEEN VITAMIN D AND HBA1C IN STUDY COHORT



that vitamin D also has important bodily functions. Target tissues of vitamin D include immune cells, heart, stomach, liver, brain, skin, pancreas, thyroid, parathyroid and adrenal glands[5]. Therefore, it is supposed that vitamin D may have inflammatory and immunologic effects. On the other hand, low serum vitamin D is a risk factor not only for diabetes mellitus but also for metabolic syndrome[2]. Another clue of association between T2DM and vitamin D insufficiency could be that obesity increases the incidence of both metabolic syndrome, T2DM and hypovitaminosis D[6].

Chiu et al suggested that lower vitamin D status was a risk factor for development of T2DM [7]. In another study, researchers found that vitamin D was more effective than metformin in ameliorating insulin resistance in subjects with metabolic syndrome[2]. Similar results have been reported in literature. Subjects with T2DM had reduced serum vitamin levels compared to healthy population in a study by Scragg et al.[8]. Authors found negative correlations between serum vitamin D and insulin secretion [9], and between vitamin D level and prevalence of diabetes [10].

Results of the studies about the effects of vitamin D in human are conflicting. Repletion of vitamin D in deficient patients caused an increase in insulin production and secretion in T2DM patients[11]. Despite oral vitamin D increased the insulin secretion following oral glucose intake in healthy subjects, its effect was neutral in patients with T2DM [2]. Moreover, deterioration of glycemic control and insulin sensitivity in response to oral vitamin D has been reported in literature[12].

We will discuss the possible reasons of the negative correlation between HbA1c and serum vitamin D. Insulin secretion of pancreatic beta cells reduced the vitamin D deficiency. In other words, insulin secretion and normal glucose tolerance is ensured by sufficient amount of vitamin D. Animal studies suggested that vitamin D deficiency lead to both glucose intolerance and even resistance to exogenous insulin[13]. Interestingly, pancreatic insulin secretion increases after vitamin D treatment[14,15]. However, a recent randomized controlled trial revealed that vitamin D supplementation was failed to alter HbA1c levels in patients with type 2 diabetes mellitus [16,17]. Negative correlation between vitamin D and HbA1c levels in this study may support other studies that found association between T2DM and vitamin D status.

Lower vitamin D levels in patients with poor diabetic control compared to better-controlled subjects should be well examined. There are studies that reported vitamin D insufficiency was not the cause but the result of diabetes mellitus. Insulin stimulates production of vitamin D, thus, insulin deficiency or inefficacy as seen in diabetes mellitus may result in a decrease in vitamin D levels[5].

Retrospective design and small study population are two main limitations of the present report. Nevertheless, the striking negative correlation between serum vitamin D and HbA1c make its results very important.

CONCLUSION

Either the cause or result, it is clear that lower vitamin D is strongly associated with worse diabetic regulation in T2DM subjects. Randomized controlled larger studies that research the relation between diabetic regulation and vitamin D status are needed to claim whether it could be a therapeutic target in future in diabetic subjects.

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RESUMO

CONTEXTO E OBJETIVO: *A deficiência de vitamina D não é apenas associada ao metabolismo ósseo, mas também ao diabetes mellitus. Procurou-se estudar a possível associação entre os níveis de concentração do soro de vitamina D e de HbA1c em pacientes com diabetes mellitus tipo 2 neste relatório retrospectivo.*

MÉTODOS: *Os pacientes com diabetes mellitus tipo 2 foram inscritos no estudo em regulada ou não regulada de acordo com os grupos de níveis de HbA1c DM2. HbA1c nível de <8% caracterizava DM2 controlada e HbA1c > 8% DM2 descontrolada.*

RESULTADOS: *Os níveis de vitamina D no soro em indivíduos com DM2 mal regulados (9,4 (4,9 a 34) ng/ml) foram significativamente menores do que o do bem regulado em doentes DM2 (13,5 (3,4-36) ng/ml) (p = 0,03). A vitamina D foi forte e inversamente correlacionada com os níveis de HbA1c (p = 0,005).*

CONCLUSÃO: *Seja qual for a causa ou o resultado do diabetes mellitus, é claro que níveis baixos de vitamina D são fortemente associados com pior regulação em indivíduos diabéticos com DM2. Maiores estudos randomizados e controlados que pesquisam a relação entre o status de vitamina D e a regulação em diabéticos são necessários para molusco se é, no futuro, poderia ser um alvo terapêutico em indivíduos diabéticos.*

PALAVRAS-CHAVE: *Vitamina D. Diabetes mellitus tipo 2. Hemoglobina A glicada.*

REFERENCES

1. Holick MF. Vitamin D: a millenium perspective. J Cell Biochem. 2003;88(2):296-307.
2. Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and β cell dysfunction. Am J Clin Nutr. 2004;79:820-5.
3. Kumar S, Davies M, Zakaria Y, Mawer EB, Gordon C, Olukoga AO, et al. Improvement in glucose tolerance and beta-cell function in a patient with vitamin D deficiency during treatment with vitamin D. Postgrad Med J. 1994;70(824):440-3.
4. Mathieu C, Gysemans C, Giulietti A, Bouillon R. Vitamin D and diabetes. Diabetologia. 2005;48(7):1247-57.
5. Bikle DD. Clinical counterpoint: vitamin D: new actions, new analogs, new therapeutic potential. Endocr Rev. 1992;13(4):765-84.
6. Palomer X, González; Clemente JM, Blanco; Vaca F, Mauricio D. Role of vitamin D in the pathogenesis of type 2 diabetes mellitus. Diabetes Obes Metab. 2008;10(3):185-97.
7. Chiu KC, Chuang LM, Yoon C. The vitamin D receptor polymorphism in the translation initiation codon is a risk factor for insulin resistance in glucose tolerant Caucasians. BMC Med Genet. 2001;2:2.
8. Scragg R, Holdaway I, Singh V, Metcalf P, Baker J, Dryson E. Serum 25-hydroxyvitamin D3 levels decreased in impaired glucose tolerance and diabetes mellitus. Diabetes Res Clin Pract. 1995;27(3):181-8.
9. Baynes KC, Boucher BJ, Feskens EJ, Kromhout D. Vitamin D, glucose tolerance and insulinaemia in elderly men. Diabetologia. 1997;40(3):344-7.
10. Scragg R, Sowers M, Bell C. Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. Diabetes Care. 2004;27(12):2813-8.
11. Borissova AM, Tankova T, Kirilov G, Dakovska L, Kovacheva R. The effect of vitamin D3 on insulin secretion and peripheral insulin sensitivity in type 2 diabetic patients. Int J Clin Pract. 2003;57(4):258-61.
12. Taylor AV, Wise PH. Vitamin D replacement in Asians with diabetes may increase insulin resistance. Postgrad Med J. 1998;74(872):365-6.
13. Ismail A, Namala R. Impaired glucose tolerance in vitamin D deficiency can be corrected by calcium. J Nutr Biochem. 2000;11(3):170-5.
14. Boucher BJ. Inadequate vitamin D status: does it contribute to the disorders comprising syndrome 'X'? Br J Nutr. 1998;79(4):315-27.
15. Zittermann A. Vitamin D in preventive medicine: are we ignoring the evidence? Br J Nutr. 2003;89(5):552-72.
16. Krul-Poel YH, Westra S, ten Boekel E, ter Wee MM, van Schoor NM, van Wijland H, et al. Effect of vitamin D supplementation on glycemic control in patients with type 2 diabetes (SUNNY trial): a randomized placebo-controlled trial. Diabetes Care. 2015;38(8):1420-6.
17. Garbossa SG, Folli F. Vitamin D, sub-inflammation and insulin resistance. A window on a potential role for the interaction between bone and glucose metabolism. Rev Endocr Metab Disord. 2017;18(2):243-58.

REVIEW ARTICLE

Cardiovascular autonomic neuropathy in type 2 diabetic patients

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SUMMARY

Diabetes is one of the most common chronic pathologies around the world, involving treatment with general clinicians, endocrinologists, cardiologists, ophthalmologists, nephrologists and a multidisciplinary team. Patients with type 2 Diabetes Mellitus (T2DM) can be affected by cardiac autonomic neuropathy, leading to increased mortality and morbidity. In this review, we will present current concepts, clinical features, diagnosis, prognosis, and possible treatment. New drugs recently developed to reduce glycemic level presented a pleiotropic effect of reducing sudden death, suggesting a potential use in patients at risk.

KEYWORDS: *Diabetes mellitus. Diabetic Neuropathies. Death, sudden.*

INTRODUCTION

Type 2 diabetes mellitus (DM2) has high morbidity and mortality and great socioeconomic impact, having affected 150 million individuals in the year 2000, with an expectancy of affecting 336 million by 2030[1]. The diabetic neuropathy, including the cardiovascular autonomic neuropathy (CAN), is a common complication of type 1 and 2 diabetes that leads to high mortality and morbidity[2].

Patients with a CAN diagnosis have an increased risk of cardiovascular mortality due to acute myocardial infarction and sudden death.[3] Although CAN is an important milestone in the history of a DM2 patient and its screening is recommended by the American Diabetes Association (ADA)[4], its present presents high variability in studies. The values found were close to 2.5% in the *Diabetes Control and Complications Trial* (DCCT)[5]; in the Oxford Community Diabetes Study[6], the prevalence of abnormal results for anatomic tests was of 20.9% in DM1 patients and 5.8% in DM2 patients. In the DiaCAN7 study, the CAN prevalence was of 16.8% in 647 DM1 patients and 22.1% in 524 DM2 patients. Despite the high heterogeneity in the epidemiological findings, it is known that the prevalence of CAN increases with age, duration of diabetes, and inadequate blood glucose control, and the presence of hepatic steatosis in patients with a DM2 diagnosis of less than a year showed a strong correlation with anatomic lesions[8]. The late stages of CAN are associated to considerable morbidity and an increase in mortality, related to postural hypotension, exercise intolerance, increase in the intraoperative instability, the incidence of silent myocardial infarction and sudden death[2].

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PATHOGENESIS

The autonomic innervation is the primary process in charge of controlling rate and performance. It must be capable of changing the rate in response to changes in the microenvironment. In the presence of sustained hyperglycemia, a neuropathic dysfunction sets in, affecting, firstly, the major and distal nerves and, secondly, the proximal nerves. The vagus nerve is the primary mechanism responsible for 75% of all parasympathetic activity, and it is the most frequently damaged. Thus, the initial manifestations of CAN involve a parasympathetic dysfunction, with an anomalous predominance of the sympathetic system[9].

The precise mechanisms of cardiovascular autonomic dysfunction are not yet well understood. Hyperglycemia increases protein glycosylation and causes a gradual increase in the advanced glycation end-products (AGEs) in the tissues. The connection of the AGEs with their receptors (Rage) creates a chronic cascade of inflammation and tissue injury, destroying the mechanisms for autonomic control. In parallel, the neural cardiovascular autonomic dysfunction perpetrates the myocardial inflammation and makes the tissue more excitable, thus stimulating the microvascular endothelial dysfunction and interstitial fibrosis[10,11].

DIAGNOSIS

Heart Rate Variability (HRV)

The earliest sign for a CAN diagnosis is the reduction of HRV. The heterogeneity with each heartbeat is regulated by a balance between the sympathetic and parasympathetic activities in response to a basal metabolic rate. In healthy individuals, the HRV is high. The reduction of HRV is linked to the severity and duration of the DM2[12].

For a diagnosis, the American Diabetes Association (ADA) task force recommends the analysis of HRV from the 24-hour Holter results[13]. The HRV indicators are: RR intervals; standard deviations of all normal RR (SDNN) during the recording; square root of the average square of the differences between consecutive RR intervals (RMSSD); and the percentage of NN intervals with a difference of over 50 milliseconds in relation to the previous interval (pNN50). The RMSSD and the pNN50 are associated with a high-frequency potency (0.15-0.4 Hz) and, thus, to the parasympathetic activity, while the SDNN is correlated to a low-frequency potency (0.04-0.15 Hz), reflecting the baroreceptor activity, an index of sympathetic and parasympathetic activities[3,14].

The CAN diagnosis using the 24-hour Holter requires an abnormal result in at least two of the following six parameters: SDNN < 50 ms, RMSSD < 15 ms, PNN50 < 0.75%, LF < 300 ms^2, HF < 300 ms^2 [15,16].

Ewing Test

This is the most classic and cheapest method of diagnosis, with a high reproducibility[16]. The CAN must be assessed by five standardized tests — three of them for a parasympathetic evaluation: responses of the heart rate to deep breathing (E/I ratio), to the orthostatic position (30s/15s ratio), and the Valsalva maneuver. The other two tests evaluate the sympathetic function: responses to arterial pressure when rising and handgrip. The results for each test must be 0 if standard; 0.5 if borderline; 1 if abnormal. A score ≥ 2 classifies the patient as CAN +[17].

Heart Rate Turbulence (HRT)

The Heart Rate Turbulence is a method first described by Schmidt et al.[18], in 1999. It consists of two analysis: the beginning of the turbulence (TI) and the turbulence slop (ST), both extracted from the 24-hour Holter records. It reflects the fluctuations of the rearranging sinus cycle after a single ventricular ectopic beat (VBP)[18]. The TI indicates the initial acceleration of the sinus rhythm, while the ST reflects the late deceleration stage of the sinus rhythm. When the TI is greater than 0 or the ST lower than 2.5 ms/RR, the diagnosis is of CAN +[16]. The TFC is a phenomenon present in low-risk patients of ischemic heart disease. The absence of this phenomenon indicates a significantly increased risk of sudden death.

CLINICAL CHARACTERISTICS

Exercise Intolerance

The autonomic dysfunction can also lead to exercise intolerance with a disproportionate increase in heart rate and arterial pressure during exercise, a reduction of the systolic volume, and slow recovery.

A late finding is a tachycardia at rest: A heart rate of approximately 100 beats per minute per minute (bpm) or more reflects a relative increase in the sympathetic tone, associated, to parasympathetic vagus damage. However, confounding factors, such as anemia, dehydration, and hyperthyroidism, must be

ruled out. A fixed heart rate that does not respond to exercises of moderate intensity, stress, or sleepiness indicates an almost total cardiac denervation and is an indicator of severe CAN[19,20].

Postural Hypertension

Some DM2 patients might present orthostatic hypotension, i.e., a reduction of over 20 mmHg in systolic arterial pressure and 10 mmHg in diastolic arterial pressure when rising. This finding occurs due to sympathetic vasomotor denervation, causing a reduction of the vasoconstriction capacity in the peripheral vascular beds. The symptoms associated with orthostatic hypotension include visual darkening, weakness, and, in more severe cases, syncope when rising. These events may be aggravated by several medications, such as diuretics, vasodilators, and tricyclic antidepressants[11,17].

Healthy individual present a reduction of the arterial pressure during sleep. CAN patients can present, in the 24-hour map, a low decrease of the nocturnal arterial pressure (lower than 10%), contributing to left ventricular hypertrophy a fatal and non-fatal cardiac events[21].

Imaging Exam

The MIBG (metaiodobenzylguanidine) is a non-metabolized marker, analogous of the norepinephrine, useful for the assessment of post-ganglionic neural fibers. Several studies have shown a decrease in the MIBG uptake in CAN patients. Some studies have shown a higher sensitivity of MIBG when compared with autonomic tests[22]. When CAN is present, the MIBG scintigraphy shows a lower uptake, especially in posterior and inferior segments of the left ventricle, up to the total absence of MIBG capitation in the advanced CAN.

PROGNOSIS

A meta-analysis with over 2,900 patients showed that mortality for a period of up to ten years was of 30.4% in diabetic patients with CAN, detected through the reduction of HRV, and 13.4% in those with no signs of CAN through HRV[10]. Whitsel et al.[23] demonstrated that patients with a CAN diagnosis presented a significant risk of prolonged QT intervals, which are associated with sudden deaths. This meta-analysis showed 2.3 times increase in the risk of prolonged QT interval in patients with diabetes and CAN.

Another publication of the Diad study showed that the CAN is an important predictor for silent myocardial ischemia, which was found in 22% of the asymptomatic DM2 patients. In addition, during exercise, diabetes patients with CAN presented a delay in the angina pain, even though the ischemic process was already underway, with a predisposition for prolonged ischemia[25].

Some trials have suggested that sudden death is the second or first most common cause of death for DM2 patients. As an example, we can mention the subanalysis of the Tecos study, which showed that sudden death was the primary cause of death of cardiovascular origin (27%), followed by AMI/CVA (21%), and heart failure (12%)[26]. In the Empa-REG study, sudden death was also the leading cause of death with a cardiovascular origin (29.9%)[27]. In the Savor-Timi 53 study, 45% of deaths due to cardiovascular origins were sudden deaths[28]. Thus, CAN must be actively investigated in DM2 patients.

CLASSIFICATION

The Ewing tests are the golden standard for CAN diagnosis. The reference values change according to age. A single test with alterations already raises the possibility of CAN. Two or more tests with alterations are a definite indicator of CAN. CAN present only after a HRV assessment is named as subclinical, while the presence of orthostatic hypotension implies severe CAN[29].

TREATMENT

The best chance for intervention and changes of prognostics for a CAN patient is the early diagnosis. The treatment is based on non-pharmacological treatments that include weight loss and improvements to insulin resistance, since both are associated with sympathetic hyperactivity, and exercises must be of low to moderate intensity[30]. Pharmacological measures, such as ACE inhibitors and beta-blockers, can contribute to the attenuation of CAN symptoms[4,31]. Therapies whose focus is, exclusively, the action of the sympathetic nervous system still require further studies, such as (i) hyperstimulation of the vagus nerve; (ii) renal sympathetic ablation; (iii) resection of the carotid body; and (iv) electrical stimulation of the baroreceptor.

Two classes of drugs for DM2 treatment, iSGLT2,

and aGLP-1, have shown a reduction in mortality due to cardiovascular disease using mechanisms not often linked to coronary artery disease. In a non-pre-specified analysis of the Empa-REG[27], it was suggested that: among patients included in the study who suffered a fatal cardiovascular event, 137 (5.3%) were in the placebo group, while 90 (3.8%) were being treated with 10 mg/d empagliflozin and 82 (3.5%) with 25 mg/d empagliflozin. Sudden death was the cause of death for 38 patients in the placebo group, 30 in the 10 mg/d empagliflozin group, and 23 in the 25 mg/d empagliflozin group. Thus, sudden death was the cause for 1.6% of all deaths in the placebo group, against 1.1% in the group treated with empagliflozin, which represented a reduction of 31.25% in deaths by sudden death in the empagliflozin group. Studies using animals have demonstrated the action of the iSGLT2 in reducing the sympathetic hyperactivity.

In another sub analysis also not pre-specified in the Leader[32] study, 278 patients progressed to death in the placebo group, against 219 patients in the treated group. Of these, sudden death was the cause of death for 74 (1.6%) of patients in the placebo group, against 51 (1,1%) in the liraglutide group, representing a 31.25% reduction in deaths by sudden death.

Thus, these two classes of drugs, iSGLT2 and aGLP1, are found to be possible attenuators of sudden death, perhaps due to their, at least partial, impact on the autonomic nervous system.

CONCLUSION

CAN is a frequent and important diagnosis achieved through the screening test recommended by the ADA. Due to its prognostic importance in DM2, the risk of sudden death and myocardial infarction and the possibility of treatment must not be neglected.

RESUMO

Diabetes é uma das mais frequentes patologias crônicas em todo o mundo, cujo tratamento envolve uma equipe multidisciplinar, médicos generalistas, endocrinologistas, cardiologistas, nefrologistas e oftalmologistas. Pacientes com diabetes mellitus tipo 2 (DMT2) podem apresentar neuropatia autonômica cardíaca (NAC), levando a aumento de mortalidade e morbidade. Nesta revisão, apresentaremos atuais conceitos, características clínicas, diagnóstico, prognóstico e possíveis tratamentos. Novas drogas recentemente desenvolvidas para redução de níveis glicêmicos apresentaram efeito pleiotrópico de redução de morte súbita, sugerindo um potencial uso neste perfil de pacientes.

PALAVRAS-CHAVE: *Diabetes mellitus. Neuropatias diabéticas. Morte súbita.*

REFERENCES

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27(5):1047-53.
2. Yun JS, Park YM, Cha SA, Ahn YB, Ko SH. Progression of cardiovascular autonomic neuropathy and cardiovascular disease in type 2 diabetes. Cardiovasc Diabetol. 2018;17(1):109.
3. Benichou T, Pereira B, Mermillod M, Tauveron I, Pfabigan D, Maqdasy S, et al. Heart rate variability in type 2 diabetes mellitus: a systematic review and meta-analysis. PLoS ONE. 2018;13(4):e0195166.
4. Pop-Busui R, Boulton AJ, Feldman EL, Bril V, Freeman R, Malik RA, et al. Diabetic neuropathy: a position statement by the American Diabetes Association. Diabetes Care. 2017;40(1):136-54.
5. The Diabetes Control and Complications Trial Research Group, Group TDC and CTR. The effect of intensive diabetes therapy on the development and progression of neuropathy. The Diabetes Control and Complications Trial Research Group. Ann Intern Med. 1995;122(8):561-8.
6. Neil HA, Thompson AV, Thorogood M, Fowler GH, Mann JI. Diabetes in the elderly: the Oxford Community Diabetes Study. Diabet Med. 1989;6(7):608-13.
7. Ziegler D, Gries FA, Mühlen H, Rathmann W, Spüler M, Lessmann F. Prevalence and clinical correlates of cardiovascular autonomic and peripheral diabetic neuropathy in patients attending diabetes centers. The Diacan Multicenter Study Group. Diabete Metab. 1993;19(1 Pt 2):143-51.
8. Ziegler D, Strom A, Kupriyanova Y, Bierwagen A, Bönhof GJ, Bódis K, et al. Association of lower cardiovagal tone and baroreflex sensitivity with higher liver fat content early in type 2 diabetes. J Clin Endocrinol Metab. 2018;103(3):1130-8.
9. Axelrod S, Lishner M, Oz O, Bernheim J, Ravid M. Spectral analysis of fluctuations in heart rate: an objective evaluation of autonomic nervous control in chronic renal failure. Nephron. 1987;45(3):202-6.
10. Maser RE, Mitchell BD, Vinik AI, Freeman R. The association between cardiovascular autonomic neuropathy and mortality in individuals with diabetes: a meta-analysis. Diabetes Care. 2003;26(6):1895-901.
11. Fisher VL, Tahrani AA. Cardiac autonomic neuropathy in patients with diabetes mellitus: current perspectives. Diabetes Metab Syndr Obes. 2017;10:419-34.
12. Gerritsen J, Dekker JM, TenVoorde BJ, Kostense PJ, Heine RJ, Bouter LM, et al. Impaired autonomic function is associated with increased mortality, especially in subjects with diabetes, hypertension, or a history of cardiovascular disease: the Hoorn Study. Diabetes Care. 2001;24(10):1793-8.
13. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: Standards of measurement, physiological interpretation and clinical use. Eur Heart J. 1996;17(3):354-81.
14. Otzenberger H, Gronfier C, Simon C, Charloux A, Ehrhart J, Piquard F, et al. Dynamic heart rate variability: a tool for exploring sympathovagal balance continuously during sleep in men. Am J Physiol. 1998;275(3 Pt 2):H946-50.
15. Balcioğlu S, Arslan U, Türkoğlu S, Ozdemir M, Cengel A. Heart rate variability and heart rate turbulence in patients with type 2 diabetes mellitus with versus without cardiac autonomic neuropathy. Am J Cardiol. 2007;100(5):890-3.

16. Lin K, Wei L, Huang Z, Zeng Q. Combination of Ewing test, heart rate variability, and heart rate turbulence analysis for early diagnosis of diabetic cardiac autonomic neuropathy. Medicine (Baltimore). 2017;96(45):e8296.

17. Spallone V, Bellavere F, Scionti L, Maule S, Quadri R, Bax G, et al. Recommendations for the use of cardiovascular tests in diagnosing diabetic autonomic neuropathy. Nutr Metab Cardiovasc Dis. 2011;21(1):69-78.

18. Schmidt G, Malik M, Barthel P, Schneider R, Ulm K, Rolnitzky L, et al. Heart-rate turbulence after ventricular premature beats as a predictor of mortality after acute myocardial infarction. Lancet. 1999;353(9162):1390-6.

19. Tesfaye S, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care. 2010;33(10):2285-93.

20. Vinik AI, Ziegler D. Diabetic cardiovascular autonomic neuropathy. Circulation. 2007;115(3):387-97.

21. Serhiyenko VA, Serhiyenko AA. Cardiac autonomic neuropathy: risk factors, diagnosis and treatment. World J Diabetes. 2018;9(1):1-24.

22. Langen KJ, Ziegler D, Weise F, Piolot R, Boy C, Hübinger A, et al. Evaluation of QT interval length, QT dispersion and myocardial m-iodobenzylguanidine uptake in insulin-dependent diabetic patients with and without autonomic neuropathy. Clin Sci (Lond). 1997;93(4):325-33.

23. Whitsel EA, Boyko EJ, Siscovick DS. Reassessing the role of QT(c) in the diagnosis of autonomic failure among patients with diabetes: a meta-analysis. Diabetes Care. 2000;23(2):241-7.

24. Heller G V, Iskandrian AE, Wittlin SD. Cardiac outcomes after screening for asymptomatic coronary artery disease in patients with type 2 diabetes: the DIAD Study: a randomized controlled trial. Trial. 2010;301(15):1547-55.

25. Ambepityia G, Kopelman PG, Ingram D, Swash M, Mills PG, Timmis AD. Exertional myocardial ischemia in diabetes: a quantitative analysis of anginal perceptual threshold and the influence of autonomic function. J Am Coll Cardiol. 1990;15(1):72-7.

26. Sharma A, Green JB, Dunning A, Lokhnygina Y, Al-Khatib SM, Lopes RD, et al. Causes of death in a contemporary cohort of patients with type 2 diabetes and atherosclerotic cardiovascular disease: insights from the TECOS trial. Diabetes Care. 2017;40(12):1763-70.

27. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med. 2015;373(22):2117-28.

28. Scirica BM, Bhatt DL, Braunwald E, Steg PG, Davidson J, Hirshberg B, et al. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. N Engl J Med. 2013;369(14):1317-26.

29. Bernardi L, Spallone V, Stevens M, Hilsted J, Frontoni S, Pop-Busui R, et al. Methods of investigation for cardiac autonomic dysfunction in human research studies. Diabetes Metab Res Rev. 2011;1-21.

30. Howorka K, Pumprla J, Haber P, Koller-Strametz J, Mondrzyk J, Schabmann A. Effects of physical training on heart rate variability in diabetic patients with various degrees of cardiovascular autonomic neuropathy. Cardiovasc Res. 1997;34(1):206-14.

31. Boulton AJM, Vinik AI, Arezzo JC, Bril V, Feldman EL, Freeman R, et al. Diabetic neuropathies: a statement by the American Diabetes Association. Diabetes Care. 2005;28(4):956-62.

32. Marso SP, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JF, Nauck MA, et al. Liraglutide and cardiovascular outcomes in type 2 diabetes. N Engl J Med. 2016;375(4):311-22.

REVIEW ARTICLE

Diabetic cardiomyopathy: factual or factoid?

