CFPA	SAAPPA / SASPA / SAT	DFTS	Winetech
Canning Fruit Producers' Assoc. Submit to: Wiehahn Victor Tel: +27 (0)21 872 1501 inmaak@mweb.co.za	Fruitgro Science Submit to: Louise Liebenberg Tel: +27 (0)21 882 8470/1 louise@fruitgro.co.za	Dried Fruit Technical Services Submit to: Dappie Smit Tel: +27 (0)21 870 2900 dappies@dtd.co.za	Submit to: Jan Booysen Tel: +27 (0)21 807 3324 booysenj@winetech.co.za

			X

Indicate (X) client(s) to whom this final report is submitted.
Replace any of these with other relevant clients if required.

FINAL REPORT
FOR 2011
PROGRAMME & PROJECT LEADER INFORMATION

	Programme leader	Project leader
Title, initials, surname	Dr DP van Velden	Prof MJ Kotze
Present position	Senior Lecturer	Senior Lecturer & Researcher
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PROJECT INFORMATION

Project number	N09/08/225
Project title	The effect of regular alcohol consumption interacting with genetic risk factors for cardiovascular
Project Keywords	Wine Brandy Cardiovascular health

Industry programme	**CFPA**	
	Deciduous	
	DFTS	
	Winetech	X
	Other	

Fruit kind(s)	Grapes
Start date (dd/mm/yyyy)	01/01/2009
End date (dd/mm/yyyy)	31/12/2011

(Note: adjust footer – insert the project number no, researcher and research institution)

FINAL REPORT

1. Executive summary

In this research project the effect of regular alcohol consumption in the form of red wine or brandy was investigated on the lipoprotein profile, oxidative stress status and inflammatory markers in 37 volunteers. We assessed the physiological effects in relation to genetic risk factors for cardiovascular disease. Nutrition intervention may be more effective to lower cardiovascular risk when the genetic background is taken into consideration for identification of gene-environment interactions.

ORIGINALITY OF THE STUDY

The effect of regular moderate alcohol consumption – wine vs brandy - on the lipoprotein profile, oxidative stress and inflammatory markers was studied for the first time in the South African population in the context of genetic risk factors known to interact with alcohol. It is envisaged that this study will lead to the development of guidelines for safer drinking practices in the local population. It is important to determine whether moderate intake of wine and brandy have similar cardiovascular protective properties. Ultimately, safe limits of wine and brandy consumption will be determined based partly on the genetic profile and other lifestyle factors studied.

2. Problem identification and objectives

The specific aims were as follows:

1. To compare the effect of moderate red wine consumption compared to brandy on the atherogenic lipoprotein profile, oxidative stress and inflammatory status.
2. To perform a genetic screen of CVD risk factors to determine individual mutation/allele frequencies in the study population
3. To correlate the presence of genetic risk factors with relevant biochemical parameters and assess gene expression in the presence and absence of known environmental triggers
4. To determine the impact of mutations/functional polymorphisms included in the genetic screen on the response to the intervention (small sample size may be a limiting factor)

3. Workplan (materials & methods)

We recruited 37 healthy adult volunteers between the ages of 18 and 70 years with or without cardiovascular risk factors that fulfilled the inclusion criteria. All specimens and data were obtained with informed consent and are kept strictly confidential.

Blood pressure, body weight, height as well as waist and hip circumferences were measured following standardized procedures on first and follow-up visits of participants to calculate the body mass index (BMI) and the waist/hip ratio (WHR). No medication was allowed at any time during the whole study period.

Study design

After a 2 week washout period, 50% of the participants consumed 250 / 175 ml red wine per day x 28 days, and 50% consumed 50 / 40 ml per day brandy x 28 days respectively for men

and women. This intervention was followed immediately by a cross-over period of wine or brandy consumption for 28 days.

