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Ingesting alcohol prior to food can alter the activity of the hypothalamic-pituitary-adrenal axis

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ABSTRACT

There is an increasing evidence that long-term alcohol intake can promote damage to most of the body's major organs. However, regular consumption of a small-moderate amount of alcohol is often recommended as being beneficial to health and of concern is that the effect of ingesting commercially available alcohol products on steroid hormone synthesis under variable nutritional conditions has not been thoroughly investigated. Many individuals consume alcohol alone prior to a meal and the aim of the present study was to assess the effect of consuming a small-moderate amount of commercially available alcohol on the level of salivary cortisol and salivary dehydroepiandrosterone sulfate (DHEAS) before and after a meal. A total of 24 males aged 19–22 years participated in the current investigation. The experimental procedure required participants to fast for 6 h before being asked to ingest either 40 g alcohol in the form of red wine ($n=8$), low alcohol and high beer ($n=8$), white wine ($n=8$) or the equivalent amount of placebo over a 135-min period before consuming food for 45-min. The level of blood alcohol, salivary cortisol and salivary DHEAS was assessed upon arrival and then at regular 45-min intervals during the 180-min experimental period. The results showed that the consumption of alcohol and placebo can significantly lower the level of salivary cortisol. However, the effect of consuming a small-moderate amount of commercially available alcohol on the level of salivary DHEAS was dependent on the nutritional content of the beverage with red wine promoting no change, white wine promoting a significant decrease, and beer having a variable effect on salivary DHEAS concentration when compared to placebo. It was concluded that the effect of commercially available alcohol on the HPA axis is not the same for all alcohol products and both the nutritional status of participants and the nutritional content of the alcoholic beverage being administered should be taken into consideration when investigating the effect of alcohol on the HPA axis.

1. Introduction

Steroid hormones under the control of the hypothalamic-pituitary-adrenal (HPA) axis have a diverse role in the body and any dysregulation in steroid synthesis could lead to the development of disease. During prolonged fasting energy is provided by the gluconeogenic pathway and an elevation in cortisol, a potent glucocorticoid, can protect against cellular damage by promoting gluconeogenesis (Choi et al., 1996). Similarly, dehydroepiandrosterone sulfate (DHEAS) is usually elevated under fasting conditions (Tegelman et al., 1986) and an elevation in DHEAS can inhibit glucose-6-phosphate dehydrogenase (G6PDH), an enzyme responsible for catalyzing the first step in the oxidative branch of the pentose phosphate pathway, an energy pathway associated with lipogenesis (Baulieu, 1996).

Both cortisol and DHEAS also serve a functional neuromodulatory role in the central nervous system (Majewska, 1992). Cortisol may alter the binding of γ-aminobutyric acid (GABA) to inhibitory $GABA_A$ receptors in a biphasic fashion, with potentiation of GABA occurring at nanomolar levels and a reduction occurring at micromolar ones (Majewska et al., 1985). Alternatively, DHEAS may modulate the action of cortisol by enhancing the magnitude of hippocampal primed burst (Diamond et al., 1996) and as a GABA antagonist at the $GABA_A$ receptor (Demirgoren et al., 1991), may potentiate the function of excitatory N-methyl-D-aspartate (NMDA) glutamate receptors (Baulieu, 1996).

It is widely accepted that alcohol administration can selectively impair functioning of NMDA receptors (Lustig et al., 1989) via potentiation of inhibitory $GABA_A$ receptors (Mehta and Ticku, 1988; Givens and Breese, 1990), which suggests that alcohol may alter the functioning of the HPA axis. However, much variability exists in the alcohol literature with respect to the effect of alcohol on the level of DHEAS (Andersson et al., 1986; Kokavec and Crowe, 2001), and cortisol (Jenkins and Connolly, 1968; Linkola et al., 1979; Davis and

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Jeffcoate, 1983; Joffe et al., 1984; Ida et al., 1992; Waltman et al., 1993; Guillaume et al., 1994; Gianoulakis et al., 1997; Kokavec and Crowe, 2001), making it difficult to determine the effect (if any) alcohol has on the HPA axis.