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SUMMARY

Although long ago described, there is no established consensus regarding the real existence of Diabetic Cardiomyopathy (CMPDM). Due to its complex pathophysiology, it has been difficult for clinical and experimental research to establish clear connections between diabetes mellitus (DM) and heart failure (HF), as well as to solve the mechanisms of the underlying myocardial disease. However, the epidemiological evidence of the relationship of these conditions is undisputed. The interest in understanding this disease has intensified due to the recent results of clinical trials evaluating new glucose-lowering drugs, such as sodium-glucose transporter inhibitors 2, which demonstrated favorable responses considering the prevention and treatment of HF in patients with DM. In this review we cover aspects of the epidemiology of CMPDM and its possible pathogenic mechanisms, as well as, present the main cardiac phenotypes of CMPDM (HF with preserved and reduced ejection fraction) and implications of the therapeutic management of this disease.

KEYWORDS: *Diabetes mellitus. Cardiomyopathies. Hypoglycemic agents. Heart failure.*

INTRODUCTION

The diagnosis of diabetic cardiomyopathy requires the association of altered glucose metabolism and exclusion of coronary, valvular, hypertensive, congenital, viral, toxic, familial or infiltrative arterial diseases. This is unquestionable from the theoretical point of view and, despite the difficulty in demonstrating in practice, the diagnosis may be more common than one might suppose. The observation that there is an association of diabetes mellitus (DM) and heart failure dates back 64 years. In the 1950s, Lundbaek hypothesized a diabetic cardiomyopathy (MCPDM) and, in 1969, published the first description of the disease. Curiously, until today there are doubts as to their actual occurrence and circumstances that may trigger it. In this narrative review, we present the current evidence of MCPDM, the possible pathogenic mechanisms, the cardiac phenotype of the disease, as well as possible ways of treating and not treating this condition.

EVIDENCE OF THE RELATIONSHIP BETWEEN DIABETES MELLITUS AND HEART FAILURE

The diagnosis of heart failure (HF) occurs more frequently in patients with diabetes mellitus (DM)[1-3] and, when it occurs, is associated with worse clinical outcomes than in non-diabetic patients with HF[2-6]. Among patients with type 2 DM, the prevalence of HF ranges from 19% to 26%[2,3] and the incidence is also high regardless of other comorbidities, as al-

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ready demonstrated by the Framingham study[7]. Diabetic men aged 45-74 years had more than double the risk of developing HF than their non-diabetic peers during follow-up of up to 18 years old. And that risk seems to be even greater in women. Diabetic women are up to 5 times greater risk than patients who do not have DM. In heart failure cohorts, there is also a higher prevalence of patients with type 2 DM, of about 28%, whether HF with ejection fraction (FE) preserved (HFEFp), or reduced (HFEFp)[6]. And the presence of DM implies a worse prognosis, leading to higher cardiovascular mortality (CV) and hospitalizations due to HF [risk ratio for HFEFp: 2.0 (1.70-2.36); and for HFEFp:1.60 (1.44-1.77); p-value for interaction: 0.0009]. There is, however, disagreement as to whether this worse prognosis would be associated with the occurrence of ischemic coronary artery disease[5].

Previous studies have shown a clear ratio between glycemic control and the incidence of heart failure. Elevations of 1% in HbA1c values are related to an 8% increase in the incidence of HF[8]. Conversely, in the UKPS study, the researchers demonstrated that for each 1% reduction in HbA1c there would be a 16% decrease in the risk of initiation of HF[9]. This bidirectional ratio occurs independently of blood pressure, body mass index, age or presence of coronary artery disease. However, the ratio is not exactly linear. Apparently, using the HbA1c cohort of 7.0 mg/dl there is a divergence of studies regarding the pattern of the glycemia ratio with the mortality of patients with HF that may be: "U" pattern[10] — in which there is an increase in mortality above and below this cohort; "J" pattern - in which there is a disproportionately greater increase for HbA1c levels above this cohort[11]; and inverse pattern - in which mortality is higher for patients with HbA1c below the cohort mentioned[12]. This ratio between glycemia and HF, as well as mortality in the HF patient, even with divergent patterns between studies, makes clear the link between DM and HF. However, these data do not allow us to distinguish whether glycemia and insulin resistance are only markers of severity or whether they actually contribute to the pathophysiology of HF. The concomitance of other CV risk factors and the complex pathophysiology of type 2 DM make it even more difficult to establish a clear and direct ratio between pathogens and cardiac and/or cardiovascular phenotypes of the diabetic patient.

POSSIBLE MECHANISMS

The impact of DM on the heart occurs by locally diverse pathways on the microcirculation and relaxation function of the sarcomere, and systemically by peripheral vascular disease, activation of the renin-angiotensin-aldosterone system (RAAS) and autonomic neuropathy. These mechanisms are established as a consequence of an exacerbated inflammatory state, endothelial lesion and perfusion abnormalities, together with unbalanced oxidative stress.

Hyperglycemia

Experimentally, endothelial cell mitochondria exposed to hyperglycemia may be injured or increase the production of reactive species of O2. Ultimately, this leads to dysfunction of nitric oxide synthases (NO), NO bioavailability, and consequently reduction of the production of the intracellular cyclic guanosine monophosphate (GMPc)[13]. Due to the decrease in intracellular kinase G (PKG) protein, there is loss of myocardial distensibility[14] mediated by hypophosphorylation of the sarcomeric titin protein[15]. In humans, similar results were observed in cardiomyocytes of patients with aortic stenosis and DM who showed improvement in stiffness when submitted to PKG administration[16]. Hyperglycaemia also results in elevation of kinase C protein (PKC) in fibroblasts, which in turn increase collagen production and deposition[16,17].

Another route of action of hyperglycemia on cardiac function occurs by glucose metabolites. A β-N-acetylglucosamine (O-G1cNAc) can bind to a myriad of proteins (on serine or threonine residues), compete for phosphorylation of molecules, and alter physiological functions[18]. A pivotal example is the modification of kinase II proteins dependent on Ca2 +/calmodulin, phospholamban and myofilaments. This effect reduces contractility and relaxation of the myocardium[19]. Mitochondrial proteins are also susceptible to modification by O-G1cNAc with impairment to cardiac function.

Insulin/hyperinsulinemia resistance

Potential mediators of the risk of HF in patients with type 2 DM are insulin and hyperinsulinemia resistance, particularly in the obese patient. The prevalence of obesity *per se* increases circulating levels of glucose and free fatty acids. In addition, insulin resistance and obesity are responsible for a constellation of signaling disorders, leading to a systemic inflam-

matory state that results in the production of reactive species of O^2 and decrease in the bioavailability of NO. Insulin resistance induces myocardial energy imbalance by increasing the use of less efficient sources to the detriment of glucose use, and this culminates with lower production of adenosine triphosphate (ATP)[20,21]. Hyperinsulinemia, in turn, activates certain pathways, such as PI3K/Akt, which is involved in hypertrophy of cardiomyocytes. This same pathway also induces the expression of a more rigid molecule of the sarcomeric titin protein, reducing cardiac distensibility [22].

Inflexibility of power supply

Diabetic patients with coronary heart disease and those with HF may already have reduced glucose utilization capacity compared to non-diabetic HF patients, despite having a similar concentration of glucose transporters (GLUT-4) in myocardial biopsies. This theory suggests that insulin resistance would limit the ability of the DM patient's myocardium to withstand ischemia and, thus, suffer worse CV outcomes[23]. Studies using hyperinsulinemic/euglycemic clamp demonstrate that elevated insulin levels increase heart rate, blood pressure and myocardial oxygen consumption (assessed by extraction of Fluordeoxyglucose[18]) in diabetic patients or not. The presence of insulin reduces the availability of non-esterified fatty acids and suppresses oxidation of fatty acids. However, the oxidation of fatty acids by the myocardium is increased. Under these conditions, there is a reduction in myocardial work efficiency, possibly due to the change in the energy substrate (from glucose to fatty acid), since the fatty acid metabolism consumes a greater amount of oxygen[24]. In diabetic patients, the rapid and drastic reduction of free fatty acids by medication intervention leads to a sudden decrease in cardiac function, corroborating this hypothesis. Cardiac function dependent on a single energy source would be completely dependent on its availability.[25]

Lipotoxicity

The uptake of cardiac free fatty acids exceeds the oxidation capacity of these molecules. This leads to accumulation of triglycerides, cardiac steatosis and eventually death of cardiomyocytes, causing LV systolic dysfunction. This process has been demonstrated in animals and is called lipotoxicity. The change in energetic substrate, due to the reduction of insulin-mediated glucose uptake, also leads to diastolic dysfunction [26]. Generation of lipid intermediates, such as diacid-glycerol[27], may be toxic to microcirculation, by effect on NO synthases and reduction of myocyte distensibility, as described above. Cardiac steatosis has also been associated with diastolic dysfunction in diabetic patients[28].

Deposition of advanced glycation end products (AGEs)

AGEs are found in smooth muscle cells of the myocardial microcirculation[16], as well as, in the extracellular matrix, between cardiomyocytes[29]. AGEs promote inflammation, extinguishing much of the NO produced locally. This would lead to a reduction in perfusion of the microcirculation at rest, but also of the coronary reserve during flow hyperemia. In addition, they trigger the production of reactive O2 species via NADPH oxidase, which may result in the activation of cellular apoptosis pathways and, therefore, systolic dysfunction[30].

Microvascular Rarefaction

One of the most striking pathophysiological features of MCPDM is the reduction of microvascular myocardial perfusion and coronary flow reserve[31]. This parameter is due to the action of AGEs in the microcirculation, but also due to rarefaction of capillaries and reduced luminal area/myocardial tissue ratio. Cardiomyocyte hypertrophy contributes to this result, raising the denominator of this ratio[32]. Capillary rarefaction *per se* reduces the bioavailability of NO to surrounding myocytes. Another result of this process is the relative tissue hypoxia associated with the production of reactive species of O^2 and, finally, cell death and systolic dysfunction.

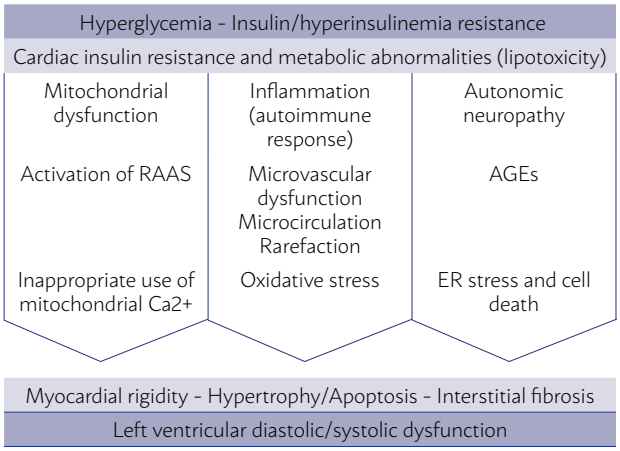
Inflammatory/autoimmune Response

Cardiac antimyosin autoantibodies were identified in type 1 diabetic patients after myocardial infarction and non-diabetic patients with autoimmune myocarditis. Diabetic patients, in general, present release of troponins that are related to HFEFr with dilatation of cardiac chambers. These findings may indicate that an autoimmune mechanism triggered by exposure to plasma troponin exists in the pathophysiology MCPDM with HFEFr[33].

Autonomic neuropathy and renin-angiotensin-aldosterone system (RAAS)

Altered glucose metabolism and insulin resistance stimulate an excessive sympathetic activity

BOARD 1. PATHOPHYSIOLOGICAL AND PATHOGENIC MECHANISMS OF DIABETIC CARDIOMYOPATHY



RAAS: Renin-angiotensin-aldosterone system, AGE: advanced glycation end products; ER: endoplasmic reticulum

that is related to hypertrophy and cardiac fibrosis[34]. In the long term, cardiac sympathetic denervation occurs, a fact that impairs β-adrenergic signaling and reduces myocardial contractility, as well as disrupts the kinetics of relaxation and diastolic distensibility[35,36]. The myocardium of DM also presents upregulation of the RAAS and local endothelin[37,38].

CARDIAC PHENOTYPE

Two types of cardiac phenotypes have been described for MCPDM[39]. The first pattern, apparently more common, is that of extracellular fibrosis, but with preserved sarcomere structure, reduced and hypertrophic left ventricle (LV), left atrial dilation, and diastolic dysfunction/restriction indicators; this pattern is clinically presented as HFEFp[40] (Figure 1). The second pattern, reported in the earliest reports of MCPDM, is cardiomyocyte apoptosis and atrophy and loss of sarcomeric structure, scar replacement, cardiac chamber dilatation, and LV systolic dilatation/dysfunction clinically recognized as HFEFr (Figure 2). In both phenotypes, microcirculation rarefaction and microvascular deposition of advanced glycation end products (AGEs) are observed[32].

TABLE 1: PATHOPHYSIOLOGICAL ASPECTS OF HFEFP AND HFEFR PHENOTYPES OF CMPDM [39]

CMPDM	HFEFp/ restrictive	HFEFr/ dilated
Hyperglycemia	+++	+
Lipotoxicity	+++	+
Deposition of AGEs	+++	+++
Microvascular rarefaction	+++	+++
Inflammatory/autoimmune response	-	+++
Insulin resistance/Hyperinsulinemia	+++	-

However, there is some controversy regarding the natural evolution of myocardial dysfunction and the interrelationship of these patterns, namely: some authors suggest that the pattern of diastolic dysfunction happens first and then develops into systolic dysfunction[41]; others argue that the phenotypes are independent and would be consequences of an individual response of each subject[39]. The latest evidence points to the second case. In a cohort of patients with HF, it was observed that significant reductions of EF occur only in very old individuals (> 80 years old) or after the occurrence of heart attacks[42] and therefore would be due to other factors not related to DM. Different phenotypic patterns imply the existence of corresponding distinct pathogens (Table 1). Thus, it is proposed that hyperglycemia, lipotoxicity and hyperinsulinemia/insulin resistance leading to microvascular coronary dysfunction are more important in the pathogenesis of CMPDM and HFEFp and that the inflammatory/autoimmune response would be more relevant for DMPDM and HFEFr.

THERAPEUTIC MANAGEMENT - POSITIVE AND NEGATIVE EFFECTS OF HYPOGLYCEMIC AGENTS

The treatment of MCPDM should follow the guidelines recommended according to the cardiac phenotype (HFEFr or HFEFp). However, evidence points to some peculiarities related to the class of drugs and its relation with MCPDM. For example, for the HFEFp phenotype, the use of beta-blockers is questionable and may even be deleterious, as it may lead to a worse clinical outcome, mediated by cardiac dysfunction, especially in women[43]. In addition, metformins have a beneficial effect by activation of adenosine monophosphate-activated protein kinase (MAPK) and increased bioavailability of NO, but there was no improvement in left ventricular diastolic function compared to pioglitazone[44]. As for sulfonylureas, they may potentially interfere with myocardial adaptive response to ischemia and are not recommended in this setting. Here are some other specificities of MCPDM treatment.

Reduced glycemia

Glycemic levels are associated with an increased risk of incident HF, as well as atherothrombotic events in patients at risk or already with DM[45]. Thus, it is reasonable to conclude that the control

of glycemic levels would reduce HF and CV events in this group of patients. In a meta-analysis including 27,049 participants from the Accord, Advance, UKPDS and Vadt studies, the authors demonstrate a 15% reduction in the relative risk of fatal/nonfatal AMI in patients with intensive glycemic control, but no reduction in hospitalization for HF, stroke, or other causes of death[46]. In a HF cohort, intensive blood glucose control in diabetic patients resulted in a worse outcome. In this study, patients who achieved HbA1c ≤ 7.2 had an increased risk of death or urgently needed cardiac transplantation[47]. Another meta-analysis of more than 95 thousand individuals revealed that the treatment with hypoglycemic drugs increased the relative risk of HF in 14%, exceeding in 5% the reduction of the relative risk of AMI in these patients[48] and without any effect on mortality. One of the possible explanations connects to the metabolic inflexibility theory of cardiomyocytes. Stimulated entry of glucose into myocytes with reduced oxidation capacity of these molecules would lead to intracellular acidosis, resulting in reduction of the distensibility of the sarcomeric protein titin and diastolic dysfunction[49].

Peroxisome proliferator-activating receptor agonists-γ (PPAR-γ)

However, the increased risk of HF generated by hypoglycemic drugs is surely linked to the specific action of each drug. This same meta-analysis demonstrated a high risk of HF related to the use of peroxisome proliferator-activating receptor agonists-γ (PPAR-γ) (or thiazolidinediones) [risk ratio: 1.42 (1.15–1.76)][48]. These drugs provoke weight gain and water retention, impairing the hemodynamic condition of the patient with HF[50,51]. There is no reduction in cardiac systolic function[51,52] using thiazolidinediones. The deleterious effect is likely to occur by stimulating renal tubular sodium reabsorption by signaling PPAR-γ pathways dependent in these cells[53] consequent fluid retention. However, the simultaneous use of other diuretic drugs may counterbalance the observed weight and fluid gain. This has been demonstrated with the combination of pioglitazone dapaglifozin in a previous clinical trial[54]. In addition, one study suggests that pioglitazone reduces diastolic dysfunction in men with uncomplicated DM and absence of coronary disease[44].

As with thiazolidinediones, dipeptidyl peptidase-4 inhibitors (iDPP-4) had a moderate risk of incident HF associated with its use unrelated to significant weight gain [risk ratio: 1.25 (1.08-1.45)][48] in the meta-analysis cited. On the other hand, the use of insulin glargine (Origin study) and weight loss by lifestyle change (Look-Ahead study), despite a favorable trend, had a neutral effect on the risk of HF.

Inhibitors of dipeptidyl peptidase-4 (iDPP-4)

In another meta-analysis (n = 79,867) including only studies using iDPP-4 (which main randomized and controlled studies were Savor-Timi 53, Examine and Tecos), the authors demonstrate a 13% increase in HF (p = 0,03) after the introduction of the drugs of this class (I^2 = 0%). The authors isolated only the main randomized controlled trials (n = 36,543) (risk ratio = 1,14 (0,97-1,32)) and the risk remained, but they lost significance due to the large heterogeneity of the results (I^2 = 42%). The Savor-Timi 53 study apparently carries the difference between results, leading to presuming that saxagliptin is the main drug (22% increase in risk) in this class responsible for the incidence of HF - whereas sitagliptin would have no effect in this sense[55]. But, despite the large sample size, it has not yet been possible to determine with statistical certainty whether there is indeed a difference of effect within the iDPP-4 class (the sample was insufficient). Anyway, this is a worrying effect, because it happens mainly in populations that, for the most part, do not have previous history of HF.

IDPP-4 class drugs are easy to administer, do not cause weight gain or hypoglycaemia and have good gastrointestinal tolerability. However, they have potential cardiac and haemodynamic deleterious effects that may be exacerbated in patients with established or subclinical HF[56]. The DPP-4 enzyme is also responsible for degrading the stromal cell-derived factor (SDF) -1 [in addition to the peptides *glucagon-like* (GLP) -1]. The increase in SDF-1, experimentally, channels mesenchymal cells to tissues undergoing overload or injury (*for example* myocardium) and promotes inflammation, regeneration, and fibrosis [57]. Another effect of SDF-1 is to increase sympathetic activity and to stimulate cyclic myocardial adenosine monophosphate (AMP-c), promoting hypertrophy and ventricular arrhythmias[58]. Finally, the natriuretic effect of iDPP-4 is unobtrusive, not leading to weight loss, and appears to be the effect localized only to the distal portion of the renal tubules.[59] The higher sodium reabsorption occurs, however, in the proximal tubules, which is

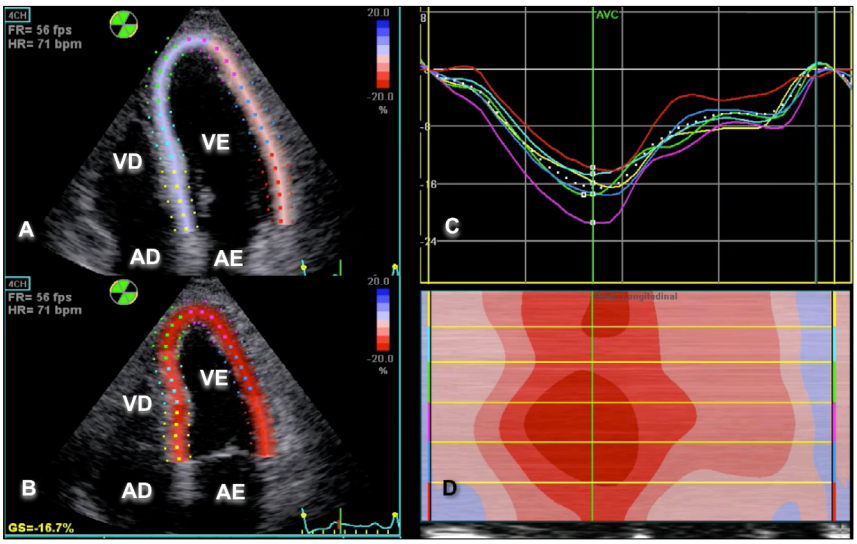
why GLP-1 receptor agonists and sodium-glucose transporter inhibitors 2 (iSGLT2) have a greater diuretic effect: they act preferentially at this site[60,61].

Sodium-glucose transporter inhibitors 2 (iSGLT2)

The Empa-REG Outcome clinical trial revealed an impressive relative risk reduction of 35% relative to the use of empaglifozine compared to the placebo group[62] in patients with established cardiovascular disease. Curiously, the results are similar for patients with previous diagnosis of HF at the beginning of the study or not. Similar findings were found in the study that evaluated the effect of canagliptin on outcomes of CV[63], a 32% reduction in hospitalizations due to HF. In parallel, "real world" results (> 180,000 patients) in a very broad population (United States and North America, Europe, the Middle East and Asia) corroborate these findings and add that this may be a class effect, as well as to be possible in individuals with no prior history of atherosclerosis[64,65]. The relative risk reduction of HF, in this case compared to other hypoglycemic agents, was similar to that found in the Empa-REG and Canvas study, about 28%.

The beneficial effects related to the use of these drugs are independent of the intensity of the glycemic reduction and may be related to significant weight loss and reduction of blood volume. However, other potential mechanisms are proposed. The use of empaglifozine deviates the use of glucose to fat

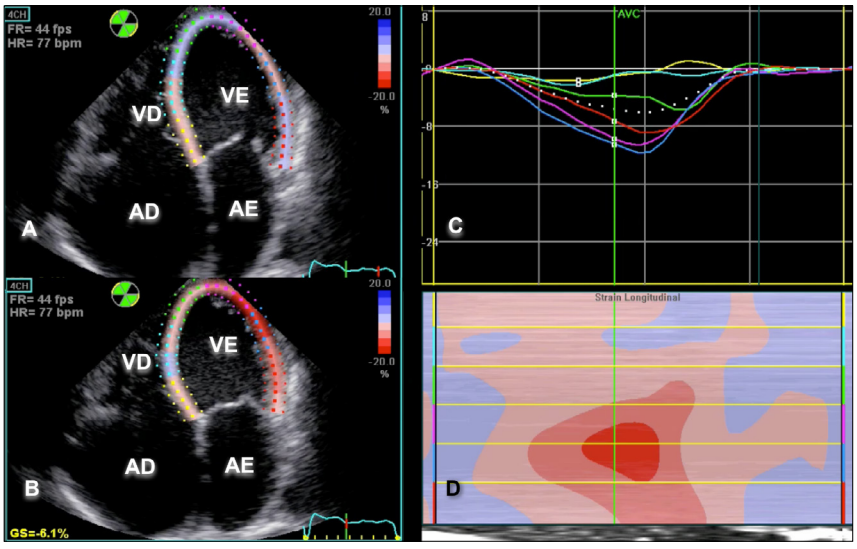
FIGURE 1: ECHOCARDIOGRAM OF PATIENT WITH CLINICAL HF AND PRESERVED LV EJECTION FRACTION



Panel A: left ventricle in diastole.
Panel B: left ventricle in systole.
Panel C: LV myocardial systolic longitudinal deformation, where each line represents a segment (Y axis) along the cardiac cycle.
Panel D: anatomic M mode of the LV in the four-chamber window: the systolic deformation of each segment (y-axis) is represented by color over time (x-axis), darker red tones are considered normal and abnormal, light tones.

AD: right atrium;
AE: left atrium;
RV: right ventricle;
LV: left ventricle;
AVC: *aortic valve closure*

FIGURE 2: ECHOCARDIOGRAM OF PATIENT WITH CLINICAL HF AND REDUCED LV EJECTION FRACTION



Panel A: left ventricle in diastole.
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as an energetic substrate by the myocardium. The homeostatic balance favors the production of glucagon in relation to insulin and therefore the release of glucose and ketogenesis[66]. One of the products of ketogenesis, β-hydroxybutyrate, is associated with a reduction in myocardial glucose uptake. Possible positive effects of ketone bodies as an energetic substrate for the myocardium include resistance to oxidative stress, reduced lipolysis, lower metabolic rate, and reduced sympathetic tone[67]. At three months of treatment, there are also signs of mass reduction and recovery of LV distensibility[68]. Other pathways in which iSGLT2 may hinder the establishment of MCPDM include: reduction of insulin resistance, blood pressure, albuminuria and uric acid, attenuation of arterial stiffness, inflammation, oxidative stress and sympathetic hyperactivity, and regulation for angiotensin receptor blockers.

Insulin

Intensive insulin use does not appear to reduce LV diastolic dysfunction in patients with MCPDM. Despite some disagreement between studies, some suggesting positive results[69] and others not, there is no evidence of improvement of left ventricular relaxation with intense glycemic control through the use of insulin[70]. The potential benefit of glycemic control may be counterbalanced by weight gain promoted by insulin use.

FINAL COMMENTS

MCPDM is a complex and difficult diagnosis condition. Its recognition has been dubious due to the simultaneity of pathogenic effects promoted by diseases and associated CV risk factors. However, the evidence has increasingly made clear the pathophysiology and clinical presentation of this cardiomyopathy, as well as demonstrated that its validity may worsen the prognosis of coexisting diseases. Many aspects of MCPDM are not yet understood, so awareness of diagnosis is necessary in the care and academic circles to create mechanisms to attenuate their ominous repercussions.

RESUMO

Apesar de há muito tempo descrita, não existe consenso estabelecido quanto à real existência da cardiomiopatia diabética (CMPDM). Devido à sua complexa fisiopatologia, tem sido árduo à pesquisa clínica e experimental estabelecer conexões claras entre diabetes mellitus (DM) e insuficiência cardíaca (IC), assim como solucionar os mecanismos da doença subjacente do miocárdio. No entanto, as evidências epidemiológicas da relação dessas condições são incontestáveis. O interesse em compreender melhor essa doença tem recrudescido devido aos recentes resultados de ensaios clínicos avaliando novos fármacos hipoglicemiantes, como os inibidores do transportador de sódio-glicose 2, que demonstraram respostas favoráveis, considerando-se a prevenção e tratamento da IC em pacientes portadores de DM. Nesta revisão, percorremos aspectos da epidemiologia da CMPDM e de seus possíveis mecanismos patogênicos, além de apresentarmos os principais fenótipos cardíacos da CMPDM (IC com fração de ejeção preservada e reduzida) e implicações do manejo terapêutico desta doença.