Wine consumption per day

Males: 250ml @ 13.5% alcohol = 33.75 g alcohol per day (13.5x 2.5 = 33.75g)
Females 175 ml @ 13.5% alcohol = 23.63 g alcohol per day (13.5x1.75 = 23.63g)
(or 0.35g alcohol/kg body mass for males and females)

Brandy consumption per day

Males: 50 ml @ 43% alcohol = 21.5 g alcohol per day (43 x 0.5 = 21.5 g)
Females: 40 ml @ 43% alcohol = 17.2 g alcohol per day (43 x 0.4 = 17.2 g)
(or 0.35g alcohol/kg body mass for males and females)

Fasting blood specimens were taken from the anterior cubital vein: 50 ml per test day:

- After the 2 week washout period (Baseline)
- After the 28 day red wine/brandy consumption period
- After the next 28 days consumption of wine/brandy consumption cross-over period

Biochemical analysis of serum/plasma

Blood were obtained from an antecubital vein in the morning after a 12 hour overnight fast. Blood tests were performed *ex vivo,* i.e. the wine or brandy was consumed by volunteers undergoing certain blood tests before and after each period of wine / brandy consumption to observe the effect that wine or brandy had on the specified biological systems (plasma levels of different lipoprotein fractions, oxidative and inflammatory status).

Clinical and biochemical parameters included total cholesterol, homocysteine, serum ferritin and oxidative stress markers. Cholesterol and triglyceride levels were determined in plasma by enzymatic methods using the RA 1000 analyzer, while the high density lipoprotein (HDL) levels were obtained after precipitation of apolipoprotein B containing lipoproteins. The cholesterol and triglyceride contents of the HDL fraction were measured, while the low density lipoprotein cholesterol was calculated using the Friedewald equation. A RA 1000 automated analyzer was used. High sensitive C-reactive protein (ELISA test kit, HELICA) and homocysteine (Araki and Sako, 1987) concentrations were analyzed in the plasma of all the participants. CRP is an important immunomodulator in host defence while elevated levels of both CRP and homocysteine have been identified as risk factors for cardiovascular diseases as well.

Biomarkers for oxidative stress and inflammation included a lipid peroxidation marker, malondialdehyde (MDA) equivalents; reduced to oxidized glutathione ratios (GSH:GSSG); oxygen radical absorbance capacity (ORAC); nitric oxide metabolic products; oxidized low density lipoproteins (Ox-LDL); C-reactive protein (CRP) among others. Full lipid profiles serum liver and kidney function indicators were also done on all study participants to exclude possible toxicity induced by the consumption of the various alcoholic beverages (red wine and brandy).

Total phenolic acid content (TP) of plasma was determined using the Folin-Ciocalteau method modified by Serafini et al (1998) to avoid plasma protein interference. Plasma antioxidant capacity was determined using the Trolox equivalent antioxidant capacity (Arendt *et al*. 2001). Sub samples of the plasma were used to determine the oxygen radical absorbance capacity (ORAC) by the method of Cao and Prior (1998); the impact on the

performance of antioxidant enzymes including superoxide dismutase (Flohe and Otting, 1984), catalase (Aebi, 1984), glutathione peroxidase (Flohe and Gunzler, 1984), the ratio of reduced to oxidised glutathione (GSH:GSSG) as an independent predictor of carotid intima-media thickness (IMT) and ultimately the presence of early atherosclerosis (Ashfaq *et al.*, 2006) and the effect on lipid peroxidation by measuring thiobarbituric acid reacting substances (Esterbauer *et al.*, 1990)

Genetic Studies

Relatively common genetic variations underlying dyslipidaemia and the development of CVD in the presence of certain lifestyle risk factors were tested to identify individuals who may not benefit from drinking alcohol as a consequence of gene-environment mismatches. DNA was extracted from whole blood for mutation screening using polymerase chain reaction (PCR)-based methods.

