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STANDARDIZATION OF HBA$_1$c IN CROATIA 2004-2013
CONTRIBUTION OF LABORATORY MEDICINE TO THE QUALITY OF DIABETES CARE IN CROATIA

M. Vučić Lovrenčić$^1$, D. Juretić$^2$, Z. Flegar-Meštrić$^3$, Ž. Metelko$^4$

Key words: HbA$_1$c, standardization, harmonization, quality, diabetes care

SUMMARY

The pivotal role of hemoglobin A$_1$c (HbA$_1$c) in the management of diabetes mellitus was established 30 years ago, followed by intensive international activities towards attaining a harmonized and affordable laboratory testing worldwide. Improvement in the quality and availability of HbA$_1$c testing in Croatia required careful and deliberate efforts of the national clinical and laboratory professional resources. Collaboration between the Vuk Vrhovac University Clinic and the Committee for External Quality Assessment of the Croatian Society of Medical Biochemists (CEQA-CSMB) resulted in development of the Croatian Program for HbA$_1$c Standardization in 2004. Various activities within the Program, lasting till December 2012, enabled successful implementation of HbA$_1$c global harmonization at the national level and significantly improved clinical utility of the test. In this paper, we review the activities and outcomes of the Croatian Program for HbA$_1$c Standardization (2004-2013) and discuss its impact on the quality of diabetes care in Croatia.

INTRODUCTION

Evidence-based clinical recommendations, generated from the results of the landmark medical studies Diabetes Control and Complications Trial (DCCT) (1) and United Kingdom Prospective Diabetes Study (UKPDS) (2), have identified hemoglobin A$_1$c (HbA$_1$c) as the ultimate biochemical marker for the assessment of diabetes treatment effectiveness, as well as the risk of developing complications in patients with type 1 and type 2 diabetes (3, 4). Recently, diagnostic utility of HbA$_1$c has been advocated, corroborated by its
practical advantages over plasma glucose, particularly in asymptomatic population screening for diabetes (3, 5).

Considering the rising prevalence and immense medical and socioeconomic burden of diabetes, implementation of effective tools for diabetes management is of utmost importance (5, 6). Thus, reliable and affordable laboratory testing of HbA1c has been one of the most prominent challenges in laboratory medicine worldwide (7).

HbA1c is a post-translationally modified adult hemoglobin, resulting from covalent binding of glucose to N-terminal-valine in the β-chain of HbA (8). The non-enzymatic nature of the glycation process and the presumed stability of circulating hemoglobin values within normal erythrocyte turnover suggest a conclusion that HbA1c concentration reflects the average glucose concentration within the preceding 2-3 months (9). The opportunity to obtain integrated information on the glycemic status for a given period of time, free from common variabilities and limitations of plasma glucose measurement, has attracted considerable scientific and clinical attention and generated new standards of diabetes research and management (3, 4, 7). However, routine HbA1c testing has long been compromised by non-standardized methodology, the need for dedicated laboratory equipment, and relatively high costs. The results of HbA1c obtained by various analytical methods differed significantly and were difficult to interpret according to evidence-based clinical target values (10, 11). Clinical harmonization of HbA1c results was achieved within the National Glycohemoglobin Standardization Program (NGSP), whereby traceability of all routine methods towards a highly-reproducible but non-specific analytical method used in the DCCT and UKPDS trials was established (12). Analytical standardization, with the analyte definition, reference method and primary reference material development was successfully accomplished by the International Federation of Clinical Chemistry (IFCC) (13). Due to specificity, the IFCC reference method yielded a significantly lower HbA1c than the NGSP aligned methods, which additionally complicated the issue of harmonization. After long-term consideration by an international consortium of relevant professional bodies, the Global Consensus on the HbA1c measurement and reporting was promulgated in 2010 (14). Briefly, the 2010 Global Consensus statement defined the IFCC reference system as the only valid analytical anchor for HbA1c methods, dual system of HbA1c reporting (% and mmol/mol as DCCT and IFCC aligned units, respectively) and inter-conversion of the results by use of IFCC-NGSP master-equation. Also, a recommendation to the editors of scientific medical journals regarding HbA1c reporting by use of a dual-unit reporting system has been proclaimed (14).

Improvement of diabetes care quality through standardization and implementation of the Global Consensus guidelines for HbA1c has become a very important agenda for the national clinical and laboratory professionals. In this paper, we review the activities and outcomes of the Croatian Program for HbA1c Standardization (CRO-AS 2004-2013), established by the Vuk Vrhovac University Clinic as the Croatian Reference Centre for Diabetes and the Committee for External Quality Assessment of the Croatian Society of Medical Biochemists (CEQA-CSMB) and discuss its impact on the quality of diabetes care in Croatia.

CRO-AS BACKGROUND AND OBJECTIVES

Results of the first national survey conducted in 1999 revealed that HbA1c testing in Croatia suffered from variable and non-standardized methodology, a lack of quality assurance, and poor availability and clinical utility (15). Considering these results, the recognized clinical importance of HbA1c testing and global standardization efforts, improvement of HbA1c testing in Croatia became one of the goals of the Croatian National Organization of Diabetes Health Care (referred to as the Croatian Model), established by the Vuk Vrhovac University Clinic, Croatian Reference Centre for Diabetes (16).
CRO-AS was designed in collaboration with the CEQA-CSMB, an expert committee with rich experience in external quality assessment of laboratory testing, and presented in 2004 with the following objectives (17):

1. Assuring the analytical quality of laboratory testing for HbA\textsubscript{1c} in Croatia.
2. Providing education to the professional community and assuring timely and consistent translation of internationally approved guidelines regarding HbA\textsubscript{1c} to the national clinical and laboratory practice.
3. Improving availability and clinical utility of HbA\textsubscript{1c} testing in Croatia.

The CRO-AS program, led and coordinated by the Department of Laboratory Medicine, Vuk Vrhovac University Clinic, was carried out till December 2012, employing specific, objective-based methods. For the sake of consistency, methodology and outcomes are presented as per objective.

**CRO-AS METHODOLOGY AND OUTCOMES**

1. Analytical quality of laboratory testing for HbA\textsubscript{1c} in Croatia

External quality assessment (EQA) for HbA\textsubscript{1c} in Croatia was established in 2005, as a dedicated module within the national EQA scheme. The aim of this module was not only to improve analytical quality, but also to provide a necessary educational tool for local implementation of the global harmonization goals regarding hemoglobin A\textsubscript{1c} testing.