PALAVRAS-CHAVE: *Diabetes mellitus. Cardiomiopatias. Hipoglicemiantes. Insuficiência cardíaca.*

REFERENCES

1. Ryden L, Armstrong PW, Cleland JG, Horowitz JD, Massie BM, Packer M, et al. Efficacy and safety of high-dose lisinopril in chronic heart failure patients at high cardiovascular risk, including those with diabetes mellitus. Results from the ATLAS trial. Eur Heart J. 2000;21(23):1967-78.
2. Shindler DM, Kostis JB, Yusuf S, Quinones MA, Pitt B, Stewart D, et al. Diabetes mellitus, a predictor of morbidity and mortality in the Studies of Left Ventricular Dysfunction (SOLVD) Trials and Registry. Am J Cardiol. 1996;77(11):1017-20.
3. Thrainsdottir IS, Aspelund T, Thorgeirsson G, Gudnason V, Hardarson T, Malmberg K, et al. The association between glucose abnormalities and heart failure in the population-based Reykjavik study. Diabetes Care. 2005;28(3):612-6.
4. Suskin N, McKelvie RS, Burns RJ, Latini R, Pericak D, Probstfield J, et al. Glucose and insulin abnormalities relate to functional capacity in patients with congestive heart failure. Eur Heart J. 2000;21(16):1368-75.
5. Domanski M, Krause-Steinrauf H, Deedwania P, Follmann D, Ghali JK, Gilbert E, et al. The effect of diabetes on outcomes of patients with advanced heart failure in the BEST trial. J Am Coll Cardiol. 2003;42(5):914-22.
6. MacDonald MR, Petrie MC, Varyani F, Ostergren J, Michelson EL, Young JB, et al. Impact of diabetes on outcomes in patients with low and preserved ejection fraction heart failure: an analysis of the Candesartan in Heart failure: Assessment of Reduction in Mortality and morbidity (CHARM) programme. Eur Heart J. 2008;29(11):1377-85.
7. Kannel WB, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: the Framingham study. Am J Cardiol. 1974;34(1):29-34.
8. Iribarren C, Karter AJ, Go AS, Ferrara A, Liu JY, Sidney S, et al. Glycemic control and heart failure among adult patients with diabetes. Circulation. 2001;103(22):2668-73.
9. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ. 2000;321(7258):405-12.
10. Aguilar D, Bozkurt B, Ramasubbu K, Deswal A. Relationship of hemoglobin A1C and mortality in heart failure patients with diabetes. J Am Coll Cardiol. 2009;54(5):422-8.
11. Goode KM, John J, Rigby AS, Kilpatrick ES, Atkin SL, Bragadeesh T, et al. Elevated glycated haemoglobin is a strong predictor of mortality in pa-

tients with left ventricular systolic dysfunction who are not receiving treatment for diabetes mellitus. Heart. 2009;95(11):917-23.

12. Eshaghian S, Horwich TB, Fonarow GC. An unexpected inverse relationship between HbA1c levels and mortality in patients with diabetes and advanced systolic heart failure. Am Heart J. 2006;151(1):91.
13. Shenouda SM, Widlansky ME, Chen K, Xu G, Holbrook M, Tabit CE, et al. Altered mitochondrial dynamics contributes to endothelial dysfunction in diabetes mellitus. Circulation. 2011;124(4):444-53.
14. van Heerebeek L, Hamdani N, Falcão-Pires I, Leite-Moreira AF, Begieneman MP, Bronzwaer JG, et al. Low myocardial protein kinase G activity in heart failure with preserved ejection fraction. Circulation. 2012;126(7):830-9.
15. Hamdani N, Franssen C, Lourenço A, Falcão-Pires I, Fontoura D, Leite S, et al. Myocardial titin hypophosphorylation importantly contributes to heart failure with preserved ejection fraction in a rat metabolic risk model. Circ Heart Fail. 2013;6(6):1239-49.
16. Falcão-Pires I, Hamdani N, Borbely A, Gavina C, Schalkwijk CG, van der Velden J, et al. Diabetes mellitus worsens diastolic left ventricular dysfunction in aortic stenosis through altered myocardial structure and cardiomyocyte stiffness. Circulation. 2011;124(10):1151-9.
17. Asbun J, Villarreal FJ. The pathogenesis of myocardial fibrosis in the setting of diabetic cardiomyopathy. J Am Coll Cardiol. 2006;47(4):693-700.
18. Slawson C, Copeland RJ, Hart GW. O-GlcNAc signaling: a metabolic link between diabetes and cancer? Trends Biochem Sci. 2010;35(10):547-55.
19. Qin CX, Sleaby R, Davidoff AJ, Bell JR, De Blasio MJ, Delbridge LM, et al. Insights into the role of maladaptive hexosamine biosynthesis and O-GlcNAcylation in development of diabetic cardiac complications. Pharmacol Res. 2017;116:45-56.
20. Murray AJ, Anderson RE, Watson GC, Radda GK, Clarke K. Uncoupling proteins in human heart. Lancet. 2004;364(9447):1786-8.
21. Boudina S, Abel ED. Mitochondrial uncoupling: a key contributor to reduced cardiac efficiency in diabetes. Physiology (Bethesda). 2006;21:250-8.
22. Kruger M, Babicz K, von Frieling-Salewsky M, Linke WA. Insulin signaling regulates cardiac titin properties in heart development and diabetic cardiomyopathy. J Mol Cell Cardiol. 2010;48(5):910-6.
23. Dutka DP, Pitt M, Pagano D, Mongillo M, Gathercole D, Bonser RS, et al. Myocardial glucose transport and utilization in patients with type 2 diabetes mellitus, left ventricular dysfunction, and coronary artery disease. J Am Coll Cardiol. 2006;48(11):2225-31.
24. Mather KJ, Hutchins GD, Perry K, Territo W, Chisholm R, Acton A, et al. Assessment of myocardial metabolic flexibility and work efficiency in human type 2 diabetes using 16-[18F]fluoro-4-thiapalmitate, a novel PET fatty acid tracer. Am J Physiol Endocrinol Metab. 2016;310(6):E452-60.
25. Tuunanen H, Engblom E, Naum A, Någren K, Hesse B, Airaksinen KE, et al. Free fatty acid depletion acutely decreases cardiac work and efficiency in cardiomyopathic heart failure. Circulation. 2006;114(20):2130-7.
26. Rijzewijk LJ, van der Meer RW, Lamb HJ, Jong HW, Lubberink M, Romijn JA, et al. Altered myocardial substrate metabolism and decreased diastolic function in nonischemic human diabetic cardiomyopathy: studies with cardiac positron emission tomography and magnetic resonance imaging. J Am Coll Cardiol. 2009;54(16):1524-32.
27. Zhang L, Ussher JR, Oka T, Cadete VJ, Wagg C, Lopaschuk GD. Cardiac diacylglycerol accumulation in high fat-fed mice is associated with impaired insulin-stimulated glucose oxidation. Cardiovasc Res 2011;89(1):148-56.
28. Rijzewijk LJ, van der Meer RW, Smit JW, Diamant M, Bax JJ, Hammer S, et al. Myocardial steatosis is an independent predictor of diastolic dysfunction in type 2 diabetes mellitus. J Am Coll Cardiol. 2008;52(22):1793-9.
29. Donaldson C, Taatjes DJ, Zile M, Palmer B, VanBuren P, Spinale F, et al. Combined immunoelectron microscopic and computer-assisted image analyses to detect advanced glycation end-products in human myocardium. Histochem Cell Biol. 2010;134(1):23-30.
30. Zhang M, Kho AL, Anilkumar N, Chibber R, Pagano PJ, Shah AM, et al. Glycated proteins stimulate reactive oxygen species production in cardiac myocytes: involvement of Nox2 (gp91phox)-containing NADPH oxidase. Circulation. 2006;113(9):1235-43.
31. Camici PG, Crea F. Coronary microvascular dysfunction. N Engl J Med. 2007;356(8):830-40.
32. van Heerebeek L, Hamdani N, Handoko ML, Falcão-Pires I, Musters RJ, Kupreishvili K, et al. Diastolic stiffness of the failing diabetic heart: importance of fibrosis, advanced glycation end products, and myocyte resting tension. Circulation. 2008;117(1):43-51.
33. Selvin E, Lazo M, Chen Y, Shen L, Rubin J, McEvoy JW, et al. Diabetes mellitus, prediabetes, and incidence of subclinical myocardial damage. Circulation. 2014;130(16):1374-82.
34. Masuo K, Rakugi H, Ogihara T, Esler MD, Lambert GW. Cardiovascular and renal complications of type 2 diabetes in obesity: role of sympathetic nerve activity and insulin resistance. Curr Diabetes Rev. 2010;6(2):58-67.
35. Schnell O, Kirsch CM, Stemplinger J, Haslbeck M, Standl E. Scintigraphic evidence for cardiac sympathetic dysinnervation in long-term IDDM patients with and without ECG-based autonomic neuropathy. Diabetologia. 1995;38(11):1345-52.
36. Schnell O, Muhr D, Weiss M, Dresel S, Haslbeck M, Standl E. Reduced myocardial 123I-metaiodobenzylguanidine uptake in newly diagnosed IDDM patients. Diabetes. 1996;45(6):801-5.
37. Kumar R, Yong QC, Thomas CM, Baker KM. Intracardiac intracellular angiotensin system in diabetes. Am J Physiol Regul Integr Comp Physiol. 2012;302(5):R510-7.
38. Di Carli MF, Janisse J, Grunberger G, Ager J. Role of chronic hyperglycemia in the pathogenesis of coronary microvascular dysfunction in diabetes. J Am Coll Cardiol. 2003;41(8):1387-93.
39. Seferovic PM, Paulus WJ. Clinical diabetic cardiomyopathy: a two-faced disease with restrictive and dilated phenotypes. Eur Heart J. 2015;36(27):1718-27, 27a-27c.
40. Marwick TH, Ritchie R, Shaw JE, Kaye D. Implications of underlying mechanisms for the recognition and management of diabetic cardiomyopathy. J Am Coll Cardiol. 2018;71(3):339-51.
41. Fang ZY, Prins JB, Marwick TH. Diabetic cardiomyopathy: evidence, mechanisms, and therapeutic implications. Endocr Rev. 2004;25(4):543-67.
42. Dunlay SM, Roger VL, Weston SA, Jiang R, Redfield MM. Longitudinal changes in ejection fraction in heart failure patients with preserved and reduced ejection fraction. Circ Heart Fail. 2012;5(6):720-6.
43. Farasat SM, Bolger DT, Shetty V, Menachery EP, Gerstenblith G, Kasper EK, et al. Effect of beta-blocker therapy on rehospitalization rates in women versus men with heart failure and preserved ejection fraction. Am J Cardiol. 2010;105(2):229-34.
44. van der Meer RW, Rijzewijk LJ, de Jong HW, Lamb HJ, Lubberink M, Romijn JA, et al. Pioglitazone improves cardiac function and alters myocardial substrate metabolism without affecting cardiac triglyceride accumulation and high-energy phosphate metabolism in patients with well-controlled type 2 diabetes mellitus. Circulation. 2009;119(15):2069-77.
45. Rao Kondapally Seshasai S, Kaptoge S, Thompson A, Di Angelantonio E, Gao P, Sarwar N, et al. Diabetes mellitus, fasting glucose, and risk of cause-specific death. N Engl J Med. 2011;364(9):829-41.
46. Control G, Turnbull FM, Abraira C, Anderson RJ, Byington RP, Chalmers JP, et al. Intensive glucose control and macrovascular outcomes in type 2 diabetes. Diabetologia. 2009;52(11):2288-98.
47. Tomova GS, Nimbal V, Horwich TB. Relation between hemoglobin a(1c) and outcomes in heart failure patients with and without diabetes mellitus. Am J Cardiol. 2012;109(12):1767-73.
48. Udell JA, Cavender MA, Bhatt DL, Chatterjee S, Farkouh ME, Scirica BM. Glucose-lowering drugs or strategies and cardiovascular outcomes in patients with or at risk for type 2 diabetes: a meta-analysis of randomised controlled trials. Lancet Diabetes Endocrinol. 2015;3(5):356-66.
49. Kotter S, Unger A, Hamdani N, Lang P, Vorgerd M, Nagel-Steger L, et al. Human myocytes are protected from titin aggregation-induced stiffening by small heat shock proteins. J Cell Biol 2014;204(2):187-202.
50. Nesto RW, Bell D, Bonow RO, Fonseca V, Grundy SM, Horton ES, et al. Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association. October 7, 2003. Circulation. 2003;108(23):2941-8.
51. Shah M, Kolandaivelu A, Fearon WF. Pioglitazone-induced heart failure despite normal left ventricular function. Am J Med. 2004;117(12):973-4.
52. St John Sutton M, Rendell M, Dandona P, Dole JF, Murphy K, Patwardhan R, et al. A comparison of the effects of rosiglitazone and glyburide on cardiovascular function and glycemic control in patients with type 2 diabetes. Diabetes Care. 2002;25(11):2058-64.
53. Zhang H, Zhang A, Kohan DE, Nelson RD, Gonzalez FJ, Yang T. Collecting duct-specific deletion of peroxisome proliferator-activated receptor gamma blocks thiazolidinedione-induced fluid retention. Proc Natl Acad Sci U S A. 2005;102(26):9406-11.
54. Rosenstock J, Vico M, Wei L, Salsali A, List JF. Effects of dapagliflozin, an SGLT2 inhibitor, on HbA(1c), body weight, and hypoglycemia risk in pa-

tients with type 2 diabetes inadequately controlled on pioglitazone monotherapy. Diabetes Care. 2012;35(7):1473-8.

55. Verma S, Goldenberg RM, Bhatt DL, Farkouh ME, Quan A, Teoh H, et al. Dipeptidyl peptidase-4 inhibitors and the risk of heart failure: a systematic review and meta-analysis. CMAJ Open. 2017;5(1):E152-E77.

56. Packer M. Worsening heart failure during the use of DPP-4 inhibitors: pathophysiological mechanisms, clinical risks, and potential influence of concomitant antidiabetic medications. JACC Heart Fail. 2018;6(6):445-51.

57. Chu PY, Mariani J, Finch S, McMullen JR, Sadoshima J, Marshall T, et al. Bone marrow-derived cells contribute to fibrosis in the chronically failing heart. Am J Pathol. 2010;176(4):1735-42.

58. Miyoshi T, Nakamura K, Yoshida M, Miura D, Oe H, Akagi S, et al. Effect of vildagliptin, a dipeptidyl peptidase 4 inhibitor, on cardiac hypertrophy induced by chronic beta-adrenergic stimulation in rats. Cardiovasc Diabetol. 2014;13:43.

59. Lovshin JA, Rajasekeran H, Lytvyn Y, Lovblom LE, Khan S, Alemu R, et al. Dipeptidyl peptidase 4 inhibition stimulates distal tubular natriuresis and increases in circulating SDF-1alpha(1-67) in patients with type 2 diabetes. Diabetes Care. 2017;40(8):1073-81.

60. Ghezzi C, Yu AS, Hirayama BA, Kepe V, Liu J, Scafoglio C, et al. Dapagliflozin binds specifically to sodium-glucose cotransporter 2 in the proximal renal tubule. J Am Soc Nephrol. 2017;28(3):802-10.

61. Muskiet MHA, Tonneijck L, Smits MM, van Baar MJB, Kramer MHH, Hoorn EJ, et al. GLP-1 and the kidney: from physiology to pharmacology and outcomes in diabetes. Nat Rev Nephrol. 2017;13(10):605-28.

62. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med. 2015;373(22):2117-28.

63. Neal B, Perkovic V, Mahaffey KW, Zeeuw D, Fulcher G, Erondu N, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. N Engl J Med. 2017;377(7):644-57.

64. Kosiborod M, Lam CSP, Kohsaka S, Kim DJ, Karasik A, Shaw J, et al. Cardiovascular events associated with SGLT-2 inhibitors versus other glucose-lowering drugs: the CVD-REAL 2 study. J Am Coll Cardiol. 2018;71(23):2628-39.

65. Kosiborod M, Cavender MA, Fu AZ, Wilding JP, Khunti K, Holl RW, et al. Lower risk of heart failure and death in patients initiated on sodium-glucose cotransporter-2 inhibitors versus other glucose-lowering drugs: the CVD-REAL study (Comparative effectiveness of cardiovascular outcomes in new users of sodium-glucose cotransporter-2 inhibitors). Circulation. 2017;136(3):249-59.

66. Ferrannini E, Baldi S, Frascerra S, Astiarraga B, Heise T, Bizzotto R, et al. Shift to fatty substrate utilization in response to sodium-glucose cotransporter 2 inhibition in subjects without diabetes and patients with type 2 diabetes. Diabetes. 2016;65(5):1190-5.

67. Newman JC, Verdin E. Ketone bodies as signaling metabolites. Trends Endocrinol Metab. 2014;25(1):42-52.

68. Verma S, Garg A, Yan AT, Gupta AK, Al-Omran M, Sabongui A, et al. Effect of empagliflozin on left ventricular mass and diastolic function in individuals with diabetes: an important clue to the EMPA-REG OUTCOME trial? Diabetes Care. 2016;39(12):e212-e3.

69. von Bibra H, Hansen A, Dounis V, Bystedt T, Malmberg K, Ryden L. Augmented metabolic control improves myocardial diastolic function and perfusion in patients with non-insulin dependent diabetes. Heart. 2004;90(12):1483-4.

70. Jarnert C, Landstedt-Hallin L, Malmberg K, Melcher A, Ohrvik J, Persson H, et al. A randomized trial of the impact of strict glycaemic control on myocardial diastolic function and perfusion reserve: a report from the DADD (Diabetes mellitus And Diastolic Dysfunction) study. Eur J Heart Fail. 2009;11(1):39-47.

REVIEW ARTICLE

Inhibition of the sodium-glucose co-transporter 2 in the elderly: clinical and mechanistic insights into safety and efficacy

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SUMMARY

The prevalence of type 2 diabetes mellitus (T2DM) in the elderly grew sharply over the last decade. Reduced insulin sensitivity and secretory capacity, weight gain, sarcopenia, and elevated adiposity are all common metabolic and body changes in the aging population that favor an increased risk of hypoglycemia, frailty syndrome, falls, and cognitive dysfunction. First line antidiabetic therapy is frequently not safe in older individuals because of its high risk of hypoglycemia and prevalent co-morbid diseases, such as chronic kidney disease, osteoporosis, cardiovascular disease, and obesity. Sodium-glucose cotransporter 2 inhibitor (SGLT2i) is a new class of antidiabetic therapy that inhibits glucose and sodium reabsorption on renal proximal convoluted tubule. Its effect is well demonstrated in various clinical scenarios in the younger population. This review and metanalysis describe particularities of the SGLT2i on the elderly, with mechanistic insights of the potential benefit and remaining challenges about the use of these drugs in this important age group. Further, we will present a meta-analysis of the main effects of SGLT2i reported in post-hoc studies in which the median age of the subgroups analyzed was over 60 years. Despite the absence of specific clinical trials for this population, our findings suggest that SGLT2i therapy on older individuals is effective to lower glucose and maintain its effect on systolic blood pressure and body weight.

KEYWORDS: *Sodium-glucose transporter/antagonists & inhibitors. Diabetes mellitus. Aging. Effectiveness.*

INTRODUCTION

The rapid increase in longevity and the prevalence of type 2 diabetes mellitus (T2DM) are among the most striking epidemiological challenges of recent decades. As a consequence, nearly 50% of individuals with T2DM are 65 years old or older[1], and 27% of those aged 65 years or older have T2DM[2]. These figures represent an increase of 62% over the last decade[3].

Over the last 25 years, population aging was the main factor for the 41% global increase in deaths due to cardiovascular disease[4]. For these older individuals, the presence of T2DM has doubled the risk of death[5]. Since these evolving changes are of particular importance, special attention must be paid to clinical and mechanistic features related to aging as well as

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to the interaction of these biological changes with hypoglycemic therapies.

Aging predisposes a person to T2DM in a number of mechanisms, which include apoptosis of beta cells, reduced insulin sensitivity from sarcopenia, decreased mitochondrial activity, and increased lipid content in cell membranes – particularly in hepatocyte and myocyte[6,7]. Beta cells of aged individuals have reduced secretory capacity. This is probably due to different mechanisms such as reduced glucose sense transporters, reduced insulin secretion related to mitochondrial activity, and impaired function of K-ATP voltage channels[8]. In clinical studies, this phenomenon has been related to reductions in the amplitude of insulin pulse secretion and glucose-stimulated insulin secretion [8,9]. Aging also leads to a progressive decline in insulin sensitivity, which is the result of processes that include increased adiposity, sarcopenia, and mitochondrial dysfunction [6,7,10,11]. A successful glucose-lowering strategy for the elderly must account for the limitations in increasing insulin secretion and the reduced effect of insulin on muscular and adipose tissues. In line with these assumptions, lowering the threshold for glycosuria has emerged as a promising therapeutic target for T2DM elderlies.

About 90% of glucose filtered by the kidney is reabsorbed by the sodium glucose co-transporter 2 (SGLT2), which is the most active co-transporter expressed at the proximal convoluted tubule [12]. In healthy individuals, virtually all filtered glucose above 180 mg/dL is excreted; however, in diabetic individuals, glucose threshold excretion is at least 20% higher, probably due to up-regulation of SGLT2 and glucose transport channels in the nephron [13].

From an evolutionary perspective, it seems reasonable that an efficient mechanism to preserve energy loss through urine has been selected [13]. On the other hand, higher glycemic levels may contribute to metabolic disturbances, such as insulin resistance and reduced insulin secretion. Therefore, an adequate balance between retaining and losing glucose through the kidney is of particular interest and certainly plays a role in helping the maintenance of a favorable metabolic profile. Accordingly, SGLT2 inhibitors (SGLT2i) reduce glycemia in an insulin-independent manner and contribute to the improvement of beta cells function [14,15] and insulin sensitivity [14-17].

Only recently, clinical trials with SGLT2i started enrolling older individuals, and many questions remain open to debate. To tackle this issue, this review presents a meta-analysis of the main effects of SGLT2i reported in post-hoc studies focused on elderly individuals (Table 1 and Table 2) and describes particularities of the SGLT2i effect on the elderly, with mechanistic insights of the potential benefits and remaining challenges about the use of these drugs in this important age group.

Methods for Systematic Review and Meta-analysis of SGLT2i Trials in Elderly

We used the methods recommended by the Cochrane guidelines to conduct the meta-analysis and reported our findings according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) statement[29]. All procedures performed for this analysis are presented in detail in the supplement.

Role of the funding source

There was no funding source for this study. The corresponding authors had full access to all the data in the study and were fully responsible for the decision to submit it for publication.

EFFICACY IN CLINICAL TRIALS

Anti-hyperglycemic effects

Recent guidelines have defined glycemic therapeutic goals that are less stringent in older individuals when compared to younger ones, based on the increased risk of hypoglycemia. Customized therapeutic targets consider functional status, comorbidities, and life expectancy[30]. More leniency with higher glucose levels, however, does not necessarily prevent hypoglycemia[31], but it could instead favor dehydration, cognitive decline, falls, and other complications[30]. Therefore, a more desirable option would be a potent antidiabetic treatment that bears a low risk for hypoglycemia. SGLT2i qualifies for this premise being associated with a risk of hypoglycemia of less than 1% in the elderly when used alone, a number three times lower than that obtained with DPP-IV inhibitors and 15 to 20 times lower than the one observed with sulphonylurea[32].

Aging may influence the efficacy of SGLT2i by either indirect or direct mechanisms. Indirectly, the age-dependent decline in glomerular filtration rate (GFR) may reduce the glucose-lowering effect of these drugs via down-regulation of the tubular

TABLE 1. CLINICAL CHARACTERISTICS OF PATIENTS ENROLLED IN THE META-ANALYSIS

	n	Follow-up time (years)	whites_	women	Age at entry (years)	CKD (%)	CKD stages III or IV (%)	Treatment arms	Publication year
Zinman et al.[18], 2015	7020	3.1	72.3	29	63.2	26	26	Placebo vs Empagliflozin 10/25mg	2015
Tikkanen et al.[19], 2015	549	0.23	93.7	39.9	60.2	0	0	Placebo vs Empagliflozin 10mg	2015
Barnett et al.[20], 2014 (CKD II)	290	1	68.3	39	62.6	100	0	Placebo vs Empagliflozin 10/25mg	2014
Barnett et al.[20], 2014 (CKD III)	374	1	56.1	43	64.9	100	100	Placebo vs Empagliflozin 25mg	2014
Barnett et al.[20], 2014 (CKD IV)	74	1	50	46	64.1	100	100	Placebo vs Empagliflozin 25mg	2014
Bolinder et al.[21], 2014	182	1.96	100	44.4	60.7	64	3	Placebo+MTF vs Dapagliflozin 10mg+MTF	2014
Matthaei et al.[22], 2015	218	0.46	95	49	61	0	0	Placebo+MTF/SFU vs Dapagliflozin 10mg+MTF/SFU	2015
Kohan et al.[23], 2014	252	1.96	86	35	67	100	96	Placebo vs Dapagliflozin 5/10mg	2014
Leiter et al.[24], 2014	962	1	84	31.7	62.9	0	0	Placebo vs Dapagliflozin 10mg	2015
Neal et al.[25], 2015	2072	1	75	34	62.7	25	NR	Placebo vs Canaglifozin 100/300mg	2015
Yale et al.[26], 2014	269	1	79.9	39.4	68.5	100	100	Placebo vs Canaglifozin 300mg	2014
Sinclair et al.[27], 2014	1085	0.5	84.4	41	67	100	100	Placebo vs Canaglifozin 100/300mg	2014
Bode et al.[28], 2014	714	1.96	77.3	44.5	64.6	0	0	Placebo vs Canaglifozin 100/300mg	2015

TABLE 2. BASELINE LABORATORY AND CLINICAL DATA OF PATIENTS ENROLLED IN THE META-ANALYSIS

	HbA1c in SGLT2i arm		SBP in SGLT2i arm		DBP in SGLT2i arm		Body weight in SGLT2i arm	
	Baseline (%)	Change* (%)	Baseline (mmHg)	Change* (mmHg)	Baseline (mmHg)	Change* (mmHg)	Baseline (Kg)	Change* (Kg)
Zinman et al.[18], 2015	8.07	-0,24	134.9	-2,6	76.6	-0,3	85.9	-1,1
Tikkanen et al.[19], 2015	7.87	-0,65	142.3	-3,78	84.1	-1,52	94.71	-1,72
Barnett et al.[20], 2014 (CKD II)	8.02	-0,65	137.4	-4,63	76.5	-3,6	92.1	-1,76
Barnett et al.[20], 2014 (CKD III)	8.02	-0,42	137.4	-4,3	76.5	-1,5	83.2	-1,17
Barnett et al.[20], 2014 (CKD IV)	8.06	0,48	145	-12,2	77.2	-5,7	77.9	-1
Bolinder et al.[21], 2014	7.19	-0,42	NR	NR	NR	NR	92.1	-2,42
Matthaei et al.[22], 2015	8.08	-0,69	134.5	-3,7	80.4	NR	88.6	-2,1
Kohan et al.[23], 2014	8.22	-0,34	133.7	-6,04	73.8	-0,36	93.2	-3,63
Leiter et al.[24], 2014	8.18	-0,4	133.5	-3	77	NR	92.6	-1,9
Neal et al.[25], 2015	8.3	-0,72	137.1	-4,9	76.3	-1,9	94.8	-3,1
Yale et al.[26], 2014	8	-0,41	136.7	-6,09	75.7	NR	90.4	-0,99
Sinclair et al.[27], 2014	7.9	-0,7	134.3	-3,6	75.9	-2,7	90.2	-1,7
Bode et al.[28], 2014	7.75	-0,55	130.8	-6,6	75.5	-2,1	88.6	-2,5

* Relative to placebo change and baseline levels. SBP: systolic blood pressure; DBP: diastolic blood pressure; NR: not reported.Figure Captions

expression of SGLT2. Directly, early and still poorly explored findings have suggested that aging may reduce the expression of SGLT2[33]. In order to verify these arguments, we assessed the effect of SGLT2i in elderly individuals and as shown in Figure 1, we found an overall decrease of 0.402% in HbA1c (95% CI: -0.432, -0.370; p<0.001; I^2=0%), which is overall comparable to findings in younger age groups[15,17,24,27].