The large degree of variability in the alcohol literature suggests that some unknown and therefore uncontrolled extraneous factor could be subtly responsible for the high degree of variability in the steroid data. We have recently reported that the prior nutritional status of the individual at the time of testing is an important factor when assessing the effect of alcohol on the HPA axis (Kokavec and Crowe, 2001). Similarly, the inconsistencies in the alcohol literature when looking at the effect of alcohol on HPA axis activity may be due to nutritional differences between the types of alcohol consumed at the time of testing.

The release of adrenocorticotrophin (ACTH) by the anterior pituitary promotes the synthesis and release of steroid hormones by the adrenal cortex (Endoh et al., 1996). Thus, any alteration in ACTH release could potentially have some influence on the synthesis of steroid hormones. Most alcoholic beverages contain varying amounts of histamine (Lonvaud-Funel, 2001) and the release of histamine can act to promote ACTH release (Knigge et al., 1988). However, while most alcohol products contain some histamine, red wine is unique in that red wine contains the highest level of histamine (Wantke et al., 1994) and (unlike other alcoholic beverages) can also promote histamine release (Intorre et al., 1996). Therefore, given that histamine can promote ACTH release (Knigge et al., 1988), the precursor for steroidgenesis (Endoh et al., 1996) it is possible that red wine could also influence the HPA axis differently to other alcoholic beverages.

Consuming alcoholic products such as beer could also have the potential to alter biochemical processes as could the use of products containing alcohol and a masking agent (e.g. orange juice). Both these alcoholic products contain carbohydrate, a nutrient that when combined with alcohol can potentiate the release of insulin (O'Keefe and Marks, 1977). Insulin, a pancreatic hormone, usually has an antagonistic relationship with cortisol (Dallman et al., 1993) and DHEAS (Kaplan and Pesce, 1996). However, a synergistic relationship between insulin and cortisol (only) has also been noted immediately following a high-carbohydrate meal (Dallman et al., 1993). The role of cortisol in this instance is to modulate the effects of insulin on glucose utilization when glycogen stores are depleted to ensure that energy stores are replenished (Goldstein et al., 1993).

There is increasing evidence that long-term alcohol misuse can promote damage to most of the body's major organs, including the brain (Victor et al., 1989). Alternatively, the consumption of a small-moderate amount of alcohol, or more specifically red wine, is thought to be beneficial to cardiac health (e.g. Brands et al., 1996; Andersen and Jensen, 2004; Szmitko and Verma, 2005; Zern and Fernandez, 2005). However, of concern is that the effect of ingesting commercially available alcohol products on steroid hormone synthesis under variable nutritional conditions is uncertain and requires further investigation. Thus the aim of the present investigation is to address this by assessing the effect of a consuming moderate dose of commercially available alcohol on the level of cortisol and DHEAS before and after a meal.

2. Method

2.1. Participants

A total of 24 adult males aged 19–22 years participated in the present series of investigations. Only young adult males were recruited due to suggestions that DHEAS synthesis can be influenced by age and sex factors (Orentreich et al., 1984). Participants were excluded if they reportedly had: a history of psychiatric illness; any neurological condition; any major physical complaint; taken any prescribed medication within the last 7 days; a history of drug use; a history of alcohol abuse; or satisfied the DSM-IV-TR criteria for alcohol abuse or dependence (American Psychiatric Association, 2000). All participants were white Caucasian of Australian or British origin and no participant was obese as the weight of all participants was within the medically recommended range for age and height. Subject participation was obtained by informed consent and no monetary incentives were provided as compensation for participation. The study was approved by La Trobe University Human Ethics Committee who determined that the methods employed complied with the National Statement on Ethical Conduct in Research Involving Humans (Commonwealth publications, 1999).

