Effects of cholesterol-reducing therapy and white wine consumption in patients with metabolic syndrome

PhD Thesis

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1. Introduction

Metabolic syndrome involves a group of cardiovascular risk factors. In addition to the impact of the individual risk factors, the complex of visceral obesity, impaired glucose metabolism, dyslipidemia and hypertension – i.e. the metabolic syndrome – increases the risk of type 2 diabetes mellitus and cardiovascular diseases to a significant extent.

Non-alcoholic fatty liver is one of the most prevalent chronic hepatic diseases worldwide. Results published so far have shown a relationship between this disease and metabolic syndrome, disorders of lipid metabolism and increased cardiovascular risk.

Moderate alcohol consumption is associated with a reduced risk of cardiovascular events. It may exert this effect through different mechanisms.

2. Objectives

Our research was aimed at *observing the prevalence* of Hungarian patients with metabolic syndrome and an elevated CV risk, as well as *examining the effects* related to the white wine consumption of individuals with metabolic syndrome. A further objective was to study the effects of cholesterol-reducing therapy in patients with NAFLD that also represents an elevated CV risk.

A. What is the prevalence of metabolic syndrome in Hungarian patients?

1. Whether the use of IDF or ATP III guidelines as a base results in diagnosing more patients with metabolic syndrome?
2. Which anomaly of metabolism is the most prevalent in Hungarian patients with metabolic syndrome?
3. Regarding the prevalence of metabolic syndrome, how is ranked Hungary in comparison to certain countries?
4. As for the prevalence of individual components of metabolic syndrome, which are the differences between Hungary and certain countries?

B. How safe and effective is ezetimibe/simvastatin combined therapy and simvastatin monotherapy in patients with NAFLD?

1. Do transaminase levels show a change during combined or monotherapy?
   If yes, is there a difference between the two treatment groups?
2. To what extent show serum lipid levels (cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride) changes in the two treatment groups? Is there any difference between the two treatment groups?
3. Does any other side effect (e.g. elevated creatine kinase) occur in the two treatment groups? If yes, is there a difference between the two treatment groups?

C. What metabolic or other effects are associated with the moderate consumption of the white wines 'rizlingszilváni' and 'pintes' in patients with metabolic syndrome?

1. Is there a change in the grade of insulin sensitivity (HOMA-IR) of patients with metabolic syndrome due to the effect of moderate white wine consumption?
2. Do the levels of parameters related to insulin sensitivity (TNF-α, CRP, IL-6, EGF, VEGF, and reductive capacity) show any change during our study?
3. Is there any change in lipid levels (cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride) due to the effect of white wine consumption?
4. Do the other parameters (BMI, abdominal circumference, blood glucose levels, systolic and diastolic blood pressure) show any change in the white wine-consuming groups?
5. Is there any change between the two white wine-consuming groups in relation to the effects exerted by the wines on the studied parameters?

3. Patients and Methods

3.1 Examination of the prevalence of metabolic syndrome in Hungary, based on the ATP III and IDF systems of criteria

A total of 13,383 adult Hungarian inhabitants (6,322 men and 7,061 women) were assessed from November to May of 2004. Their age varied from 20 to 90 year (main: 59.4 ± 12.5 year). A total of 114 family doctors participated in the study from different places of the country. *Exclusion* criteria were: hepatic and renal diseases, hematologic or neoplastic diseases, hormone therapy, and pregnancy.

Inclusion was based on the ATP III and IDF criteria for metabolic syndrome. Margins of error for measurements of body mass and body height were 0.1 kg and 0.1 cm respectively. Body mass index (BMI) was calculated by

dividing the body mass in meter by the square of body weight in kilogram (kg/m^2). The mean values of successive blood pressure measurements were taken as a base in the study.

Blood samples were taken from the patients after a fasting period of 12 hours. Samples were stored at 4°C and processed within 24 hours. Blood glucose levels were determined with glucose oxidase method. Total cholesterol and triglyceride levels were measured by enzymatic methods.

Mean values and distributions were obtained from weighed data. Ages are given in mean and standard deviation (SD) values.

3.2 Efficacy and safety of ezetimibe/simvastatin combined therapy and simvastatin monotherapy in patients with non-alcoholic fatty liver disease

In a retrospective study, we summarized the clinical and epidemiological data of 45 patients with a controlled type 2 diabetes and NAFLD. Participants had metabolic syndrome based on the ATP III system of criteria.

The diagnosis of NAFLD was based on hepatic steatosis described by abdominal ultrasonography; ALT and AST values were also taken into consideration for an inclusion. For male patients alanine aminotransferase (ALT) was >40 U/L, and aspartate aminotransferase (AST) was >37 U/L; for female patients both ALT and AST values were >31 U/L.