Blood Pressure Reduction

Although the coexistence of hypertension and T2DM could be related to their increased prevalence later in life, it may also stem from many common contributors such as aging, diabetic nephropathy, volume expansion, hyperinsulinemia, increased arterial stiffness, hyperglycemia[34,35]. More than 50% of hypertensive individuals are older than 60 years; and among individuals older than 60 years, up to 67% have hypertension[36]. Typically, isolated systolic hypertension (ISH) is the most frequent type of age-related hypertension and is responsible for up to 90% of cases in individuals older than 70[37].

Arterial stiffness seems to be the main link between aging and ISH, and its mechanistic basis is strongly related to elastin fracture and increased collagen deposition in blood vessel walls[38]. Among diabetic individuals, the process of arterial stiffening is accelerated by the deposition of advanced glycation products like glyoxal and methylglyoxal, which are responsible for a number of collagen cross-links[39]. The binding of these glycation products with endothelial cells also induces cell signaling that results in oxidative stress, increased expression of cytokines and adhesion molecules, and activation of nuclear factor-kappa B (NF-kB)[40]. Similarly, modifiable causes such as endothelial dysfunction or those related to the metabolism of uric acid, calcium or potassium may influence the stiffness of conductance arteries leaving room for therapeutic interventions[41]. In fact, treatment with empagliflozin, which attenuates oxidative stress and improves endo-

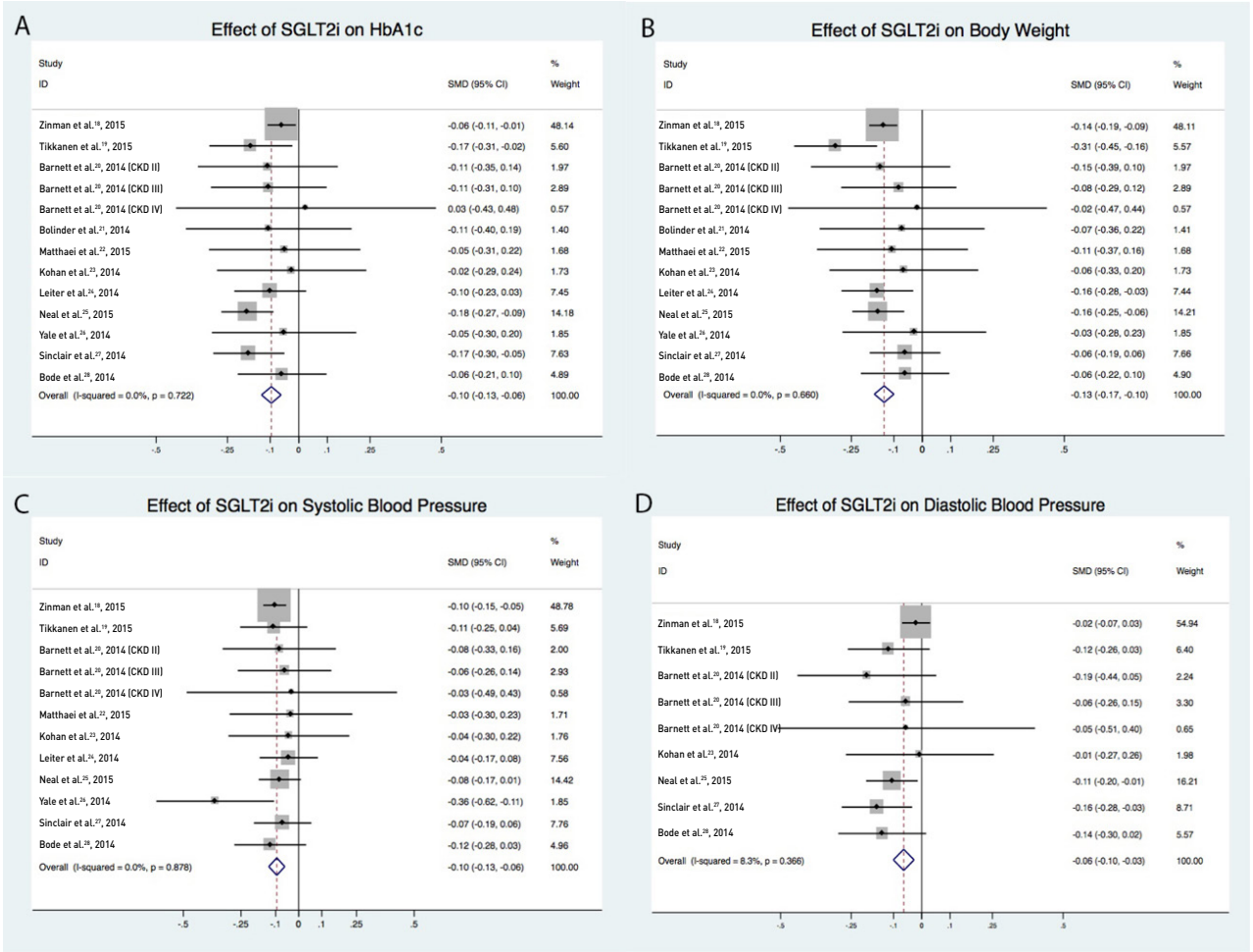

FIGURE 1. Meta-analysis of clinical trials mostly represented by individuals >60 years-old comparing SGLT2 inhibitors vs. placebo on (A) HbA1c, (B) Body weight, (C) Systolic blood pressure (SBP) and (D) Diastolic blood pressure (DBP).

thelial function, has led to a reduction in both arterial stiffness and vascular resistency[42-44].

In several studies, including some with older individuals, SGLT2i has reduced systolic BP[17,27,28,42]. For example, canagliflozin 300 mg has led to a 7.5 mmHg (95% CI −9.8, −5.2) reduction in systolic BP compared to the placebo over a 104 weeks treatment[17,27,28], which was consistent with further studies in individuals over 75 years-old treated with empagliflozin[42]. It is noteworthy that such an effect was independent of background therapies, such as metformin, sulphonylurea, GLP-1 agonists, and insulin[22,25,45]. Importantly, the magnitude of the effect achieved with SGLT2i is approximately half of that obtained with hydrochlorothiazide 25 mg[46], reaching a mean reduction in systolic BP of 3.45 mmHg (95% CI: (-4.10, -2.81); p <0.001; I^2=43.2%). Although this effect may not reach clinical benefit alone, it could solve the problem of the increasing number of drugs or dose in the antihypertensive therapy[47].

The effect of SGLT2i therapy on BP was tested on two specific trials, though none of these were exclusively performed on elders. The EMPA-REG BP trial assessed the efficacy of empagliflozin on BP reduction on hypertensive patients monitored by ambulatory BP monitoring (ABPM) using up to two hypotensive medications. The mean 24h systolic BP on empagliflozin 25 mg reduced by 4.16 mmHg (95% CI: -5.5, -2.83; p<0.001) and diastolic BP by -1.72 mmHg (95% CI: -2.51, -0.93; p<0.0001) compared to the placebo[19]. In a similar trial performed on hypertensive diabetic patients using on angiotensin-converting-enzyme inhibitor (ACEi) or angiotensin II receptor blockers (ARB) plus other anti-hypertensive medication, dapagliflozin 10 mg was associated to a mean 24-h systolic BP reduction of 4.28 mmHg (95% CI: –6.54, –2.02)[48]. This effect was similar across others antihypertensive medications such as thiazide diuretic, a calcium-channel blocker, and beta-blocker, though less intensive when dapagliflozin was added to thiazide diuretic[48].

Different factors may contribute to BP reduction on SGLT2i therapy[35]. In the early stage, increased natriuresis and osmotic diuresis favor systolic BP reduction[35]. This is likely the reason why a reduction of BP was observed as soon as 1 week after treatment with dapagliflozin[23]. Plasma volume contraction of -7.3% on dapagliflozin and -5.4% on canagliflozin was observed early on during the treatment[46,49]. Paradoxically, this effect on BP is maintained while sodium excretion and diuresis tend to return to previous levels over the first 12 weeks[19,49].

In addition to these effects, weight loss and glycemic control may also take part in the observed effect SGLT2i on BP[35]. Hyperglycemia is associated with up-regulation of SGLT2 and activation of both renin-angiotensin-aldosterone axis and sympathetic system[35]. Even weight loss obtained exclusively by dietary treatment can reduce BP[50], contributing in up to 28% of systolic BP reduction[51].

Therefore, it seems possible that SGLT2i therapy could contribute to the control of the hypertension burden in older individuals. It is unlikely that this effect in BP will be the determinant factor for choosing these medications in the future, but they could pose as ancillary therapy.

2.3 Weight loss

Weight loss in older individuals is a debating theme. In the general population, obesity has been extensively associated with multiple comorbidities and an increased incidence of cardiovascular events. In the elderly, observational studies have reported that weight loss is associated with falls, disability[52], increased morbidity[53] and mortality[52,54]. Multiple adjustments have been made in these studies in order to mitigate the interference of confounders. Still, the inability to exclude the potential interaction by unapparent or unknown disease-mechanisms underlying such spontaneous weight loss has carried on this controversy.

Intentional weight loss was not associated with increased mortality during a 12-years follow-up in obese individuals older than 65 years[55]. In a cohort of older adults, weight loss due to psychosocial stress was not associated with increased mortality, though an adverse outcome was still associated with spontaneous weight loss[56]. Hence, it is conceivable that spontaneous and induced weight loss could not be the same; the latter may even be considered as a therapeutic target.

Studies with induced weight loss on elders have found improvement in both lipid profile[57,58] and glucose metabolism[57,59]. In such studies, two-thirds of the weight loss is from fat and a third from lean tissue. While one might assume that this loss of lean body mass can trigger or intensify a loss of physical function, the results available indicate otherwise. In fact, the improvement of physical function is related to the amount of fat mass lost regardless of the amount of

lean mass lost [60]. The muscle's fat content is inversely related to its strength and directly related to its decline over time[61-64]. Thus, reduced muscle strength is a reliable marker of mortality and loss of mobility independently of lean body mass[65,66]. Also, the proportional increase in lean mass is associated with a gain in bone mineral density, an improvement regarding frailty syndrome[67] and a reduction in mortality[68].

Although weight loss goals have not yet been established, this effect of SGLT2i in the elderly has been tested in different backgrounds. As seen in Figure 1, the pooled effect of SGLT2i therapy across trials with the elderly was of - 1.72 kg (95% CI: -2.48, -0.97; p<0.0001; I^2=38.3%). For instance, canagliflozin 300 mg was associated with 3.0% (-2.7 kg) weight loss at 26 weeks[28], which was maintained over 104 weeks[17]. Clinically relevant weight reduction (considered as 5% of weight reduction) increased by 23% with this treatment[17,27]. On individuals ≥65 years, dapagliflozin induced a progressive weight reduction that reached -3.4 kg at 52 weeks[24].

This is also observed in individuals using medications associated with weight gain such as sulphonylurea or insulin, the addition of SGLT2i reduced body weight significantly[22,25,69,70]. Moreover, this effect is additive for other therapies associated with weight loss such as GLP-1 agonists[45].

The amount of glucose lost through urine provides a deficit of 200–400 kcal/day. With such caloric loss during a long-term follow-up, one could expect that the weight loss would be more significant than what was actually verified. The increase in caloric intake71 partially explains this reduced expected effect[1]. Such a compensatory increase in the caloric intake is proportional to the glucose excretion[71]. Potentially, the association between SGLT2i and appetite suppression would provide a higher and longer weight loss than the use of these drugs as monotherapy.

Besides hyperphagia, SGLT2i treatment in mice was associated with a decrease in oxygen consumption and brown adipose tissue thermogenesis via down-regulation of an inter-organ neural network consisting of the common hepatic vagal branch and sympathetic nerves[72]. Together, the reduction of energy consumption and increased caloric intake promote a balance to caloric loss through urine that keeps the initial weight loss achieved with SGLT2i in the first few weeks till up to 208 weeks[73].

Regarding the effect of SGLT2i in body composition, over 102 weeks of dapagliflozin treatment, fat mass changed by -1.34 kg (-2.44, -0.23) and lean mass changed by -0.4 kg (-1.0, 0.2)[23]. This led to a proportional change in body composition, with a decrease of −1.5% (−2.1, −0.8) of fat mass and an increase of 1.3% (0.5, 2.1) of lean mass[21]. In the mechanistic point of view, one possible explanation could be the effect of SGLT2i in increasing insulin sensitivity, thus favoring the anabolism of the muscle tissue[14,74].

Another issue yet to be clarified is the preferred effect of fat mass loss after SGLT2i in visceral or subcutaneous tissue. In a study using magnetic resonance imaging, the decrease in visceral adipose tissue (-258.4 cm^3 (-448.1, -68.6)) tended to be higher than that on subcutaneous adipose tissue (-184.9 cm^3 (-359.7, -10.1)), though this difference did not reach statistical significance in up to 102 weeks of treatment[21,74].

EFFECT ON KIDNEY AND RENAL DISEASE PROGRESSION

As a result of the natural decline in glomerular filtration rate (GFR), the elderly often present clinically relevant renal dysfunction, particularly with diabetes. Indeed, chronic kidney disease (CKD) affects more than 50% of individuals over 70 years[75]. Although less than 2% of stage 3 CKD patients require renal replacement in mid-term follow-up (8 years)[76], almost half end-stage kidney disease is attributed to DM2[77]. The relative risk of death and progression of end-stage renal disease is increased in elders with low GFR and high albuminuria, though its corresponding effect on mortality wanes at an older age[78].

Aging induces changes in the kidney as compared to a disease that occurs in some but not all individuals. The microanatomical structural changes of the kidney with older age include a decreased number of functional glomeruli from an increased prevalence of glomerulosclerosis, arteriosclerosis and tubular atrophy with interstitial fibrosis and compensatory hypertrophy of remaining nephrons. Among carefully screened healthy kidney donors, glomerular filtration rate (GFR) declines at a rate of 6.3 mL/min/1.73 m^2 per decade. The elderly have less kidney functional reserve when they do actually develop CKD, and they are at higher risk for diabetic kidney disease and its progression[79]. Diabetes accelerates these age-related changes leading to a higher functional decline and precocity[80,81]. In fact, senescent tubular phenotype cells could be induced by high glucose concentrations[82], and these alterations on tubular proximal cultured

cells are associated with increased expression of SGLT2[83].

Increased glomerular filtration is one of the earlier markers of diabetic nephropathy. The renin-angiotensin system (RAS) plays a significant role in glomerular hyperfiltration on diabetic nephropathy. Glucose can induce angiotensin II (AngII) generation by local activation of RAS[84], constriction of efferent arteriole thus influencing sodium reabsorption and increasing glomerular permeability[85].

Similarly, the increased sodium reabsorption through SGLT2 may also contribute to renal hyperfiltration. SGLT2 expression is increased at the proximal tubule of diabetic experimental models[86] and in diabetic patients[87]. This promotes a reduction of sodium on macula densa, thus increasing vasodilation on afferent arteriole[88]. The therapeutic use of SGLT2i increases the sodium delivery to the macula densa, thus decreasing GFR[89]. In parallel, other mediators participate in the regulation of renal hemodynamics, such as adenosine, nitric oxide, and calcium influx, contributing to the glomerular filtration rate. Thus, the hyperfiltration of diabetic kidney disease and its control will depend on the outcome of the balance of this set of players.

On aging CKD patients, an increased rate of nephron loss units is observed. In parallel, there is an adaptive decrease in SGTL2 transcription rate[90] and, consequently, a decrease in SGLT2i effectiveness on CKD patients, as SGLT2i acts on the extracellular surface of the tubule lumen cell[91]. Therefore, clinical trials on SGLT2i observed reduced effectiveness on glycemic control. A progressive decline on HbA1c effectiveness was reported on empagliflozin 25mg in individuals with CKD stage 2 (-0.68%), to stage 3 (-0.42%) and to stage 4 (+0.04%)[20]. An investigation of dapagliflozin effect on CKD individuals indicated that the cut-off point for the decline of SGLT2i glucose-lowering effect is GFR ≤45mL/min[23]. In the general population, the concomitant inhibition of SGLT1 and SGLT2 increases the glycosuria as compared with the isolated inhibition of SGLT2[92]. In CKD patients, however, a head-to-head comparison of their effect is unavailable.

Hypertension is the most common comorbidity among CKD patients and its prevalence increases as renal function worsens[93]. Particularly in this population, BP controlling could attenuate the progression of kidney disease[94]. On stage 3 and 4 CKD, SGLT2i therapy reduced SBP by - 5.46 mmHg (95% CI: -7.83, -3.07; p=0.001; I^2=0%). SGLT2i therapy was associated with a reduction on SBP and DBP by 52[20,23,26] and 104 weeks[23] among CKD patients. As with glycemia, empagliflozin effects on BP wane as renal insufficiency worsens[20].

On CKD patients, not only the above-cited mechanisms could be beneficial; body weight management is associated with hindering proteinuria and the prevention of GFR decline[95]. Indeed, SGLT2i therapy is associated with body weight reduction in CKD patients, which is inversely proportional to the severity of renal dysfunction[92]. This coupling is consistent with the reduced tubular expression of SGLT2 and the glycosuria induced by SGLT2i, which is proportional to the decline in GFR.

Probably as a consequence of constriction of the proximal arteriole, eGFR decreases by approximately 4 mL/min/1.73 m^2 on the general population and older patients[96]. The magnitude of the reduction on GFR during SGLT2i therapy is similar to that observed after distal arteriole dilation by ACEi[88,97]. This effect is observed as soon as after 1 week[98] and tended to return to baseline values during follow-up[17,96,98], though persistent reductions can be observed[25]. During SGLT2i therapy, older patients had similar or slightly higher reductions of eGFR compared to their younger counterpart[27,96,98]. However, even though aging does not significantly influence the variation in absolute GFR values, the percent loss of renal function may be higher due to preexisting renal dysfunction often found in the elderly[96,99]. Even though, a clinically significant decrease of GFR (at least 50%) is infrequent even in Stage 3 CKD (12.2%)[96].

Since the approval of the first SGL2i in March 2013 until October 2015, the FDA received reports of 101 confirmed cases of acute kidney injury (canagliflozin=73, dapagliflozin=28). Hospitalization and need of dialysis were required for a selected number of patients. Most improved with the discontinuation of the drug. There were no signs of direct drug toxicity, and most cases were observed in patients with predisposing factors to pre-renal AKI: decreased blood volume, chronic kidney insufficiency, congestive heart failure, concomitant medications such as diuretics, ACEi, ARBs, NSAID. The warning reinforces that the patient's kidney function should be assessed prior to starting treatment and be monitored periodically after that before being started on SGLT2 therapy. Also, one should consider temporarily discontinuing treatment in any setting of severe acute illness, prolonged fast-

ing, or severe fluid losses. If acute kidney injury occurs, the drug should be discontinued promptly.

SAFETY CONCERNS

Volume-depletion-related events

Adverse events (AE) related to volume depletion are of particular concern of this drug class. As it would be expectable, SGLT2i therapy induces plasma volume contraction through its effect on the proximal tubular cells. An increase in 24-h urine output of approximately 370 mL and up to 10% contraction of plasma volume have been reported on this treatment[46,49,100,101]. Whether or not and in what degree the plasma volume returns to baseline levels during therapy is still a debated theme[46,49]. However, safety concern on volume depletion has been assessed in recent clinical trials.

In safety clinical trials, volume depletion has been characterized by postural dizziness, orthostatic hypotension, increased in heart rate, dehydration, hypotension, orthostatic hypotension, pre-syncope, and syncope. In 104 weeks, the incidence is reported to be 5.9% on canagliflozin[17].

Probably as a consequence of plasma volume contraction, RAS activity and aldosterone levels are increased during SGLT2i therapy[46]. Actually, an elegant study demonstrated that during SGLT2i therapy there is an increase in both systemic and intrarenal RAS activity[97]. It is established the proatherosclerotic role of the RAS axis activation via a spectrum of mechanisms including inflammatory pathways, insulin resistance, hypertension, and oxidative stress[102]. The long-term effects of these pathways' activation with SGLT2i remain to be assessed. In the only published study in which the cardiovascular effect of SGLT2i was tested, about 80% of patients were on concomitant use of RAS inhibitors[18]. By presumption, it would be gainful to associate RAS inhibitor therapy with SGLT2i[48,49].

The incidence of volume-depletion-related AE is low, but it may increase as renal function worsens. For example, the incidence after empagliflozin is 1.0% on stage 2, 3.7% on stage 3 CKD and 5.4% on stage 4[20]. Likewise, the incidence of volume depletion AE is dose-dependent[103] and increased overtime[26,99]. There is no apparent influence of the concomitant use of anti-hypertensive medications[19] or even thiazide therapy on the incidence of these AE[48,104]. Among the elderly, special attention must be paid to orthostatic hypotension, whose incidence is increased in up to 9% after SGLT2i[19].

Osmotic diuresis-related AE

Since long-term therapy with osmotic diuresis became available for clinical practice for the first time after SGLT2i development, the pros and cons of this therapy remain theoretical. Potential AE includes pollakiuria, polyuria, dry mouth, dry throat, micturition urgency, nocturia, polydipsia, and increased thirst, among others. So, particular attention must be paid on elderly individuals to be treated by SGLT2i whose baseline pre-treatment condition is already associated with some of these symptoms. Osmotic diuresis symptoms are dose-related[28], GFR-influenced[23,26,96,99,103] and time-dependent, raising up to 12.3% over 104 weeks[17]. Also in patients in use of therapies associated with sodium retention such as insulin and sulfonylurea, similar rates of osmotic diuresis AE have been reported[25,45]. However, a SGLT2i-induced 3-fold rise (from 0.1 to 0.3%) in the incidence of volume-related AE were previously reported[98].

Diabetes Ketoacidosis

A particular type of diabetic ketoacidosis (DKA) has been reported during SGLT2i therapy which differs from the usual form by the attenuated expression of hyperglycemia and ketonuria; the called euglycemic DKA (eDKA). On a pooled analysis, eDKA frequency was overall low but slightly higher on SGLT2i (2-3 times) than with other anti-diabetic therapies[18,105].

SGLT2i therapy increases ketonemia in a dose-dependent manner via a spectrum of mechanisms[106]. In animal and cell models, SGLT2i therapy induces ketone production by directly inducing glucagon secretion by alpha pancreatic cells[107]. Consistently, in clinical studies, hepatic glucose production is up-regulated after SGLT2i treatment[14,16], at least in part due to the increase in glucagon levels stimulating hepatic ketogenesis and gluconeogenesis[108]. Likewise, SGLT2i increases the risk of eDKA by augmenting ketone absorption and reducing ketonuria[108].

Among the reported eDKA cases, a high proportion of individuals had concurrent conditions which may increase their susceptibility, such as of autoimmune diabetes, reduction of insulin background therapy and acute illness[105]. Moreover, the eDKA cases were one decade older than their counterparts, and at least 50% had late autoimmune diabetes on adults (LADA)[109], while the prevalence of LADA is up

to 10% on diabetic population[110,111]. Thus, although the incidence of eDKA during SGLT2i is low (1.3/1000 patients-year), the possibility of this diagnosis must be borne in mind particularly among the elderly and those insulin-requiring[109].

Hypoglycemia

In elderly diabetics, the casual detection of hypoglycemia indicates a fourfold increase in the risk of death, which is a risk predictor even greater than pre-existing ischemic heart disease[112]. Beyond that, in this age group hypoglycemia is associated with an increased risk of fall[113], fracture[114], and cognitive decline[115,116].

From a pathophysiological point of view, direct and indirect effects of hypoglycemia ensues a set of metabolic, thrombotic, inflammatory and vasomotor effects, favoring the remodeling of atherosclerotic plaques to an unstable phenotype and its thrombotic occlusion. In the short term, hypoglycemia can reduce the energy source for myocardial cells, particularly in individuals with diabetes or ischemic heart disease, prolonging QT interval, a substrate for life-threatening ventricular arrhythmias. In the elderly, impaired counter-regulatory mechanisms may result in higher susceptibility to hypoglycemia's duration and its deleterious effects[117]. Consistent with the short-term effect, while the blood glucose decreased by about 16% the risk of nonfatal myocardial infarction, the incidence of cardiovascular death is not reduced.

SGLT2i therapy in the elderly did not increase the risk of hypoglycemia. The relative risk of hypoglycemia was 1.11 (95% CI: 0.84, 1.45; p=0.554; I^2=0%) across SGLT2i and comparators on patients on a background therapy not prone to hypoglycemia, as seen in figure 2A. Hypoglycemia was not described slightly more often when SGLT2i was added to a background prone to hypoglycemia (insulin or sulphonylurea), posing a RR of 1.05 (95% CI: 0.99, 1.11; p = 0.198; I^2=39.1%) (Figure 2B), although considerable heterogeneity was observed. Although hypoglycemic adverse events were higher on SGLT2 inhibitors when compared to the placebo, these AE were much higher on insulin or sulphonylurea therapy backgrounds. It is also described as an increase of hypoglycemic AE when renal function worsens. However, specifically on the elderly, there are no head-a-head trials of other hypoglycemic therapies *vs.* SGLT2i. Therefore, although it is possible that SGLT2i could reduce hypoglycemia rates in comparison to other drugs on older individuals, more studies are needed to assess this issue.

In stage 3 or 4 CKD individuals, hypoglycemia rate was similar among SGLT2i and placebo on individuals not on hypoglycemia prone therapy, with RR of 1.01 (95% CI 0.75, 1.37; p=0.688; I^2=0%), though on insulin or sulphonylurea background therapy RR was 1.05 (95% CI: 0.85, 1,30; p=0.311; I^2=57%) with substantial heterogeneity among trials in this last comparison. Comparing the effect of empagliflozin on different stages of CKD, progressively renal impairment was associated with higher rates of hypoglycemia[20]. In fact, stage 4 CKD on empagliflozin had an incidence of 37.8% of hypoglycemic AE *vs.* 32.4% on placebo, but lower incidence of AE were noted on stage 3 CKD (27.8% *vs.* 28.3% on empagliflozin and placebo, respectively) or stage 2 CKD (22.7% *vs.* 26.5% *vs.* 24.2% on empagliflozin 25 and 10 mg and placebo, respectively)[20].

Genital urinary-tract infections

Urinary tract infections (UTI) incidence is higher on older TSDM females, specifically those with poorly controlled diabetes[118]. Actually, in T2DM, moderate to severe glycosuria is associated with asymptomatic bacteriuria and also to pyelonephritis[119]. As a concern of diabetic and older individuals and because of increased glycosuria on SGLT2i therapy, most of the clinical trials and reviews involving SGLT2 inhibitors reported UTI and GTI incidences[117-122].