2.2. Materials and measures

2.2.1. Biochemical analysis

Salivary cortisol was assessed using COBAS ELECSYS 2010 immunoassay (Roche Diagnostics, Indianopolis, IN, USA). The intra assay coefficient of variation was 6.1% at 4.68 nmol/L, 2.7% at 11.5 nmol/L, 1.5% at 15.9 nmol/L, and 2.8% at 19.8 nmol/L. Quality controls for the assessment of cortisol in saliva were performed using Bio Rad Unassayed Liquidcheck Chemistry Control (Bio Rad, USA). The level of salivary DHEAS was determined using the DSL-2700-S double antibody radioimmunoassay kit (Diagnostic Systems Laboratories, Inc. Texas, USA). Sensitivity of the assay was 0.3 ng/mL. Assessment of DHEAS was carried out in accordance with the manufacturer's instructions. Hormonal readings for salivary cortisol and salivary DHEAS were obtained for each time point using a single saliva sample. All hormonal analysis was performed by staff employed by Analytical Reference Laboratories (Melbourne, Australia), naïve to the experimental conditions. Semi-quantitative urinalysis was performed using Labstix™ (Bayer Australia Limited), in order to measure ketones (sensitivity was 0.5–1.0 mmol/L acetoacetic acid), glucose (sensitivity was 4–7 mmol/L glucose), blood (sensitivity was 150–620 μg/L haemoglobin), and protein (sensitivity was 0.15–0.30 g/L albumin). Blood alcohol readings (BAL) were made using a Lion alcolmeter™ (Lion laboratories Limited, Cardiff. UK).

2.2.2. Test beverages

2.2.2.1. Red wine trial (n=8). Penfolds Rawson's Retreat 2004 cabernet sauvignon red wine (Penfolds wines, Magill, South Australia), containing 14% alcohol (285 kJ per standard unit) or the equivalent volume of placebo in the form of red non-alcoholic grape juice containing 235 kJ per 100 mL (Berri Limited, Carlton, Victoria, Australia).

2.2.2.2. White wine trial (n=8). Hardy's™ Medium Dry white wine (Hardy Wine Company, Reynella, South Australia) containing 12% alcohol (315 kJ per standard unit or the equivalent volume of placebo in the form of white non-alcoholic grape drink containing 285 kJ per 100 mL units (Berri Limited, Carlton, Victoria, Australia).

2.2.2.3. Beer trial (n=8). High alcohol beer in the form of Fosters® beer (Foster's Group Limited, Victoria, Australia, 3006), containing 4.9% alcohol (168 kJ and 3.1 g carbohydrate per 100 mL) or the equivalent volume of light beer in the form of Fosters Light® beer containing 2.3% alcohol and 3.5 g carbohydrate per 100 mL. Total amount of alcohol consumed in the low alcohol beer trial was 2.1 standard units (21 g alcohol).

2.3. Procedure

Participation in all trials was preceded by a six hour fast commencing at 1100 h Eastern Standard Time (EST) and testing began at 1700 h EST. Upon arrival all participants were breathalyzed and asked to provide a 50 mL urine sample for urinalysis in order to determine

that all individuals had adhered to the fasting requirements and were alcohol free prior to testing. Participants assumed that all trials involved the ingestion of alcohol. Participants were recruited on separate occasions for participation in one of three paired alcohol trials (white wine/white grape, red wine/red grape, high alcohol beer/low alcohol beer). The same experimental methodology was utilized for all trials. Participants were required to provide a 50 mL urine sample and a 5 mL saliva sample for measurement of DHEAS and cortisol before consuming a total of 4 standard units of alcohol (40 g alcohol) or the equivalent volume of low alcohol beer (20 g alcohol) and/or placebo slowly over a 135-min period. Saliva samples for the assessment of DHEAS and cortisol were taken; urinalysis was performed; and blood alcohol level (BAL) was assessed regularly at 45-min intervals between 1700 h and 1915 h EST. Food and a non-alcoholic beverage in the form of non-vegetarian pizza and Coca-Cola soft drink was provided at 1915 h, and readings for all parameters were taken once participants had finished eating at 2000 h. All individuals reported small-moderate feelings of intoxication at the completion of the alcohol trials and no participant experienced gastrointestinal or other distress at any stage during the alcohol or placebo trials.