Patients with monogenic CVD subtypes such as familial hypercholesterolaemia (FH) and familial defective apolipoprotein B100 (FDB) were excluded from the study based on the lipid profile and a strong family history of early-onset CHD. The genetic analysis included screening for mutations / functional polymorphisms the ApoE, MTHFR and HFE genes known to influence CVD risk the effect of alcohol intake, using previously described methods (Kotze et al. 2003; Kotze and Thiart 2003) with some modification to allow high-throughput analysis. Direct DNA sequencing of PCR-amplified DNA was performed to verify the results obtained with real-time TaqMan technology applied in high-throughput genotyping.

STATISTICAL ANALYSIS

The statistical analysis will be performed by Professor Martin Kidd, the chief statistician at the Centre for Statistical Consultation, University of Stellenbosch.

ETHICAL CONSIDERATIONS

Prior to this study, study participants were informed of the fact that E4 allele of the Apo E gene included in the genetic analysis does not only increase the risk of coronary heart disease in the presence of environmental risk factors such as smoking and a high-saturated fat diet, but also increase the risk of Alzheimer's disease, with a specific gene-alcohol effect documented in various studies. This has to be considered before the results of the genetic testing can be requested by the participants and/or provided by the attending clinician to participants.

4. Results and discussion

State results obtained and list any benefits to the industry. Include a short discussion if applicable to your results.
This final discussion must cover ALL accumulated results from the start of the project, but please limit it to *essential* information.

All the data obtained during the study related to the lipoprotein profile, oxidative stress, inflammatory markers and genetic risk factors have been captured in an electronic database. The statistical analysis have been performed in four phases and discussed afterwards at project meetings arranged with the statistician. Four members of the research team – Dr DP van Velden, Dr Dee Blackhurst, Prof M Marnewick, and Prof MJ Kotze attended these meetings – and provided their academic input. After the final meeting held on the 13th of March additional genetic analyses was requested that was received on the 15th of March 2012. These results will be used towards the completion of several students involved in this study.

Milestone	**Achievement**
1. Recruitment	37 participants completed the study
2. Biochemical determinations	Completed in 37 subjects
3. Genetic testing	8 SNPs performed
4. Statistical analysis	Completed

Lipid Profile

The most significant finding related to the response in the lipid profile was the effect of alcohol consumption on HDL-cholesterol (good cholesterol) levels. Both the wine and brandy (p=0.00002) intervention resulted in a significant increase in HDL-cholesterol (Figure 1), known to have a cardiovascular protective effect.

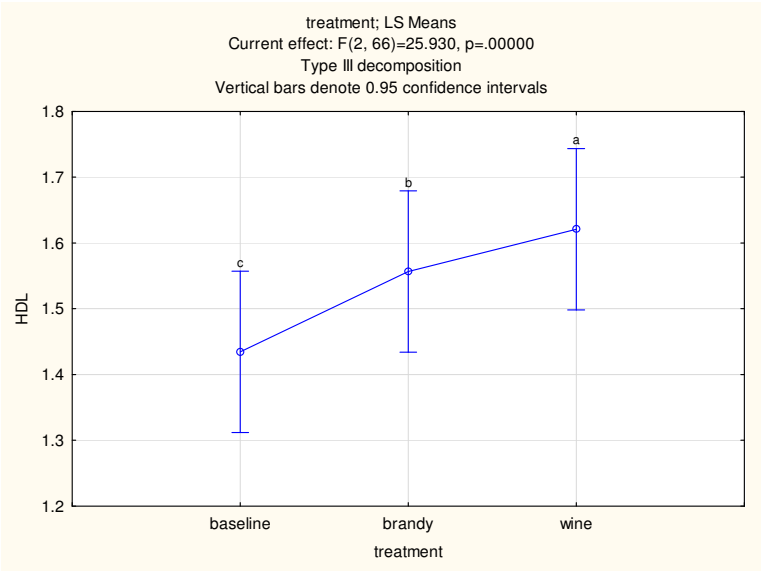

Figure1. Effect of alcohol consumption on HDL-cholesterol levels.