Commercially available lyophilizates with declared target values according to both IFCC and NGSP reference systems for a wide range of methods/reagents were selected as control materials. These were sent by mail with appropriate result-forms containing sample-handling information. The participants were asked to return their results together with data on the method/analyzer/manufacturer used in their laboratory and, as of 2009, reporting system/units (DCCT, IFCC). Results were evaluated by calculating total, as well as the subgroup imprecision(s) according to the method/analyzer/manufacturer, as appropriate. Biases related to targets declared by the manufacturer in respect to the method/analyzer/manufacturer were used to assess the quality of clinical performance according to the European Reference Laboratory for Glycohemoglobin (ERL) criteria (18).

Twenty-eight laboratories participated in the first pilot EQA cycle in 2005, and the number almost doubled (N=53) by the end of 2012. The collected data revealed automated immunoassays as the most prevalent methodology in Croatian laboratories. The results demonstrated continuous improvement in analytical quality as regards both imprecision and deviation from the target, reflecting both the manufacturers’ compliance with global harmonization standards and improved level of laboratory service quality (Table 1 and Figure 1). The Croatian EQA scheme for HbA\textsubscript{1c} gained international recognition as a valuable contribution to quality improvement of laboratory and diabetes care services (19, 20).

<table>
<thead>
<tr>
<th>Year</th>
<th>EQA cycles/year (n)</th>
<th>Laboratories/ cycle (n)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>1</td>
<td>28</td>
<td>9.6</td>
</tr>
<tr>
<td>2006</td>
<td>3</td>
<td>29</td>
<td>12.6</td>
</tr>
<tr>
<td>2007</td>
<td>2</td>
<td>34</td>
<td>6.1</td>
</tr>
<tr>
<td>2008</td>
<td>2</td>
<td>36</td>
<td>6.5</td>
</tr>
<tr>
<td>2009</td>
<td>2</td>
<td>43</td>
<td>7.6</td>
</tr>
<tr>
<td>2010</td>
<td>2</td>
<td>49</td>
<td>5.9</td>
</tr>
<tr>
<td>2011</td>
<td>2</td>
<td>51</td>
<td>5.4</td>
</tr>
<tr>
<td>2012</td>
<td>2</td>
<td>53</td>
<td>5.2</td>
</tr>
</tbody>
</table>
2. Education and translation of internationally approved HbA1c guidelines to the national clinical and laboratory practice

Education of laboratory professionals was carried out through a number of dedicated workshops and courses organized in collaboration with the Croatian Chamber of Medical Biochemists (CCMB), as well as presentations at respective national conferences (Table 2). Apart from this, continuous consultation service to laboratory and medical professionals regarding HbA1c methodology, interpretation and practical problem solving has been provided by the Department of Laboratory Medicine, Vuk Vrhovac University Clinic. Translation of the Global Consensus guidelines and implementation of the recommended HbA1c dual reporting system were announced with a respective paper (21) published simultaneously in the leading national journals covering laboratory medicine and diabetology topics and the dedicated guideline of the CCMB (22). The beneficial effect of these activities contributed significantly to the improved quality and clinical availability of HbA1c testing in Croatia, as well as comparability of the results between laboratories and safety for use in clinical research and practice (22-27).

3. Reaching appropriate availability and clinical utility of HbA1c testing in Croatia

Despite formal classification of HbA1c within the general medical biochemistry tests, which took part in 2003 (28), a lack of formal contracting for HbA1c in primary health care laboratories remained the major obstacle to achieve the recommended level of clinical test utilization in terms of frequency and availability. The activities and results of CRO-AS contributed significantly to the negotiation process that took part between CCMB and the Croatian Health Insurance Fund (CHIF), leading eventually to contracting and full implementation of HbA1c testing in primary health care laboratories as of 2013. At the time, not only analytical quality and harmonization had been achieved, but also laboratory professionals in Croatia had reached the appropriate level of education and

Table 2. Educational activities on HbA1c testing, harmonization and quality improvement carried out within the CRO-AS program (22-26)

<table>
<thead>
<tr>
<th>Year</th>
<th>Activity</th>
<th>Organizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>Workshop: Laboratory diagnosis and monitoring of diabetes mellitus</td>
<td>Vuk Vrhovac University Clinic, Croatian Reference Centre for Diabetes</td>
</tr>
<tr>
<td>2006</td>
<td>Scientific workshops/symposia on external quality control/inter-laboratory comparisons within the 5th, 6th and 7th Croatian Congresses of Medical Biochemists</td>
<td>Croatian Society of Medical Biochemists</td>
</tr>
<tr>
<td>2009</td>
<td>Continuous education courses: - Harmonization in laboratory medicine - The role of laboratory in the diagnosis and monitoring of diabetes mellitus</td>
<td>Croatian Chamber of Medical Biochemists</td>
</tr>
<tr>
<td>2012</td>
<td></td>
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</tbody>
</table>
competences for successful implementation of HbA\textsubscript{1c} testing in routine laboratory process in general primary care laboratories. The importance of the process is best illustrated by the subsequent doubling of laboratories routinely performing HbA\textsubscript{1c} (29) and the establishment of HbA\textsubscript{1c} based quality indicator of the primary health care system efficiency assessment in Croatia in 2014 (30).

**CONCLUSIONS**

The CRO-AS was terminated in December 2012, after having successfully reached the set objectives. As of 2013, HbA\textsubscript{1c} in Croatia has become a widely available and harmonized laboratory test performed by educated and competent laboratory professionals and complying with the globally recognized clinical and laboratory guidelines. The regular analytical quality assessment within the CRO-AS established and dedicated EQA module is continued by the Croatian Centre for Quality Assessment in Laboratory Medicine (CROQALM), having succeeded the CEQA in 2013.

The CRO-AS represents a unique, carefully tailored and conducted process aimed to improve diabetes care in Croatia by the establishment of harmonized HbA\textsubscript{1c} testing, traceable to the globally recognized standards, available to the patients and clinical professionals, and recognized by the national health authorities as a powerful tool in health care quality assessment. CRO-AS also has significantly contributed to the process of harmonization of laboratory medicine in Croatia (31). However, considering that the global harmonization process has left an unsolved issue regarding reporting units, the national clinical consensus on this matter is needed (32). As for laboratories, the next step towards improvement of quality and compliance with the international guidelines would be accreditation of HbA\textsubscript{1c} testing, as proposed by the international guidelines (33). Despite the undisputable evidence on its beneficial effects in respect to quality and efficiency of laboratory services (34), accreditation according to the ISO151989 Medical Laboratories – Requirements for Quality and Competence is currently present in only 8 Croatian laboratories, including Clinical Department of Medical Biochemistry and Laboratory Medicine, Merkur University Hospital, where the Department of Laboratory Medicine of the Vuk Vrhovac University Clinic is operating today. The experience and outcomes of the CRO-AS point to HbA\textsubscript{1c} testing as a paradigmatic tool in establishment of an internationally acclaimed quality management system for medical laboratories that should be implemented in order to improve the quality of diabetes care, proclaimed as one of the key areas of the health care system in Croatia (35).
REFERENCES