As observed in Figure 3, SGLT2i therapy did not increase the incidence of UTI events in trials with elderly individuals. The RR of uncomplicated and complicated UTI on SGLT2i therapy was respectively 1.04 (0.95, 1.14; p=0.186; I^2=24.9%) and 0.93 (0.66, 1.31; p=0.745; I^2=0%). However, as expected on SGLT2i therapy, females had a higher risk of GTI, with a RR of 4.13 (2.96, 5.76; p<0.001; I^2=32.6%), while males had a RR of 4.02 (2.91, 5.57; p<0.001; I^2=0%). However, few participants discontinued medication due to this AE, and the majority of GTI and UTI are benign conditions that were resolved with appropriate medication.

The overall incidence of UTI was higher on EMPAREG trial than others (on females, 40.6% on placebo and 36.4% on empagliflozin; on male individuals, 9.4% on placebo and 10.4% on empagliflozin). Paradoxically, the placebo branch had higher rates of complicated urinary tract infections than the empagliflozin (1.8% *vs.* 1.7%), though urosepsis developed on 17 individuals on empagliflozin and 3 on placebo[18]. The RR of UTI on CKD patients was 1.09 (0.83, 1.44;

p=0.517). Similar to that of subjects without renal disease, RR of GTI was 2.31 (95% CI: 1.13, 4.75; p=0.22) on males and 4.00 (95% CI: 1.62, 9.86; p=0.003) on females on SGLT2i therapy.

Bone Metabolism

From the fifth decade of life, there is a progressive loss of bone mass, which may reach the osteoporosis degree according to the peak of bone mass achieved,

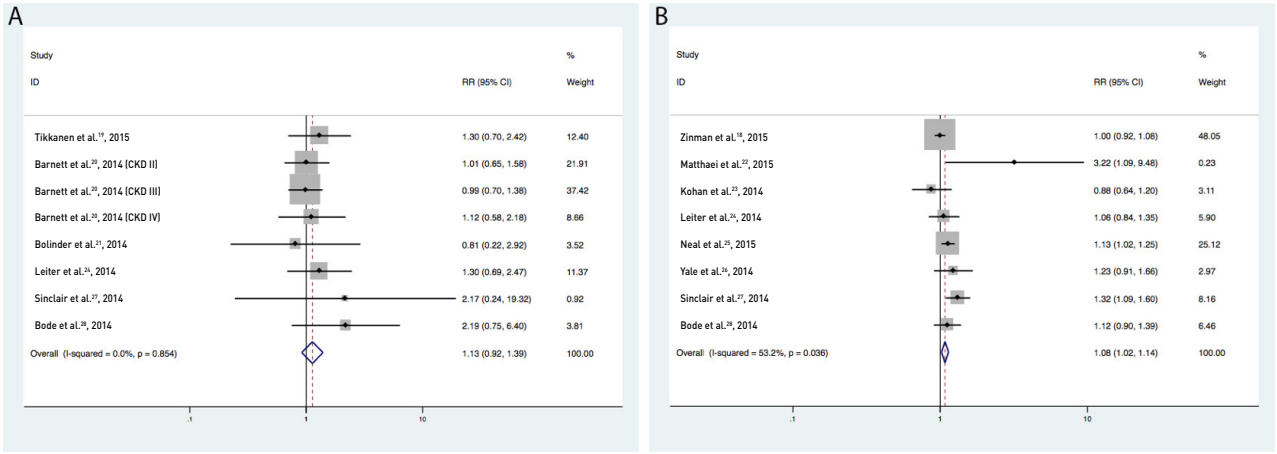

FIGURE 2. Meta-analysis of clinical trials in individuals >60 years-old comparing SGLT2 inhibitors vs. placebo on (A) Hypoglycemic events in patients not on background insulin or sulphonylurea therapy, (B) Hypoglycemic events in patients on background insulin or sulphonylurea therapy.

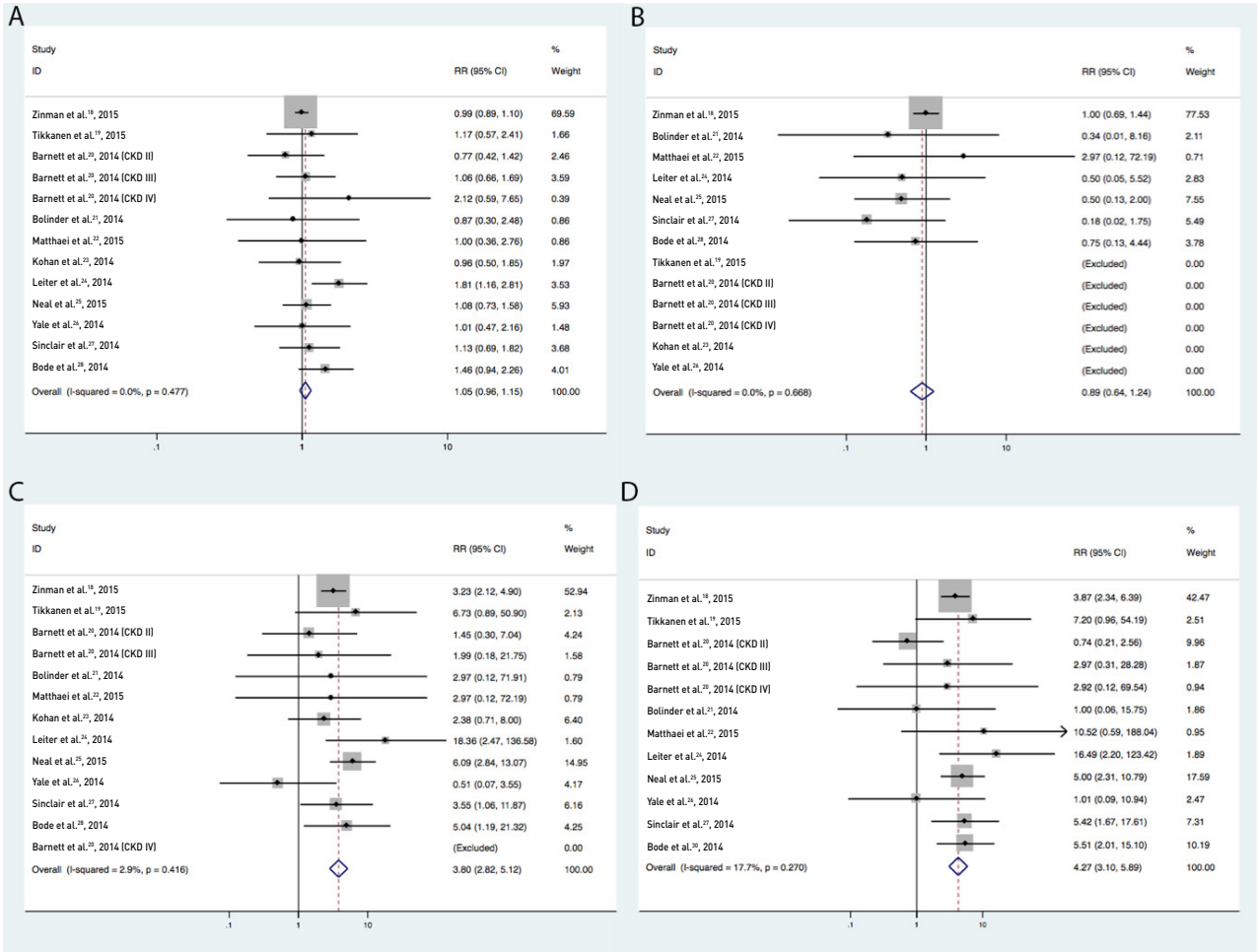

FIGURE 3. Meta-analysis of clinical trials in individuals >60 years-old comparing SGLT2 inhibitors vs. placebo on (A) uncomplicated urinary tract infection (UTI), (B) complicated UTI events, genital tract infection (GTI) events in males (C) and females. (D)

the rate of bone loss and longevity. Genetic factors, hormonal status, physical inactivity, low calcium intake, low sun exposure, smoking and comorbidities such as CKD and DM2 may all contribute to an accelerated deterioration of bone mass; thus, they may favor the risk for osteoporotic fragility fracture. In parallel, regardless of the bone mineral density (BMD), individuals with T2DM may have an osteopathic disease with significant changes in bone quality and architecture[123]. Finally, T2DM is also associated with increased risk of fractures among the older population due to peripheral neuropathy and increased risk of falls[124].

In regards to pharmacodynamics of SGLT2i, this therapy may favor mineral and electrolytes disturbances, which can further contribute to the rate of bone loss by increasing phosphate, calciuria and production of parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23)[125]. In up to 50 weeks, SGLT2i treatment on subjects ≥60 years presented no significant differences between SGLT2i therapy and the placebo on markers of bone reabsorption, such as procollagen type 1 N-terminal propeptide and C-terminal cross-linking telopeptides of type I collagen[126]. However, on a longer follow-up of 104 weeks, different bone turnover markers such as β-carboxy-telopeptide and osteocalcin were increased with SGLT2i therapy[127]. However, the increased bone turnover does not seem to impact on BMD of the femoral neck, lumbar spine, and total hip after 50 weeks[126] or 104 weeks[21] of SGLT2i.

In a recent trial performed on individuals between 55-80 years, canagliflozin was associated with a 1.2% decrease in total hip BMD over 104 weeks, though not on other sites measured[127]. Despite the hypothesis-generating nature of the study, the possibility of a more significant impact on the decline in bone mass after SGLT2i was raised by these findings. It is not plausible that it could be a direct action of the drug since SLGT2 is not expressed on bone cell types[128]. It is possible that the decrease in BMD is attributable to weight loss. On a post hoc analysis, 40% of BMD decline could be attributed to weight loss[127]. Similar results were observed in other weight loss situations[129].

In the animal model, prolonged treatment with canagliflozin was associated with trabecular bone deterioration[125]. However, in subjects with T2DM treated for a long time, improved glycemic control and insulin resistance attenuation can compensate an initial loss in bone calcification. In fact, oral hypoglycemic drugs are generally safe on clinical trials[129]. Few clinical trials have assessed bone health and the majority has reported fracture as an adverse event, posing a limitation to accurate analysis. Thus, a clearer picture of the mechanisms and clinical implications of the interaction between the inhibition of SGLT2 receptors and bone metabolism is still pending.

Another aspect to be considered in this scenario is diabetic osteopathy leading to weakness from BMD-independent structural changes in the bone[130]. Thus, the hard endpoint to be considered during clinical trials or in real life situations must be the incidence of fractures. In patients ≥65 years, the incidence of fractures was higher on the placebo branch than on dapagliflozin (2.7% *vs.* 0.4%, respectively)[24]. In a pooled analysis of canagliflozin trials, the incidence of fractures was similar in both canagliflozin and the placebo, 2.7% *vs.* 1.9 (HR 1.32 [1.00–1.74]), respectively. However, in a subpopulation of high cardiovascular risk patients, canagliflozin was associated with a higher incidence of fracture (4% *vs.* 2.6%), possibly due to increased incidence of falls, as volume-related AE were more frequent on canagliflozin than on placebo (HR 1.76 (1.27–2.44))[131]. Possibly endorsing fall-related fractures, this incidence was higher early on the beginning of treatment, and the fracture sites were the fists and feet. A sensitivity analysis including only osteoporotic fractures showed a similar risk increase of with canagliflozin[131]. It was also observed on the SGLT2i group a tendency (RR 1.30 (1.00-1.68)) to increase the risk of stroke, and this could be related to the risk of falls, especially for the elderly[132]. Therefore, it is possible that fractures related to SGLT2i therapy are associated with volume depletion.

In individuals with increased susceptibility to fractures due to osteoporotic fragility such as CKD patients, there is no clear evidence of an adverse effect of SGLT2i therapy. The incidence of fractures on CKD patients was indeed lower on canagliflozin 300 mg (1.1%) than on the placebo (2.2%) over 104 weeks[26]. Patients on empagliflozin had a reduced number of fractures compared to those on the placebo with progressive renal failure from stage 2 to 3 CKD[20]. Although one study observed higher rates of fractures with dapagliflozin (7.7%), all reported events were related to trauma, and only 2 were considered severe adverse events[23].

In conclusion, although the available data are insufficient to confirm or exclude a specific deleterious

effect of these drugs on bone metabolism, falls due to hypovolemia were reported and the risk of falling should be considered in therapy indication with SGLT2 or SGLT2 / SGLT1 in elderly individuals.

Effect on mortality

Recently, the EMPA-REG Outcome trial showed a decrease in all-cause and cardiovascular mortality after the short-term use of SGLT2i in high-risk diabetic individuals[18]. Although SGLT2i could have a positive influence on cardiovascular risk factors (i.e., HbA1c, systolic BP, weight excess), the role of SGLT2i on survival improvement via atherosclerotic risk is unlikely. In this trial, for each non-fatal myocardial infarction prevented with empagliflozin three cardiovascular deaths were spared. This number speaks for itself against the attenuation of atherosclerosis as the primary mechanism of benefit. In fact, in statin trials, for each nonfatal myocardial infarction prevented 0.5 cardiovascular deaths are spared[133]. Moreover, the early opening of the survival curves in about one month is unlikely a consequence of complex, long-lasting phenotypic changes such as stabilization of atherosclerotic plaques.

Potentially, a change in the heart-kidney crosstalk would favor the SGLT2i effect on cardiovascular mortality. Several mediators for this crosstalk have been reported, many with apparent impact on survival. Fibroblast growth factor 23 (FGF23)[134], renalase[135], asymmetric dimethylarginine (ADMA), erythropoietin, trimethylamine-N-Oxide (TMAO) and PTH are among the most studied players. As noted above, with aging there is a progressive decline in GFR generating an imbalance in the heart-kidney crosstalk in favor of increased cardiovascular risk. In fact, in the EMPA-REG trial[18], there was a significant interaction between age and cardiovascular benefit provided by SGLT2 inhibition, which was higher among those with 65 years or older.

Looking from a different perspective, as a co-transporter of sodium and glucose, its inhibition can hypothetically reduce the sodium influx into cardiomyocytes reducing the propensity for life-threatening ventricular arrhythmias[136]. Consistent with this finding, the risk of sudden death was about 30% lower in those treated with SGLT2i[18]. Both aging[137] and T2DM[138] increase the incidence of sudden cardiac death[8]; the severity of coronary artery disease and hypoglycemia are mediators of the risk in both cases. In disagreement with this assumption, although the evidence is still scarce, studies in humans have indicated that SGLT1 is the predominant receptor in the myocardium and small intestine, while SGLT2 is predominant in the kidney[139]. We still do not know if the proportion of SGLT1 and SGLT2 in the myocardium changes during the life course or under different stimuli. So far, aside from canagliflozin, other SGLT inhibitors have had minimal effect on the SGLT1[140].

In subjects with T2D[141] or heart failures (HF)[142], the liver converts abundant plasma FFA into ketone bodies (KB) such as acetoacetate and 3-hydroxybutyrate. This KB excess in plasma is highly absorbed in the myocardium and in a dose-dependent manner is converted to acetyl-CoA[143]. In rodents, KB is used as the primary source of energy by cardiac cells[144], and such use might hypothetically occur in individuals with T2D and heart failure, favoring the improvement in energy reserves[141]. Nevertheless, it is also possible that overfeeding myocardium with KB may induce insulin resistance and block the citrate cycle, thus reducing the power supply[143]. This controversy has been highlighted recently with the confrontation of two SGLT2i effects: increasing plasma KB and decreasing cardiovascular mortality in individuals with T2D and HF. In healthy elderly individuals, the KB output is similar to that in younger adults[145]. However, as commented above, among diabetic elderlies, the KB production tends to be increased due to insulin deficiency and malnutrition[109]. Hence, if this mechanism is in fact involved in the SGLT2i effects, elderly diabetic patients will be among the most improved populations.

Effect on stroke

A neutral or even beneficial effect has been reported with hypoglycemic therapies in stroke risk[146]. The possibility of benefits concerning stroke risk becomes even more likely if we consider the reduction of systolic blood pressure during treatment with SGLT2i[147]. However, despite the clear cardiovascular benefit SGLT2i, some concern was raised with a tendency to increase the incidence of stroke. In the EMPA-REG Outcome trial, the incidence of stroke tended to increase by 24% (95% CI 0.92, 1.67; p = 0.16)[18]. Considering the EMPA-REG trials outcome together with phase 2 or 3 studies, the risk of stroke increased by 30% (95% CI: 1.00, 1.68; p=0.049)[132]. An overall set of mechanisms that may be behind this potential adverse effect is unclear. However, ortho-

static hypotension[148], hemoconcentration[149]and increased activity of renin-angiotensin-aldosterone system (RAAS)[150] are among consequences of SGLT2i therapy that would favor stroke incidence.

In the elderly, this potential adverse effect may have greater significance for the natural propensity to hemoconcentration and orthostatic hypotension. To date, however, the relationship between the SGLT2i and the risk of stroke remains hypothetical as the number of events is small and only one prospective outcome-driven trial analysis is published. Two other studies are underway with dapagliflozin and canagliflozin, and its completion will help to discern more clearly this finding.

CONCLUSION

Few studies performed pre-specified analysis on the elderly. Therefore, to accurately assess its effects in older individuals, prospective studies are necessary. SGLT2i therapy is associated with glycemic effects on older individuals that are similar to younger ones. Similar effects on BP and BW have also been observed. In fact, as intentional body weight is not associated with higher AE, it is plausible that drug-induced decrease on BW would have no increase in mortality. One limitation of these new agents is the higher prevalence of CKD on older individuals that could, in turn, reduce effectiveness. Other safety concerns are being outlined recently. DKA could influence SGLT2i prescription on insulin users but is rare on other individuals. Few cases of urosepsis were described on the largest SGLT2i trial to this date, and it is possible that this low number will be maintained in the long-term. The forthcoming studies regarding the cardiovascular safety of these new agents may establish this class as a top second- or first-line option in diabetes therapy.

RESUMO

A prevalência da diabetes mellitus tipo 2 em idosos cresceu muito na última década. A redução na sensibilidade à insulina e na capacidade secretora, ganho de peso, sarcopenia e adiposidade elevada são todas alterações metabólicas e corporais comuns entre a população idosa. Essas mudanças críticas favorecem o aumento no risco de hipoglicemia, síndrome de fragilidade, quedas e disfunções cognitivas. A primeira linha de tratamento contra a diabete muitas vezes não é segura para indivíduos mais velhos devido ao alto risco de hipoglicemia e a prevalência de comorbidades patogênicas, como doença renal crônica, osteoporose, doença cardiovascular e obesidade. Os inibidores do cotransportador sódio-glicose 2 (SGLT2) são uma nova classe de tratamento contra a diabete que inibe reabsorção de glicose e sódio na parte convoluta do túbulo proximal. Seu efeito é claramente demonstrado em diversos cenários clínicos em populações mais jovens. Esta revisão e meta-análise descreve as particularidades dos SGLT2 na população idosa, abordando os mecanismos dos potenciais benefícios e desafios ainda presentes do uso destes medicamentos nesse grupo etário tão importante. Além disso, apresentaremos uma meta-análise dos principais efeitos dos SGLT2 encontrados em estudos post-hoc nos quais a idade média dos subgrupos analisados foi acima de 60 anos. Apesar da ausência de ensaios clínicos que incluem essa população, os dados encontrados sugerem que o tratamento com SGLT2 em idosos é eficaz para diminuir os níveis de glicose e tem efeitos na pressão arterial sistólica e no peso corporal.

PALAVRAS-CHAVE: *Transportador 2 de glucose-sódio/antagonistas e inibidores. Diabetes mellitus. Idoso. Eficácia.*

REFERENCES

1. Viljoen A, Sinclair AJ. Diabetes and insulin resistance in older people. Med Clin North Am. 2011;95(3):615-29.
2. Corriere M, Rooparinesingh N, Kalyani RR. Epidemiology of diabetes and diabetes complications in the elderly: an emerging public health burden. Curr Diab Rep. 2013;13(6):805-13.
3. Sloan FA, Bethel MA, Ruiz D Jr., Shea AM, Feinglos MN. The growing burden of diabetes mellitus in the US elderly population. Arch Intern Med. 2008;168(2):192-9.
4. Roth GA, Forouzanfar MH, Moran AE, Barber R, Nguyen G, Feigin VL, et al. Demographic and epidemiologic drivers of global cardiovascular mortality. N Engl J Med. 2015;372(14):1333-41.
5. Sinclair A, Dunning T, Rodriguez-Manas L. Diabetes in older people: new insights and remaining challenges. Lancet Diabetes Endocrinol. 2015;3(4):275-85.
6. Johannsen DL, Conley KE, Bajpeyi S, Punyanitya M, Gallagher D, Zhang Z, et al. Ectopic lipid accumulation and reduced glucose tolerance in elderly adults are accompanied by altered skeletal muscle mitochondrial activity. J Clin Endocrinol Metab. 2012;97(1):242-50.
7. Cnop M, Igoillo-Esteve M, Hughes SJ, Walker JN, Cnop I, Clark A. Longevity of human islet alpha- and beta-cells. Diabetes Obes Metab. 2011;13(Suppl 1):39-46.
8. Gong Z, Muzumdar RH. Pancreatic function, type 2 diabetes, and metabolism in aging. Int J Endocrinol. 2012;2012:320482.
9. Meneilly GS, Ryan AS, Veldhuis JD, Elahi D. Increased disorderliness of basal insulin release, attenuated insulin secretory burst mass, and reduced ultradian rhythmicity of insulin secretion in older individuals. J Clin Endocrinol Metab. 1997;82(12):4088-93.
10. Amati F, Dube JJ, Coen PM, Stefanovic-Racic M, Toledo FG, Goodpaster BH. Physical inactivity and obesity underlie the insulin resistance of aging. Diabetes Care. 2009;32(8):1547-9.
11. Bryhni B, Jenssen TG, Olafsen K, Eikrem JH. Age or waist as determinant of insulin action? Metabolism. 2003;52(7):850-7.

12. DeFronzo RA, Hompesch M, Kasichayanula S, Liu X, Hong Y, Pfister M, et al. Characterization of renal glucose reabsorption in response to dapagliflozin in healthy subjects and subjects with type 2 diabetes. Diabetes Care. 2013;36(10):3169-76.
13. DeFronzo RA, Davidson JA, Del Prato S. The role of the kidneys in glucose homeostasis: a new path towards normalizing glycaemia. Diabetes Obes Metab. 2012;14(1):5-14.
14. Merovci A, Solis-Herrera C, Daniele G, Eldor R, Fiorentino TV, Tripathy D, et al. Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. J Clin Invest. 2014;124(2):509-14.
15. Cefalu WT, Leiter LA, Bruin TW, Gause-Nilsson I, Sugg J, Parikh SJ. Dapagliflozin's effects on glycemia and cardiovascular risk factors in high-risk patients with type 2 diabetes: a 24-week, multicenter, randomized, double-blind, placebo-controlled study with a 28-week extension. Diabetes Care. 2015;38(7):1218-27.
16. Ferrannini E, Muscelli E, Frascerra S, Baldi S, Mari A, Heise T, et al. Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients. J Clin Invest. 2014;124(2):499-508.
17. Bode B, Stenlof K, Harris S, Sullivan D, Fung A, Usiskin K, et al. Long-term efficacy and safety of canagliflozin over 104 weeks in patients aged 55-80 years with type 2 diabetes. Diabetes Obes Metab. 2015;17(3):294-303.
18. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med. 2015;373(22):2117-28.
19. Tikkanen I, Narko K, Zeller C, Green A, Salsali A, Broedl UC, et al. Empagliflozin reduces blood pressure in patients with type 2 diabetes and hypertension. Diabetes Care. 2015;38(3):420-8.
20. Barnett AH, Mithal A, Manassie J, Jones R, Rattunde H, Woerle HJ, et al. Efficacy and safety of empagliflozin added to existing antidiabetes treatment in patients with type 2 diabetes and chronic kidney disease: a randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol. 2014;2(5):369-84.
21. Bolinder J, Ljunggren O, Johansson L, Wilding J, Langkilde AM, Sjöström CD, et al. Dapagliflozin maintains glycaemic control while reducing weight and body fat mass over 2 years in patients with type 2 diabetes mellitus inadequately controlled on metformin. Diabetes Obes Metab. 2014;16(2):159-69.
22. Matthaei S, Bowering K, Rohwedder K, Sugg J, Parikh S, Johnsson E; et al. Durability and tolerability of dapagliflozin over 52 weeks as add-on to metformin and sulphonylurea in type 2 diabetes. Diabetes Obes Metab. 2015;17(11):1075-84.
23. Kohan DE, Fioretto P, Tang W, List JF. Long-term study of patients with type 2 diabetes and moderate renal impairment shows that dapagliflozin reduces weight and blood pressure but does not improve glycemic control. Kidney Int. 2014;85(4):962-71.
24. Leiter LA, Cefalu WT, Bruin TW, Gause-Nilsson I, Sugg J, Parikh SJ. Dapagliflozin added to usual care in individuals with type 2 diabetes mellitus with preexisting cardiovascular disease: a 24-week, multicenter, randomized, double-blind, placebo-controlled study with a 28-week extension. J Am Geriatr Soc. 2014;62(7):1252-62.
25. Neal B, Perkovic V, Zeeuw D, Mahaffey KW, Fulcher G, Ways K, et al. Efficacy and safety of canagliflozin, an inhibitor of sodium-glucose cotransporter 2, when used in conjunction with insulin therapy in patients with type 2 diabetes. Diabetes Care. 2015;38(3):403-11.
26. Yale JF, Bakris G, Cariou B, Nieto J, David-Neto E, Yue D, et al. Efficacy and safety of canagliflozin over 52 weeks in patients with type 2 diabetes mellitus and chronic kidney disease. Diabetes Obes Metab. 2014;16(10):1016-27.
27. Sinclair A, Bode B, Harris S, Vijapurkar U, Mayer C, Fung A, et al. Efficacy and safety of canagliflozin compared with placebo in older patients with type 2 diabetes mellitus: a pooled analysis of clinical studies. BMC Endocr Disord. 2014;14:37.
28. Bode B, Stenlof K, Sullivan D, Fung A, Usiskin K. Efficacy and safety of canagliflozin treatment in older subjects with type 2 diabetes mellitus: a randomized trial. Hosp Pract. 2013;41(2):72-84.
29. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097.
30. Du YF, Ou HY, Beverly EA, Chiu CJ. Achieving glycemic control in elderly patients with type 2 diabetes: a critical comparison of current options. Clin Interv Aging. 2014;9:1963-80.
31. Munshi MN, Segal AR, Suhl E, Staum E, Desrochers L, Sternthal A, et al. Frequent hypoglycemia among elderly patients with poor glycemic control. Arch Intern Med. 2011;171(4):362-4.
32. Farahani P. Non-severe hypoglycemia risk difference between sulfonylurea and sodium-glucose cotransporter-2 inhibitors (SGLT2-I) as an add-on to metformin in randomized controlled trials. J Popul Ther Clin Pharmacol. 2017;24(2):e32-e40.
33. Treves C, Favilli F, Stio M, Iantomasi T, Vincenzini MT. Changes in metabolite transport by small intestine and kidney of young and old rats. Mech Ageing Dev. 1990;52(2-3):263-76.
34. Laffin LJ, Bakris GL. Update on blood pressure goals in diabetes mellitus. Curr Cardiol Rep. 2015;17(6):37.
35. Maliha G, Townsend RR. SGLT2 inhibitors: their potential reduction in blood pressure. J Am Soc Hypertens. 2015;9(1):48-53.
36. Wright JT Jr., Fine LJ, Lackland DT, Ogedegbe G, Dennison Himmelfarb CR. Evidence supporting a systolic blood pressure goal of less than 150 mm Hg in patients aged 60 years or older: the minority view. Ann Intern Med. 2014;160(7):499-503.
37. Aronow WS, Fleg JL, Pepine CJ, Artinian NT, Bakris G, Brown AS, et al. ACCF/AHA 2011 expert consensus document on hypertension in the elderly: a report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus documents developed in collaboration with the American Academy of Neurology, American Geriatrics Society, American Society for Preventive Cardiology, American Society of Hypertension, American Society of Nephrology, Association of Black Cardiologists, and European Society of Hypertension. J Am Coll Cardiol. 2011;57(20):2037-114.
38. Palombo C, Kozakova M. Arterial stiffness, atherosclerosis and cardiovascular risk: pathophysiologic mechanisms and emerging clinical indications. Vascul Pharmacol. 2016;77:1-7.
39. Xue M, Rabbani N, Thornalley PJ. Glyoxalase in ageing. Semin Cell Dev Biol. 2011;22(3):293-301.
40. Choi KM, Yoo HJ, Kim HY, Lee KW, Seo JA, Kim SG, et al. Association between endogenous secretory RAGE, inflammatory markers and arterial stiffness. Int J Cardiol. 2009;132(1):96-101.
41. Elewa U, Fernandez-Fernandez B, Alegre R, Sanchez-Niño MD, Mahillo-Fernández I Perez-Gomez MV, et al. Modifiable risk factors for increased arterial stiffness in outpatient nephrology. PLoS One. 2015;10(4):e0123903.
42. Chilton R, Tikkanen I, Cannon CP, Crowe S, Woerle HJ, Broedl UC, et al. Effects of empagliflozin on blood pressure and markers of arterial stiffness and vascular resistance in patients with type 2 diabetes. Diabetes Obes Metab. 2015;17(12):1180-93.
43. Cherney DZ, Perkins BA, Soleymanlou N, Har R, Fagan N, Johansen OE, et al. The effect of empagliflozin on arterial stiffness and heart rate variability in subjects with uncomplicated type 1 diabetes mellitus. Cardiovasc Diabetol. 2014;13:28.
44. Oelze M, Kroller-Schon S, Welschof P, Jansen T, Hausding M, Mikhed Y, et al. The sodium-glucose co-transporter 2 inhibitor empagliflozin improves diabetes-induced vascular dysfunction in the streptozotocin diabetes rat model by interfering with oxidative stress and glucotoxicity. PloS One. 2014;9(11):e112394.
45. Fulcher G, Matthews DR, Perkovic V, Zeeuw D, Mahaffey KW, Mathieu C, et al. Efficacy and safety of canagliflozin when used in conjunction with incretin-mimetic therapy in patients with type 2 diabetes. Diabetes Obes Metab. 2016;18(1):82-91.
46. Lambers Heerspink HJ, Zeeuw D, Wie L, Leslie B, List J. Dapagliflozin a glucose-regulating drug with diuretic properties in subjects with type 2 diabetes. Diabetes Obes Metab. 2013;15(9):853-62.
47. Basile J. Hypertension in the elderly: a review of the importance of systolic blood pressure elevation. J Clin Hypertens (Greenwich). 2002;4(2):108-12.
48. Weber MA, Mansfield TA, Cain VA, Iqbal N, Parikh S, Ptaszynska A. Blood pressure and glycaemic effects of dapagliflozin versus placebo in patients with type 2 diabetes on combination antihypertensive therapy: a randomised, double-blind, placebo-controlled, phase 3 study. Lancet Diabetes Endocrinol. 2016;4(3):211-20.
49. Sha S, Polidori D, Heise T, Natarajan J, Farrell K, Wang SS, et al. Effect of the sodium glucose co-transporter 2 inhibitor canagliflozin on plasma volume in patients with type 2 diabetes mellitus. Diabetes Obes Metab. 2014;16(11):1087-95.
50. Siebenhofer A, Jeitler K, Berghold A, Waltering A, Hemkens LG, Semlitsch T, et al. Long-term effects of weight-reducing diets in hypertensive patients. Cochrane Database Syst Rev. 2011:(9):CD008274.

