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ORIGINAL RESEARCH ARTICLES

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SUMMARY

This was an 18-month prospective observational study aiming to assess the effect of treatment with glucagon-like peptide-1 receptor agonists (GLP-1 RAs) on hepatic fat quantity estimated by fatty liver index (FLI) in patients with type 2 diabetes mellitus (T2DM). The study enrolled 75 T2DM patients aged 58±8 years with diabetes duration of 12±6 years, body mass index (BMI) 38.9±5.1 kg/m² and HbA1c level 8.3±1.2%, randomized to either liraglutide (n=39; 48%) or exenatide (n=36; 52%) continuing their oral hypoglycemic agents, as well as lipid lowering and antihypertensive therapy. After 18-month follow up, BMI, waist circumference, HbA1c, triglycerides, C-reactive protein and FLI were significantly lower compared to baseline values in both groups. However, after inter-group comparison, exenatide was superior to liraglutide in BMI (p=0.001), HbA1c (p=0.026) and FLI (p=0.001) reduction. The introduction of GLP-1 analogues in T2DM therapy may have positive effect on hepatic fat metabolism, with a more favorable effect of exenatide compared to liraglutide, which requires further study evaluation.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most common liver diseases affecting up to 30% of the Western hemisphere general population (1). It is defined as a chronic liver condition characterized by insulin resistance and hepatic fat accumulation, in the absence of other identifiable causes such as alcohol abuse, viral or autoimmune hepatitis, alpha-1 antitrypsin deficiency, medications like corticosteroids and estrogens, and other conditions (2). Liver biopsy is the gold standard for quantification of liver steatosis that is not routinely performed because it is an invasive procedure with a certain degree of sampling error (3). Instead, several conventional radiologic imaging methods such as ultrasound, computed tomography and magnetic resonance can be used (4). Alanine aminotransferase (ALT) >30 IU/L was usually used as the cut-off level in screening for NAFLD, but normal ALT does not exclude steatosis (5, 6). Fatty liver index (FLI) is a formula including body mass index (BMI), waist circumference, triglycerides (TG)
and gamma glutamyltransferase (GGT), which has shown good agreement with abdominal ultrasound in liver fat quantification (7).

Nonalcoholic fatty liver disease may range from indolent fat deposition in the liver to severe lipotoxocity-induced steatohepatitis with necroinflammation called nonalcoholic steatohepatitis (NASH) with consequential fibrosis, and approximately 10% of patients develop cirrhosis or even hepatocellular carcinoma (8, 9). NAFLD is closely associated with metabolic syndrome and type 2 diabetes mellitus (T2DM); according to data from the Edinburgh Type 2 Diabetes Study, up to 43% of T2DM patients have NAFLD-related steatosis (10). Additionally, several studies have demonstrated that it is associated with a higher prevalence of cardiovascular disease and greater burden of diabetic complications in T2DM population (11, 12).

Glucagon-like peptide-1 (GLP-1) is a gut peptide secreted in response to food intake that stimulates glucose-dependent insulin secretion in pancreatic β cells, inhibits postprandial glucagon release in pancreatic α cells and delays gastric emptying (13). As GLP-1 secretion was found to be impaired in T2DM, GLP-1 receptor agonists found their place in clinical practice as novel glucose lowering agents (14-18). Additionally, the decrease in glycated hemoglobin (HbA1c) levels in T2DM patients treated with exenatide was shown to correlate with hepatic fat reduction (19). Recent studies have reported the presence of GLP-1 receptor on human hepatocytes and the possible direct exenatide modulation of intrahepatic lipid metabolism and insulin signaling with consequential improvement in hepatic steatosis (20).

The aim of our study was to evaluate the effect of two GLP-1 receptor agonists most frequently used in clinical practice, exenatide and liraglutide, on hepatic biomarkers (AST, ALT, GGT, alkaline phosphatase (AP) and FLI, as well as on BMI, waist circumference and HbA1c levels in T2DM patients.

**PATIENTS AND METHODS**

**Study design and participants**

This was an open-label two arm-parallel-group uncontrolled 18-month study carried out at the Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Zagreb, Croatia, according to the Declaration of Helsinki (accessed at www.ich.org), and an informed consent was obtained from study participants before enrolment.