THE ROLE OF METABOLIC SYNDROME IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

M. Mornar Jelavić1, Z. Babić2, H. Pintarić3

Key words: metabolic syndrome, anthropometry, myocardial infarction, percutaneous coronary intervention, sick leave

SUMMARY

This study investigated the role of metabolic syndrome (MetS) in clinical severity and prognosis of acute ST-elevation myocardial infarction (STEMI). We prospectively analyzed 250 acute STEMI patients treated with primary percutaneous coronary intervention (September 2011 – September 2012). They were classified into two groups (with/without MetS), which were analyzed by baseline data (medical history, demography, and anthropometry) and by the parameters of severity (clinical, laboratory, echocardiography, coronary angiography, and in-hospital complications) and prognosis (major adverse cardiovascular events and sick leave duration during 12-month follow up). MetS patients (n=136, 54.4%) had longer hospital stay, higher number of significantly stenosed coronary arteries, wider stents, higher rates of total in-hospital complications and significant proximal/middle coronary segment stenosis, as well as longer sick leave duration (p<0.05); MetS increased the risk of total in-hospital complications and ≥2 significantly stenosed coronary arteries (p<0.05). In conclusion, MetS has a role in predicting acute STEMI severity (total in-hospital complications and severity of coronary artery disease), but not in prognosis. MetS patients have longer sick leave duration.

INTRODUCTION

Metabolic syndrome (MetS) is defined as a group of interrelated factors (hyperglycemia, abdominal obesity, atherogenic dyslipidemia, hypertension, prothrombotic and proinflammatory states), which significantly increase the risk of coronary artery disease (CAD) and other forms of atherosclerotic cardiovascular diseases (CVD), impaired fasting glucose and diabetes mellitus type 2 (DMT2), cardiovascular and all-cause mortality (1, 2).

MetS is a worldwide problem with rapid growth. It could be explained by the parallel rise of obesity prevalence. Approximately one-quarter of adult Europeans have MetS, depending on geographic location, age and characteristics of study population...
(3, 4). Its prevalence increases with age, markedly from age 30, and peaks around age 60-75, but generally with no gender differences (5-7).

There are several definitions of MetS published by international organizations and expert groups (1, 2). In the time of planning and conducting this research, the revised National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) definition was the most widely accepted and cited in the literature because it provides a relatively simple approach for the diagnosis of MetS (1). According to the revised NCEP-ATP III definition, one of the MetS constitutive parameters is increased waist circumference (WC), which is a measure of abdominal obesity and an independent risk factor for CVD. It is usually associated with at least one more risk factor, such as arterial hypertension, DMT2 and dyslipidemia (1, 2). They all are independently, as well as synergistically associated with an increased incidence of myocardial infarction (MI) (8-11). So, it is not surprising that MetS is common among patients with CAD. Moreover, it is highly prevalent among patients with acute ST-elevation myocardial infarction (STEMI) (12-14).

The main objectives of this study were to evaluate the severity and prognosis of acute STEMI in patients with/without MetS (revised NCEP-ATP III) treated with primary percutaneous coronary intervention (PCI). Also, for the first time, we investigated involvement of coronary artery (CAs) segments with significant stenosis and sick leave duration (SLD) in these patients.

MATERIALS AND METHODS

We prospectively analyzed 250 consecutive patients with acute STEMI treated with primary PCI at Department of Cardiology, Sestre milosrdnice University Hospital Center, Zagreb, Croatia, from September 2011 till September 2012. The study was approved by the Sestre milosrdnice University Hospital Center Ethics Committee.

The inclusion criteria were as follows: presenting within 12 h from the onset of symptoms (history of chest pain/discomfort lasting for 10-20 minutes or more, not responding fully to nitroglycerine), persistent ST-segment elevation on electrocardiography (ECG) in at least two consecutive leads or (presumed) new left bundle branch block (LBBB), and elevated cardiac laboratory biomarkers (cardiac troponin T (cTnT) and creatine kinase (CK)). The diagnosis of acute STEMI was established and primary PCI performed using the European Society of Cardiology criteria (15, 16).

After primary PCI, patients were classified into two groups (with/without MetS). We used revised NCEP-ATP III criteria for the diagnosis of MetS and its components (1), as follows:

1) abdominal obesity: WC ≥102 cm in males and ≥88 cm in females;
2) hypertriglyceridemia: triglycerides ≥150 mg/dL (1.7 mmol/L), or on medication for elevated triglycerides;
3) low HDL-cholesterol: <40 mg/dL (1.04 mmol/L) in males or <50 mg/dL (1.29 mmol/L) in females, or on medication for low HDL-cholesterol;
4) arterial hypertension: blood pressure ≥130/85 mm Hg, or on medication for hypertension;
5) hyperglycemia: fasting plasma glucose ≥100 mg/dL (5.5 mmol/L), or on medication for hyperglycemia; and
6) MetS: presence of any of three or more of the above mentioned five criteria.

The groups were analyzed by baseline data, and by the severity and prognostic parameters of acute STEMI, as follows:

1) Baseline demographic and medical history parameters included gender, age, smoking, known family history of cardiovascular events (MI, cerebrovascular insult (CVI)), previous MI, previous PCI and coronary artery bypass grafting (CABG). Abdominal obesity, hypertriglyceridemia, low HDL-cholesterol, arterial hypertension and hyperglycemia were diagnosed by revised NCEP-ATP III criteria.
Anthropometric data included measurement of body mass index (BMI), WC and waist-to-hip ratio (WHR) (1, 8).

2) The severity of acute STEMI was estimated by clinical presentation (angina pectoris, dyspnea, and length of hospital stay), in-hospital complications (arrhythmias, conduction disturbances, reperfusion arrhythmias, heart failure, cardiogenic shock, cardiac arrest, mechanical ventilation, reinfarction, repeated PCI, mortality, and total in-hospital complications), coronary angiography, laboratory (maximal cTnT and CK) and echocardiography (left ventricular ejection fraction, LVEF) findings. Coronary angiography was performed by applying a monoplane system (Axiom Artis, Siemens, Erlangen, Germany) using a common technique as recommended in the current guidelines (16). Patients received 70 IE/kg unfractionated heparin, 300 mg aspirin, a loading dose of 600 mg clopidogrel, and a GPIIb/IIIa inhibitor according to judgment of interventional cardiologist. CAs stenosis of more than 50% was considered clinically significant. We analyzed the number of significantly narrowed CAs, number, length and diameter of used stents. Additionally, for the first time, we analyzed significantly stenosed segments of CAs. For that purpose, and according to the modified American Heart Association classification (17), CAs were divided into 16 segments. Segments were classified into two groups, as follows:

A) proximal and middle CAs segments: segment 1 (right coronary artery (RCA), proximal), segment 2 (RCA, mid), segment 5 (main stem), segment 6 (left anterior descending coronary artery (LAD), proximal), segment 7 (LAD, mid), segment 9 (first diagonal, D1), segment 11 (left circumflex artery (LCX), proximal), segment 12 (obtuse marginal, OM); and

B) distal CAs segments: segment 3 (RCA, distal), segment 4 (right posterior descendens), segment 8 (LAD, distal), segment 10 (second diagonal, D2), segment 13 (LCX, distal), segment 14 (LCX, posterolateral branch), segment 15 (LCX, posterodescendens branch), segment 16 (RCA, posterolateral branch).