The HDL-cholesterol raising effect varied significantly according to Apo E genotype (allele E4 p=0.027, allele E2 p=0.51). The HDL-cholesterol levels increased with alcohol intake in both the presence and absence of the Apo E4 allele. In the group that consumed brandy first the HDL-cholesterol levels increased throughout the intervention period irrespective of Apo E genotype. However, in the group that consumed wine first, it seems that this alcohol effect was suppressed in the Apo E4-positive study group. In the wine-first group without the Apo E4 allele HDL-cholesterol levels increased while the brandy had no further effect. Although HDL-cholesterol raising effect of alcohol was slightly modified by genetic variation in the MTHFR gene (p=0.048), the HDL-cholesterol raising effect was confirmed in both mutation-positive and –negative individuals with alcohol intake (Figure 2).

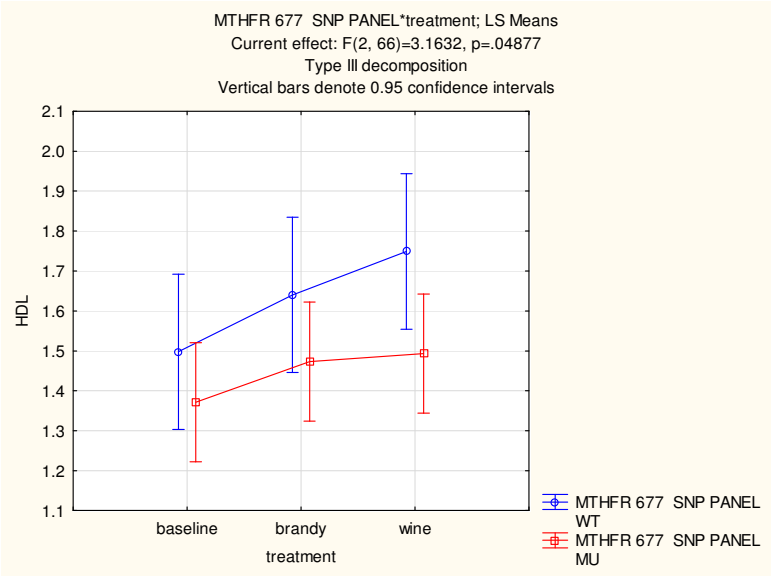

Figure 2. Effect of alcohol consumption on HDL-cholesterol levels in relation to genetic variation on the MTHFR gene.

An important finding was that the statistically significant increase in total cholesterol levels observed after the alcohol intervention (p=0.034) (Figure 3a) was only found in individuals with the C282Y and/or H63D mutations in the HFE gene (p=0.016) (Figure 3b).

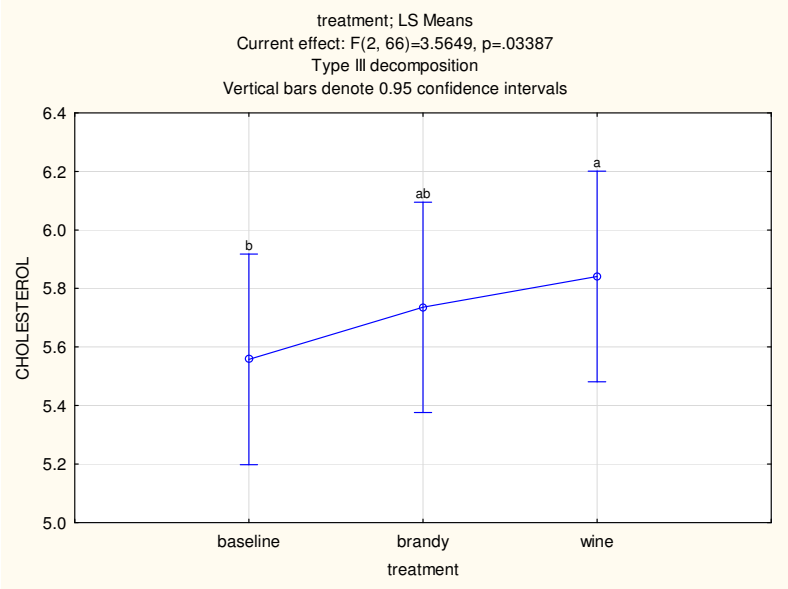

Figure 3a. Effect of alcohol consumption on total cholesterol levels.