2.4. Statistical analyses

Two-way repeated measures Analysis of Variance (ANOVA) with Time (0-min, 45-min, 90-min, and 135-min) and Trial (alcohol and placebo) as 'within subjects' factors was used to compare the level of salivary cortisol and salivary DHEAS in each paired trial (i.e. red wine/red grape, white wine/white grape, high alcohol beer/low alcohol beer). Similarly, two-way repeated measures ANOVA with Time (135-min and 180-min) and Trial (alcohol and placebo) as 'within subjects' factors was used to compare the level of salivary cortisol and salivary DHEAS in each paired trial following food intake. A 2×4 mixed design ANOVA with Time (0-min, 45-min, 90-min, and 135-min) as the 'within subjects' factor and Trial (alcohol and placebo) as the 'between subjects' factors was used to compare steroid findings in the high alcohol beer trial and low alcohol beer trial with those obtained by a statistically matched group of participants administered white grape drink. Similarly, a 2×2 mixed design ANOVA with Time (135-min and 180-min) as the 'within subjects' factor and Trial (alcohol and placebo) as the 'between subjects' factor assessed the level of salivary cortisol and salivary DHEAS before and after food intake in the low alcohol and high alcohol beer trials. Results were classed as significant if the calculated probability was $P<.05$. Any violation of assumptions was corrected using the Huynh Feldt correction.

3. Results

Upon arrival participants were breathalyzed and all registered a BAL of zero. Urinalysis revealed the presence of ketone bodies in all participants, which confirmed that individuals had adhered to the fasting requirements of the investigation in all trials.

3.1. Red wine trial

The average BAL increased steadily during red wine consumption reaching a mean peak of 0.06 mg/100 mL, with little variability (SEM $<\pm$ 0.01 mg/100 mL), after four standard units of red wine (40 g alcohol) had been consumed at 135-min. The mean level of salivary cortisol and salivary DHEAS observed in the red wine trial is graphically presented in Fig. 1A and B, respectively.

The level of salivary cortisol in the red wine and placebo trials was assessed across time points using two-way repeated measures ANOVA. The results showed a significant main effect for Time, $F(3, 21)=6.88$, $P<.01$, and a non-significant main effect for Trial, $F(1, 7)=.47$, $P=.51$. The Trial $\times$ Time interaction was non-significant, $F(3, 21)=.75$, $P=.53$. Assessment of salivary cortisol before and after food intake using two-way repeated measures ANOVA revealed a non-significant main effect for Time, $F(1, 7)=4.82$, $P=.06$, and Trial, $F(1, 7)=.78$, $P=.41$. The Time $\times$ Trial interaction was also non-significant, $F(1, 7)=.07$, $P=.80$.

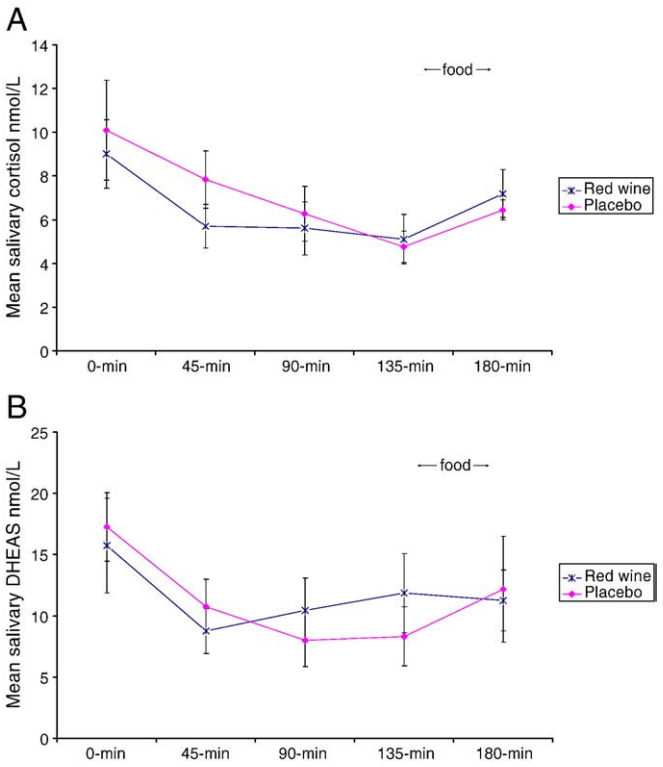

Fig. 1. Mean salivary cortisol (A) and salivary DHEAS (B) before (0-min) and after ingestion of red wine containing 40 g alcohol or placebo (45–135-min), and after food (180-min). Data are shown as the mean $\pm$ S.E.M ($N=8$).