Serum CK activity was measured by spectrophotometry (Olympus 680, Beckman Coulter Inc., California, USA). Serum cTnT levels were measured by electrochemiluminescence (ECL) assay (Cobas e411, Roche Diagnostics, Sussex, UK). During hospitalization, echocardiography was performed in all patients (Acuson Sequoia 512, Siemens, Munich, Germany) according to clinical standards and current echocardiography guidelines (18).

3) The prognosis of acute STEMI was estimated using major adverse cardiovascular events (MACE) parameters (reinfarction, CAs restenosis and new stenosis, cardiac and non-cardiac rehospitalization, CVI, urgent CAGB, mortality, total MACE) during 12-month follow up. Data were collected by medical examination, checking medical documentation, or telephone contact with patients, family members or home physicians. In addition, during the same follow up period, we collected data on SLD in working population.

Statistical analysis

Qualitative data were presented in absolute number and percentage. We used $\chi^2$-test with Yates correction. Quantitative data were presented with median and corresponding interquartile range. Differences between the two groups were tested by Mann-Whitney U test. The $\chi^2$-test and logistic regression analysis were used to investigate the relationship between one dependent and one or more independent variables that may influence or predict the value of the dependent variable. The level of statistical significance was set at $p<0.05$. Processing was done using the STATISTICA 6.0 for Windows software.
RESULTS

Of the total of 250 patients, there were 136 (54.4%) and 114 (45.6%) patients with and without MetS, respectively. We got the following results:

1) There were no significant between-group differences in most of the baseline parameters, except for the expected higher rates of arterial hypertension (130 (95.6%) vs. 51 (44.7%), p=0.000), hypertriglyceridemia (78 (57.4%) vs. 38 (33.3%), p=0.000), low-HDL cholesterol (96 (70.6%) vs. 50 (43.9%), p=0.000), hyperglycemia (54 (39.7%) vs. 7 (6.1%), p=0.000), WC ≥102/88 cm (117 (86.0%) vs. 32 (28.1%), p=0.000) and BMI ≥30 kg/m² (63 (46.3%) vs. 9 (7.9%), p=0.000), as well lower rates of BMI <25.0 (19 (14.0%) vs. 41 (36.0%), p=0.000) and BMI 25.0-29.9 kg/m² (54 (39.7%) vs. 64 (56.1%), p=0.014) in the group of patients with MetS.

2) Patients with MetS had longer hospital stay, higher rate of total in-hospital complications, higher number of significantly stenosed CAs and wider stents, higher rate of significantly stenosed proximal and middle CAs segments, and longer SLD (p<0.05) (Table 1).

3) Investigating the influence of MetS and its individual constitutive parameters (central obesity, hypertriglyceridemia, low HDL-cholesterol, hypertension, and hyperglycemia) on the severity and prognosis of acute STEMI showed that MetS was independently associated with a higher risk of total in-hospital complications (47.8% vs. 34.2%) (odds ratio (OR) 1.76, confidence interval (CI) [1.05-2.94], p=0.031) and ≥2 significantly stenosed CAs (57.4% vs. 43.9%) (OR 1.72, CI [1.04-2.84], p=0.034), while hyperglycemia was independently associated with a higher risk of heart failure (36.1% vs. 22.2%) (OR 1.97, CI [1.05-3.70] p=0.033).

Furthermore, BMI <25.0 kg/m² was independently associated with higher (41.7% vs. 26.3%) (OR 2.00, CI [1.10-3.67], p=0.025) and BMI 25.0-29.9 kg/m² with lower risk of dyspnea (22.9% vs. 36.4%) (OR 0.51, CI [0.29-0.90], p=0.020).

DISCUSSION

According to several authors, the prevalence of MetS (NCEP-ATP III) in hospitalized patients with acute STEMI varies between 46.0% and 59.4%, with a higher rate in females. Patients with MetS are more frequently non-smokers (13, 14). In our study, the prevalence of MetS (NCEP-ATP III) in patients with acute STEMI was 54.4%, with a nonsignificantly higher rate in females. We had an almost equal rate of smokers in both groups. Like other authors, we also excluded significant differences in medical history (family history of cardiovascular events, previous MI, PCI and CABG) between patients with and without MetS (13).

In patients with acute MI, MetS is associated with severe heart failure, higher in-hospital mortality and higher rate of overall in-hospital complications (13, 14, 19-21). Also, MetS patients have more serious CAD, i.e. more often three-vessel CAD, greater number of implanted stents without difference in their length, but with difference in their diameter. To our knowledge, this was the first study that investigated involvement of CA segments with significant stenosis in patients with MetS and acute STEMI. We found MetS patients to have longer hospital stay, higher number of significantly stenosed CAs and wider stents, as well as a higher rate of significantly stenosed proximal and middle CA segments.

MetS is an independent prognostic factor for 12-month MACE and target vessel revascularization (TVR) (21). MetS patients did not show an increased risk of TVR or higher MACE rate compared with controls during 12-month follow up after PCI (22, 23). During the same follow up period, our MetS patients had worse results in total MACE and some MACE parameters (new stenosis, cardiac and non-cardiac rehospitalization, CVI, and urgent CABG), but without statistical significance. A statistically significant difference was only recorded in the mean SLD, which was longer in patients with MetS. It is one of the most important characteristics of our study because there are no literature data on SLD in patients with MetS and acute STEMI.
Table 1. Severity and prognosis of acute ST-elevation myocardial infarction