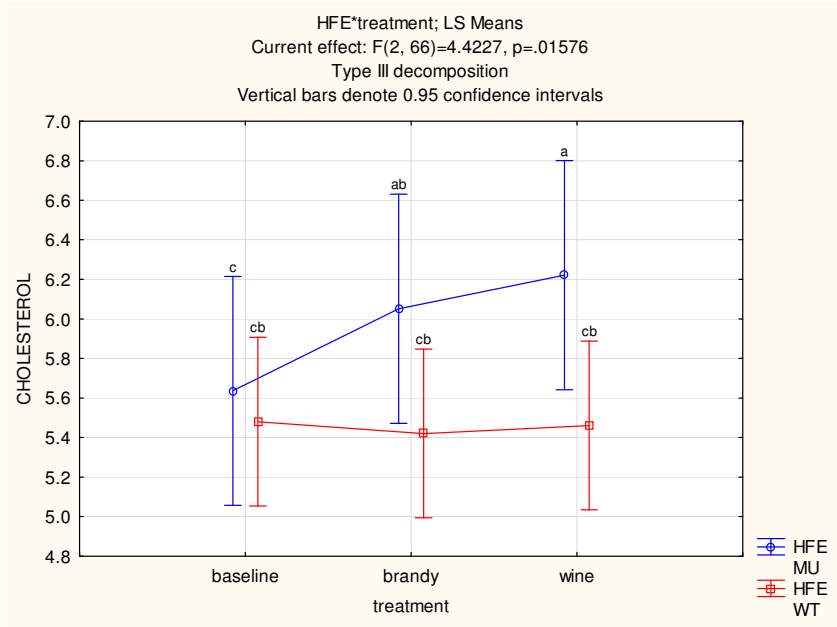

Figure 3b. Effect of alcohol consumption on total cholesterol levels in relation to genetic variation on the HFE (mutations C282Y and H63D) gene.

An increase of triglyceride levels was observed with alcohol intake, which was only seen in HFE mutation-positive individuals (p=0.02) (Figure 4). This finding demonstrates an important gene-environment interaction and may explain why some individuals in the general population, but not all, experience an increase in triglyceride levels with alcohol intake.

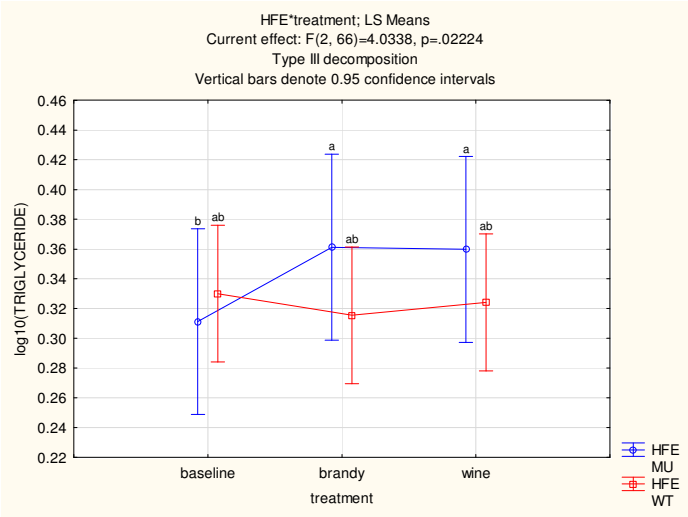

Figure 4. Effect of alcohol consumption on triglyceride levels in relation to genetic variation on the HFE (mutations C282Y and H63D) gene.