The study included T2DM patients on stable doses of oral hypoglycemic agents (OHA) metformin or/and sulfonylurea when starting exenatide or liraglutide therapy. Patients were randomized to receive either exenatide (started as a 5 µg twice daily dose and increased to 10 µg twice daily if needed) or liraglutide (started as a 0.6 mg/day dose and increased to 1.2 mg/day after 14 days) in addition to continuing their antidiabetic background treatment. Also, the antihypertensive and lipid-lowering drugs were unchanged during the study period. Randomization procedures were performed by principal investigator or his/her delegate by physical method of randomization using shuffle sealed envelopes with treatment allocations inside. Patients of both groups were instructed to strictly maintain dietary habits and daily activities during the study. The inclusion criteria were: age >18 years to <80 years, T2DM on stable doses of sulfonylurea and/or metformin, BMI >35 kg/m², and no evidence for other causes of liver disease. Exclusion criteria were: clinical signs of cirrhosis as evidenced by any of the following: spider angiomatas, splenomegaly, ascites, jaundice, encephalopathy, INR >1.2, platelet count <100,000/mL, serum albumin <3.0 g/dL, other liver diseases including chronic viral hepatitis (B or C), alcohol abuse, hemochromatosis, alpha-1 antitrypsin deficiency, autoimmune hepatitis, Wilson’s disease, primary sclerosing cholangitis or primary biliary cirrhosis, current use of >20 g of alcohol per day, or unwillingness to avoid alcohol during the study, AST
or ALT >10 times the upper limit of normal, and initiation or change in the dose of hypolipidemic drugs within 6 months of enrolment.

**Study measurements**

All subjects were studied in the morning after an overnight fast at the study entry and after 18 months. Basic anthropometric measurements were performed on all study subjects. Fasting venous blood samples were collected in the morning between 08:00 and 09:30 AM after an overnight fast for determination of complete blood count and biochemistry panel, triglycerides, HbA1c and liver biochemistry. FLI was calculated according to the formula: FLI = \( \frac{e^{0.953\times\log_{10}(\text{triglycerides})} + 0.139\times\text{BMI} + 0.718\times\log_{10}(\text{ggt}) + 0.053\times\text{waist circumference} - 15.745}{1 + e^{0.953\times\log_{10}(\text{triglycerides})} + 0.139\times\text{BMI} + 0.718\times\log_{10}(\text{ggt}) + 0.053\times\text{waist circumference} - 15.745} \times 100 \) (21).

**Statistical analysis**

Statistical evaluation of data was carried out using the SPSS statistical package, version 17.0 for Windows. Baseline data were reported using descriptive statistics. Normality of distribution for continuous variables was analyzed using Kolmogorov-Smirnov test. Normally distributed variables were described with mean and standard deviation (SD). Nominal variables were reported with absolute numbers and percentages. To assess differences in pre- and post-treatment measurements we used paired sample T-test in parametric and Wilcoxon signed rank test in non-parametric statistics. The level of significance was set at p<0.05.

**RESULTS**

Out of 75 T2DM patients included in the study, 33 (44%) were male (mean age 58±8 years, mean diabetes duration 12±6 years, mean BMI 38.9±5.1 kg/m² and mean HbA1c level 8.3±1.2%). Six patients were taking liraglutide 1.2 mg/daily alone; 19 patients liraglutide 1.2 mg/daily + 2 g metformin; ten patients liraglutide 1.2 mg/daily + 2 g metformin + 60 mg gliclazide/daily; and four patients liraglutide 1.2 mg + 2 g metformin + 60 mg gliclazide/daily. Nine patients were taking exenatide 10 mcg/twice daily alone; 16 patients exenatide 10 mcg/twice daily + 2 g metformin; and ten patients exenatide 10 mcg/twice daily + 2 g metformin + 60 mg gliclazide/day. Liraglutide treated group included 39 (52%) and exenatide group 36 (48%) patients. There were no between-group differences according to age (58±9 vs 57±7 years), baseline BMI (39.15±5.44 vs. 38.58 kg/m²) or glycemic control based on HbA1c level (8.12±1.00 vs. 8.58±1.33). Diabetes duration was also comparable between the two groups (12±6 vs. 11±6 years). After 18-month follow up, both groups showed significant BMI and waist circumference reduction. The mean BMI change in the liraglutide group was -1.6±0.03 kg/m² and in the exenatide group -1.7±0.04 kg/m², i.e., significantly in favor of the exenatide treated group (Table 1), whereas neither of the treated groups showed superiority regarding waist circumference. Both groups showed a statistically significant change in CRP, triglycerides and FLI (Table 1). The mean FLI change in the liraglutide group was -1.15±0.7 and in the exenatide group -3.28±1.71. Exenatide showed significant superiority to liraglutide in FLI reduction.

