The Short-term Influence of a Mediterranean-type Diet and Mild Exercise with and without Red Wine on Patients with the Metabolic Syndrome

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The metabolic syndrome is a target for the dietary prevention of cardiovascular disease. The effect of adding red wine to the diet has not been fully investigated. This study examined whether a Mediterranean-type diet complemented with red wine and mild exercise had an impact on patients with the metabolic syndrome in the short term. Twelve patients with the metabolic syndrome consumed a Mediterranean-type diet for four weeks without and with red wine respectively and performed mild exercise. We implemented the diagnostic criteria for the metabolic syndrome as formulated by the Adult Treatment Panel III (ATP III) in 2001. The patients were also screened for multiple genetic markers implicated in cardiovascular disease. Weight, body mass index, abdominal circumference and blood pressure were measured, as well as various biochemical, haematological and inflammatory markers. There was a significant decrease in the body weight (p = 0.04) and an increase in ORAC value (p = 0.035) after the dietary intervention. A significant decrease in systolic blood pressure (p = 0.045) was observed. Red wine had no additional benefits. Although diet reduced weight and blood pressure, the lipoprotein and pro-coagulant profiles of patients with the metabolic syndrome were not affected in this study. These findings may be explained partly by the diverse genetic profile identified among the study participants, as 50% had mutations involved in lipid metabolism that may influence the response to dietary intervention and alcohol consumption.

INTRODUCTION

The metabolic syndrome is an important cluster of coronary heart disease risk factors linked to insulin resistance. It has been estimated that approximately 24% of American adults have the syndrome, and there is reason to believe that there is a worldwide increase in the incidence and prevalence of this atherogenic phenotype (Ford et al., 2002). People with the metabolic syndrome are at increased risk for developing diabetes mellitus (Haffner et al., 1992) and cardiovascular disease (CVD) (Isomaa et al., 2001), and face an increased risk of mortality from CVD and all causes (Trevisan et al., 1998). A working definition of this syndrome was formulated by the recently released Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (National Institutes of Health, 2001).

The hallmarks of the metabolic syndrome include increased body weight and waist circumference (central and abdominal obesity), elevated plasma glucose, an atherogenic lipoprotein profile (high blood TG and small, dense LDL-C levels and low blood levels of HDL-C), and raised blood pressure.

Therapeutic lifestyle changes have been shown to reverse the pathophysiology of the metabolic syndrome, improve biomarkers of the risk factors and reduce the prevalence of the associated cardiovascular risk (Esposito et al., 2004). Mediterranean-type dietary changes combined with mild exercise and weight loss are key therapeutic objectives in the prevention and treatment of the metabolic syndrome. Modest weight reduction has been associated with clinically significant improvements in diabetes-associated mortality (Heilbronn et al., 1999), hypertension (Tuck et al., 1981), lipid abnormalities (Dattilo and Kris-Etherton, 1992), and glycaemic control.

It is well documented that dietary changes comprising increased servings of fruits, vegetables and high-fibre whole grains, legumes, walnuts, olive oil and fish, and a moderate alcohol intake, are inversely correlated with coronary heart disease, whereas foods of animal origin are directly correlated (Menotti et al., 1999). Populations consuming a Mediterranean diet (MD) had the lowest mortality among the populations of seven countries included in the study of Menotti et al. (1999).

The Lyon Diet Heart Study convincingly demonstrated that the Mediterranean α-linolenic acid-rich diet achieved greater reduc-
tions in the risk of all-cause mortality and coronary heart disease mortality in a secondary prevention trial than any of the cholesterol-lowering studies to date (De Logeril et al., 1999).