<table>
<thead>
<tr>
<th>Finding</th>
<th>Parameter</th>
<th>MetS* (n=136)</th>
<th>No-MetS (n=114)</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical presentation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Angina pectoris, n (%)‡</td>
<td>134 (98.5)</td>
<td>111 (97.4)</td>
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</tr>
<tr>
<td></td>
<td>Dyspnea, n (%)‡</td>
<td>45 (33.1)</td>
<td>30 (26.3)</td>
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<tr>
<td></td>
<td>Hospital stay (days)§</td>
<td>9 (3-31)</td>
<td>8 (2-32)</td>
<td>0.042</td>
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<tr>
<td><strong>In-hospital complications</strong></td>
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<tr>
<td></td>
<td>Arrhythmias, n (%)‡</td>
<td>29 (21.3)</td>
<td>14 (12.3)</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td>Conduction disturbances, n (%)‡</td>
<td>12 (8.8)</td>
<td>4 (3.5)</td>
<td>0.087</td>
</tr>
<tr>
<td></td>
<td>Reperfusion arrhythmias, n (%)‡</td>
<td>11 (8.1)</td>
<td>7 (6.1)</td>
<td>0.553</td>
</tr>
<tr>
<td></td>
<td>Heart failure, n (%)‡</td>
<td>41 (30.2)</td>
<td>23 (20.2)</td>
<td>0.072</td>
</tr>
<tr>
<td></td>
<td>Cardiogenic shock, n (%)‡</td>
<td>10 (7.4)</td>
<td>8 (7.0)</td>
<td>0.919</td>
</tr>
<tr>
<td></td>
<td>Cardiac arrest, n (%)‡</td>
<td>23 (16.9)</td>
<td>13 (11.4)</td>
<td>0.217</td>
</tr>
<tr>
<td></td>
<td>Mechanical ventilation, n (%)‡</td>
<td>7 (5.2)</td>
<td>3 (2.6)</td>
<td>0.312</td>
</tr>
<tr>
<td></td>
<td>Reinfarction, n (%)‡</td>
<td>1 (0.7)</td>
<td>0 (0)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Repeated PCI, n (%)‡</td>
<td>2 (1.5)</td>
<td>2 (1.8)</td>
<td>0.859</td>
</tr>
<tr>
<td></td>
<td>Mortality, n (%)‡</td>
<td>11 (8.2)</td>
<td>8 (7.1)</td>
<td>0.740</td>
</tr>
<tr>
<td></td>
<td>Total, n (%)‡</td>
<td>65 (47.8)</td>
<td>39 (34.2)</td>
<td>0.041</td>
</tr>
<tr>
<td><strong>Laboratory</strong></td>
<td>Maximal cTnT (ng/mL)§</td>
<td>3.04 (0.02-10.0)</td>
<td>3.12 (0.02-10.0)</td>
<td>0.802</td>
</tr>
<tr>
<td></td>
<td>Maximal CK (U/L)§</td>
<td>1834 (25-14094)</td>
<td>1897 (70-15617)</td>
<td>0.710</td>
</tr>
<tr>
<td><strong>ECHO</strong></td>
<td>Left ventricular ejection fraction (%)§</td>
<td>50 (25-70)</td>
<td>54 (30-76)</td>
<td>0.350</td>
</tr>
<tr>
<td></td>
<td>Number of significantly stenosed CAs§</td>
<td>2 (1-4)</td>
<td>1 (1-4)</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>Significant stenosis of ≥2 CAs, n (%)‡</td>
<td>78 (57.4)</td>
<td>50 (43.9)</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>Number of stents§</td>
<td>1 (1-3)</td>
<td>1 (1-4)</td>
<td>0.213</td>
</tr>
<tr>
<td></td>
<td>Diameter of stents (mm)§</td>
<td>3.5 (2.3-4.0)</td>
<td>3.0 (2.8-4.0)</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>Length of stents (mm)§</td>
<td>20 (8-38)</td>
<td>20 (8-38)</td>
<td>0.615</td>
</tr>
<tr>
<td></td>
<td>Proximal/middle CA stenosis, n (%)‡</td>
<td>128 (94.1)</td>
<td>98 (86.7)</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>Distal CA stenosis, n (%)‡</td>
<td>53 (39.0)</td>
<td>44 (38.9)</td>
<td>0.996</td>
</tr>
<tr>
<td></td>
<td>Reinfarction, n (%)‡</td>
<td>1 (0.8)</td>
<td>1 (1.0)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Restenosis, n (%)‡</td>
<td>3 (2.4)</td>
<td>4 (3.8)</td>
<td>0.541</td>
</tr>
<tr>
<td></td>
<td>New stenosis, n (%)‡</td>
<td>4 (3.2)</td>
<td>3 (2.9)</td>
<td>0.873</td>
</tr>
<tr>
<td></td>
<td>Cardiac rehospitalization, n (%)‡</td>
<td>20 (16.3)</td>
<td>16 (15.4)</td>
<td>0.857</td>
</tr>
<tr>
<td><strong>MACE</strong></td>
<td>Non-cardiac rehospitalization, n (%)‡</td>
<td>5 (4.1)</td>
<td>4 (3.8)</td>
<td>0.933</td>
</tr>
<tr>
<td></td>
<td>CVI, n (%)‡</td>
<td>1 (0.8)</td>
<td>0 (0)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Urgent CABG, n (%)‡</td>
<td>4 (3.2)</td>
<td>2 (1.9)</td>
<td>0.534</td>
</tr>
<tr>
<td></td>
<td>Mortality, n (%)‡</td>
<td>2 (1.6)</td>
<td>2 (1.9)</td>
<td>0.865</td>
</tr>
<tr>
<td></td>
<td>Total, n (%)‡</td>
<td>26 (20.8)</td>
<td>21 (20.0)</td>
<td>0.881</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>Sick leave duration (weeks)§</td>
<td>16 (2-52)</td>
<td>10 (1-48)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

CAs = coronary arteries; CABG = coronary artery bypass graft; CK = creatine kinase; cTnT = cardiac troponin T; CVI = cerebrovascular insult; ECHO = echocardiography; MACE = major adverse cardiovascular events; MetS = metabolic syndrome; PCI = percutaneous coronary intervention; †statistical significance defined as p<0.05; ‡data are expressed as absolute number and percentage, compared by χ²-test; §data are expressed as median and interquartile range, compared by Mann-Whitney U test.
In our study, MetS was independently associated with total in-hospital complications and severity of CA disease, but without its influence on other components of severity and prognosis. Except for hyperglycemia, which was independently associated with a higher risk of heart failure, none of the MetS components per se had any significant influence on clinical severity and prognosis in our patients with acute STEMI. Hyperglycemia is the main correlate of the risk of developing severe heart failure during AMI. Our result confirms the most important fact that MetS as a pathophysiological concept is relevant and superior in risk prediction in patients with acute STEMI urgently treated with PCI.

In this study, we found no significant influence of anthropometric parameters on clinical severity and prognosis of acute STEMI, except for BMI <25.0 kg/m² that increased the risk of dyspnea, which could be explained by the overall ‘obesity paradox’.