**DISCUSSION**

In this open label parallel-group uncontrolled 18-month study, we assessed changes in hepatic biomarkers, FLI, BMI, waist circumference, HbA1c and TG levels in two groups of T2DM patients, one treated with exenatide and another one treated with liraglutide. Data from our study clearly suggested that the introduction of GLP-1 analogues to the existing T2DM therapy resulted in reduction of hepatic biomarker levels and intrahepatic fat quantity as calculated by FLI. We recorded BMI, waist circumference, TG and HbA1c reduction in both liraglutide and exenatide groups, however, exenatide proved superior to liraglutide in most of the observed parameters.

A similar effect of GLP-1 agonists (exenatide and liraglutide) was observed by Cuthbertson et al. (19). They report a significant intrahepatic liver fat and biomarker reduction following 6-month GLP-1
agonist therapy in 25 T2DM patients. The reduction did not correlate with weight reduction but did correlate with HbA1c reduction (19). Data from the studies by Klonoff et al. and Buse et al. suggest that exenatide treatment in T2DM patients leads to significant ALT and intrahepatic fat reduction in correlation to insulin resistance decrease (22, 23). Sathyanarayana et al. performed an uncontrolled observational study with exenatide and pioglitazone and highlighted the beneficial effect of exenatide on intrahepatic fat content independently of BMI reduction (24). These data clearly suggest that exenatide therapy could reduce or even reverse hepatic fat accumulation. The question that arises from these results is whether exenatide has a direct effect on intrahepatic liver fat reduction or it is mediated by weight reduction and abdominal fat decrease, as well as HbA1c decrease, since weight reduction itself has shown to be effective in NAFLD therapy (25).

The reduction in hepatic fat content could be partially explained by reduced caloric intake, which is one of the main therapeutic contributions of this drug class.