51. Sjostrom CD, Hashemi M, Sugg J, Ptaszynska A, Johnsson E. Dapagliflozin-induced weight loss affects 24-week glycated haemoglobin and blood pressure levels. Diabetes Obes Metab. 2015;17(8):809-12.

52. Iqbal J, Denvir M, Gunn J. Frailty assessment in elderly people. Lancet. 2013;381(9882):1985-6.

53. Fabbri E, Tanaka T, An Y, Zoli M, Bandinelli S, Guralnik JM, et al. Loss of weight in obese older adults: a biomarker of impending expansion of multimorbidity? J Am Geriatr Soc. 2015;63(9):1791-7.

54. Newman AB, Yanez D, Harris T, Duxbury A, Enright PL, Fried LP, et al. Weight change in old age and its association with mortality. J Am Geriatr Soc. 2001;49(10):1309-18.

55. Shea MK, Nicklas BJ, Houston DK, Miller ME, Davis CC, Kitzman DW, et al. The effect of intentional weight loss on all-cause mortality in older adults: results of a randomized controlled weight-loss trial. Am J Clin Nutr. 2011;94(3):839-46.

56. Wijnhoven HA, van Zon SK, Twisk J, Visser M. Attribution of causes of weight loss and weight gain to 3-year mortality in older adults: results from the Longitudinal Aging Study Amsterdam. J Gerontol A Biol Sci Med Sci. 2014;69(10):1236-43.

57. Katzel LI, Bleecker ER, Colman EG, Rogus EM, Sorkin JD, Goldberg AP. Effects of weight loss vs aerobic exercise training on risk factors for coronary disease in healthy, obese, middle-aged and older men. A randomized controlled trial. JAMA. 1995;274(24):1915-21.

58. Purnell JQ, Kahn SE, Albers JJ, Nevin DN, Brunzell JD, Schwartz RS. Effect of weight loss with reduction of intra-abdominal fat on lipid metabolism in older men. J Clin Endocrinol Metab. 2000;85(3):977-82.

59. Dengel DR, Pratley RE, Hagberg JM, Rogus EM, Goldberg AP. Distinct effects of aerobic exercise training and weight loss on glucose homeostasis in obese sedentary men. J Appl Physiol. 1996;81(1):318-25.

60. Beavers KM, Miller ME, Rejeski WJ, Nicklas BJ, Kritchevsky SB. Fat mass loss predicts gain in physical function with intentional weight loss in older adults. J Gerontol A Biol Sci Med Sci. 2013;68(1):80-6.

61. Sipila S, Koskinen SO, Taaffe DR, Takala TE, Cheng S, Rantanen T, et al. Determinants of lower-body muscle power in early postmenopausal women. J Am Geriatr Soc. 2004;52(6):939-44.

62. Sipila S, Suominen H. Knee extension strength and walking speed in relation to quadriceps muscle composition and training in elderly women. Clin Physiol. 1994;14(4):433-42.

63. Goodpaster BH, Carlson CL, Visser M, Kelley DE, Scherzinger A, Harris TB, et al. Attenuation of skeletal muscle and strength in the elderly: the Health ABC Study. J Appl Physiol. 2001;90(6):2157-65.

64. Visser M, Kritchevsky SB, Goodpaster BH, Newman AB, Nevitt M, Stamm E, et al. Leg muscle mass and composition in relation to lower extremity performance in men and women aged 70 to 79: the health, aging and body composition study. J Am Geriatr Soc. 2002;50(5):897-904.

65. Newman AB, Kupelian V, Visser M, Simonsick EM, Goodpaster BH, Kritchevsky SB, et al. Strength, but not muscle mass, is associated with mortality in the health, aging and body composition study cohort. J Gerontol A Biol Sci Med Sci. 2006;61(1):72-7.

66. Visser M, Goodpaster BH, Kritchevsky SB, Newman AB, Nevitt M, Rubin SM, et al. Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident mobility limitations in well-functioning older persons. J Gerontol A Biol Sci Med Sci. 2005;60(3):324-33.

67. Aguirre L, Napoli N, Waters D, Qualls C, Villareal DT, Armamento-Villareal R. Increasing adiposity is associated with higher adipokine levels and lower bone mineral density in obese older adults. J Clin Endocrinol Metab. 2014;99(9):3290-7.

68. Jahangir E, De Schutter A, Lavie CJ. Low weight and overweightness in older adults: risk and clinical management. Prog Cardiovasc Dis. 2014;57(2):127-33.

69. Matthaei S, Bowering K, Rohwedder K, Grohl A, Parikh S, Study 05 Group. Dapagliflozin improves glycemic control and reduces body weight as add-on therapy to metformin plus sulfonylurea: a 24-week randomized, double-blind clinical trial. Diabetes Care. 2015;38(3):365-72.

70. Fulcher G, Matthews DR, Perkovic V, Zeeuw D, Mahaffey KW, Weiss R, et al. Efficacy and safety of canagliflozin used in conjunction with sulfonylurea in patients with type 2 diabetes mellitus: a randomized, controlled trial. Diabetes Ther. 2015;6(3):289-302.

71. Ferrannini G, Hach T, Crowe S, Sanghvi A, Hall KD, Ferrannini E. Energy balance after sodium-glucose cotransporter 2 inhibition. Diabetes Care. 2015;38(9):1730-5.

72. Chiba Y, Yamada T, Tsukita S, Takahashi K, Munakata Y, Shirai Y, et al. Dapagliflozin, a sodium-glucose co-transporter 2 inhibitor, acutely reduces energy expenditure in BAT via neural signals in mice. PloS One. 2016;11(3):e0150756.

73. Del Prato S, Nauck M, Duran-Garcia S, Maffei L, Rohwedder K, Theuerkauf A, et al. Long-term glycaemic response and tolerability of dapagliflozin versus a sulphonylurea as add-on therapy to metformin in patients with type 2 diabetes: 4-year data. Diabetes Obes Metab. 2015;17:581-90.

74. Lee CG, Boyko EJ, Barrett-Connor E, Miljkovic I, Hoffman AR, Everson-Rose SA, et al. Insulin sensitizers may attenuate lean mass loss in older men with diabetes. Diabetes Care. 2011;34(11):2381-6.

75. Levey AS, Inker LA, Coresh J. Chronic kidney disease in older people. JAMA. 2015;314(6):557-8.

76. Hallan SI, Dahl K, Oien CM, Grootendorst DC, Aasberg A, Holmen J, et al. Screening strategies for chronic kidney disease in the general population: follow-up of cross sectional health survey. BMJ. 2006;333(7577):1047.

77. Tuttle KR, Bakris GL, Bilous RW, Chiang JL, Boer IH, Goldstein-Fuchs J, et al. Diabetic kidney disease: a report from an ADA Consensus Conference. Am J Kidney Dis. 2014;64(4):510-33.

78. Hallan SI, Matsushita K, Sang Y, Mahmoodi BK, Black C, Ishani A, et al. Age and association of kidney measures with mortality and end-stage renal disease. JAMA. 2012;308(22):2349-60.

79. Denic A, Glassock RJ, Rule AD. Structural and functional changes with the aging kidney. Adv Chronic Kidney Dis. 2016;23(1):19-28.

80. Blazer S, Khankin E, Segev Y, Ofir R, Yalon-Hacohen M, Kra-Oz Z, et al. High glucose-induced replicative senescence: point of no return and effect of telomerase. Biochem Biophys Res Commun. 2002;296(1):93-101.

81. Yokoi T, Fukuo K, Yasuda O, Hotta M, Miyazaki J, Takemura Y, et al. Apoptosis signal-regulating kinase 1 mediates cellular senescence induced by high glucose in endothelial cells. Diabetes. 2006;55(6):1660-5.

82. Verzola D, Gandolfo MT, Gaetani G, Ferraris A, Mangerini R, Ferrario F, et al. Accelerated senescence in the kidneys of patients with type 2 diabetic nephropathy. Am J Physiol Renal Physiol. 2008;295(5):F1563-73.

83. Kitada K, Nakano D, Ohsaki H, Hitomi H, Minamino T, Yatabe J, et al. Hyperglycemia causes cellular senescence via a SGLT2- and p21-dependent pathway in proximal tubules in the early stage of diabetic nephropathy. J Diabetes Complications. 2014;28(5):604-11.

84. De Nicola L, Gabbai FB, Liberti ME, Sagliocca A, Conte G, Minutolo R. Sodium/glucose cotransporter 2 inhibitors and prevention of diabetic nephropathy: targeting the renal tubule in diabetes. Am J Kidney Dis. 2014;64:16-24.

85. Gallagher H, Suckling RJ. Diabetic nephropathy: where are we on the journey from pathophysiology to treatment? Diabetes Obes Metab. 2016;18(7):641-7.

86. Freitas HS, Anhe GF, Melo KF, Okamoto MM, Oliveira-Souza M, Bordin S, et al. Na(+) -glucose transporter-2 messenger ribonucleic acid expression in kidney of diabetic rats correlates with glycemic levels: involvement of hepatocyte nuclear factor-1alpha expression and activity. Endocrinology. 2008;149(2):717-24.

87. Rahmoune H, Thompson PW, Ward JM, Smith CD, Hong G, Brown J. Glucose transporters in human renal proximal tubular cells isolated from the urine of patients with non-insulin-dependent diabetes. Diabetes. 2005;54(12):3427-34.

88. Cherney DZ, Perkins BA, Soleymanlou N, Maione M, Lai V, Lee A, et al. Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. Circulation. 2014;129(5):587-97.

89. Skrtic M, Cherney DZ. Sodium-glucose cotransporter-2 inhibition and the potential for renal protection in diabetic nephropathy. Curr Opin Nephrol Hypertens. 2015;24(1):96-103.

90. Nakamura N, Masuda S, Takahashi K, Saito H, Okuda M, Inui K. Decreased expression of glucose and peptide transporters in rat remnant kidney. Drug Metab Pharmacokinet. 2004;19(1):41-7.

91. Ghezzi C, Hirayama BA, Gorraitz E, Loo DD, Liang Y, Wright EM. SGLT2 inhibitors act from the extracellular surface of the cell membrane. Physiol Rep. 2014;2(6).

92. Sha S, Polidori D, Farrell K, Ghosh A, Natarajan J, Vaccaro N, et al. Pharmacodynamic differences between canagliflozin and dapagliflozin: results of a randomized, double-blind, crossover study. Diabetes Obes Metab. 2015;17(2):188-97.

93. Townsend RR, Taler SJ. Management of hypertension in chronic kidney disease. Nat Rev Nephrol. 2015;11(9):555-63.

94. Palmer SC, Mavridis D, Navarese E, Craig JC, Tonelli M, Salanti G, et al. Comparative efficacy and safety of blood pressure-lowering agents in

adults with diabetes and kidney disease: a network meta-analysis. Lancet. 2015;385(9982):2047-56.

95. Navaneethan SD, Yehnert H, Moustarah F, Schreiber MJ, Schauer PR, Beddhu S. Weight loss interventions in chronic kidney disease: a systematic review and meta-analysis. Clin J Am Soc Nephrol. 2009;4(10):1565-74.

96. Perkovic V, Jardine M, Vijapurkar U, Meininger G. Renal effects of canagliflozin in type 2 diabetes mellitus. Curr Med Res Opin. 2015;31(12):2219-31.

97. Cherney DZ, Perkins BA, Soleymanlou N, Xiao F, Zimpelmann J, Woerle HJ, et al. Sodium glucose cotransport-2 inhibition and intrarenal RAS activity in people with type 1 diabetes. Kidney Int. 2014;86(5):1057-8.

98. Kohan DE, Fioretto P, Johnsson K, Parikh S, Ptaszynska A, Ying L. The effect of dapagliflozin on renal function in patients with type 2 diabetes. J Nephrol. 2016;29(3):391-400.

99. Yale JF, Bakris G, Cariou B, Yue D, David-Neto E, Xi L, et al. Efficacy and safety of canagliflozin in subjects with type 2 diabetes and chronic kidney disease. Diabetes Obes Metab. 2013;15(5):463-73.

100. Wilding JP, Norwood P, T'Joen C, Bastien A, List JF, Fiedorek FT. A study of dapagliflozin in patients with type 2 diabetes receiving high doses of insulin plus insulin sensitizers: applicability of a novel insulin-independent treatment. Diabetes Care. 2009;32(9):1656-62.

101. List JF, Woo V, Morales E, Tang W, Fiedorek FT. Sodium-glucose cotransport inhibition with dapagliflozin in type 2 diabetes. Diabetes Care. 2009;32(4):650-7.

102. Hayashi T, Takai S, Yamashita C. Impact of the renin-angiotensin-aldosterone-system on cardiovascular and renal complications in diabetes mellitus. Curr Vasc Pharmacol. 2010;8(2):189-97.

103. Yamout H, Perkovic V, Davies M, Woo V, Zeeuw D, Mayer C, et al. Efficacy and safety of canagliflozin in patients with type 2 diabetes and stage 3 nephropathy. Am J Nephrol. 2014;40(1):64-74.

104. Devineni D, Vaccaro N, Polidori D, Rusch S, Wajs E. Effects of hydrochlorothiazide on the pharmacokinetics, pharmacodynamics, and tolerability of canagliflozin, a sodium glucose co-transporter 2 inhibitor, in healthy participants. Clin Ther. 2014;36(5):698-710.

105. Erondu N, Desai M, Ways K, Meininger G. Diabetic ketoacidosis and related events in the canagliflozin type 2 diabetes clinical program. Diabetes Care. 2015;38(9):1680-6.

106. Kaku K, Watada H, Iwamoto Y, Utsunomiya K, Terauchi Y, Tobe K, et al. Efficacy and safety of monotherapy with the novel sodium/glucose cotransporter-2 inhibitor tofogliflozin in Japanese patients with type 2 diabetes mellitus: a combined Phase 2 and 3 randomized, placebo-controlled, double-blind, parallel-group comparative study. Cardiovasc Diabetol. 2014;13:65.

107. Bonner C, Kerr-Conte J, Gmyr V, Queniat G, Moerman E, Thévenet J, et al. Inhibition of the glucose transporter SGLT2 with dapagliflozin in pancreatic alpha cells triggers glucagon secretion. Nat Med. 2015;21(5):512-7.

108. Taylor SI, Blau JE, Rother KI. Perspective: SGLT2 inhibitors may predispose to ketoacidosis. J Clin Endocrinol Metab. 2015;100(8):2849-52.

109. Rosenstock J, Ferrannini E. Euglycemic diabetic ketoacidosis: a predictable, detectable, and preventable safety concern with SGLT2 inhibitors. Diabetes Care 2015;38(9):1638-42.

110. Tuomi T, Santoro N, Caprio S, Cai M, Weng J, Groop L. The many faces of diabetes: a disease with increasing heterogeneity. Lancet. 2014;383(9922):1084-94.

111. Itariu BK, Stulnig TM. Autoimmune aspects of type 2 diabetes mellitus: a mini-review. Gerontology. 2014;60(3):189-96.

112. Duckworth W, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, et al. Glucose control and vascular complications in veterans with type 2 diabetes. N Engl J Med. 2009;360(2):129-39.

113. Signorovitch JE, Macaulay D, Diener M, Yan Y, Wu EQ, Gruenberger JB, et al. Hypoglycaemia and accident risk in people with type 2 diabetes mellitus treated with non-insulin antidiabetes drugs. Diabetes Obes Metab. 2013;15(4):335-41.

114. Johnston SS, Conner C, Aagren M, Ruiz K, Bouchard J. Association between hypoglycaemic events and fall-related fractures in Medicare-covered patients with type 2 diabetes. Diabetes Obes Metab. 2012;14(7):634-43.

115. Feinkohl I, Aung PP, Keller M, Robertson CM, Morling JR, McLachlan S, et al. Severe hypoglycemia and cognitive decline in older people with type 2 diabetes: the Edinburgh type 2 diabetes study. Diabetes Care. 2014;37(2):507-15.

116. Mattishent K, Loke YK. Bi-directional interaction between hypoglycaemia and cognitive impairment in elderly patients treated with glucose lowering agents: systematic review and meta-analysis. Diabetes Obes Metab. 2016;18(2):135-41.

117. Lipska KJ, Montori VM. Glucose control in older adults with diabetes mellitus: more harm than good? JAMA Intern Med. 2013;173(14):1306-7.

118. Hirji I, Guo Z, Andersson SW, Hammar N, Gomez-Caminero A. Incidence of urinary tract infection among patients with type 2 diabetes in the UK General Practice Research Database (GPRD). J Diabetes Complications. 2012;26(6):513-6.

119. Geerlings S, Fonseca V, Castro-Diaz D, List J, Parikh S. Genital and urinary tract infections in diabetes: impact of pharmacologically-induced glucosuria. Diabetes Res Clin Pract. 2014;103(3):373-81.

120. Johnsson KM, Ptaszynska A, Schmitz B, Sugg J, Parikh SJ, List JF. Vulvovaginitis and balanitis in patients with diabetes treated with dapagliflozin. J Diabetes Complications. 2013;27(5):479-84.

121. Liakos A, Karagiannis T, Athanasiadou E, Sarigianni M, Mainou M, Papatheodorou K, et al. Efficacy and safety of empagliflozin for type 2 diabetes: a systematic review and meta-analysis. Diabetes Obes Metab. 2014;16(10):984-93.

122. Nyirjesy P, Sobel JD, Fung A, Mayer C, Capuano G, Ways K, et al. Genital mycotic infections with canagliflozin, a sodium glucose co-transporter 2 inhibitor, in patients with type 2 diabetes mellitus: a pooled analysis of clinical studies. Curr Med Res Opin. 2014;30(6):1109-19.

123. Jackuliak P, Payer J. Osteoporosis, fractures, and diabetes. Int J Endocrinol. 2014;2014:820615.

124. Strotmeyer ES, Cauley JA, Schwartz AV, Nevitt MC, Resnick HE, Bauer DC, et al. Nontraumatic fracture risk with diabetes mellitus and impaired fasting glucose in older white and black adults: the health, aging, and body composition study. Arch Intern Med. 2005;165(14):1612-7.

125. Thrailkill KM, Clay Bunn R, Nyman JS, Rettiganti MR, Cockrell GE, Wahl EC, et al. SGLT2 inhibitor therapy improves blood glucose but does not prevent diabetic bone disease in diabetic DBA/2J male mice. Bone 2016;82:101-7.

126. Ljunggren O, Bolinder J, Johansson L, Wilding J, Langkilde AM, Sjöström CD, et al. Dapagliflozin has no effect on markers of bone formation and resorption or bone mineral density in patients with inadequately controlled type 2 diabetes mellitus on metformin. Diabetes Obes Metab. 2012;14(11):990-9.

127. Bilezikian JP, Watts NB, Usiskin K, Polidori D, Fung A, Sullivan D, et al. Evaluation of bone mineral density and bone biomarkers in patients with type 2 diabetes treated with canagliflozin. J Clin Endocrinol Metab. 2016;101(1):44-51.

128. Alba M, Xie J, Fung A, Desai M. The effects of canagliflozin, a sodium glucose co-transporter 2 inhibitor, on mineral metabolism and bone in patients with type 2 diabetes mellitus. Curr Med Res Opin. 2016;32(8):1375-85.

129. Palermo A, D'Onofrio L, Eastell R, Schwartz AV, Pozzilli P, Napoli N. Oral anti-diabetic drugs and fracture risk, cut to the bone: safe or dangerous? A narrative review. Osteoporos Int. 2015;26(8):2073-89.

130. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes: a meta-analysis. Osteoporos Int. 2007;18(4):427-44.

131. Watts NB, Bilezikian JP, Usiskin K, Edwards R, Desai M, Law G, et al. Effects of canagliflozin on fracture risk in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab. 2016;101(1):157-66.

132. Wu JH, Foote C, Blomster J, Toyama T, Perkovic V, Sundström J, et al. Effects of sodium-glucose cotransporter-2 inhibitors on cardiovascular events, death, and major safety outcomes in adults with type 2 diabetes: a systematic review and meta-analysis. Lancet Diabetes Endocrinol. 2016;4(5):411-9.

133. Cholesterol Treatment Trialists' (CTT) Collaborators, Kearney PM, Blackwell L, Collins R, Keech A, Simes J, Peto R, et al. Efficacy of cholesterol-lowering therapy in 18,686 people with diabetes in 14 randomised trials of statins: a meta-analysis. Lancet. 2008;371(9607):117-25.

134. Brandenburg VM, Kleber ME, Vervloet MG, Tomaschitz A, Pilz S, Stojakovic T, et al. Fibroblast growth factor 23 (FGF23) and mortality: the Ludwigshafen Risk and Cardiovascular Health Study. Atherosclerosis. 2014;237(1):53-9.

135. Yin J, Lu Z, Wang F, Jiang Z, Lu L, Miao N, et al. Renalase attenuates hypertension, renal injury and cardiac remodelling in rats with subtotal nephrectomy. J Cell Mol Med. 2016;20(6):1106-17.

136. Anzawa R, Bernard M, Tamareille S, Baetz D, Confort-Gouny S, Gascard JP, et al. Intracellular sodium increase and susceptibility to

ischaemia in hearts from type 2 diabetic db/db mice. Diabetologia. 2006;49(3):598-606.

137. Tung P, Albert CM. Causes and prevention of sudden cardiac death in the elderly. Nat Rev Cardiol. 2013;10(3):135-42.

138. El-Menyar AA. Dysrhythmia and electrocardiographic changes in diabetes mellitus: pathophysiology and impact on the incidence of sudden cardiac death. J Cardiovasc Med. 2006;7(8):580-5.

139. Banerjee SK, McGaffin KR, Pastor-Soler NM, Ahmad F. SGLT1 is a novel cardiac glucose transporter that is perturbed in disease states. Cardiovasc Res 2009;84(1):111-8.

140. Scheen AJ. Pharmacokinetic and pharmacodynamic profile of empagliflozin, a sodium glucose co-transporter 2 inhibitor. Clin Pharmacokinet. 2014;53(3):213-25.

141. Mudaliar S, Alloju S, Henry RR. Can a shift in fuel energetics explain the beneficial cardiorenal outcomes in the EMPA-REG OUTCOME study? A unifying hypothesis. Diabetes Care. 2016;39(7):1115-22.

142. Kupari M, Lommi J, Ventila M, Karjalainen U. Breath acetone in congestive heart failure. Am J Cardiol. 1995;76(14):1076-8.

143. Ashrafian H, Frenneaux MP, Opie LH. Metabolic mechanisms in heart failure. Circulation. 2007;116(4):434-48.

144. Jeffrey FM, Diczku V, Sherry AD, Malloy CR. Substrate selection in the isolated working rat heart: effects of reperfusion, afterload, and concentration. Basic Res Cardiol. 1995;90(5):388-96.

145. Freemantle E, Vandal M, Tremblay Mercier J, Plourde M, Poirier J, Cunnane SC. Metabolic response to a ketogenic breakfast in the healthy elderly. J Nutr Health Aging. 2009;13(4):293-8.