Two-way repeated measures ANOVA was used to compare the level of salivary DHEAS across time points in the red wine and placebo trials. The ANOVA analysis revealed a significant main effect for Time, $F(3, 21)=15.13$, $P<.01$, and a non-significant main effect for Trial, $F(1, 7)=.14$, $P=.72$. The Time $\times$ Trial interaction was non-significant, $F(3, 21)=1.71$, $P=.20$. Assessment of salivary DHEAS before and after food intake using two-way repeated measures ANOVA revealed a non-significant main effect for Time, $F(1, 7)=.41$, $P=.54$, and Trial, $F(1, 7)=.33$, $P=.58$. The Time $\times$ Trial interaction was also non-significant, $F(1, 7)=2.31$, $P=.17$.

3.2. High alcohol beer versus low alcohol beer

The consumption of high alcohol beer resulted in BAL increasing steadily reaching a mean peak of 0.06 mg/100 mL at 135-min with little variability (S.E.M $=\pm$ .01). Alternatively, the consumption of an equivalent volume of low alcohol beer resulted in BAL increasing only slightly, reaching a mean peak of 0.02 mg/100 mL at 135-min, with little variability (S.E.M $<\pm$ .01). The mean level of salivary cortisol and salivary DHEAS from the high alcohol beer and low alcohol beer trials is graphically presented in Fig. 2A and B, respectively.

The level of salivary cortisol was compared across time points in the high alcohol and low alcohol beer trials using two-way repeated measures ANOVA. The results showed a significant main effect for Time, $F(3, 21)=9.53$, $P<.01$, and non-significant main effect for Trial, $F(1, 7)=.01$, $P=.92$. The Trial $\times$ Time interaction was also non-significant, $F(3, 21)=.72$, $P=.55$.

Comparison of salivary DHEAS concentration across time points in the high alcohol and low alcohol beer trials using two-way repeated

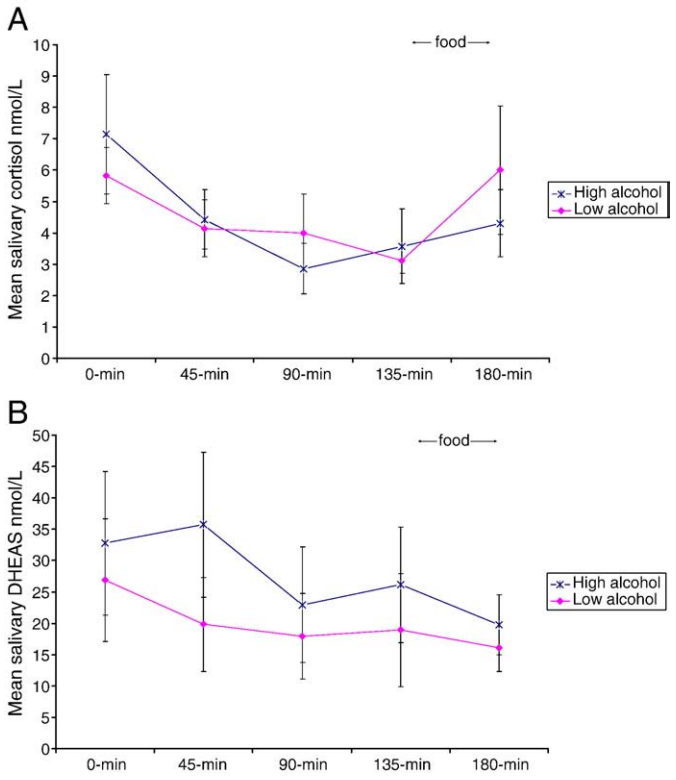

Fig. 2. Mean salivary cortisol (A) and salivary DHEAS (B) before (0-min) and after ingestion of high alcohol beer containing 40 g alcohol and low alcohol beer containing 20 g alcohol (45–135-min), and after food (180-min). Data are shown as the mean ± S.E.M ($N=8$).

measures ANOVA revealed a significant main effect for Time, $F(3, 21)=6.24$, $P<.01$, and non-significant main effect for Trial, $F(1, 7)<.01$, $P=.98$. The Trial×Time interaction was also non-significant, $F(3, 21)=1.03$, $P=.40$.