### Table 1. Differences in laboratory parameters between study entry and 18-month values in liraglutide- and exenatide-treated groups of patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Liraglutide group (n=39)</th>
<th>P*</th>
<th>Exenatide group (n=36)</th>
<th>P*</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study entry</td>
<td>39.15±5.44</td>
<td>0.000</td>
<td>38.58±4.65</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td>18-month follow up</td>
<td>36.33±6.07</td>
<td></td>
<td>35.78±4.35</td>
<td></td>
<td></td>
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<tr>
<td>Waist circumference (cm)</td>
<td></td>
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</tr>
<tr>
<td>Study entry</td>
<td>121.5±12.44</td>
<td>0.003</td>
<td>119.1±12.5</td>
<td>0.001</td>
<td>0.771</td>
</tr>
<tr>
<td>18-month follow up</td>
<td>116.6±14.01</td>
<td></td>
<td>114.92±10.3</td>
<td></td>
<td></td>
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<tr>
<td>HbA1c (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study entry</td>
<td>8.12±1.00</td>
<td>0.881</td>
<td>8.58±1.33</td>
<td>0.036</td>
<td>0.181</td>
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<tr>
<td>18-month follow up</td>
<td>8.11±1.17</td>
<td></td>
<td>7.95±1.49</td>
<td></td>
<td></td>
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<tr>
<td>Triglycerides (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study entry</td>
<td>2.71±2.54</td>
<td>0.483</td>
<td>2.24±1.33</td>
<td>0.820</td>
<td>0.953</td>
</tr>
<tr>
<td>18-month follow up</td>
<td>3.08±2.73</td>
<td></td>
<td>2.18±1.3</td>
<td></td>
<td></td>
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<tr>
<td>CRP (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study entry</td>
<td>6.35±7.97</td>
<td>0.007</td>
<td>6.68±7.88</td>
<td>0.000</td>
<td>0.026</td>
</tr>
<tr>
<td>18-month follow up</td>
<td>5.35±6.73</td>
<td></td>
<td>3.76±6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Study entry</td>
<td>25.85±11.52</td>
<td>0.566</td>
<td>24.33±8.27</td>
<td>0.217</td>
<td>0.161</td>
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<tr>
<td>18-month follow up</td>
<td>26.16±12.59</td>
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<td>22.67±7.8</td>
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<tr>
<td>ALT (IU/L)</td>
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<td></td>
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<tr>
<td>Study entry</td>
<td>31.23±14.57</td>
<td>0.224</td>
<td>34.42±17.08</td>
<td>0.049</td>
<td>0.370</td>
</tr>
<tr>
<td>18-month follow up</td>
<td>32.71±13.67</td>
<td></td>
<td>29.44±15.86</td>
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<td></td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td></td>
<td></td>
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<tr>
<td>Study entry</td>
<td>77.56±23.75</td>
<td>0.858</td>
<td>84.64±24.47</td>
<td>0.114</td>
<td>0.054</td>
</tr>
<tr>
<td>18-month follow up</td>
<td>83.96±25.33</td>
<td></td>
<td>79.08±21.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study entry</td>
<td>36.36±18.88</td>
<td>0.127</td>
<td>45.55±29.03</td>
<td>0.004</td>
<td>0.037</td>
</tr>
<tr>
<td>18-month follow up</td>
<td>43.1±27.98</td>
<td></td>
<td>35.75±19.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilirubin (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study entry</td>
<td>12.16±5.86</td>
<td>0.086</td>
<td>11.83±4.25</td>
<td>0.094</td>
<td>0.076</td>
</tr>
<tr>
<td>18-month follow up</td>
<td>11.29±5.52</td>
<td></td>
<td>11.11±4.67</td>
<td></td>
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<tr>
<td>FLI</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Study entry</td>
<td>36.77±8.13</td>
<td>0.010</td>
<td>33.77±3.67</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>18-month follow up</td>
<td>29.04±8.41</td>
<td></td>
<td>21.98±9.84</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Intra-group comparison (at study entry and at 18-month follow up); **Inter-group comparison; level of significance p<0.05
This is in concordance with a decrease in TG levels in both study groups and also consistent with the BMI and waist circumference reduction, which was greater in the exenatide group. However, patient food intake was not controlled, and both groups had a decrease in BMI, as well as in waist circumference, so the effect of reduced caloric intake could be argued.

There are several studies explaining the molecular pleiotropic effect of GLP-1 analogues on human hepatocytes and hepatic fat reduction. Experimental studies by Samson et al. showed that exenatide increased adiponectin levels in diet induced obese mice, and adiponectin levels are reported to negatively correlate with hepatic fat content in experimental animal models, as well as in humans (24, 26-28). Adiponectin activates AMP-activated protein kinase and enhances mitochondrial fat oxidation and it could be an important exenatide mediator in reducing hepatic lipid content (29).

Recent studies suggest that a direct effect of GLP-1 analogues on hepatic lipogenesis and lipid oxidation cannot be ruled out. GLP-1 treatment in mice resulted in significant reduction in mRNA expression of stearoyl-CoA desaturase 1 and genes associated with fatty acid synthesis (26). Additionally, the presence of GLP-1 receptor on human hepatocytes has been confirmed, as well as the activation of signal transduction cascades that lead to decrease in hepatic steatosis by modulation of insulin signaling pathway after exenatide binding (20). Consistent with this, exenatide plays a direct important role in hepatic fat metabolism but the responsible molecular mechanism needs further research.