146. Kernan WN, Viscoli CM, Furie KL, Young LH, Inzucchi SE, Gorman M, et al. Pioglitazone after ischemic stroke or transient ischemic attack. N Engl J Med. 2016;374(14):1321-31.

147. Emdin CA, Rahimi K, Neal B, Callender T, Perkovic V, Patel A. Blood pressure lowering in type 2 diabetes: a systematic review and meta-analysis. JAMA 2015;313(6):603-15.

148. Ricci F, Fedorowski A, Radico F, Romanello M, Tatasciore A, Di Nicola M, et al. Cardiovascular morbidity and mortality related to orthostatic hypotension: a meta-analysis of prospective observational studies. Eur Heart J. 2015;36(25):1609-17.

149. Laragh JH, Sealey JE. Abnormal sodium metabolism and plasma renin activity (renal renin secretion) and the vasoconstriction volume hypothesis: implications for pathogenesis and treatment of hypertension and its vascular consequences (heart attack, stroke). Clin Chem. 1991;37(10 pt 2):1820-7.

150. Mollsten A, Stegmayr B, Wiklund PG. Genetic polymorphisms in the renin-angiotensin system confer increased risk of stroke independently of blood pressure: a nested case-control study. J Hypertens. 2008;26(7):1367-72.

REVIEW ARTICLE

Central role of obesity in endothelial cell dysfunction and cardiovascular risk

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SUMMARY

Atherosclerosis is the leading cause of mortality in the contemporary world. The critical role of the endothelial cells (EC) in vascular homeostasis, the metabolic changes that take place when the cell is activated, and the elements involved in these processes have been widely explored over the past years. Obesity and its impact, promoting a rise in blood levels of free fatty acids (FAs) are often associated with atherosclerosis and cardiovascular mortality. However, the mechanisms that promote cardiovascular structural changes and adaptive changes in the ECs, particularly in the context of obesity, are little known. Here, we reviewed studies that assessed the metabolic adaptations of healthy and dysfunctional ECs during exposure to FAs, as well as the epidemiological perspectives of cardiovascular structural changes in obesity. Finally, we explored the role of new agents – sphingolipids, dietary unsaturated fatty acids and sodium-glucose cotransporter-2 inhibitors (iSGLT2) – in atherosclerosis and their relationship with obesity.

KEYWORDS: *Obesity. Risk factors. Atherosclerosis. Endothelium.*

INTRODUCTION

Atherosclerosis is the leading cause of mortality worldwide and is associated with obesity. This disease has a physiopathological component key to the dysfunction of ECs, the cells that make up the luminal surface of blood vessels[1]. The activation of the endothelial cell by different biochemical or biomechanical stimuli results in an inflammatory phenotype, with loss of the homeostatic function as a micro-barrier, expression of adhesion molecules and prothrombotic molecules on its surface, as well as the generation of reactive oxygen species (ROS), which results in the progression of the atherosclerotic injury in the vessel wall[2]. This review focused on epidemiological aspects involving the association between obesity and atherosclerosis and how the basic understanding of metabolism and the ECs signaling during their exposure to FAs or other atherogenic components of the obese environment have contributed in providing

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insights and identifying potential therapeutic targets for the obesity/atherosclerosis binomial. In the final part of the review, we will discuss the roles of molecules or other substances identified more recently or fewer studies, sphingolipids, dietary unsaturated fatty acids and sodium-glucose cotransporter-2 inhibitors (iSGLT2), and their potential involvement in the physiopathology or therapy of the cardiovascular changes that accompany obesity.

OBESITY AND RISK OF CARDIOVASCULAR DISEASE

The rapid increase in the prevalence of obesity in Brasil and in the world over the last few decades was accompanied by a parallel increase in other comorbidities associated with obesity[3]. The epidemiological evidence establishes an association between obesity and mortality; however, there are controversies in the relationship between obesity and cardiovascular risk, and that seems to be associated with the pattern of body fat distribution and metabolic factors that involve insulin sensitivity, the profile of hormone secretion of the adipocytes and cardiorespiratory fitness[4].

The heterogeneity of obesity and the concept of "obesity paradox," in which individuals with overweight and obesity present an improved cardiometabolic prognosis in comparison with eutrophic individuals, are fertile ground for an investigation of the mechanistic and physiological fundamentals of the diversity of effects of obesity on cardiovascular health[5,6].

Although widely used in epidemiological studies and the clinical routine, the body mass index (BMI) is unable to distinguish areas of concentration of white adipose tissue. The local distribution of white adipose tissue and its impact on the cardiometabolic risk have been described since the 1940s, and the deposits of ectopic fat – deposits in visceral organs – and in the abdominal cavity are significantly correlated with cardiometabolic abnormalities[7].

The deposits of visceral fat are more resistant to the insulin action and release free FAs in a higher proportion in comparison to the subcutaneous adipose tissue. The excess of free FAs in the bloodstream is closely related with the onset of inflammation, notedly observed through the increase in the serum levels of C-reactive protein, interleukin-6, and tumor necrosis factor- α (TNF-α) in peripheral tissues[4]. Additionally, the excess of circulating FAs and their influx into the hepatic portal system triggers a higher production of VLDL by the liver. In this context, although many obese individuals present standard levels of low-density lipoproteins (LDL-c), most of the LDL-c produced are small and dense particles, classically more atherogenic[8]. In rodents, perivascular fat and the role of the resistance to the effect of insulin on vascular cells during atherosclerosis are well documented. These studies demonstrate that the resistance to the effect of insulin on vascular cells reduces the bioavailability of nitric oxide and favors the adhesion and infiltration of immune cells, compromising the vasodilation properties and favoring the endothelial dysfunction[9,10].

Taken together, the mechanisms mentioned above constitute some of the pillars of the heterogeneity of obesity and the physiopathology of cardiovascular diseases associated with obesity. The following topics discuss these interfaces in greater detail.

METABOLIC ADAPTATIONS IN THE ENDOTHELIAL CELL IN OBESITY – BIOCHEMICAL PERSPECTIVES

In healthy conditions, the endothelium coordinates the formation of blood vessels and keeps the vascular homeostasis and its structure. In pathological conditions, however, the metabolic changes that take place in the ECs can promote dysfunction and be a trigger event for atherosclerosis[1]. ECs support different cellular processes through several metabolic pathways, which can have different signatures according to bad metabolic adaptations, of which the understanding might help us prevent injuries and identify new therapeutic targets[11]. Initially, we present the fundamental aspects of the endothelial metabolism of blood glucose and Fas,[12], both the most critical energy-generating pathways in healthy ECs, before exploring which fundamental changes in these mechanisms are present in the endothelial cells of obese individuals.

Counter-intuitively, in ECs that are very close to an environment where there is direct exposure to high blood flow, the ATP generation dos not rely, primarily, on the oxidative phosphorylation (Oxphos). ECs have a low mitochondrial density, and their generation of adenosine triphosphate (ATP) occurs mostly through anaerobic glycolysis, a process that occurs at a similar rate to the the one in cancer cells,

for example, in which the glycolytic flux also prevails in relation to the oxidation of FAs and blood glucose (> 200x). Thus, even though the ATP generation through anaerobic glycolysis is less efficient than Oxphos, in a cellular environment where there is abundant availability of glucose, glycolysis becomes a efficient option[12,13]. Besides, that non-oxidative cellular approach decreases the generation of reactive oxygen species associated with Oxphos, as well as reduces the use of oxygen, making it available, primarily, for perivascular cells. Furthermore, this process allows the ECs to use the lactate protectively, as a molecule for controlling the angiogenesis[12,14]. In parallel, an intermediary of the glycolytic flux feeds the pentose phosphate pathway, which results in the generation of glutathione (GSH), a molecule with anti-oxidant potential (ROS scavenging)[1,15].

FAs represent another energetic pathway for the production of energy in the ECs (approximately 5%); however, the primary use of the FAs that enter an EC is not generating ATP[12]. However, in the absence of glucose, the metabolic flow is diverted to the oxidation of the FAs (FAO) in a process regulated by the AMPK (*adenosine monophosphate-activated protein kinase*), a cellular metabolic sensor[16]. The plasma FAs enter the cell through passive diffusion or FAs translocase, which also acts as acyl-CoA synthetase, leading to the formation of fatty acyl-CoA. The fatty acyl-CoA is conjugated to carnitine through carnitine palmitoyltransferase 1A (CPTIA) before being transported to the mitochondria by the carnitine/acylcarnitine shuttle for β-oxidation. After entering the mitochondrial matrix, enzymes of the CPT2 family catalyze the formation of acylcarnitines back to the fatty acyl-CoAs, which enter the β-oxidation pathway[17]. Thus, one of the regulators that play an important role in FAO is the CPT1A, limiting the FA flux destined to β-oxidation. For example, the EC-specific gene silencing of CPT1A causes defects in cell proliferation (though it does not cause changes in other homeostatic elements, such as cell migration) and EC angiogenesis[18,19]. Such changes occur because, in ECs, FAO is crucial for the synthesis of deoxyribonucleotide, since the FAs are a source of carbon as critical as glucose and glutamine to the citric acid cycle in the ECs[19]. In this context, occurs the generation of aspartate and glutamate, precursors of nucleotides, which, in turn, are critical for the DNA (deoxyribonucleic acid) synthesis during cell proliferation[1].

Obesity is associated with a high level of circulating FAs, and that increase in supply is a metabolic challenge for the ECs since this process is also associated with the generation of reactive oxygen species and is deleterious to the cells (ROS)[20]. Most endothelial cells in adults are in quiescent form (QEC), unlike the cells in proliferation (PECs). The QECs are continuously exposed to an environment rich in oxygen in the peripheral blood, and, consequently, to the oxidative stress promoted by the ROs, capable of promoting endothelial dysfunction through the decoupling of the vasoprotective function of the endothelial nitric oxide synthase (eNOS)[21]. Studies have sought evidence of the mechanisms through which these cells protect themselves to keep the vascular homeostasis, which could lead to the identification of new therapeutic targets that promote vascular protection in a FA-rich environment, like obese individuals[18]. According to what has been stated above, PECs use the β-oxidation of FAs as a source of carbon for the synthesis of nucleotides during the proliferative stage of the angiogenesis[19]. Recently, Kalucka et al.[22] demonstrated that QECs are not hypometabolic, as was believed.

On the contrary, QECs increase the FAO flux when they become quiescent. Surprisingly, they use the β-oxidation of FAs not for the synthesis of nucleotides, such as in PECs, but to keep the redox homeostasis through the regeneration of antioxidant molecules [22]. That understanding certainly contributes to the view that EC adopts protective mechanisms during stress situations and the use of FAs to maintain redox homeostasis is a key mechanism.

This view that there are adaptive mechanisms that protect against the excess of FAs has been recently described. In general, it is known that highly acute levels of free FAs induce a bad adaptation and a consequent endothelial dysfunction in vivo in humans due to a worsen vasodilation mediated by nitric oxide[23], as well as promotes the activation of the inflammasome and the of inflammatory signaling cascade controlled by the factor nuclear kappa B (NF-kB)[24]. Similarly, obesity/insulin resistance also promotes in vivo endothelial dysfunction in humans[25]. In the same way, FAs promote apoptosis through a mechanism dependent on the generation of ROS using the NAD(P)H oxidase[26], as well as the membrane saturation of the endoplasmic reticulum and the consequent endoplasmic reticulum stress, which also activates inflammatory pathways[27].

On the contrary, increasing FAO through the in-

duction of the CPT1A upregulation or the promotion of other metabolic sensors upregulation, such as the peroxisome-proliferator-activator-receptor (PPAR), is capable of reducing endothelial dysfunction and the EC apoptosis, therefore, being a possible therapeutic target for endothelial protection in situations in which there is an increased supply of FAs in the plasma[24,28]. Besides, bariatric surgery can reduce markers of systemic inflammation and endothelial activation[29] significantly. This improvement in the endothelial dysfunction might be associated to better management of fatty acids by lean tissue after the surgery since there is a decrease of the systemic lipolysis during the intravenous overload of lipids, as well as better disposal of triglycerides and production of acylcarnitine after the bariatric surgery[30].

CARDIOVASCULAR STRUCTURAL ADJUSTMENTS IN OBESITY

Obesity is a well-known risk factor; however, part of its effect on the cardiovascular structure is caused by the concomitance of other risk factors. The isolated expression of the excess of white fat, thus, would happen only in so-called metabolic-healthy obese individuals. The analysis of cohorts featuring individuals with these characteristics would allow us to understand which cardiovascular phenotype is related to obesity. The largest cohort, so far, in number of patients (n = 3,500,000), suggests that this action leads to an adjusted risk of heart failure two times greater (Risk ratio: 1.96; confidence interval of 95%: 1.86–2.06) and 50% higher chance of coronary artery disease (Risk ratio: 1.49; confidence interval of 95%: 1.45–1.54).0.46-1.81). This same study revealed a lower risk, but not insignificant, of cerebrovascular disease (Risk ratio: 1.07; confidence interval of 95%: 1.04–1.11) also linked to obesity. The average follow-up time for these outcomes to occur was 5.4[31]. Another meta-analysis confirms these findings in different populations that differ only on whether or not there is an increase in the overall mortality of these patients in comparison with the general population[32,34].

These adverse cardiovascular outcomes are mediated by structural changes that precede them. The most important of these outcomes seems to be heart failure. The association of obesity with diastolic dysfunction and left ventricular hypertrophy is well established[35,36], and both factors precede this outcome. The increase in mass and the deficit of left ventricle relaxation seem to be related with an increase in the total peripheral vascular resistance determined by a chronic increase of blood volume, already suggested by the Framingham study[37]. Also associated with heart failure, the left ventricular systolic dysfunction, as the diastolic, is more frequent in obese individuals. There is a clear linear correlation between BMI and the ejection fraction of the left ventricle, as well as between BMI and the E/e' ratio (marker of the diastolic dysfunction), and these relationships follow the increase or reduction of body weight in a individual, thus showing the strength of the association between these parameters[38]. The reduction in the ejection fraction, however, seems to be slightly more connected to hyperglycemia and insulin resistance concomitant with the increase in adiposity. Hyperglycemia can increase the intra and extracellular glycation of proteins, increasing the stress oxidation, inflammation, and injuries to the myocardium, culminating in the rigidity and reduction of contractility[39]. However, there are also descriptions of ventricular systolic dysfunction in obese individuals with standard blood glucose and glycated hemoglobin, indicating there must be other mediating mechanisms.

In fact, obese individuals with no insulin resistance (Homa-IR < 2.5 and blood glucose < 100 mg/dl) or other components of metabolic syndrome present heart disease more often than non-obese patients. Subclinical atherosclerosis assessed by the coronary calcium score is two times more frequent in this group (Prevalence ratio: 2.26; confidence interval of 95%: 1.48–3.43), as shown by a cross-sectional study[40]. This same study, however, suggests that the diagnosis parameters for metabolic syndrome are linked to a higher calcium score, even though they are below the cutoff values, especially when considered with the values for LDL-cholesterol (also within the normal range in the study). This set of evidence suggests that the connection of the metabolic parameters of obese patients and coronary disease might not have a threshold below which the risk would be similar to the general healthy population.

The association between obesity and cerebrovascular disease, however, seems less evident. Large cohort studies have shown relationships[31], but others were not able to confirm them[41], in very distinct population, it is important to note. However, this disagreement may be due to the dichotomization of the classification of "healthy" and "unhealthy" obese

individuals, since there is a clear correlation between cerebrovascular accident and adiposity when considering the parameters of systolic blood pressure ≥ 130 mmHg and/or diastolic ≥ 85 mmHg, or hypertension treatment, fasting glucose > 100 mg/dl, or diabetes mellitus treatment, and total cholesterol > 240 mg/dl or dyslipidemia treatment[41]. The set of data indicates, thus, that the metabolic changes are necessary for the outcome of cerebrovascular disease to occur, unlike what happens with heart failure and, possibly, coronary artery disease.

NEW AND OLD MOLECULES IN THE OBESITY AND ATHEROSCLEROSIS INTERFACE

iSGLT2, obesity, and atherosclerosis

The iSGLT2 are a class of anti-diabetic drugs that inhibit the absorption of sodium and glucose in the proximal convoluted tubule, promoting osmotic diuresis and the renal glucose excretion[42]. Among the main effects of these drugs, are the reduction of blood glucose, arterial pressure (AP), and weight. All these effects can contribute to the reduction of atherosclerosis.

From the studies on safety requested by the American Food and Drug Administration (FDA) since 2009, interesting effects of anti-diabetic drugs have been observed in DM2 patients with high cardiovascular risk[43]. The use of empagliflozin in the Empla-REG study was associated with a 14% reduction (Risk ratio of 0.86; CI 95%: 0.74–0.99; p = 0.04 in the incidence of major cardiovascular events (Mace) after an average of 3.1 years, an effect induced, mostly, by the 38% reduction in mortality due to cardiovascular diseases (risk ratio of 0.62; CI 95%: 0.49–0.77; p < 0.001) and of 35% in admissions due to heart failure (risk ratio 0.65; CI 95%: 0.50–0.85; p=0.002)[44]. A similar result was observed in the Canvas study, using canagliflozin with a 14% reduction (risk ratio 0.86; CI 95%: 0.75–0.97; p = 0.02) of Mace incidence after 3.6 years[45]. In uncontrolled studies conducted from databases, the so-called real-world studies, the lower incidence of events was observed with more intensity, with a 51% reduction of mortality due to all causes (risk ratio: 0.49; CI 95%: 0.41–0.57; p < 0.001)[46]. The reasons behind this reduction remain unclear; however, it might be associated with the effects the iSGLT2 have on cardiovascular risk factors.

Gliflozins are associated with dose/response weight loss[47], with an average reduction of 2.1 kg (CI 95%: –2.3 – –2.0; p < 0.01) in comparison with the placebo after12 weeks of use[48] and an average reduction of 2.9 kg (CI 95%: –3.72 – –2.07; p < 0.001) after two years, in comparison with other medications[49]. Still, the weight loss is predominantly of adipose tissue (in a 2:1 ratio)[50], particularly of visceral adipose tissue. The weight reduction is due, mostly, to glycosuria, with a estimated calorie deficit of 50 kcal/day[51], despite the possible contribution of other mechanisms, such as the activation of brown adipose tissue and insulin reduction[52].

The gliflozins promote a reduction of 3-5 mmHg in the systolic AP and 1-3 mmHg in the diastolic[53]. Mechanistically, the osmotic diuresis induced by the glycosuria contributes to the blood pressure reduction; however, other effects, such as the improvement of endothelial function[54], arterial stiffness[55], sympathetic tone[56], and increase in natriuresis might be associated[57].

Some studies have linked treatments with gliflozin to lower levels of inflammatory factors associated with atherosclerosis, such as the TNF-alpha[58], interleukin 6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1)[59]. In animal models, the treatment with iSGLT2 was capable of reducing the formation of atheromatous plaques in the aortic arch[60], as well as the formation of foam cells in the atheromatous plaque[61]. However, the iSGLT2 are associated with an increase in LDL, perhaps due to the reduction of the hepatic metabolism of these proteins[62], and have a neutral effect on HDL. Still, no alterations were observed in the functions of HDL and the enzymes associated with this protein[47,63]. Studies on obese patients with no DM2 were not conducted. It is possible, however, that these effects are found mainly on DM2 patients, but not on those with regular blood glucose levels.

Sphingolipids, lipid profile, and atherosclerosis

The increase in FA levels results, also, from a reshaping of the synthesis of bioactive lipids, which might be involved in the physiopathology of conditions associated with obesity, such as diabetes and cardiovascular diseases. Among these bioactive lipids, the sphingolipids have been noted as an important structural membrane element and as molecules with cell signaling functions[64]. The sphingolipid de novo biosynthesis uses as a rate-limiting enzyme the serine palmitoyltransferase, whose catalytic activity has a significant increase with the increase of

availability of palmitoyl-CoA, derived from a higher concentration of palmitate. Thus, a higher enzyme activity of the serine palmitoyltransferase results in a greater formation of several sphingolipids. Among them, the most involved in cellular processes are the ceramide and the sphingosine-1-phosphate, which implies directly in a larger pool fo these molecules in conditions of a higher supply of FAs. Such reshaping of sphingolipids can affect several tissues[64,65].

Recently, plasma lipidomics data in the investigation of such bioactive lipids as biomarkers for unstable coronary artery disease in the retrospective cohort Lipid identified several lipid classes positively associated with future cardiovascular events, such as ceramides and sphingolipids, while lysophosphatidylcholine and diacylglycerol were negatively associated[66], which awakens an interest in other bioactive molecules as possible effectors of lipid signaling. In total, 53 lipid species were associated with cardiovascular events. Atherogenic lipoproteins, such as LDL and VLDL, are enriched in sphingolipids, which might also be associated with cardiovascular risk. However, the sphingolipids that are also associated with risk in the Lipid cohort had already been positively associated previously with unfavorable metabolic conditions, including dihydroceramide and different kinds of ceramides. These results suggest that the regulation might occur in a ceramide-synthase level, which has a distinct expression according to the tissue[66].

Even though there are few divergent data, overall, the studies on lipidomic profile have shown a positive association between ceramides and several variables associated with metabolic disorders, such as atherosclerosis and cardiomyopathy due to lipotoxicity. Mechanisms of action of the ceramides intracellularly involve, classically, the induction of apoptosis, insulin resistance, endoplasmic reticulum stress, and opening of the permeability transition pore in the mitochondria. On the contrary, the inhibition of the biosynthesis of ceramides results in an improvement of several metabolic disorders in animal models, such as the decrease in the formation of atherosclerotic plaque[67].

The effector signaling of the sphingolipids sphingosine-1-phosphate deserves attention for its sophisticated and still not fully understood behavior (Figure 1). Initially, as the class of sphingolipids in general, during obesity in animal models, there is a positive regulation of the enzyme apparatus for the synthesis of ceramides with an increase of the sphingolipids and sphingosine-1-phosphate both in the plasma of obese mice as well as in adipocytes cultured in vitro. This increase culminates in the positive regulation of the cytokines involved in the inflammatory and pro-thrombotic status of obesity, possibly mediating atherosclerotic complications associated with weight gain[70]. Such findings are corroborated by the positive association of the sphingosine-1-phosphate (S1P) with obesity in humans[71] and by the experimental finding that it positively regulates the inflammatory pathway triggered by the TNF-α receptor, a vital element of the meta-inflammation triggered by obesity[72]. Despite that, the S1P has a dual role[73], since the signaling cascade triggered by the S1P in the vascular endothelium and in the cardiomyocyte has a protective role, regulating the vascular development, migration, angiogenesis, vasodilation signaling, and microvascular barrier function, especially when the S1P é chaperoned by the HDL or the apolipoprotein M[74,75], a condition in which the HDL/S1P induces a complex with the β-arrestin in order to suppress the NF-κB activation induced by cytokines. Besides, the signaling of the S1P1 receptor is vessel-protective, since its EC-specific super expression protects from the formation of plaques in atherosclerosis models in a diet rich in fat[75]. Unfortunately, there is still a gap concerning the specific reshaping of these sphingolipids in obesity and its role in the vascular endothelium during obesity, although some light has been shed on the protective role of the S1P1 and one of its ligands (S1P carried by the HDL or ApoM). However, it is also reasonable to extend this atherogenic environment to the changes in the content of the dysfunctional HDL S1P of both diabetic and coronary artery disease patients[76,77], who have a decreased capacity to carry S1P. Indeed, since the biosynthesis pathway of sphingolipids is interconnected, the therapeutic modulation of any element would be complicated, and so far unpredictable, and the ceramide-synthase would have a pathogenic role in a given context[64], or even on how the entire system would behave in the endothelium of an obese individual.

Unsaturated fatty acids, diet, and atherosclerosis

Lipids have been widely studied in the context of cardiovascular diseases, and the dietary recommendations for the ingestion of the macronutrients have emphasized the replacement of saturated FAs

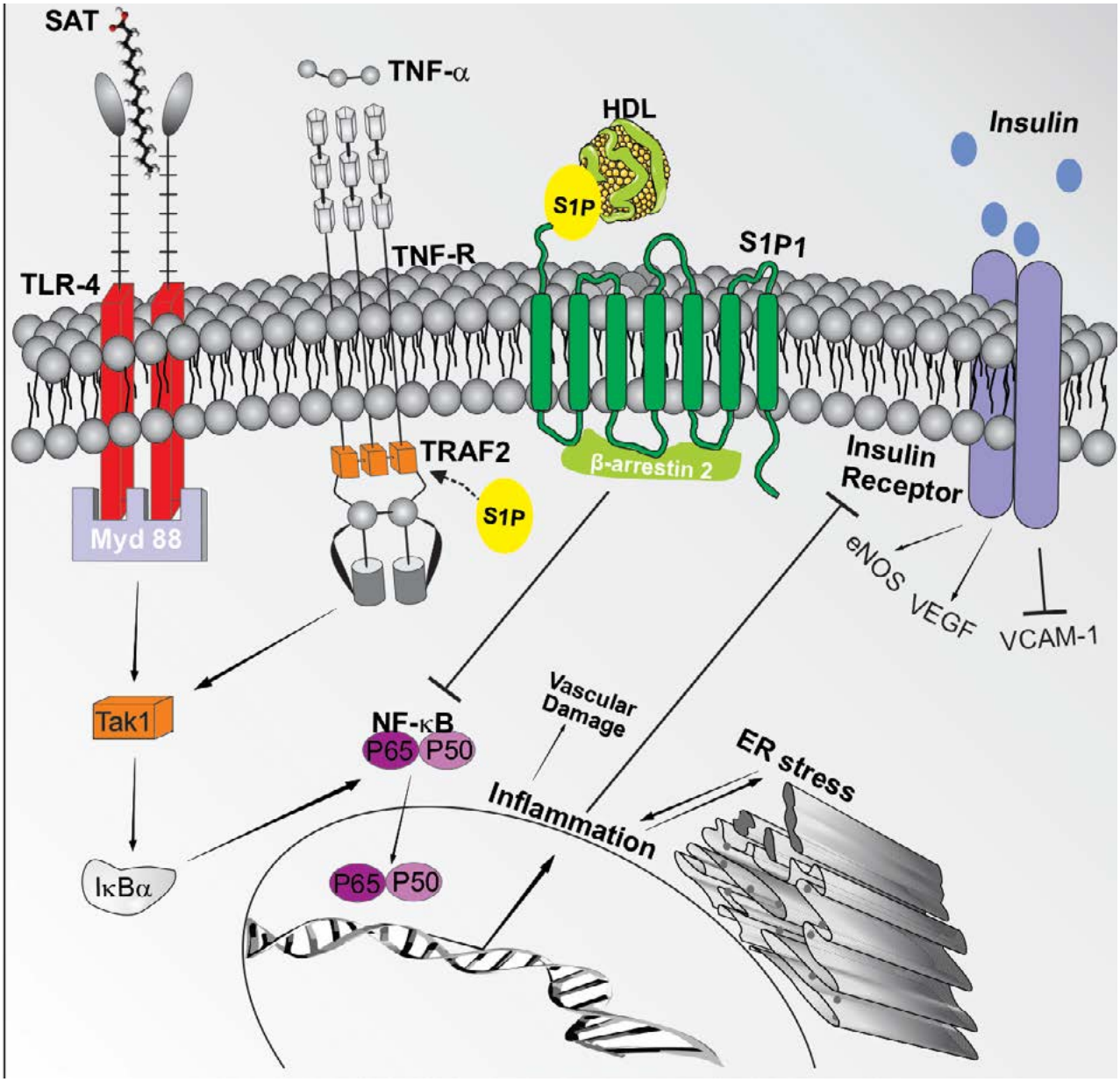

FIGURE 1 (adapted from Moura-Assis et al.[68]). Activation of inflammatory signaling by saturated fatty acids in conditions associated with obesity and their interaction with the S1P The TAK1 (Transforming growth factor b-activated kinase 1) is activated by several stimuli, including cytokines, TLRs, and factors associated with the TNF receptor (Traf), which culminates in the activation of the central pathway *IkB kinase (IKK)–nuclear factor-kB (NF-kB)*, responsible for the activation of transcription of several inflammatory genes[69] and the blocking of the insulin receptor. This scheme also demonstrates that the S1Pmolecule, when generated intracellularly, acts as a secondary lipidic messenger, connecting itself to the Traf2 and promoting changes in the complex with Traf2 that are crucial to the inflammatory activation of the IKK- *NF-kB*[70]. When S1P is carried by HDL or ApoM, when it connects to the endothelial cell, it promotes the formation of a S1P1-β-arrestin 2 complex that inhibits the activation of *NF-kB* and blocks vascular inflammation.

for unsaturated FAs since the 1960s. The debates around the recommendations for the ingestion of FAs occupy a prominent position on the agenda of most dietary guides and, according to the American guide, the ingestion of saturated FAs should no exceed the daily limit of 10% of the total energetic value. The dietary guidelines for the Brazilian population, in turn, was structured to stimulate dietary patterns and healthy behavior to the detriment of recommendations of individual nutrients. Such action is necessary since nutrients *per se* seem to not represent a *sine qua non*-condition for the development of diseases. However, even within the perspective of the Brazilian dietary guide, there is an instruction towards the reduction of saturated fats, substituting them for unsaturated ones.