Epidemiologic studies have consistently shown an inverse correlation between HDL-C levels and the risk of cardiovascular disease (Brewer, 2004; Alsheikh-Ali et al., 2005). In the past decade, high-density lipoproteins (HDL) have emerged as a new potential therapeutic target for the treatment of CVD. The key role of HDL-C is that of a carrier of excess cellular cholesterol in the reverse cholesterol transport pathway, a process by which free cholesterol is removed from peripheral tissue, including blood vessels, for transfer back to the liver and, ultimately, excretion in the bile. HDL-C and Apo-A1 have also been shown to protect LDL particles against oxidative modification and reverse the inhibitory effects of oxidised LDL on endothelial function (Mackness et al., 1993; Naval et al., 2002). HDL-C may also slow the progression of lesions by selectively decreasing the production of endothelial cell-adhesion molecules that facilitate the uptake of cells into the vessel wall (Barter et al., 2002). Other studies have also demonstrated direct antioxidant, anti-inflammatory, anti-thrombotic and pro-fibrinolytic effects of HDL (Shah et al., 2001; Kuvin and Karas, 2003).

Aerobic exercise and weight loss increase HDL-C (O’Connor et al., 1995). Aerobic exercise and weight loss have also been demonstrated to improve insulin sensitivity and lower blood pressure, thus correcting many abnormalities associated with the insulin resistance syndrome (Hunter and Garvey, 1998; Despres, 2005). Recent studies demonstrate that dietary modification and enhanced physical activity (Schoeller et al., 1997) may delay or prevent the transition from impaired glucose tolerance to type 2 diabetes mellitus (Goodpaster et al., 2003). It is well documented that low cardio-respiratory fitness is an important risk marker for the metabolic syndrome, and an increase in physical activity/exercise is essential to reverse the abnormal metabolic changes in this atherogenic phenotype. It has also been demonstrated that, provided the stimulus is adequate, regular physical activity and endurance training can promote body fat loss and a mobilisation of abdominal and visceral adipose tissue, increase muscle mass and insulin sensitivity, and reverse the atherogenic lipoprotein profile, as well as other features of the metabolic syndrome, including inflammation (Despres et al., 2001).

Moderate alcohol consumption may have beneficial effects by decreasing insulin resistance, increasing HDL-C (Gaziano et al., 1993) and reducing platelet aggregation (Richer et al., 1997). Ethanol in the diet reduces the risk of coronary heart disease and diabetes. In healthy adults, ethanol causes hypoglycaemia through the inhibition of gluconeogenesis and glycogen catabolism. Ethanol also augments insulin release, similar to the sulfonylurea drugs, leading to a transient hyperinsulinaemia, which can also serve to accelerate clearance of glucose from the blood (Bisson et al., 1995). It has been demonstrated that alcohol consumption is associated with lower levels of inflammation, as measured by C-reactive protein, WBC, fibrinogen, VIIc and albumin (Mukamal et al., 2004).

**Objectives**

In this study, we aimed to evaluate the added effect of moderate red wine consumption and mild exercise with the Mediterranean-type diet on CVD risk factors in subjects with the metabolic syndrome during a two-month intervention period. The end points were observed changes in risk factors (clinical variables and biochemical markers) after one month of the diet and after one month of the diet plus wine intervention.

**MATERIALS AND METHODS**

Our study included 12 non-smoking subjects, on no medication influencing platelet aggregation and lipoprotein profile, between the ages of 32 and 60 years and with the diagnostic criteria of the metabolic syndrome. As detailed in the ATP III report (executive summary), we used the following criteria to diagnose the metabolic syndrome: abdominal obesity: waist circumference >102 cm in men and >88 cm in women; hypertriglyceridaemia: ≥1.69 mmol/L; low HDL-C: <1.0 mmol/L in men and <1.3 mmol/L in women; high blood pressure: ≥130/85 mm Hg, and high fasting glucose: ≥6.1 mmol/L (National Institutes of Health, 2001). Participants having three or more of these criteria were defined as having the metabolic syndrome.