In conclusion, the introduction of GLP-1 agonists in T2DM patient therapy seems to have positive pleiotropic effect on the liver, especially on the hepatic fat metabolism, although so far it has been mostly studied for its effect on weight reduction, glucose dependent insulin secretion with consequential glucoregulation, and positive cardiovascular effects. However, whether detection of a decrease in hepatic fat content in T2DM patients treated with GLP-1 based therapy is due to direct effect of this drug class needs to be assessed in further follow up studies.
REFERENCES


KEY WORDS: diabetes, exercise, glycemic control

SUMMARY

Physical activity, both aerobic and anaerobic exercise, has significant influence on glycemic control and plays a major role in metabolic regulation in diabetic patients. It reduces glycated hemoglobin (A1C) values by approximately 0.5 to 0.7 percentage points. Despite the fact that beneficial effects of physical activity are known for a long time, the majority of diabetic patients do not practice sufficient physically activity and their compliance with prescribed physical activity is very poor. Physicians have to prescribe exercise as a therapeutic modality to every person with diabetes mellitus, and always remind patients of the importance of exercise. Current recommendations include a combination of at least 150 minutes of moderate-intensity aerobic exercise per week and resistance training at least twice per week, but even smaller amounts of exercise provide health benefits.

INTRODUCTION

In 1870, more than 3000 years after diabetes had been first described in ancient Egypt, Bouchardat was the first to notice the beneficial effect of physical activity on the disease regulation (1). In 1916, Elliott Joslin as the leading diabetologist in the USA wrote in his book entitled The Treatment of Diabetes Mellitus that physical activity and diet significantly reduced the risk of death in people with diabetes mellitus (2).

DIABETES AND EXERCISE

Despite the fact that beneficial effects of physical activity are known for a long time, data show that the majority of diabetic patients are inadequately physically active (3). Beneficial effect of exercise on the regulation of diabetes was later on well described and documented in numerous clinical studies. Exercise is utilizing muscle glycogen. Also, exercising muscle takes glucose from the circulation. Fall in blood glucose concentration inhibits insulin secretion and raises glucagon secretion, which leads to an increased hepatic glucose production due to glycogenolysis and gluconeogenesis. Further exercise leads to the rise in counter-regulatory hormones that stimulate lipolysis (4). Exercise has numerous effects on muscle
metabolism including increased translocation of insulin-responsive glucose transporters (GLUT4) from intracellular stores to the cell surface, which promotes glucose uptake and increases insulin sensitivity (5). Even regular 7-day exercise program has an effect on insulin sensitivity (6). Resistance training increases insulin receptor substrate (IRS1) expression, which also increases insulin sensitivity (7).

Both aerobic and anaerobic exercise has a significant influence on glycemic control. Exercise training reduces glycated hemoglobin (A1C) values by approximately 0.5 to 0.7 percentage points (8-10). At first, for a long time only the effects of traditional aerobic exercise on glycemic control were studied. Since the study by Eriksson et al. showed a good impact of resistance training on glycemic control in T2DM patients (11), numerous studies have confirmed the favorable effect of anaerobic exercise on glycemic control. A study by van Dijk et al. showed that a single session of resistance or endurance-type exercise substantially reduced the prevalence of hyperglycemia during the subsequent 24-h period in individuals with impaired glucose tolerance, and in insulin-treated and non-insulin treated type 2 diabetic patients (12). In their analysis, Thomas et al. included 14 randomized controlled trials comparing exercise against no exercise in type 2 diabetes involving 377 participants. Trials ranged from 8-week to 12-month duration. Compared with controls, the exercise intervention significantly improved glycemic control as indicated by a 0.6% decrease in glycated hemoglobin levels. The result was both statistically and clinically significant (13). In another study, 16 weeks of progressive resistance training reduced HbA1c from 8.7 to 7.6 percent (14).

Exercise has beneficial effect on almost every aspect of metabolic syndrome. First, it improves weight loss and has an important role in maintaining weight loss after weight reduction (15). Furthermore, physical inactivity is an independent risk factor for the development of coronary heart disease (16), and physically inactive individuals have twice the risk of mortality regardless of body mass index (17). In a study including 2896 diabetic adults, those that walked for at least two hours per week had a significantly lower cardiovascular mortality rate (18). Evidence from numerous studies led to inclusion of regular exercise in current recommendations for prevention of coronary heart disease both as a primary and secondary measure (19). Regular exercise significantly reduces blood pressure up to 15 mm Hg (7,14,20). Studies also showed improvements in serum lipid concentrations in patients practicing exercise (21).