All patients with any other liver disease (hepatitis B and C, hepatic impairment due to alcohol consumption [>20 g/day] or induced by drugs) were *excluded*.

In addition, patients with known cardiovascular diseases; uncontrolled type 2 diabetes (HbA1c [hemoglobin A1c] >7%); type 1 diabetes, thyroid disease (abnormal TSH [thyroid-stimulating hormone] value and/or treated thyroid disease) or renal disease (GFR [glomerular filtration rate] <60 mL/min/1.73m²) were also *excluded*.

Patients had hypercholesterolemia in all cases, diagnosed and treated at the Outpatient Clinics of Diabetology in the Health Center of Budaörs. When serum levels of LDL-cholesterol were >2.6 mmol/L, the patient was regarded as having dyslipidemia.

Patients made no change in their lifestyle during the study. Also their antidiabetic and antihypertensive therapy showed no change during the studied period.

We assessed the following parameters before and after the treatment: serum ALT, AST, creatine kinase (CK), and serum lipid (cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol) levels, body mass, BMI, abdominal circumference, and HbA1c value. HbA1c values are given in %, according to the DCCT/NGSP (Diabetes Control and Complications Trial/National Glycohemoglobin Standardization Program).

A part of patients (n = 26) received simvastatin (20mg daily), while the other part (n = 19) received ezetimibe/simvastatin (10/10mg daily) for 6 months.

Blood samples were taken in a fasting state in the morning. Plasma glucose levels were determined with hexokinase method (Beckman Coulter, Olympus Diagnostica 2700, Hungary). AST and ALT levels were measured by IFCC reference method, and GGT levels were measured with kinetic colorimetric method (Diagnosticum, Olympus Diagnostica 2700, Hungary). Measurements of serum

total, HDL-, LDL-cholesterol and triglyceride were performed in an enzymatic way with Olympus AU640 Clinical Chemistry Analyzer (Diagnosticum, Olympus Diagnostica 2700, Hungary).
Descriptive statistical methods were used for continuous variables; given as mean ± SD and sample size. One-way ANOVA was used for comparing the treatment groups. Tukey's test was used for post-hoc analysis. Differences between two groups were examined by Student's test or nonparametric Wilcoxon test. Values of $p<0.05$ were considered as significant.

3.3 Effects of moderate white wine consumption on insulin sensitivity in patients with metabolic syndrome

A total of 32 individuals with metabolic syndrome took part in our prospective, double-blind study. They included 26 men (28–72-year-old) and 6 women (31–67-year-old), chosen from the central data base of the Health Center of Budaörs. Metabolic syndrome was diagnosed on the base of ATP III definition.
Exclusion criteria for our clinical study were: uncontrolled type 2 diabetes mellitus (HbA1c >7%); uncontrolled hypertension (systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg); type 1 diabetes mellitus; thyroid disease (abnormal TSH level or treated thyroid disease); liver disease (transaminase levels ≥3x upper limit of normal), kidney disease (GFR<60 mL/min/1.73m²).
Patients made no changes in their lifestyle or medicine-taking habits during the studied period.

Participants of the prospective study were randomized to consume either Rizlingszilváni (n=14) or Pintes (n=18) for 4 weeks. The Hungarian wines originated from the Veress winery; the vintages of Rizlingszilváni and Pintes were 2007 and 2008 respectively. The alcohol content of the two white wines was identical, namely each 100 mL corresponded to approx. 10 g of alcohol. Men consumed 300 mL (30g) daily, and women consumed 200 mL (20g) daily. Blood samples were taken in the morning, after 12 hours of fasting. Plasma glucose levels were determined with hexokinase method (Beckman Coulter, Olympus Diagnostica 2700, Hungary). Plasma insulin levels were measured with luminescent enhanced enzymatic immunoassay method (Diagnosticum, Immulite) and chromatography (Diagnosticum, Adams Hungary). AST and ALT levels were measured by IFCC reference method, and GGT levels were measured with kinetic colorimetric method (Diagnosticum, Olympus Diagnostica 2700, Hungary). Measurements of serum total, HDL-, LDL-cholesterol and triglyceride were performed in an enzymatic way with Olympus AU640 Clinical Chemistry Analyzer (Diagnosticum, Olympus Diagnostica 2700, Hungary). Plasma CRP concentrations were determined with turbidimetry (Beckman Coulter, Olympus Diagnostica 2700, Hungary).

HOMA-IR was calculated with fasting blood glucose levels and plasma insulin levels using the following formula: HOMA-IR=fasting glucose (mmol/L) x insulin (μU/L)/22.5.

Levels of IL-6, TNF-α, vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF) were

measured with radioimmunoassay (Evidence Biochip Array Analyser, Randox).