Assessment of salivary cortisol before and after food intake using two-way repeated measures ANOVA revealed a non-significant main effect for Time, $F(1, 7)=3.02$, $P=.13$, and Trial, $F(1, 7)<.01$, $P=.97$. The Time×Trial interaction was also non-significant, $F(1, 7)=.22$, $P=.65$. Moreover, the level of salivary DHEAS before and after food intake was statistically similar when beer trials were compared. A non-significant main effect for Time, $F(1, 7)<.01$, $P=.96$, and Trial, $F(1, 7)=.09$, $P=.78$ and non-significant Time×Trial interaction, $F(1, 7)=.53$, $P=.49$, was noted.

3.2.1. High alcohol beer versus placebo

The mean level of salivary cortisol and salivary DHEAS from the high alcohol beer and placebo (white grape juice) trials is graphically presented in Fig. 3A and B, respectively.

The level of salivary cortisol across time points during high alcohol beer consumption and placebo was compared using a 2×4 mixed design ANOVA. The results showed a significant main effect for Time, $F(3, 42)=16.87$, $P<.01$, and a non-significant main effect for Trial, $F(1, 14)=.10$, $P=.76$. The Trial×Time interaction was also non-significant, $F(3, 42)=.98$, $P=.41$. Assessment of salivary cortisol before and after food intake using a 2×2 mixed design ANOVA revealed a significant main effect for Time, $F(1, 14)=19.01$, $P<.01$, and non-significant main effect for Trial, $F(1, 7)=3.33$, $P=.09$. The Time×Trial interaction was also non-significant, $F(1, 7)=4.13$, $P=.06$.

A 2×4 mixed design ANOVA was used to compare the level of salivary DHEAS across time points in the high alcohol beer and placebo trial. The ANOVA analysis revealed a significant main effect for Time, $F(3, 42)=15.06$, $P<.01$, and a non-significant main effect for Trial, $F(1, 14)=.34$, $P=.57$. However, this was modified by a significant Time×Trial interaction, $F(3, 42)=8.81$, $P<.01$. Inspection of the raw DHEAS data suggests that the effect of high alcohol beer on DHEAS rhythmicity at 45-min, 90-min and 135-min may be opposite to that of placebo. Assessment of salivary DHEAS before and after food intake using 2×2 mixed design ANOVA revealed a significant main effect for Time, $F(1, 7)=5.10$, $P=.04$, and non-significant main effect for Trial, $F(1, 7)=.42$, $P=.53$. The Time×Trial interaction was also non-significant, $F(1, 7)=1.91$, $P=.19$.

3.2.2. Low alcohol beer versus placebo

The mean level of salivary cortisol and salivary DHEAS under low alcohol beer and placebo conditions is graphically presented in Fig. 4A and B, respectively.

The level of salivary cortisol in the low alcohol beer and white grape juice trials was compared across time points using a 2×4 mixed design ANOVA. The results showed a significant main effect for Time, $F(3, 42)=7.65$, $P<.01$, and a non-significant main effect for Trial, $F(1, 14)=.29$, $P=.60$. The Trial×Time interaction was also non-significant, $F(3, 42)=.29$, $P=.83$. Assessment of salivary cortisol before and after food intake using a 2×2 mixed design ANOVA revealed a significant main effect for Time, $F(1, 14)=19.97$, $P<.01$, and non-significant main effect for Trial, $F(1, 7)=2.54$, $P=.13$. The Time×Trial interaction was also non-significant, $F(1, 7)=2.80$, $P=.12$.

A 2×4 mixed design ANOVA was used to assess the level of salivary DHEAS across time points in the low alcohol beer trial. The ANOVA analysis revealed a significant main effect for Time, $F(3, 42)=14.07$, $P<.01$, and a non-significant main effect for Trial, $F(1, 14)=.27$, $P=.61$. However, this was modified by a significant Time×Trial interaction, $F(3, 42)=3.47$, $P=.03$. Inspection of the raw DHEAS data suggests that consuming low alcohol beer may have the opposite effect on DHEAS rhythmicity at 90-min and 135-min when compared to placebo. Assessment of salivary DHEAS before and after food intake