In 2007, DARE study was published, which was the first adequately powered and controlled study comparing aerobic training, resistance training, or both with change in HbA1c in individuals with type 2 diabetes. All exercise groups had a reduction in HbA1c compared with control group, but the combination group had a larger reduction (-1.0%) compared with the resistance training (-0.4%) and aerobic (-0.5%) groups. However, it was unclear whether the additional benefit observed was due to the combination of resistance and aerobic training or to the extra exercise time (22). Later studies confirmed the best influence of a combination of aerobic and anaerobic exercise on glycemic control (23, 24). As a result, current recommendations for persons with type 1 and 2 diabetes mellitus include at least 150 minutes of moderate intensity aerobic exercise per week in the absence of contraindications, and patients should be encouraged to perform resistance training (exercise with free weights or weight machines) at least twice per week (25). Yet, studies clearly showed that higher levels of exercise intensity were associated with greater improvements in HbA1C, so it is advisable to encourage patients to perform more exercise if possible (8).

Recently, some new forms of exercise such as high intensity/low volume exercise in patients with type 2 diabetes have been studied. This included for example cycling at 90% of maximal heart rate for 1 minute followed by 1 minute of rest, repeated 10 times (26). Another study investigated the influence of small doses of intense exercise before each main meal, so called ‘exercise snacks’, on glycemic control (27). Although this new approach showed good impact on glycemic control, the studies were short-termed and
included small numbers of patients, therefore additional studies with this type of exercise are needed.

**DISCUSSION**

The basic principles of diabetes mellitus treatment include dietary measures and exercise. Modern pharmacotherapy of diabetes is so effective that sometimes we forget (or not think enough) where the root of the problem is. Thousands years ago, when our ancestors wanted to have lunch, they had to go to the woods and catch the food. The food had one or two pairs of running legs, and to catch it, man had to run a lot. Despite the effort, man frequently remained hungry (rabbit escaped). Modern hunter ‘catches’ food in supermarkets and then goes home by car. As a result, the prevalence of obesity and diabetes has exploded. It is important to understand that our body and our metabolism, over a long period of our evolution, were made to run, not to seat in front of TV and eat snacks.

All studies clearly show that the role of physical activity is essential in the prevention of diabetes and regulation of diabetes. According to current recommendations, the best effect on health has a combination of aerobic and anaerobic exercise. Considering the way our ancestors had lived for centuries, it seems quite logical because their way of life included both. However, when advising patients about physical activity, we must always consider their interests and lifestyle, and the best way to achieve permanent success is to advise patients to do physical activity they like. Furthermore, although the combination of aerobic and anaerobic exercise is the best, virtually all types of exercise are beneficial for health. Sometimes, 150 minutes of exercise are hard to accomplish, but even 60 minutes of exercise per week provides health benefits (28).

High intensity/low volume exercise is a promising concept for the regulation of diabetes; however, additional studies with more patients and in comparison to aerobic and anaerobic forms of exercise are needed to evaluate the impact of this form of exercise on glycemic control.

Despite the fact that the role of physical activity in diabetes regulation is most important, compliance with prescribed physical activity is very poor. In a 10-year study including 255 diabetic patients enrolled in a diabetes education program that emphasized exercise, compliance fell from 80 percent at six weeks to less than 50 percent at three months and to less than 20 percent at one year (29). So, it is essential that physicians always remind patients of physical activity and emphasize the importance of exercise in the regulation of diabetes and health in general.

**CONCLUSION**

Physical activity plays a major role in the regulation of diabetes mellitus and health in general. Current recommendations include a combination of at least 150 minutes of moderate intensity aerobic exercise per week and resistance training at least twice per week, but even smaller amounts of exercise provide health benefits. Some new forms of exercise such as high intensity low volume exercise have shown positive influence on glycemic control, but additional studies are needed to evaluate its efficiency. Still, the majority of diabetic patients are not adequately physically active, and compliance with prescribed physical activity is very poor. Physicians must prescribe exercise as therapy to every person with diabetes mellitus, and always remind the patients of the importance of exercise in the regulation of diabetes and health in general.
REFERENCES