The participants consumed a Mediterranean-type diet for four weeks without and with red wine respectively. During the experimental periods, the subjects increased their intake of vegetables, cereals, fruit, mono-unsaturated fatty acids and fish at the expense of red meat and dairy products (Figure 1). The dietary guidelines for the Mediterranean-type diet included the following: wholegrain/brown bread and whole-wheat cereal products should preferably be consumed; fish should be included in the diet at least twice per week and at least one portion should be fatty fish; chicken without skin can be included in the diet; not more than two portions of red meat (2 x 150 g for men and 2 x 120 g for women, cooked weight) should be consumed per week; no more than 60 g hard cheese (e.g. Cheddar) should be consumed per week; canola or olive oil should be used for food preparation, within the fat allowance; canola or olive oil margarine should be used as bread spread, and red wine consumption should be 250 mL for men and 180 mL for women per day. We supplied the candidates with a variety of good quality South African red wines with a mild antioxidant potential.

Dietary information was collected by means of three-day weighed and estimated dietary records (including one weekend day), covering the last three days of the baseline period, and during the third week of the different experimental periods. Subjects received verbal and written instructions before the start of the study on record keeping and estimating and weighing food portions. Dietary records were checked after three days by a registered dietician for completeness and for compliance with the dietary prescriptions. The National Research Institute for Nutritional Diseases (NRIND) Food Composition Tables (Gouws and Langenhoven, 1986) were used for encoding the type of food eaten and, if the food was not weighed, the NRIND Food Composition Tables (Gouws et al., 2001) were used to convert the amount of food reported in household measures into grams of food eaten. Dietary data were analysed for energy, macronutrients, cholesterol and fatty acid intake.

The subjects were motivated to include 20 to 30 min of mild exercise (brisk walking) in their daily routine. For practical reasons, it was not possible to monitor the exercise sessions of each individual, and we had to rely on their cooperation in this regard.

During a two-week washout period prior to the study, our subjects were instructed to consume their habitual diet with no alco-
holic beverages and to take no medications that may influence the platelet aggregation and lipoprotein profile. After the subjects had fasted overnight, plasma lipid concentrations, platelet aggregation, fasting glucose and insulin levels, uric acid and vascular inflammatory marker concentrations were determined at baseline, after four weeks of diet and after four weeks of diet plus wine, as well as body weight, abdominal circumference and blood pressure.

To determine the atherogenic lipoprotein profile associated with the metabolic syndrome, various biochemical and inflammatory parameters were determined in all 12 subjects. Fasting blood samples were analysed for TC, TG, HDLc, LDLc, TC/HDL ratio and uric acid. Insulin resistance was measured by determining fasting blood glucose and insulin concentrations, and determining the glucose/insulin ratio. Hypersensitive C-reactive protein (hs-CRP), FVIII, fibrinogen and vWF were measured to determine vascular inflammation as a marker of atherogenesis. The changes in the pro-coagulants and anti-coagulants in our participants during the experimental period were measured by determining the platelet count, platelet coagulation, platelet membrane fluidity, plasma ThBD, tPA, PAI-1 (fibrinolytic markers), and FVII (affected by dietary triglycerides). The samples were analysed by PathCare Laboratories, Cape Town, with a Beckman Coulter LX 20 analyser.

To measure the antioxidant effect of the diet and wine interventions respectively, the oxygen radical absorbance capacity (ORAC) (Ou et al., 2001) was determined on the plasma of all the subjects at baseline, after the four-week period on the diet without wine, and after the four-week period on the diet with red wine.