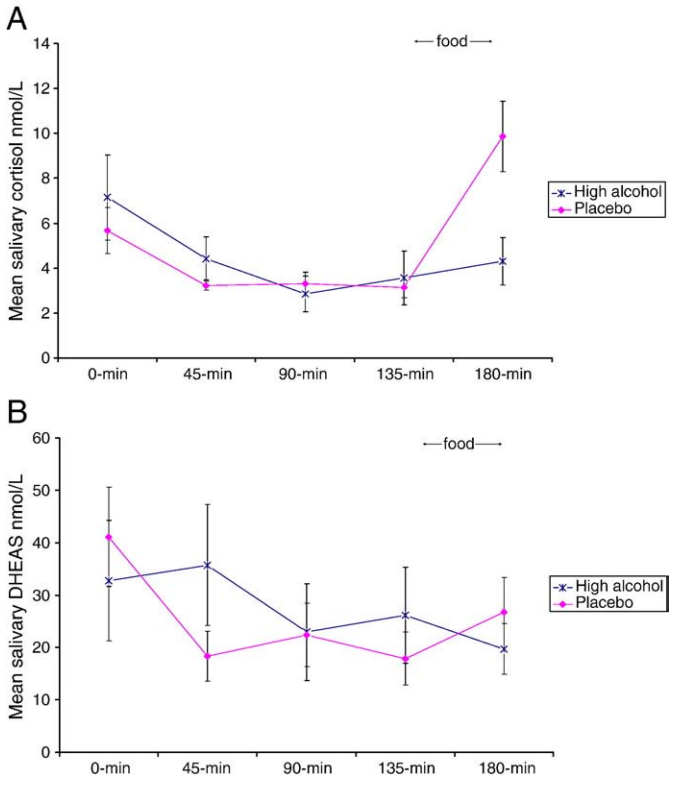

Fig. 3. Mean salivary cortisol (A) and salivary DHEAS (B) before (0-min) and after ingestion of high alcohol beer containing 40 g alcohol or placebo (45–135-min), and after food (180-min). Data are shown as the mean ± S.E.M ($N=16$).

using 2×2 mixed design ANOVA revealed a non-significant main effect for Time, $F(1, 7) = .65$, $P = .44$, and non-significant main effect for Trial, $F(1, 7) = .58$, $P = .46$. The Time×Trial interaction was also non-significant, $F(1, 7) = 2.14$, $P = .17$.

3.3. White wine

During white wine consumption BAL increased steadily reaching a mean peak of .06 mg/100 mL (S.E.M<±.01 mg/100 mL), with little variability after 40 g of alcohol had been consumed over 135-min. The mean level of salivary cortisol and salivary DHEAS under experimental and placebo conditions is graphically presented in Fig. 5A and B, respectively.

The level of salivary cortisol in the white wine and placebo trial was compared across time points using two-way repeated measures ANOVA. The ANOVA analysis produced a significant main effect for Time, $F(3, 21) = 11.84$, $P<.01$, and a non-significant main effect for Trial, $F(1, 7) = .60$, $P = .47$. The Trial×Time interaction was also non-significant, $F(3, 21) = .80$, $P = .51$. Assessment of salivary cortisol concentration before and after food intake using two-way repeated measures ANOVA revealed a significant main effect for Time, $F(1, 7) = 34.51$, $P<.01$, and Trial, $F(1, 7) = 10.83$, $P = .01$. However, this was modified by a significant Time×Trial interaction, $F(1, 7) = 5.42$, $P = .05$. Post hoc analysis confirmed that the level of cortisol is significantly lower at 135-min and 180-min when white wine is consumed.

Two-way repeated measures ANOVA was used to compare salivary DHEAS concentration across time points. The ANOVA analysis revealed a significant main effect for Time, $F(3, 21) = 67.60$, $P<.01$, and a non-significant main effect for Trial, $F(1, 7) = .42$, $P = .54$. The Time×Trial interaction was non-significant, $F(3, 21) = .45$, $P = .72$. Assessment of salivary DHEAS before and after food intake using two-way repeated measures ANOVA revealed a non-significant main effect for Time, $F(1, 7) = 1.02$, $P = .35$, and Trial, $F(1, 7) = 2.73$, $P = .14$. However, this was modified by a significant Time×Trial interaction, $F(1, 7) = 10.01$, $P = .02$. Post hoc analysis revealed that the level of DHEAS at 180-min is significantly lower when white wine is consumed.