Since the publication of the 2012 statement concerning the sodium-glucose transporter-2 (SGLT-2), these agents are now widely used as an adjunctive treatment in managing type 2 diabetes due to their oral administration route, potent hemoglobin A1c reduction, minimal risk of hypoglycemia, and significant weight loss. This new class of agents is included as a reasonable choice for second-line, third-line therapy or even first-line therapy in the updated statements (1, 2). Moreover, because its metabolic effects do not depend on an insulin-dependent mechanism, these agents can be proposed for use throughout the natural history of type 2 diabetes, and are effective when used with other agents including combination with insulin therapy (3). The most important symptomatic side effects are genitourinary, including frequent urination, discomfort with urination, and vaginal or penile mycotic infections. A novel approach to SGLT inhibition also involves blockade of SGLT-1, the primary transporter for glucose uptake from intestinal lumen. Recently, data on a novel dual inhibitor of SGLT-1 and SGLT-2 (LX4211) in type 2 diabetes were presented and these data suggest that dual inhibition of SGLT-1 and SGLT-2 produced a significant dose-related improvement in glucose control that was not correlated with glycosuria and was associated with reductions in weight and systolic blood pressure (4). Although all the data presented on SGLT-2 inhibition were very encouraging, there is still a reason for caution because these drugs are new and the duration of studies available at present is limited, and the benefit versus risk balance is still not well understood.

The US Food and Drug Administration (FDA) warning released on May 15, 2015 indicated that SGLT-2 inhibitors used to treat type 2 diabetes may lead to ketoacidosis requiring hospitalization (5). The warning includes the SGLT-2 inhibitors canagliflozin (Invokana, Johnson & Johnson), dapagliflozin (Forxiga, AstraZeneca), and empagliflozin (Jardiance, Lilly/Boehringer) because search of the FDA Adverse Event Reporting System database identified 20 cases of acidosis reported as diabetic ketoacidosis (DKA), ketoacidosis, or ketosis in patients treated with SGLT-2 inhibitors from March 2013 to June 6, 2014. DKA is a serious acute complication of diabetes mellitus often developing in individuals with poorly controlled type 1 diabetes and rarely in those with type 2 diabetes, along with external stress such as infection, injury, or surgery. Although DKA is typically associated with marked hyperglycemia and dehydration, in patients...
treated with SGLT-2 inhibitors the DKA case presentations were atypical in that glucose levels were only mildly elevated to less than 11 mmol/L in some reports. This uncommon form of DKA is also known as euglycemic DKA (6). However, besides atypical glucose levels, those patients had other typical symptoms of DKA: high anion-gap metabolic acidosis accompanied by elevated blood or urine ketones, difficulty breathing, nausea, vomiting, abdominal pain, confusion, and unusual fatigue and sleepiness. In all cases, hospitalization was needed to treat the episode. A temporal association with SGLT2-inhibitor initiation was noted in all cases and median time to onset of symptoms after initiation of drug therapy was 2 weeks (1-175 days). The potential DKA-triggering factors identified in some cases included acute illness changes such as infection (urinary tract infection, gastroenteritis, influenza, trauma), urosepsis, reduced caloric or fluid intake, reduced insulin dose, hypovolemia, acute renal impairment, hypoxemia, reduced oral intake, and a history of alcohol use. However, in approximately half of the cases, the triggering factor for DKA was not identified.

The first case report of DKA by a SGLT-2 inhibitor ipragliflozin was described in a 32-year-old Japanese woman with Prader-Willi syndrome and diabetes mellitus (7). This patient had followed a low-carbohydrate diet with an estimated carbohydrate intake of 66 g/day, which also reduces circulating insulin levels and triggers a ketogenic metabolic state, and had been treated with a combination of a sulfonylurea, a DPP4 inhibitor, and a biguanide. However, this regimen was switched to monotherapy with the SGLT-2 inhibitor and after 13 days she developed DKA with a blood glucose level of 10.6 mmol/L and an undetectable urinary level of C-peptide.