A genetic screen for genetic markers that can be triggered by lifestyle risk factors such as smoking, physical inactivity or unhealthy food choices that manifest as CVD was carried out on all the participants to support the clinical diagnosis (Kotze and Badenhorst, 2005). The genetic profile was also built to determine the polymorphisms of genes involved in lipoprotein metabolism or atherosclerosis and to search for interactions between gene polymorphisms and response to diet and alcohol. A risk assessment, linked to nutrition and lifestyle, which targeted the interaction between genes and the environment was done for each participant. By combining genetic testing for both monogenic and multi-factorial conditions, it is possible to distinguish between patients with familial hypercholesterolemia (FH), which requires lifelong drug treatment, and those at increased CVD risk as a consequence of other risk factors that can be managed effectively by dietary and other lifestyle modifications. (Kotze and Badenhorst, 2005). Genetic testing was performed as previously described, using a reverse-hybridisation strip-assay technique (Kotze et al., 2003). This technique involves (1) DNA isolation from anticoagulated blood, (2) amplification of relevant gene sequences and terminal labelling with a reporter molecule, and (3) selective hybridisation of the amplification products to allele-specific (wild-type or mutant) oligonucleotide probes in separate cavities of a micro-well plate and detection by immunoreaction. The FH strip-assay (Kotze et al., 2003) was used in combination with a multi-gene CVD assay (Kotze et al., 2003), which includes 12 functional genetic changes in 10 different genes. These include mutation 10708G→A in the apolipoprotein B (ApoB) gene, mutations 4075C→T (E2 allele) and 3937T→C (E4 allele) in the apolipoprotein E (Apo E) gene, 677C→T and 1298A→C in the methylenetetrahydrofolate reductase (MTHFR) gene, 20210G→A in the factor II (prothrombin) gene, 1691G→A in the factor V gene, 103G→T in the factor XIII gene, 455G→A in the β-fibrinogen gene, 1565T→C in the glycoprotein IIb/IIIa (GP IIb/IIIa) gene, the 4G/5G polymorphism in the plasminogen activator inhibitor-1 (PAI-1) gene, and mutation 845G→A in the HFE gene involved in iron overload. The 4G/5G polymorphism in the plasminogen activator inhibitor (PAI-1) gene is also associated with metabolic syndrome, insulin resistance and type II diabetes, and protein levels are increased by emotional or psychosocial stress and inflammation.

For statistical analysis of the data, repeated measures of analysis of variance were used. In all cases, normal probability plots of the residuals were inspected for possible outliers. A 5% significance level was used as guideline for determining significant differences.

RESULTS
In this study, statistically significant changes were found in the anthropometric parameters relating to the measurement of body weight (Figure 2) and systolic blood pressure. Body weight decreased significantly (p = 0.04), and systolic blood pressure (Figure 3) decreased (p = 0.045) during the experimental period. A trend for the abdominal circumference to decrease (p = 0.059) was observed.

Participants demonstrated a statistically significant weight loss over the two-month intervention period. The error bars on the graph indicate 95% confidence intervals for the estimated means.
There was a statistically significant increase in the ORAC (Figure 4) after the four-week diet intervention period, from 7.5 ± 1.4 mmol/L trolox equivalents (TE) to 9.3 ± 2.5 mmol/L TE, p = 0.035. The ORAC decreased when wine was added to the diet (to 8.5 ± 1.1 mmol/L TE, but this was not statistically significant (p = 0.342), and the increase in the ORAC was still significantly different from baseline levels (p = 0.04) (Table 1).

The atherogenic and prothrombotic serum lipoprotein profiles, uric acid, insulin and fasting glucose did not change significantly during the study period, nor did the inflammatory markers compared to baseline values. The addition of red wine to the Mediterranean-type diet did not add any beneficial or detrimental changes to the measured biochemical markers of the metabolic syndrome.

The genetic assessment demonstrated different combinations of genetic risk factors that may interact with diet and lifestyle factors, unique to each individual tested. All 12 study participants had one or more genetic risk factors involved in lipid metabolism, folate metabolism (high homocysteine levels), haemostasis or insulin resistance. Notably, two subjects included in the study have FH and four tested positive for the E4 allele of the apolipoprotein E (Apo E) polymorphism that interacts with smoking, alcohol and dietary factors to increase the risk of CVD. Detection of both the factor V Leiden (1691G→A) and prothrombin mutations (20210G→A) in one individual was of special concern, as the combined effect of these two mutations is associated with a 40-fold increased risk of venous thrombosis. The risk is further increased in the presence of obesity, which was noted in the subject, and the low physical activity reported.

DISCUSSION

The Mediterranean-type diet had a protective effect against the metabolic syndrome, by significantly lowering the body weight and systolic blood pressure after the diet and the diet with wine period. The Mediterranean diet influenced the antioxidant potential of the serum, whereas red wine had no significant additional in vivo antioxidant effects.