The presence of increased WC is associated with greater myocardial necrosis and worse LVEF in patients with acute MI. Other authors report on the central ‘obesity paradox’, i.e. a protective role of increased WC for development of significant angiographic CAD. They conclude that subcutaneous fat component is probably mainly responsible for the paradoxical protective effect of abdominal obesity, whereas visceral fat may have an opposing effect and increase the risk of angiographic CAD. WC does not add prognostic information for prediction of six-month mortality or myocardial reinfarction in patients with acute MI. WHR may indicate better distribution of body fat, i.e. the variation in WC correlates with the variation in subcutaneous and abdominal visceral fat, whereas hip circumference incorporates pelvic structure, gluteal muscle and gluteal subcutaneous fat. Furthermore, WHR is associated with significant coronary stenosis, but not with the extent of CAD, i.e. the number of CAs with significant stenosis. Finally, WHR is an independent predictor of six-month mortality in acute STEMI.

BMI is inferior to WC and WHR in the evaluation of obesity and cardiovascular risk. Several studies describe a paradoxical clinical effect of elevated BMI on improved survival after PCI in patients with acute MI, i.e. the overall ‘obesity paradox’. Furthermore, by using the American Association of Clinical Endocrinologists/American College of Endocrinology definition of MetS in patients with acute STEMI, Babić et al. found no significant differences in severity and prognosis. The authors concluded that, among other problems, anthropometry (using BMI instead of WC or WHR) was the most important reason for that.

In conclusion, the main finding of this study was more frequent involvement of proximal/middle CA segments with significant stenosis and longer SLD in MetS patients with acute STEMI. Finally, MetS was found to have a more superior role in predicting acute STEMI severity than its components and anthropometry, but without significant differences between them in prognosis.
REFERENCES


GLP-1 RECEPTOR AGONISTS: EFFECTS ON NONALCOHOLIC FATTY LIVER DISEASE

T. Bulum¹, K. Blaslov¹, A. Pavić Ljubičić¹, L. Duvnjak¹,²

Key words: nonalcoholic fatty liver disease, fatty liver, glucagon-like peptide-1, liraglutide, exenatide

SUMMARY

Nonalcoholic fatty liver disease (NAFLD), previously also named diabetes hepatitis, is characterized by accumulation of fat in the liver and refers to a spectrum of disorders ranging from simple hepatic steatosis to more severe manifestations including nonalcoholic steatohepatitis. Although the etiology is still unclear, NAFLD is strongly associated with hepatic and adipose tissue insulin resistance. Glucagon-like peptide 1 (GLP-1) receptor agonists represent a novel class of therapies for the treatment of type 2 diabetes with a potent blood glucose-lowering action mediated via its ability to induce insulin secretion and reduce glucagon secretion in a glucose-dependent manner. In addition, GLP-1-based therapy has direct effects on decreasing hepatic steatosis in vitro by modulating elements of the insulin signaling pathway via functional GLP-1 receptor, resulting in the regulation of gene expression associated with insulin resistance and lipid metabolism, and the suppression of oxidative stress in liver cells.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) affects up to 30% of the general population worldwide. It is considered to be the leading cause of chronic liver damage in western countries and it is estimated that NAFLD increases healthcare costs by 26% and will be the leading cause of liver transplantation by 2020 (1). NAFLD, previously also known as diabetes hepatitis, is characterized by accumulation of fat in the liver and refers to a spectrum of disorders ranging from simple hepatic steatosis to more severe manifestations, including nonalcoholic steatohepatitis (NASH), which can progress to fibrosis, cirrhosis, and liver failure, in the absence of substantial alcohol consumption or other causes of liver disease such as viral hepatitis (2, 3). Although the etiology is still unclear, it is considered as a hepatic manifestation of metabolic syndrome, a condition closely linked to obesity and type 2 diabetes (T2DM). Insulin resistance is the
central pathophysiological phenomenon of metabolic syndrome (4). NAFLD is usually clinically expressed as an incidental finding of elevated aminotransferase levels or radiographic studies suggesting the liver is fat (5). It has been shown that NAFLD, as manifested by elevated alanine transaminase (ALT) levels, predicts future development of T2DM (6). On the other hand, one autopsy series showed that individuals with diabetes had a 2.6-fold increased risk of steatohepatitis (7).

The liver plays an important role in maintaining normal glucose concentrations and it is also a major site of insulin clearance. NAFLD is considered as hepatic expression of insulin resistance and metabolic syndrome, responsible for the risk of advanced liver disease observed in these patients (8-12). The underlying metabolic condition favoring the occurrence of NAFLD is hepatic insulin resistance leading to ectopic accumulation of fat in the liver, almost a universal finding in NAFLD (13, 14). However, it is not clear whether insulin resistance causes hepatic steatosis or the accumulation of fat in the liver is the primary event leading to hepatic and later peripheral insulin resistance (15). The current treatment of NAFLD principally includes weight loss, insulin sensitivity and serum lipid improvement by lifestyle modifications (16-18). Treatment with insulin sensitizing agents such as metformin and thiazolidinediones has modest efficacy in NAFLD treatment (19, 20).

Incretins are gut hormones which are secreted into the circulation in response to nutrient ingestion and induce insulin secretion and reduce glucagon secretion in a glucose-dependent manner. Incretins also increase pancreatic β cell mass and suppress appetite and delay in gastric emptying, resulting in weight loss (21). The glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), the two incretin hormones identified so far, contribute equally to incretin effect (22). However, since metabolic effects of GIP are blunted in T2DM, only GLP-1 remains for the treatment of T2DM and related disorders (22). Since recent studies suggest that GLP-1 receptor agonists have beneficial effects on NAFLD, in this review we investigated the relationship between GLP-1 receptor agonists and NAFLD and explored the possible mechanisms and direct effects of GLP-1 based therapies on NAFLD.

GLP-1 RECEPTOR AGONISTS

Glucagon-like peptide-1 receptor agonists represent a novel class of therapies for T2DM treatment with a potent blood glucose-lowering action mediated via its ability to induce insulin secretion and reduce glucagon secretion in a glucose-dependent manner, but they also increase pancreatic β cell mass and suppress appetite and delay in gastric emptying, resulting in weight loss (21). There are currently four GLP-1 receptor agonists approved for use in the United States (US) and five approved for use in the European Union: exenatide twice daily, exenatide once weekly, liraglutide once daily, albiglutide once weekly, and lixisenatide once daily. Dulaglutide once weekly has been submitted for approval in both the US and Europe. All agents within the class have demonstrated significant reductions in hemoglobin A1c (HbA1c) and weight with minimal risk of hypoglycemia. However, the use of GLP-1 receptor agonists may be limited by the adverse effects (mostly gastrointestinal, i.e. nausea, vomiting, and diarrhea), and the need for subcutaneous administration. Most clinical experience is with exenatide twice daily, liraglutide once daily, and exenatide once weekly. Head-to-head studies suggest that liraglutide may have the largest HbA1c lowering capability of the three, followed by exenatide once weekly and then exenatide twice daily (23-26). However, the occurrence of gastrointestinal side effects appears to be less in patients taking exenatide once weekly compared with exenatide twice daily and liraglutide once daily (23-26).