The results of the blood biomarkers indicative of antioxidant activity and content, lipid peroxidation and redox status (GSH:GSSG) are shown in Table 1. When considering the plasma antioxidant capacity (ORAC) and the level of total polyphenols, no significant changes after the wine or brandy interventions were detected when compared to the baseline. This could be expected as the plasma used was fasting samples and polyphenolic compounds have a relative short half life (1-5 hrs) in the plasma. No significant changes in

plasma markers for lipid peroxidation (CDs, TBARS) when comparing the different interventions with the baseline were detected in this study. The level of total glutathione was significantly (P=0.038) decreased after the brandy intervention when compared to the baseline (Figure 5), while the wine intervention did not cause any significant decrease and showed similar values compared to the baseline.

Table 1: Antioxidant status and oxidative stress parameters

Group	Conjugated dienes (umol/L)	TBARS (umol/L)	tGSH (µmol/L)	GSSG (µmol/L)	GSH:GSSG ratio	ORAC (µmol/L)	Total polyphenols (mg/L)
Baseline	128.1± 18.8a	0.89 ± 0.17a	1006 ± 177a	18.8 ± 30.5a	145 ± 108a	6193 ± 683a	158 ± 15a
Wine	133.5 ± 19.0a	0.87 ± 0.14a	995 ± 157a	20.1 ± 14.1b (P=0.019)	84 ± 63b (P=0.0009)	6147 ± 794a	155 ± 15a
Brandy	130.1 ± 20.7a	0.87 ± 0.17a	964 ± 144b (P=0.0007	27.4 ± 23.1b (P=0.0004)	76 ± 90b (P=0.0002)	5926 ± 580a	153 ± 16a

Values in columns are averages ± SD. Values followed by the same letter indicates P>0.05 (non-significance), while when letters differ indicates P<0.05 (significance)

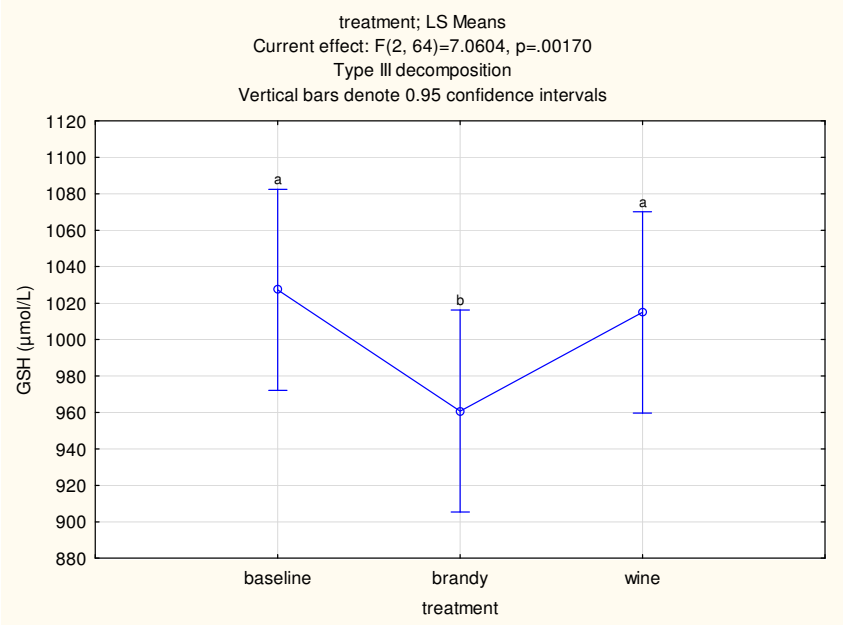

Figure 5. Effect of brandy and wine interventions on total glutathione levels in the blood compared to baseline levels

Significantly increased levels of oxidised glutathione (GSSG) were shown for both interventions when compared with the baseline (Figure 6), thus causing a significant decrease in the ratio of GSH: GSSG (an indicator of oxidative stress status).