Another group of authors have recently reported 13 episodes of DKA associated with mild hyperglycemia or normoglycemia in nine individuals treated with the SGLT-2 inhibitor canagliflozin (8). However, seven of these nine individuals had type 1 diabetes mellitus, which is not an on-label indication for this class of drugs. The remaining two individuals had type 2 diabetes and had recently undergone surgery. Hine et al. have reported two cases of euglycemic DKA in individuals diagnosed with type 2 diabetes mellitus and treated with the SGLT2 inhibitor dapagliflozin (9). However, both had undiagnosed pancreatic insufficiency: low insulin and C-peptide concentrations with negative autoantibodies. One patient had a history of pancreatitis, with subsequent pancreatic atrophy, while another underwent distal pancreatectomy and developed metabolic acidosis within 24 hours of dapagliflozin therapy introduction. In Japan, six SGLT-2 inhibitors (ipragliflozin, dapagliflozin, luseogliflozin, tofogliflozin, canagliflozin, and empagliflozin) are on the market, and as of July 2015, a total of 28 cases of DKA or ketoacidosis had been reported (6, 7). However, the higher incidence of DKA in Japanese can be explained with the fact that individuals with type 2 diabetes in East Asia tend to be leaner and their disease to be more largely attributable to β-cell insufficiency (10).

A recently published study assessed the incidence of serious adverse events of DKA among more than 17,000 type 2 diabetic patients treated with canagliflozin (11). They found that serious adverse events of DKA and related events were reported in only 12 patients. Furthermore, most patients with DKA and related events were on insulin, and had DKA-precipitating factors, including some with type 1 diabetes/latent autoimmune diabetes of adulthood (LADA). The authors concluded that DKA and related events occurred at a low frequency in the canagliflozin type 2 diabetes program. In type 1 diabetic patients treated with SGLT-2 inhibitors, DKA occurred only in patients in whom total insulin dose was reduced by over 50%, and in those with technical complications (insulin pump failure) or acute illness (gastroenteritis) (12, 13). Most important, DKA did not occur in a study that included 70 adults with type 1 diabetes treated with SGLT-2 inhibitor dapagliflozin without insulin reduction at randomization (14). In addition, in a phase 2 trial with dual SGLT-1 and SGLT-2 inhibitor sotagliflozin in type 1 diabetes, two patients experienced DKA. Each patient was managed with insulin pump therapy and in each case the investigators considered DKA to be insulin pump-related and not due to sotagliflozin (15).
The SGLT-2 inhibitors trigger multiple mechanisms that could predispose to DKA (16). When SGLT-2 inhibitors are combined with insulin, it is often necessary to decrease insulin dose to avoid hypoglycemia. However, since insulin normally suppresses lipolysis and ketogenesis, it is possible that the lower dose of insulin may be insufficient to suppress lipolysis and ketogenesis and consequently stimulate the production of free fatty acids, which are converted to ketone bodies by β-oxidation in the liver. In addition, insulin stimulates the activity of acetyl-CoA carboxylase, which produces malonyl-CoA, a potent inhibitor of carnitine palmitoyltransferase-I (CPT-I). Since CPT-I promotes the transport of fatty acids into mitochondria and increases the rate of β-oxidation, its activation via decreased circulating level of insulin promotes the production of ketone bodies (6). Furthermore, SGLT-2 receptors are expressed in pancreatic α-cells, and SGLT-2 inhibitors promote glucagon secretion, which may lead to increased hepatic ketogenesis and decreased endogenous insulin because glucagon inhibits acetyl-CoA carboxylase and thereby increases CPT-I activity in the liver. Finally, phlorizin, a nonselective inhibitor of SGLT family transporters, decreases renal clearance of ketone bodies. SGLT-2 inhibitors could act in a similar way, leading to increased ketone body levels in the blood.

Euglycemic DKA might be missed based on clinical signs, given that it is not necessarily associated with typical manifestations of DKA such as dehydration induced by marked hyperglycemia. In patients treated with SGLT-2 inhibitors, closer monitoring for ketones might be essential to prevent DKA, a potential life-threatening complication. Since insulinopenia is the most important underlying risk factor for DKA in patients treated with SGLT-2 inhibitors, accurate diagnosis of diabetes type is essential in cases where SGLT inhibition is being considered, to avoid inappropriate patient exposure to these drugs. In my opinion, if we follow guidelines and indication for SGLT-2 inhibitors and prescribe those agents only to type 2 diabetic patients with preserved beta-cell function (not type 1, LADA or patients with secondary diabetes), without kidney failure, acute illness, genitourinary infection and volume depletion, the risk of DKA will be minimal or absent. However, if we do not follow those guidelines, the risk of DKA with SGLT-2 inhibitors will be the reality as described in previous case reports.
REFERENCES


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