Exenatide, originally derived from the salivary glands of the Gila monster with 53% homology with human GLP-1, was the first GLP-1 receptor agonist introduced in clinical practice. In clinical trials, exenatide significantly reduced HbA1c and these improvements in HbA1c (about 1.1%) were comparable with insulin therapy (24, 27-29). Exenatide once weekly reduced HbA1c significantly more compared with the twice daily formulation,
while body weight decreased similarly in the two groups throughout the 30-week study (23). Head-to-head studies suggest similar HbA1c reductions between another GLP-1 receptor agonist lixisenatide and exenatide twice daily while showing more weight loss with exenatide twice daily and less gastrointestinal side effects with lixisenatide (30).

The once daily human GLP-1 receptor agonist liraglutide has 97% homology with human GLP-1 with a single amino acid substitution extending its half-life up to 13 hours (31). Data from a large prospective Liraglutide Effect and Action in Diabetes (LEAD) program have demonstrated that liraglutide significantly reduces HbA1c up to 1.5% when used either as monotherapy or as an add-on in combination with other oral hypoglycemic agents and insulin (32, 33). Liraglutide reduced HbA1c significantly more than exenatide twice daily and exenatide once weekly, while the percentage of subjects achieving weight loss and overall weight loss was similar between the groups (25). However, one study showed more weight loss with liraglutide compared with exenatide once weekly (26). Albiglutide once weekly was less efficacious in both lowering HbA1c and lowering weight compared with liraglutide, but had less gastrointestinal side effects (34). Dulaglutide, once weekly GLP-1 receptor agonist, was noninferior to liraglutide in lowering HbA1c with greater weight loss with liraglutide compared to dulaglutide (35, 36).

THE PATHOGENESIS OF NAFLD AND ASSOCIATION WITH GLP-1 SIGNALING

Nonalcoholic fatty liver disease is strongly associated with hepatic and adipose tissue insulin resistance, as well as reduced whole-body insulin sensitivity (10). The underlying metabolic condition favoring the occurrence of NAFLD is hepatic insulin resistance, associated with obesity, dyslipidemia and ectopic accumulation of fat in the liver, almost a universal finding in NAFLD (13, 14, 37). Insulin resistance increases free fatty acid flux to the liver by decreasing inhibition of lipolysis and also by increasing de novo lipogenesis (15). Hepatic gene expression of sterol regulatory element-binding protein (SREBP) 1c, the key transcriptional activator of lipogenic genes, as well as acetyl-CoA carboxylases (ACCs), fatty acid synthase (FAS), and the activity of lipogenic enzyme stearoyl-CoA desaturase 1 (SCD1) is increased in subjects with NAFLD compared to healthy controls (38, 39). In insulin resistance state, the ability of insulin to inhibit hepatic glucose production is impaired leading not only to higher fasting plasma glucose concentration and hyperinsulinemia but also the insulin resistant liver overproduces triglyceride rich very-low-density lipoprotein (VLDL) in the fasting state, which then leads to hypertriglyceridemia and low high density lipoprotein (HDL) concentration that can be found in subjects with metabolic syndrome and NAFLD (4, 40, 41). Moreover, although insulin resistance is not an underlying cause of some other diseases such as autoimmune type 1 diabetes, the presence of insulin resistance in these subjects is independently associated with markers of NAFLD (42). The use of insulin sensitizers such as thiazolidinediones, the peroxisome-proliferator-activated receptor γ (PPARγ) agonists has been shown to decrease liver fat content by up to 40% (43).

The GLP-1 receptor has been detected in human hepatocytes at both the mRNA and protein level and GLP-1 based proteins should be analyzed as insulin sensitizing agents in hepatocytes (44, 45). Recently, an association of defective GLP-1 signaling with NAFLD has been documented. The glucose-induced GLP-1 secretion is markedly decreased in patients with NAFLD or NASH compared to controls, suggesting a deficiency of GLP-1 signaling in NAFLD (46). Moreover, enzyme dipeptidyl peptidase-4 (DPP4), which inactivates intact GLP-1, is upregulated in liver biopsy samples of patients with NAFLD compared to healthy subjects and serum DPP-IV activity and DPP-IV expression in the human liver correlate with histopathologic grade and insulin resistance in patients with NAFLD (47-49). Hepatic GLP-1 receptor expression in human and animal models of NASH was significantly lower compared to healthy controls (48). It has been suggested that GLP-1-based agents may have direct effects in decreasing hepatic steatosis in vitro by modulating elements of the insulin signaling pathways.
GLP-1 RECEPTOR AGONISTS: AN INDIRECT ACTION

All GLP-1 receptor agonist agents have demonstrated significant reductions in HbA1c with minimal risk of hypoglycemia. Head-to-head studies suggest that liraglutide may have the largest HbA1c lowering capability, followed by exenatide once weekly and then exenatide twice daily (23-26). In clinical trials, exenatide significantly reduced HbA1c and these improvements in HbA1c (about 1.1%) were comparable with insulin therapy (24, 27-29). Data from the LEAD program have demonstrated that liraglutide significantly reduces HbA1c by up to 1.5% when used either as monotherapy or as an add-on in combination with other oral hypoglycemic agents and insulin (32, 33). Lower fasting glucose level results in lower fasting insulin level and consequently lower insulin resistance, the underlying metabolic condition favoring the occurrence of NAFLD. Moreover, the relative reduction in intrahepatic lipid in patients treated with exenatide and liraglutide was 42% and significantly correlated with the relative reduction in HbA1c (52).

Beyond glucose-lowering effects, GLP-1 receptor agonists also have a pleiotropic effect on appetite, weight, blood pressure, cardiovascular function and central nervous system (53-55). GLP-1 receptor activation in hypothalamus as well as delay in gastric emptying reduces appetite and leads to weight loss of 1.9 to 3 kg following liraglutide and exenatide therapy (56-58). Even modest weight loss of 5%-10% of body weight decreases liver fat by up to 40%-80% in diabetic and nondiabetic subjects (59). Several studies also suggest that GLP-1 receptor agonists increase glucose uptake independently of changes in insulin secretion and directly affect peripheral insulin sensitivity (60).