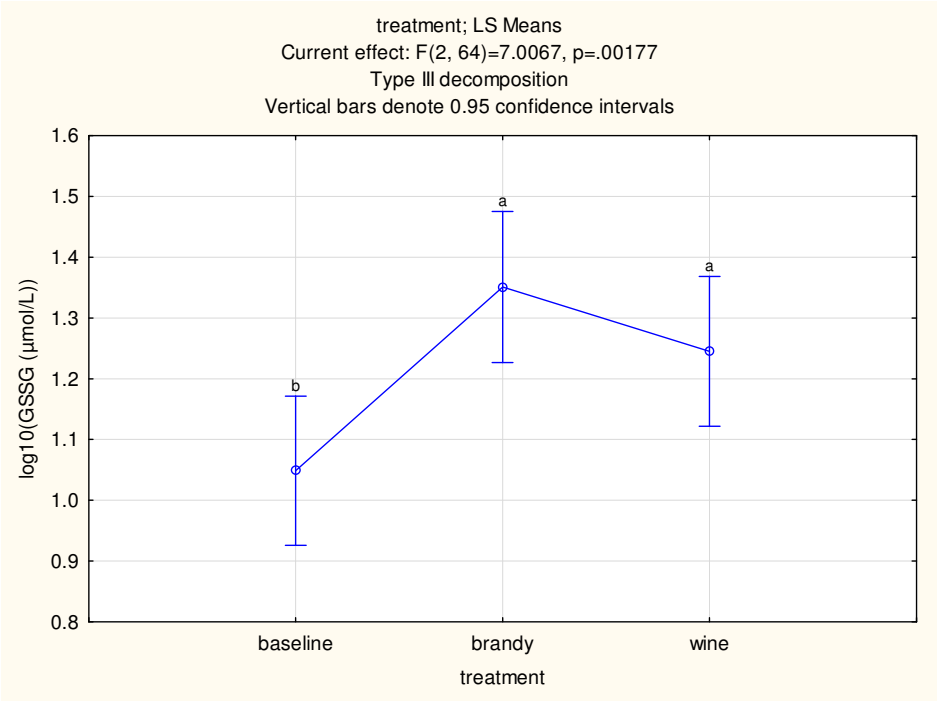

Figure 6. Effect of brandy and wine interventions on oxidised glutathione levels in the blood compared to baseline levels.

Whole blood GSH levels were measured as it is an important circulating endogenous antioxidant. Reduced glutathione is a powerful intracellular antioxidant that plays a vital role in stabilizing various enzymes and could also be considered a good marker for tissue antioxidant capacity [Wang & Jiao, 2000; Van Acker et al., 2000]. Several clinical conditions are associated with a decrease in cellular GSH levels that may result in a lowered cellular redox potential [Exner, et al., 2000]. A recent study by Campolo et al. [2007] also suggested blood glutathione analyses to be included in dietary supplementation trials to assess the thiol redox status especially in chronic heart failure (CHF) patients, as increased free radical production in these patients could result from abnormalities in intracellular GSH cycling, that was associated with increased lipid peroxidation (measured as MDA) in CHF [Campolo et al., 2007]. In this study, it was only the brandy consumption that adversely affected the level of glutathione and not the red wine. No other increases in oxidative stress parameters could be shown after brandy intervention. Previously, the ability of red wine to enhance the red blood cell's reduced glutathione level (GSH) has been reported (Urquiaga et al., 2010).

References:

Wang, S.Y.; Jiao, H. Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals and singlet oxygen. *J. Agric. Food Chem.* 48:677-684; 2000.

Van Acker, F.; Schouten, O.; Haenen, G.R.M.; Van der Vijg, W.J.F.; Bast, A. Flavonoids can replace R-tocopherol as antioxidant. *Fed. Euro. Biochem. Soc. Lett*. 473:145-148; 2000.

Exner, R.; Wessner, B.; Manhart, N.; Roth, E. Therapeutic potential of glutathione. *Wien. Klin. Wochenschr*. 112:610-616; 2000.

Campolo, J.; De Maria, R.; Caruso.; Accinni, R.; Turazza, F.; Parolini, M.; Roubina, E.; De Chiara, B.; Cighetti, G.; Frigerio, M.; Vitali, E.; Parodi, O. Blood glutathione as independent marker of lipid peroxidation in heart failure. *Intl. J. Cardiol.* 117:45-50; 2007.