Even though there are multiple associations between lipid consumption and the development of

cardiovascular diseases, so far, no evidence has been established through well-controlled clinical trials. Besides, the massive divergence between dietary patterns between countries makes it substantially more challenging to create guidelines.

Some studies designed to assess the effect of the replacement of saturated FAs by carbohydrates indicate there are no overall differences in the markers for cardiovascular risk prediction such as LDL-c e HDL-c[78]. However, some studies that emphasized the replacement of saturated FAs by carbohydrates with low glycemic indexes found a reduction of LDL-c and increase in HDL-c[79]. These opposite effects emphasize that specifying the source of foods that make up the replacement list is mandatory and the use of carbohydrates with low glycemic index as replacements for saturated FAs confers cardiovascular benefits. A recent analysis of the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS) estimated that the isocaloric replacement of 5% of the saturated FAs by polyunsaturated, monounsaturated Fas, and carbohydrates with low glycemic index represented a reduction of 25%, 15%, and 9% of cardiovascular risk, respectively[80].

Since the epidemiological findings on the low incidence of cardiovascular mortality among the Inuit of Greenland, the consumption of fish and the omega-3 FAs have been extensively studied, In general, prospective studies suggest a lower risk of coronary disease in individuals with no previous cardiovascular disease who have a higher intake of polyunsaturated or fish FAs[81]. The randomized clinical trials who did find benefits in the supplementation with fish oil in individuals with cardiovascular disease present certain limitations and need to be interpreted with caution. Individuals with cardiovascular disease already receive, in general, pharmacological treatment (statins, for example), and the supplementation with fish oil hardly potentializes the action of these drugs. In addition, the benefits of the omega-3 FAs seem to have a threshold, and additional doses do not confer an increment to the cardiovascular protection in individuals who already have an adequate intake of these FAs in their diet.

Some mechanistic studies in rodents have dissected with greater precision the effects of different types of FAs in the metabolism and in their effect as signaling molecules. Polyunsaturated FAs can increase the expression of genes involved in the oxidation of lipids (PPARα) and decrease the expression of those involved in the hepatic lipogenesis (SREBP-1c), decreasing atherosclerotic injuries[82,83]. Additionally, the partial and isocaloric replacement of saturated FAs by unsaturated FAs decreases inflammation and the endoplasmic reticulum stress in the aorta of obese and insulin-resistant mice[68]. On the other hand, saturated FAs have been associated with an increase of endothelial inflammation and greater atherosclerotic injury in diet-induced obese mice[82]. Such effects seem to be related with the activation of the pattern recognition toll-like receptor 4 (TLR4) of the innate immune system and its inflammatory cascade[84].

Beyond the consensual difficulties, adherence to the traditional Mediterranean diet has been associated with lower cardiovascular risk and is widely encouraged for the high-risk population. Monounsaturated FAs, especially the oleic acid, represent between 16-29% of the total energetic value[85] and their inclusion in diet, as a replacement for simple carbohydrates, is significantly associated with a reduction in mortality[81]. Additionally, the Predimed study (Prevención con Dieta Mediterránea) found a reduction in cardiovascular events in groups that received supplementation with extra-virgin olive oil or oilseeds in comparison with the control group in a five-year follow-up with men and women with no cardiovascular disease but high-risk. The individuals placed in the supplement group were instructed to consume at least 50 grams of olive oil or 30 grams of oilseeds, including nuts, almonds, and hazelnuts[86]. Finally, the impact of this diet on effector mechanisms for the protection of the activated endothelial cells in obese individuals is still not clear.

CONCLUSIONS

The increase in the overall prevalence of obesity is associated with an increase in cardiovascular risk. There is piling evidence that demonstrates that obesity promotes macrostructural cardiovascular changes and bad cellular metabolic adjustments in the vascular endothelium, a vital element of the onset of the atherosclerotic process. Endothelial activation is triggered, initially, by the plasma content of free fatty acids in obese individuals, but also promotes a series of metabolic adjustments in several tissues from the reshaping of the bioactive lipid pool, which are capable of controlling other pathways of cellular and protective stress. The scientific advancement in this

is due to the incorporation of several tools that allow for the study of lipids at a omic perspective, as well for the integration of such knowledge to a knowledge of cell signaling and population data, which has allowed for the progress concerning the identification of new biomarkers and new therapeutic targets. Indeed, over the next years, there will probably be more pieces available to this network of interdisciplinary knowledge that goes beyond the from bench to bedside limitations.

RESUMO

A aterosclerose é a causa líder de mortalidade no mundo contemporâneo. O papel central da célula endotelial (EC) na homeostase vascular, as alterações metabólicas que ocorrem quando a célula se torna ativada e os elementos envolvidos nesses processos vêm sendo bastante explorados nos últimos anos. A obesidade e o seu impacto, promovendo uma elevação dos níveis sanguíneos de ácidos graxos (FAs) livres, é bastante associada à aterosclerose e à mortalidade cardiovascular. Entretanto, os mecanismos que promovem alterações estruturais cardiovasculares e alterações adaptativas nas ECs, particularmente no contexto da obesidade, são pouco conhecidos. Aqui, nós revisamos estudos que avaliaram as adaptações metabólicas das ECs normais e disfuncionais durante exposição a FAs, bem como as perspectivas epidemiológicas das alterações cardiovasculares estruturais na obesidade. Finalmente, exploramos o papel de novos atores — esfingolípides, ácidos graxos insaturados da dieta e inibidores do cotransportador de sódio-glucose 2 (iSGLT2) — na aterosclerose e sua relação com a obesidade.

PALAVRAS-CHAVE: *Obesidade. Fatores de risco. Aterosclerose. Endotélio.*

REFERENCES

1. Pircher A, Treps L, Bodrug N, Carmeliet P. Endothelial cell metabolism: a novel player in atherosclerosis? Basic principles and therapeutic opportunities. Atherosclerosis. 2016;253:247-57.
2. Gimbrone Jr MA, García-Cardeña G. Endothelial cell dysfunction and the pathobiology of atherosclerosis. Circ Res. 2016;118(4):620-36.
3. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. Lancet. 2017;390(10113):2627-42.
4. Neeland IJ, Poirier P, Després JP. Cardiovascular and metabolic heterogeneity of obesity: clinical challenges and implications for management. Circulation. 2018;137(13):1391-406.
5. Kim SH, Després JP, Koh KK. Obesity and cardiovascular disease: friend or foe? Eur Heart J. 2016;37(48):3560-8.
6. Neeland IJ, Turer AT, Ayers CR, Berry JD, Rohatgi A, Das SR, et al. Body fat distribution and incident cardiovascular disease in obese adults. J Am Coll Cardiol. 2015;65(19):2150-1.
7. Després JP. Body fat distribution and risk of cardiovascular disease: an update. Circulation. 2012;126(10):1301-13.
8. Cepeda-Valery B, Pressman GS, Figueredo VM, Romero-Corral A. Impact of obesity on total and cardiovascular mortality: fat or fiction? Nat Rev Cardiol. 2011;8(4):233-7.
9. Rask-Madsen C, King GL. Vascular complications of diabetes: mechanisms of injury and protective factors. Cell Metab. 2013;17(1):20-33.
10. King GL, Park K, Li Q. Selective insulin resistance and the development of cardiovascular diseases in diabetes: the 2015 Edwin Bierman Award Lecture. Diabetes. 2016;65(6):1462-71.
11. Bierhansl L, Conradi LC, Treps L, Dewerchin M, Carmeliet P. Central role of metabolism in endothelial cell function and vascular disease. Physiology (Bethesda). 2017;32(2):126-40.
12. Eelen G, de Zeeuw P, Simons M, Carmeliet P. Endothelial cell metabolism in normal and diseased vasculature. Circ Res. 2015;116(7):1231-44.
13. Groschner LN, Waldeck-Weiermair M, Malli R, Graier WF. Endothelial mitochondria: less respiration, more integration. Pflugers Arch. 2012;464(1):63-76.
14. Lee DC, Sohn HA, Park ZY, Oh S, Kang YK, Lee KM, et al. A lactate-induced response to hypoxia. Cell. 2015;161(3):595-609.
15. Krützfeldt A, Spahr R, Mertens S, Siegmund B, Piper HM. Metabolism of exogenous substrates by coronary endothelial cells in culture. J Mol Cell Cardiol. 1990;22(12):1393-404.
16. Dagher Z, Ruderman N, Tornheim K, Ido Y. Acute regulation of fatty acid oxidation and amp-activated protein kinase in human umbilical vein endothelial cells. Circ Res. 2001;88(12):1276-82.
17. Bastin J. Regulation of mitochondrial fatty acid β-oxidation in human: what can we learn from inborn fatty acid β-oxidation deficiencies? Biochimie. 2014;96:113-20.
18. Eelen G, Zeeuw P, Treps L, Harjes U, Wong BW, Carmeliet P. Endothelial cell metabolism. Physiol Rev. 2018;98(1):3-58.
19. Schoors S, Bruning U, Missiaen R, Queiroz KC, Borgers G, Elia I, et al. Fatty acid carbon is essential for dNTP synthesis in endothelial cells. Nature. 2015;520(7542):192-7.
20. Goldberg IJ, Bornfeldt KE. Lipids and the endothelium: bidirectional interactions. Curr Atheroscler Rep. 2013;15(11):365.
21. Wilhelm K, Happel K, Eelen G, Schoors S, Oellerich MF, Lim R, et al. FOXO1 couples metabolic activity and growth state in the vascular endothelium. Nature. 2016;529(7585):216-20.
22. Kalucka J, Bierhansl L, Conchinha NV, Missiaen R, Elia I, Brüning U, et al. Quiescent endothelial cells upregulate fatty acid β-oxidation for vasculoprotection via redox homeostasis. Cell Metab. 2018;S1550-4131(18)30459-5.
23. Steinberg HO, Tarshoby M, Monestel R, Hook G, Cronin J, Johnson A, et al. Elevated circulating free fatty acid levels impair endothelium-dependent vasodilation. J Clin Invest. 1997;100(5):1230-9.
24. Theodorou K, Boon RA. Endothelial cell metabolism in atherosclerosis. Front Cell Dev Biol. 2018;6:82.
25. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. J Clin Invest. 1996;97(11):2601-10.
26. Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, et al. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C- dependent activation of NAD(P)H oxidase in cultured vascular cells. Diabetes. 2000;49(11):1939-45.
27. Li X, Gonzalez O, Shen X, Barnhart S, Kramer F, Kanter JE, et al. Endothelial acyl-CoA synthetase 1 is not required for inflammatory and apoptotic effects of a saturated fatty acid-rich environment. Arterioscler Thromb Vasc Biol. 2013;33(2):232-40.
28. Won JC, Park JY, Kim YM, Koh EH, Seol S, Jeon BH, et al. Peroxisome proliferator-activated receptor-gamma coactivator 1-alpha overexpression prevents endothelial apoptosis by increasing ATP/ADP translocase activity. Arterioscler Thromb Vasc Biol. 2010;30(2):290-7.
29. Stolberg CR, Mundbjerg LH, Funch-Jensen P, Gram B, Bladbjerg EM, Juhl CB. Effects of gastric bypass surgery followed by supervised physical train-

ing on inflammation and endothelial function: a randomized controlled trial. Atherosclerosis. 2018;273:37-44.

30. Grenier-Larouche T, Carreau AM, Geloën A, Frisch F, Biertho L, Marceau S, et al. Fatty acid metabolic remodeling during type 2 diabetes remission after bariatric surgery. Diabetes. 2017;66(11):2743-55.

31. Caleyachetty R, Thomas GN, Toulis KA, Mohammed N, Gokhale KM, Balachandran K, et al. Metabolically healthy obese and incident cardiovascular disease events among 3.5 million men and women. J Am Coll Cardiol. 2017;70(12):1429-37.

32. Zheng R, Zhou D, Zhu Y. The long-term prognosis of cardiovascular disease and all-cause mortality for metabolically healthy obesity: a systematic review and meta-analysis. J Epidemiol Community Health. 2016;70(10):1024-31.

33. Kramer CK, Zinman B, Retnakaran R. Are metabolically healthy overweight and obesity benign conditions? A systematic review and meta-analysis. Ann Intern Med. 2013;159(11):758-69.

34. Fan J, Song Y, Chen Y, Hui R, Zhang W. Combined effect of obesity and cardio-metabolic abnormality on the risk of cardiovascular disease: a meta-analysis of prospective cohort studies. Int J Cardiol. 2013;168(5):4761-8.

35. Cil H, Bulur S, Turker Y, Kaya A, Alemdar R, Karabacak A, et al. Impact of body mass index on left ventricular diastolic dysfunction. Echocardiography. 2012;29(6):647-51.

36. Ng ACT, Prevedello F, Dolci G, Roos CJ, Djaberi R, Bertini M, et al. Impact of diabetes and increasing body mass index category on left ventricular systolic and diastolic function. J Am Soc Echocardiogr. 2018;31(8):916-25.

37. Cheng S, Xanthakis V, Sullivan LM, Lieb W, Massaro J, Aragam J, et al. Correlates of echocardiographic indices of cardiac remodeling over the adult life course: longitudinal observations from the Framingham Heart Study. Circulation. 2010;122(6):570-8.

38. Blomstrand P, Sjoblom P, Nilsson M, Wijkman M, Engvall M, Länne T, et al. Overweight and obesity impair left ventricular systolic function as measured by left ventricular ejection fraction and global longitudinal strain. Cardiovasc Diabetol. 2018;17:113.

39. Berg TJ, Snorgaard O, Faber J, Torjesen PA, Hildebrandt P, Mehlsen J, et al. Serum levels of advanced glycation end products are associated with left ventricular diastolic function in patients with type 1 diabetes. Diabetes Care. 1999;22(7):1186-90.

40. Chang Y, Kim BK, Yun KE, Cho J, Zhang Y, Rampal S, et al. Metabolically-healthy obesity and coronary artery calcification. J Am Coll Cardiol. 2014;63(24):2679-86.

41. Lee HJ, Choi EK, Lee SH, Kim YJ, Han KD, Oh S. Risk of ischemic stroke in metabolically healthy obesity: a nationwide population-based study. PLoS One. 2018;13(3):e0195210.

42. Zelniker TA, Braunwald E. Cardiac and renal effects of sodium-glucose co-transporter 2 inhibitors in diabetes: JACC state-of-the-art review. J Am Coll Cardiol. 2018;72(15):1845-55.

43. Lim S, Eckel RH, Koh KK. Clinical implications of current cardiovascular outcome trials with sodium glucose cotransporter-2 (SGLT2) inhibitors. Atherosclerosis. 2018;272:33-40.

44. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med. 2015;373(22):2117-28.

45. Neal B, Perkovic V, Matthews DR. Canagliflozin and cardiovascular and renal events in type 2 diabetes. N Engl J Med. 2017;377(21):2099.

46. Kosiborod M, Cavender MA, Fu AZ, Wilding JP, Khunti K, Holl RW, et al. Lower risk of heart failure and death in patients initiated on sodium-glucose cotransporter-2 inhibitors versus other glucose-lowering drugs: the CVD-REAL Study (Comparative effectiveness of cardiovascular outcomes in new users of sodium-glucose cotransporter-2 inhibitors). Circulation. 2017;136(3):249-59.

47. Fadini GP, Bonora BM, Mayur S, Rigato M, Avogaro A. Dipeptidyl peptidase-4 inhibitors moderate the risk of genitourinary tract infections associated with sodium-glucose co-transporter-2 inhibitors. Diabetes Obes Metab. 2018;20(3):740-4.

48. Storgaard H, Gluud LL, Bennett C, Grøndahl MF, Christensen MB, Knop FK, et al. Benefits and harms of sodium-glucose co-transporter 2 inhibitors in patients with type 2 diabetes: a systematic review and meta-analysis. PLoS One. 2016;11(11):e0166125.

49. Zhang XL, Zhu QQ, Chen YH, Li XL, Chen F, Huang JA, et al. Cardiovascular safety, long-term noncardiovascular safety, and efficacy of sodium-glucose cotransporter 2 inhibitors in patients with type 2 diabetes mellitus: a systemic review and meta-analysis with trial sequential analysis. J Am Heart Assoc. 2018;7(2):pii: e007165.

50. Bolinder J, Ljunggren Ö, Kullberg J, Johansson L, Wilding J, Langkilde AM, et al. Effects of dapagliflozin on body weight, total fat mass, and regional adipose tissue distribution in patients with type 2 diabetes mellitus with inadequate glycemic control on metformin. J Clin Endocrinol Metab. 2012;97(3):1020-31.

51. Ferrannini G, Hach T, Crowe S, Sanghvi A, Hall KD, Ferrannini E. Energy balance after sodium-glucose cotransporter 2 inhibition. Diabetes Care. 2015;38(9):1730-5.

52. Xu L, Nagata N, Nagashimada M, Zhuge F, Ni Y, Chen G, et al. SGLT2 Inhibition by empagliflozin promotes fat utilization and browning and attenuates inflammation and insulin resistance by polarizing m2 macrophages in diet-induced obese mice. EBioMedicine. 2017;20:137-49.

53. Sarafidis PA, Lazaridis AA, Ruiz-Hurtado G, Ruilope LM. Blood pressure reduction in diabetes: lessons from ACCORD, SPRINT and EMPA-REG OUTCOME. Nat Rev Endocrinol. 2017;13(6):365-74.

54. Shigiyama F, Kumashiro N, Miyagi M, Ikehara K, Kanda E, Uchino H, et al. Effectiveness of dapagliflozin on vascular endothelial function and glycemic control in patients with early-stage type 2 diabetes mellitus: DEFENCE study. Cardiovasc Diabetol. 2017;16(1):84.

55. Pfeifer M, Townsend RR, Davies MJ, Vijapurkar U, Ren J. Effects of canagliflozin, a sodium glucose co-transporter 2 inhibitor, on blood pressure and markers of arterial stiffness in patients with type 2 diabetes mellitus: a post hoc analysis. Cardiovasc Diabetol. 2017;16(1):29.

56. Wan N, Rahman A, Hitomi H, Nishiyama A. The effects of sodium-glucose cotransporter 2 inhibitors on sympathetic nervous activity. Front Endocrinol (Lausanne). 2018;9:421.

57. León Jiménez D, Cherney DZI, Bjornstad P, Castilla Guerra L, Miramontes González JP. Antihyperglycemic agents as novel natriuretic therapies in diabetic kidney disease. Am J Physiol Renal Physiol. 2018. doi: 10.1152/ajprenal.00384.2017.

58. Birnbaum Y, Bajaj M, Yang HC, Ye Y. Combined SGLT2 and DPP4 inhibition reduces the activation of the Nlrp3/asc inflammasome and attenuates the development of diabetic nephropathy in mice with type 2 diabetes. Cardiovasc Drugs Ther. 2018;32(2):135-45.

59. Mancini SJ, Boyd D, Katwan OJ, Strembitska A, Almabrouk TA, Kennedy S, et al. Canagliflozin inhibits interleukin-1β-stimulated cytokine and chemokine secretion in vascular endothelial cells by AMP-activated protein kinase-dependent and -independent mechanisms. Sci Rep. 2018;8(1):5276.

60. Nasiri-Ansari **N**, Dimitriadis GK, Agrogiannis G, Perrea D, Kostakis ID, Kaltsas G, et al. Canagliflozin attenuates the progression of atherosclerosis and inflammation process in APOE knockout mice. Cardiovasc Diabetol. 2018;17(1):106.

61. Terasaki M, Hiromura M, Mori Y, Kohashi K, Nagashima M, Kushima H, et al. Amelioration of hyperglycemia with a sodium-glucose cotransporter 2 inhibitor prevents macrophage-driven atherosclerosis through macrophage foam cell formation suppression in type 1 and type 2 diabetic mice. PLoS One. 2015;10(11):e0143396.

62. Briand F, Mayoux E, Brousseau E, Burr N, Urbain I, Costard C, et al. Empagliflozin, via switching metabolism toward lipid utilization, moderately increases LDL cholesterol levels through reduced LDL catabolism. Diabetes. 2016;65(7):2032-8.

63. Fadini GP, Bonora BM, Zatti G, Vitturi N, Iori E, Marescotti MC, et al. Effects of the SGLT2 inhibitor dapagliflozin on HDL cholesterol, particle size, and cholesterol efflux capacity in patients with type 2 diabetes: a randomized placebo-controlled trial. Cardiovasc Diabetol. 2017;16(1):42.

64. Cowart LA. Sphingolipids: players in the pathology of metabolic disease. Trends Endocrinol Metab. 2009;20(1):34-42.

65. Choi S, Snider AJ. Sphingolipids in high fat diet and obesity-related diseases. Mediators Inflamm. 2015;2015:520618.

66. Mundra PA, Barlow CK, Nestel PJ, Barnes EH, Kirby A, Thompson P, et al. Large-scale plasma lipidomic profiling identifies lipids that predict cardiovascular events in secondary prevention. JCI Insight. 2018;3(17).

67. Chaurasia B, Summers SA. Ceramides: lipotoxic inducers of metabolic disorders. Trends Endocrinol Metab. 2015;26(10):538-50.

68. Moura-Assis A, Afonso MS, Oliveira V, Morari J, Santos GA, Koike M, et al. Flaxseed oil rich in omega-3 protects aorta against inflammation and endoplasmic reticulum stress partially mediated by GPR120 receptor in obese, diabetic and dyslipidemic mice models. J Nutr Biochem. 2018;53:9-19.

69. Ajibade AA, Wang HY, Wang RF. Cell type-specific function of TAK1 in

innate immune signaling. Trends Immunol. 2013;34(7):307-16.

70. Samad F, Hester KD, Yang G, Hannun YA, Bielawski J. Altered adipose and plasma sphingolipid metabolism in obesity: a potential mechanism for cardiovascular and metabolic risk. Diabetes. 2006;55(9):2579-87.

71. Kowalski GM, Carey AL, Selathurai A, Kingwell BA, Bruce CR. Plasma sphingosine-1-phosphate is elevated in obesity. PLoS One. 2013;8(9):e72449.

72. Alvarez SE, Harikumar KB, Hait NC, Allegood J, Strub GM, Kim EY, et al. Sphingosine-1-phosphate is a missing cofactor for the E3 ubiquitin ligase TRAF2. Nature. 2010;465(7301):1084-8.

73. Lee JF, Zeng Q, Ozaki H, Wang L, Hand AR, Hla T, et al. Dual roles of tight junction-associated protein, zonula occludens-1, in sphingosine 1-phosphate-mediated endothelial chemotaxis and barrier integrity. J Biol Chem. 2006;281(39):29190-200.

74. Proia RL, Hla T. Emerging biology of sphingosine-1-phosphate: its role in pathogenesis and therapy. J Clin Invest. 2015;125(4):1379-87.

75. Galvani S, Sanson M, Blaho VA, Swendeman SL, Obinata H, Conger H, et al. HDL-bound sphingosine 1-phosphate acts as a biased agonist for the endothelial cell receptor S1P1 to limit vascular inflammation. Sci Signal. 2015;8(389):ra79.

76. Keul P, Polzin A, Kaiser K, Gräler M, Dannenberg L, Daum G, et al. Potent anti-inflammatory properties of HDL in vascular smooth muscle cells mediated by HDL-S1P and their impairment in coronary artery disease due to lower HDL-S1P: a new aspect of HDL dysfunction and its therapy. FASEB J. 2018:fj201801245R.

77. Frej C, Mendez AJ, Ruiz M, Castillo M, Hughes TA, Dahlbäck B, et al. A shift in ApoM/S1P between HDL-particles in women with type 1 diabetes mellitus is associated with impaired anti-inflammatory effects of the ApoM/S1P complex. Arterioscler Thromb Vasc Biol. 2017;37(6):1194-205.

78. Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr. 2003;77(5):1146-55.

79. Levitan EB, Cook NR, Stampfer MJ, Ridker PM, Rexrode KM, Buring JE, et al. Dietary glycemic index, dietary glycemic load, blood lipids, and C-reactive protein. Metabolism. 2008;57(3):437-43.

80. Li Y, Hruby A, Bernstein AM, Ley SH, Wang DD, Chiuve SE, et al. Saturated fats compared with unsaturated fats and sources of carbohydrates concerning risk of coronary heart disease: a prospective cohort study. J Am Coll Cardiol. 2015;66(14):1538-48.

81. Wang DD, Li Y, Chiuve SE, Stampfer MJ, Manson JE, Rimm EB, et al. Association of specific dietary fats with total and cause-specific mortality. JAMA Intern Med. 2016;176(8):1134-45.

82. Lottenberg AM, Afonso MS, Lavrador MS, Machado RM, Nakandakare ER. The role of dietary fatty acids in the pathology of metabolic syndrome. J Nutr Biochem. 2012;23(9):1027-40.

83. Afonso MS, Castilho G, Lavrador MS, Passarelli M, Nakandakare ER, Lottenberg SA, et al. The impact of dietary fatty acids on macrophage cholesterol homeostasis. J Nutr Biochem. 2014;25(2):95-103.

84. Velloso LA, Folli F, Saad MJ. TLR4 at the crossroads of nutrients, gut microbiota, and metabolic inflammation. Endocr Rev. 2015;36(3):245-71.

85. Wang DD, Hu FB. Dietary fat and risk of cardiovascular disease: recent controversies and advances. Annu Rev Nutr. 2017;37:423-46.

86. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, et al. Primary prevention of cardiovascular disease with a mediterranean diet supplemented with extra-virgin olive oil or nuts. N Engl J Med. 2018;378(25):e34.