GLP-1 RECEPTOR AGONISTS: A DIRECT ACTION

It is well known and documented that GLP-1-based therapies lead to weight loss and glycemic improvement, which are both important for the improvement of NAFLD. However, recent reports have shown that GLP-1-based therapies also have direct effects on the liver (Figure 1). Treatment with exenatide significantly reduced ALT levels, the most important marker of liver steatosis, and hepatic fat content (61, 62). In our study, we also demonstrated significant improvement in fatty liver index on exenatide therapy, independently of changes in body weight and HbA1c (63).

Figure 1. Potential roles of glucagon-like peptide-1 receptor agonists in nonalcoholic fatty liver disease

GLP-1: glucagon-like peptide-1; GLP-1R: glucagon-like peptide-1 receptor; DPP-4: dipeptidyl peptidase-4; NAFLD: nonalcoholic fatty liver disease; AMPK: AMP-activated protein kinase; PDK1: phosphoinositide-dependent kinase-1; PKC: protein kinase C; Akt: protein kinase B; GLUT-1: glucose transporter-1; JNK: c-Jun N-terminal protein kinase; SREBP-1: sterol regulatory element-binding protein 1; ACC: acetyl-CoA carboxylase; FAS: fatty acid synthase; PPAR: Peroxisome proliferator activator receptor; LDL-R: low-density lipoprotein receptor.
significantly reduces hepatic lipid stores evaluated by histological improvement and improved ALT values via direct action on hepatocytes including gene profile that is conducive in reduction of fatty acid synthesis and triglyceride storage in hepatocytes: increased mRNA for both PPARα along with decreased mRNA expression for SREBP-1c and ACC1 (64). In diet induced obesity mice model, treatment with exendin-4 reverses hepatic steatosis and decreases hepatic expression of genes involved in de novo fatty acid synthesis, including ACC1, FAS and SCD1 (65). Exendin-4 treatment also improves the expression of PPARα and its downstream target genes: acyl-Coenzyme A oxidase (ACOX) and carnitine palmitoyltransferase 1 A (CPT1A) in hepatocytes isolated from rats with NASH (44). GLP-1 receptor agonists also improve hepatic steatosis by modulating fibroblast growth factor-21 (FGF-21) signalization. FGF-21 is predominantly produced in the liver, where it increases adipocyte insulin sensitivity and glucose intolerance and regulates lipolysis in white adipose tissue, but also enhances hepatic fat oxidation and reduces hepatic steatosis partially through its effects on AMP-activated protein kinase (AMPK) activity (66). AMPK in the liver activates fatty acid oxidation via activation of PGC1α and indirectly other enzymes of lipogenesis (e.g., SREBP and FAS) (67). In human and animal models of NAFLD, liver FGF-21 protein levels and RNA are increased and FGF-21 signaling in the liver and white adipose tissue is impaired in association with hepatic steatosis (68, 69). Treatment with exenatide in T2DM and diet induced obese mouse is associated with a decrease in FGF-21 and hepatic fat, and an increase in hepatic AMPK and ACC phosphorylation (70). Moreover, exenatide regulatory effect on the expression of genes related to fatty acid β-oxidation was abolished by PKA or AMPK inhibitors, confirming that the effects of exenatide on lipid metabolism in the liver are mediated via PKA and AMPK signaling pathway (44).

Therapy with another GLP-1 agonist, liraglutide, in T2DM is associated with improvement of liver inflammation, alteration of liver fibrosis, and also reduced steatosis (71). Efficacy and safety of liraglutide therapy on liver parameters in comparison with controls were investigated in the LEAD program on 2241 patients with abnormal elevation of plasma ALT levels at baseline. Liraglutide dose-dependently reduced the ALT levels in these patients, and in the LEAD-2 sub-study, where hepatic steatosis was measured by computerized tomography scan, liraglutide therapy improved hepatic steatosis compared with placebo, independently of reduction in body weight and HbA1c (72). In an animal model, hepatic steatosis improved histologically, and the liver total lipid content was dramatically reduced by 8-week liraglutide treatment in hypoadiponectinemia and high fat and high fructose diet-induced NAFLD mice model (73, 74). Liraglutide treatment was able to enhance activation of AMPK, a critical signal molecule involved in the regulation of hepatic insulin sensitivity, and also completely or partially restored the deterioration in insulin resistance and the alteration of regulatory factors involved in hepatic insulin sensitivity (74-76). Treatment with liraglutide and exenatide therapy suppressed c-Jun N-terminal protein kinase signaling pathway, a key mechanism involved in hepatic oxidative stress, suggesting that GLP-1 receptor agonists attenuate liver cell oxidative stress, one of the essential steps in the progression of NAFLD or NASH (44, 74). Liraglutide therapy also downregulated the expression of ACC and FAS in the liver of NAFLD mice and restored hepatic transcription of PPAR-α, low-density lipoprotein receptor and insulin-induced geng-2, downregulated in a NAFLD model (74, 76).

Since human studies investigating the effects of GLP-1 receptor agonists on liver injury have been limited to case reports, a protocol for a 48-week multicenter, double-blind, placebo-controlled randomized clinical trial LEAN (Liraglutide efficacy and action in NASH) of treatment with liraglutide for adults with biopsy-proven NASH has been published (77). The primary outcome measure will be analysis of the proportion of evaluable patients achieving an improvement in liver histology between liver biopsies at baseline and after 48-week treatment. First results that were presented this year at the International Liver Congress showed that NASH had resolved with no worsening of fibrosis in 39% of the patients (9/23).
having received liraglutide compared with 9% of the patients on placebo (2/22). Moreover, 82.6% of the patients treated with liraglutide showed improvement in the liver fat content (78). Similar results were found in a Japanese pilot study where patients treated with liraglutide showed decreased histological inflammation as determined by NASH activity score and stage determined by Brunt classification (79).

CONCLUSION

Nonalcoholic fatty liver disease affects up to 30% of the general population worldwide and it is considered to be the leading cause of chronic liver damage in western countries. NAFLD is strongly associated with hepatic and adipose tissue insulin resistance, as well as reduced whole-body insulin sensitivity. GLP-1 receptor agonists have a potent blood glucose-lowering action mediated via their ability to induce insulin secretion and reduce glucagon secretion but they also suppress appetite and delay in gastric emptying, resulting in weight loss. The GLP-1 receptor has been detected in human hepatocytes at both the mRNA and protein level and GLP-1-based agents have direct effects in decreasing hepatic steatosis in vitro by modulating elements of the insulin signaling pathway resulting in the regulation of gene expression associated with insulin resistance and lipid metabolism, and suppression of oxidative stress in liver cells. Results published to date and ongoing placebo-controlled randomized clinical trials have confirmed that therapy with GLP-1 receptor agonist improves fat content of the liver and decreases histological inflammation and fibrosis.
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