Urquiaga et al., 2010: Mediterranean diet and red wine protect against oxidative damage in young volunteers. Atheroclerosis: 21, 694-699.

5. Accumulated outputs

List ALL the outputs from the start of the project.
The year of each output must also be indicated.

A set of standard operating procedures (SOPs) were developed for the SNPs analysed, which formed part of student training: Yandiswa Yako, registered PhD student. She was appointed and a research assistant for this project.

The results of the study will be presented at an international Winehealth Congress in Australia during 2013 and submitted for publication in a peer reviewed journal.

Technology development, products and patents

Indicate the commercial potential of this project (intellectual property rights or a commercial product(s)).
The industry will benefit from the results of this study in the following areas:

- The comparison of the effect of moderate red wine consumption to brandy on the atherogenic lipoprotein profile, oxidative stress and inflammatory status has enabled us to document the effect of brandy in relation to the well documented health benefits of moderate wine consumption.

- The genetic screen of CVD risk factors identified the proportion of individuals who may not benefit from drinking alcohol as a consequence of gene-environment mismatches, based on the mutation/allele frequencies in the study population.

- With this information the presence of genetic risk factors can in future be correlated with relevant biochemical parameters to compare gene expression in the presence and absence of known environmental triggers.

- We were also be able to determine the impact of mutations/functional polymorphisms included in the genetic screen on the response to the intervention to ultimately develop guidelines for safe drinking habits.

Human resources development/training

Indicate the number and level (e.g. MSc, PhD, post doc) of students/support personnel that were trained as well as their cost to industry through this project. Add in more lines if necessary.

	Student level (BSc, MSc, PhD, Post doc)	Cost to project (R)
1.	Y Yako, PhD	R 67 000 (SOPs development, extractions)
2.	L Fisher, MSc	R 5 000 (ABI-lightcycler mutation screening
3.	S Spagni, MSc	R 5 000 (THRIP project participation)
4.	L Nutt, matric	R 25 000 (admin support, consent forms)
5.		

Publications (popular, press releases, semi-scientific, scientific)

Books:

Van Velden D P, "Die Dokter as Vennoot" Protea Uitgewers 2011

Articles:

1. van Velden DP, Kotze MJ, Blackhurst D, Kidd M. Health claims on the benefits of moderate alcohol consumption in relation to genetic profiles. Journal of Wine Research, 2011, Vol 22, pp. 123-129.
2. Van Velden DP. Gesondheidsvoordele van wyn is afhanklik van interaksie tussen gene en omgewing. Wynboer, Augustus 2010; 106-107.
3. Kotze MJ, van Velden DP. Waar staan ons nou met alkohol en gesondheid? Wynboer, Oktober 2011
4. van Velden DP van der Merwe S, Fourie E, Blackhurst DM, Kidd M, Kotze MJ, Mansvelt EPG. The influence of a Mediterranean-like diet with and without red wine on patients with the metabolic syndrome. S Afr J Enol Vitic. 2007;28 (1): 44-49.
5. Mansvelt EPG, Fourie E, Blackhurst D, Kotze T, Stofberg H, van der Merwe S. Kotze MJ, van Velden DP. The influence of a Mediterranean Diet with and without red wine on the haemostatic and inflammatory parameters of subjects with the metabolic syndrome. S Afr J Enol Vitic. 2007;28 (1): 37-43

Presentations/papers delivered

4th International Wine and Health Conference, 3-6 October 2010, Friuli, Italy. Invited speaker: Health claims on the benefits of moderate alcohol consumption in relation to genetic profiles

4. Total cost summary of project

	Year
Total cost in real terms for year 1	
Total cost in real terms for year 2	
Total cost in real terms for year 3	
Total cost in real terms for year 4	
Total cost in real terms for year 5	
TOTAL	

CFPA	Deciduous	DFTS	Winetech	THRIP	Other	TOTAL