In a pilot study on healthy subjects, we found that an intervention with the Mediterranean-type diet and red wine could influence the impaired response to the physiological effects of insulin, including those on glucose and lipid metabolism. In these subjects there was a statistically significant weight loss, an increase in HDL-C and a reduction in blood glucose levels during the diet and wine intervention period.

In contrast to these findings, we found no significant statistical differences in any of the biochemical parameters that were measured in our subjects during the short intervention study period. Our subjects with the features of the metabolic syndrome, who were at high global risk for CVD (hypertension, hyperglycaemia, atherogenic dyslipidemia, abdominal obesity and physical inactivity), were remarkably resistant to changes in the biochemical parameters. We can speculate that the biochemical abnormalities of patients with the metabolic syndrome may need a longer intervention period to change (Esposito et al., 2004) All the participants in this study demonstrated features of insulin resistance, which could also contribute to the resistance to improvement in the biochemical parameters of the metabolic syndrome. The metabolic abnormalities associated with the metabolic syndrome may induce upregulation of the vascular inflammatory-thrombogenic state.
and this may be responsible for the inefficiency of the anti-thrombotic and anti-atherogenic effects of the Mediterranean-type diet and red wine intervention.

Genetic testing of our subjects demonstrated three different genotypes that may respond differently to diet and alcohol interventions. The two subjects with FH require lifelong drug therapy. The four ApoE4 allele carriers did not experience an increase in HDL-C associated with alcohol intake, and were more responsive to diet and less responsive to statin therapy than ApoE2 allele carriers (Djousse, 2004).

By combining genetic testing for both monogenic and multifactorial conditions, it was possible to distinguish between patients with FH requiring lifelong drug treatment and those at increased CVD risk as a consequence of other risk factors that can be managed effectively by dietary and other lifestyle modifications. Failure to demonstrate similar effects following dietary intervention in healthy individuals compared with the current study group of patients with the metabolic syndrome highlights the importance of genetic assessments as part of intervention programmes. Treatment requirements or therapeutic responses may differ due to the genetic background of the individual. Our findings may be explained partly by the diverse genetic profile identified among the study participants, as 50% of our cases had mutations involved in lipid metabolism that may influence the response to dietary intervention and alcohol consumption.

The limitation of this study is that we had only a small cohort of subjects, and we monitored them over only an eight-week intervention period. Because wine was not given randomly in the first or second period, it could be that the compliance in the second period was reduced in comparison to the first, which could obscure the effects of wine. Our subjects also demonstrated three different genotypes that respond differently to diet and alcohol interventions. Subjects with FH require lifelong drug therapy. ApoE4 allele carriers do not experience an increase in HDL associated with alcohol intake, are more responsive to diet and are less responsive to statin therapy than ApoE2 allele carriers (Mukamal et al., 2004).

People demonstrating the features of the metabolic syndrome are habitually physically inactive, and it is known that the clustering atherogenic and diabetogenic abnormalities of the metabolic syndrome are highly prevalent in sedentary populations. An increase in physical activity in these patients is probably the most difficult factor to deal with on the epidemiological scale. We did not accurately control the amount and intensity of the exercise prescription, and all participants probably did not adhere to our recommendations to include 20 to 30 min of moderate-intensity exercise in their daily routine.

The significant weight loss, slight decrease in abdominal circumference, and significant reduction in systolic BP are encouraging signs and support the view that the abnormal clinical features of the metabolic syndrome do respond rapidly to lifestyle interventions such as dietary modifications and mild exercise.

There was a statistically significant increase in the ORAC after the four-week diet intervention period. This may be attributed to the olive oil-based, alpha-linolenic acid-rich, Mediterranean-type diet. The ORAC decreased slightly when wine was added to the diet, but this was not statistically significant and the increase in the ORAC was still significantly different from baseline levels.