When determining plasma reducing capacity, we examined the patients' redox homeostasis. Plasma reducing capacity was determined according to Oyaizu. Reducing capacity was given ascorbic acid equivalent (eqAS). The reducing power of a sample with a unit volume (1mL) is one ascorbic acid equivalent when its effect is equivalent with that of 1μmol ascorbic acid.

As the patients made no changes in their lifestyle and medicine-taking habits, thus the significant changes can be attributed to the effects of wine consumption. Statistical analyses were performed for the whole group of patients. A significant level of difference was determined with the use of Student's two-sample t-test, two-sample Kolmogorov-Smirnov test and Wilcoxon signed-rank test at a 5% level of significance for all tests.

4. Results

4.1 Examination of the prevalence of metabolic syndrome in Hungary, based on the ATP III and IDF systems of criteria

The prevalence of metabolic syndrome showed a rise after the age of 50 years in both men and women, according to both systems of criteria (***Table 1*****).** While among men more patients with metabolic syndrome were detected on the base of the IDF definition, among women more such patients were found according to the ATP III.

Table 1: **Prevalence of the metabolic syndrome (%) based on the ATP III and the IDF systems of criteria**

ATP III – Adult Treatment Panel III; IDF – International Diabetes Federation

Age	Metabolic syndrome	
	ATP III	IDF
men	%	%
20-29	5.8	2.6
30-39	16.2	8.6
40-49	22.6	14.7
50-59	30.3	20.4
60-69	16.2	12.0
≥ 70	8.6	41.3
all men	6.7	14.9
women	%	%
20-29	0.0	4.0
30-39	3.5	4.0
40-49	12.6	18.1
50-59	24.9	31.6
60-69	46.0	24.7
≥ 70	12.8	17.2
all women	9.8	8.6
total	8.3	11.5

Obesity, hypertension and hypertriglyceridemia were the three most frequent components of the metabolic syndrome according to both the ATP III and the IDF criteria.

4.2 Efficacy and safety of ezetimibe/simvastatin combined therapy and simvastatin monotherapy in patients with non-alcoholic fatty liver disease

Neither group showed any significant change of BMI, abdominal circumference, CK and HbA1c levels upon therapy. Significant reductions were seen in ALT ($p<0.0001$), AST ($p<0.0001$), cholesterol ($p<0.0001$), and LDL-cholesterol ($p<0.0001$) levels after 6 months of ezetimibe/simvastatin treatment. Triglyceride levels also decreased significantly ($p<0.0001$), while HDL-cholesterol levels increased to a significant extent ($p<0.0001$) upon the combined therapy. Similar results were obtained after 6 months of simvastatin treatment. ALT ($p<0.0001$) and AST ($p<0.0001$) values also decreased significantly. Upon the monotherapy, cholesterol ($p<0.0001$), LDL-cholesterol ($p<0.0001$), and triglyceride ($p<0.0001$) concentrations decreased significantly as well. HDL-cholesterol concentrations showed a significant increase ($p<0.0001$) in comparison to the baseline.

When comparing the results of the two therapeutic groups, we found that simvastatin monotherapy reduced ALT ($p<0.0112$) and AST ($p\leq0.0001$) levels to a significantly higher extent. There were no relevant differences between the results of the two therapeutic groups regarding to the cholesterol ($p=0.2134$), triglyceride ($p=0.4671$), and HDL-cholesterol ($p=0.1029$) levels after the 6 months of

treatment. LDL-cholesterol levels decreased to a significantly higher extent (p=0.0063) after ezetinibe/simvastatin therapy as compared to the monotherapy.

4.3 Effects of moderate white wine consumption on insulin sensitivity in patients with metabolic syndrome

No relevant difference was found in BMI, waist girth, systolic and diastolic blood pressure when comparing their values before and after the regular consumption of white wines ‘rizlingszilváni’ and ‘pintes’. There were no relevant differences in HDL-cholesterol and triglyceride levels between baseline and post-consumption values, in both groups alike.

In patients who consumed rizlingszilváni, levels of both total cholesterol (p=0.006) and LDL-cholesterol (p=0.04) showed a significant decrease; however no significant changes were found in these parameters (p=0.25 and p=0.5) in the group which consumed pintes. CRP levels increased to a slight degree in those who consumed rizlingszilváni; contrarily it decreased significantly in the pintes group. TNF-α levels showed a moderate rise in the rizlingszilváni group, however it decreased moderately in the pintes group. IL-6 decreased to a non-significant extent in both groups, and VEGF levels showed no relevant change. EGF increased significantly in both groups of patients with metabolic syndrome. Plasma reducing capacity increased significantly upon the consumption of both sorts of wine.