The latter finding was unexpected, because the red wines used in this project had a very high concentration of polyphenols (2.6 g gallic acid/L) on average. Wine polyphenols have proven in vitro antioxidant properties. The total antioxidant capacity of the 12 red wines used in this project was 19.02 mM TE. The high alcohol content (12 to 15%) could negate the antioxidant effects of the polyphenols, because ethanol on its own demonstrates oxidative properties, and we had a 33% Apo E4 polymorphism among our test subjects, which correlates well with the finding of Kotze et al. (2003).

A more feasible explanation of this phenomenon could be that, despite their potent in vitro actions as antioxidants, anti-inflammatory and anti-cancer agents, the effective concentrations of wine polyphenols are relatively unattainable by virtue of their poor bioavailability and/or their conjugation prior to absorption (Hollman et al., 1997). The increase in the antioxidant potential in the serum could mostly be ascribed to the high intake of fruits, grains, vegetables and extra virgin olive oil, and the decrease in red meat consumption in the Mediterranean-type diet during the intervention period, and is unrelated to the red wine intake in our study.

This research suggests that the addition of red wine to the Mediterranean-type diet does not demonstrate any additional beneficial or detrimental effects apart from the diet on its own on any of the biochemical parameters that we measured during this short intervention period. Although the antioxidant potential of the serum did increase significantly during the diet intervention, this had no sufficient influence on the inflammatory markers, insulin sensitivity or lipid profile during this short intervention period. In order to demonstrate sustainable benefits, we will have to follow the subjects over a longer period of time to observe the beneficial effects of these lifestyle interventions on the features of the metabolic syndrome.

Furthermore, because wine is arguably the healthiest form of alcoholic beverage, its moderate consumption should be considered an important component of a healthy diet and lifestyle. Moderate alcohol consumption may be a marker of a healthy lifestyle, and should therefore not be considered in isolation from other aspects of lifestyle, such as cigarette smoking, inactivity and diet. The limited epidemiological data linking wine consumption to superior health outcomes compared with drinkers of other alcoholic beverages warrants further research to identify a plausible mechanism.

**CONCLUSIONS**

This study indicates that the Mediterranean diet can beneficially influence the early clinical diagnostic criteria relating to the metabolic syndrome during a short-term intervention period. The addition of red wine to the diet demonstrated no additional health benefits or detrimental effects.

It must be acknowledged that the aetiology of coronary heart disease is multi-factorial, that risk factors have a multiplicative effect, and that physicians have to deal holistically with patients, and not with isolated risk factors. At the same time, the prevention of coronary heart disease is multifaceted, and wine cannot be seen in isolation from other preventative strategies such as exercise, healthy diet and weight loss. The influence of wine on health should therefore not be seen in isolation, but as part of a healthy lifestyle.

The molecular mechanisms through which the additional benefits of the polyphenols and other substances in wine are realised, as well as the tendency of wine to be consumed regularly, with
meals and in moderation, need to be explained by continued and focused scientific research. More investigations are needed on the bioavailability of the polyphenols in wine before any health claims relating to the non-alcoholic components of wine can be made.

A genetic screen should be done on patients with dyslipidaemia to clarify the interplay between genotype and environment in defining susceptibility to illness and determining the appropriate therapy. It will also demonstrate how the genotype varies amongst cohorts of patients, and especially the role of such variation in the causation of important illnesses and in responses to pharmaceuticals and dietary interventions. Physicians should incorporate genetic testing as an integral part of clinical and laboratory assessments to identify patients with very early evidence of reversible disease, as manifested by endothelial dysfunction, early markers of chronic inflammation, or symptoms and signs of the metabolic syndrome.

Additional investigation is needed to determine the influence of wine and alcohol on improving endothelial dysfunction, combating the damaging effects of low-grade chronic inflammation of the endothelium, and preventing the premature onset of the metabolic syndrome. The challenge will be to identify symptom-free, high-risk individuals and act to reduce their risk factor levels through lifestyle, environmental, social and economic interventions. Wine, as part of a healthy, Mediterranean-type diet and lifestyle, could play a role in this regard, but may not be beneficial for individuals with certain genetic predispositions.

**LITERATURE CITED**