Fasting blood glucose values showed no relevant change in either group. On the contrary, fasting insulin levels decreased significantly in both groups. HOMA-IR values

decreased significantly after the consumption of both wines (in the rizlingszilváni group: 1.85 ± 2.1 vs. 1.06 ± 0.6; p=0.03; in the pintes group: 2.28 ± 2.04 vs. 1.08 ± 0.6; p=0.002) as compared to the baseline (***figure 1***).

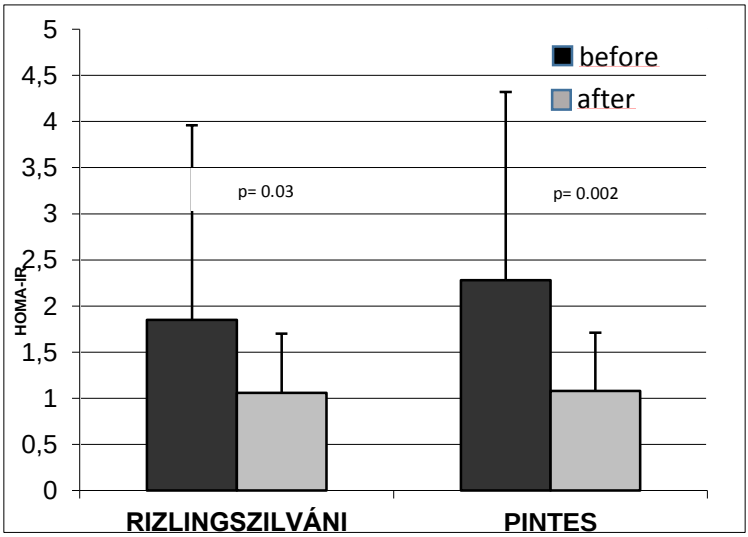

Figure 1: **Changes of HOMA-IR upon the consumption of the two sorts of wine** (level of significance: p=0.05)

5. Conclusions

5.1 When studying the prevalence of the metabolic syndrome in Hungary, we detected more patients with metabolic syndrome among men based on the IDF guideline, while we found more such patients among women according to the ATP III

recommendations. In our country, obesity (abnormal abdominal circumference/waist girth) was the most frequent abnormity in comparison to the other components of the metabolic syndrome.

5.2 Based on the result of our study, obtained according to the ATP III system of criteria; the prevalence of metabolic syndrome has proven to be low in comparison to some countries. Hungarian men and women have a leading place in relation to elevated blood glucose levels, obesity and hypercholesterolemia as well.

5.3 When our study was published, in 2006, very few data were yet available in relation to the prevalence of metabolic syndrome in the Central and Eastern European countries. Our study was among the first ones in Hungary and it was one of the largest assessments in this region.

5.4 In our retrospective study in type 2 diabetic patients with NAFLD and hypercholesterolemia, hepatic transaminase concentrations showed a significant decrease upon ezetimibe/simvastatin combined therapy and simvastatin monotherapy. When comparing the two therapeutic groups, transaminase levels decreased to a significantly greater extent during simvastatin therapy.

5.5 Serum lipid levels decreased significantly on both combined and monotherapy. LDL-cholesterol levels decreased to a significantly greater extent upon ezetimibe/simvastatin combined therapy as compared to simvastatin therapy.

5.6 Our study confirmed that simvastatin and ezetimibe therapy is safe and effective in type 2 diabetic patients with NAFLD and hypercholesterolemia.

5.7 As for the consumption of white wines rizlingszilváni and pintes, we found no relevant difference between BMI, abdominal circumference, systolic and diastolic blood pressure values obtained before and after the regular consumption of the wines.

5.8 There were no relevant differences between HDL-cholesterol and triglyceride concentrations measured at baseline and after wine consumption. In patients who consumed rizlingszilváni, levels of both total cholesterol and LDL-cholesterol showed a significant decrease; however no significant changes were found in these parameters in the group which consumed pintes.

5.9 GGT levels showed a slight decrease in both groups; ALT and AST concentrations, however, decreased significantly.

5.10 Plasma reducing capacity increased to a significant degree upon the consumption of both sorts of wine.

5.11 Fasting blood glucose values showed no relevant change in either group. Contrarily, fasting insulin levels decreased moderately in the rizlingszilváni group, and the change showed an already significant decrease in the pintes group.

5.12 In our study we demonstrated firstly that the moderate consumption of Hungarian white wines (pintes, rizlingszilváni) increases the insulin sensitivity in patients with metabolic syndrome. The decrease of CRP and IL-6 levels as well as the increase of EGF levels observed in our study may also play a role in the mechanism of this process.

6. List of the own publications

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