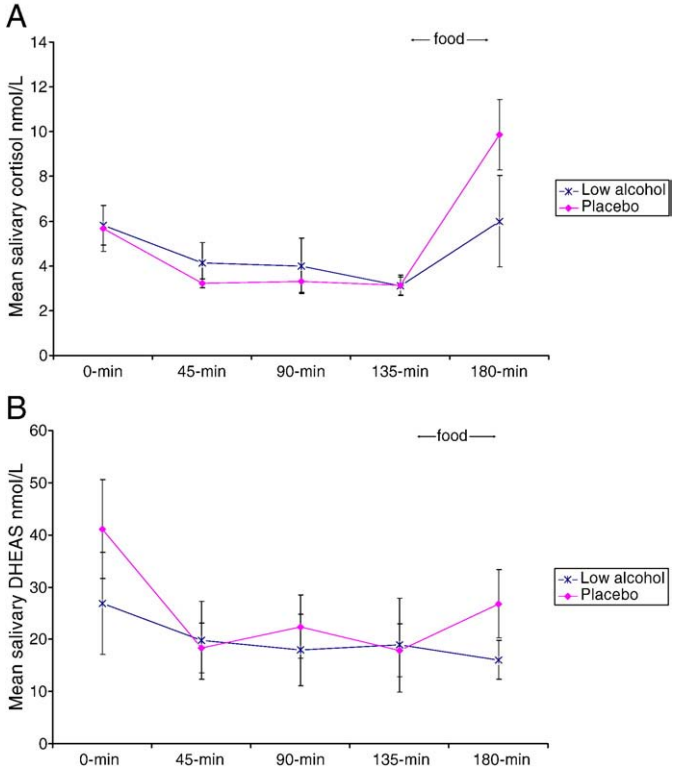

Fig. 4. Mean salivary cortisol (A) and salivary DHEAS (B) before (0-min) and after ingestion of low alcohol beer containing 20 g alcohol or placebo (45–135-min), and after food (180-min). Data are shown as the mean ± S.E.M ($N = 16$).

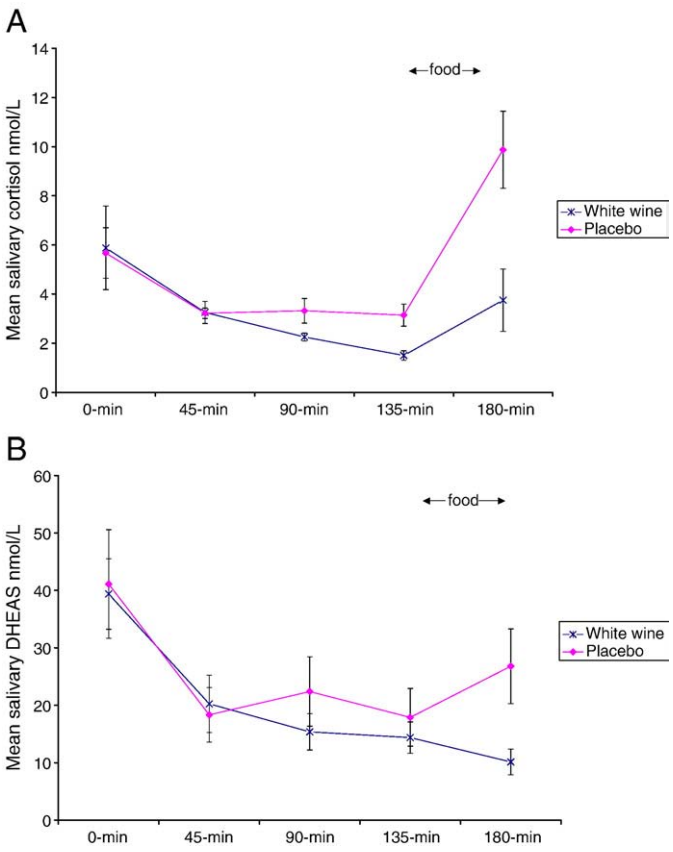

Fig. 5. Mean salivary cortisol (A) and salivary DHEAS (B) before (0-min) and after ingestion of white wine containing 40 g alcohol or placebo (45–135-min), and after food (180-min). Data are shown as the mean ± S.E.M ($N = 8$).

4. Discussion

The results from this series of trials investigating the effect of a small-moderate dose of commercially available alcohol on the HPA axis showed that consuming alcohol and/or placebo prior to food can promote a significant alteration in cortisol and DHEAS concentration. However, while a significant decrease in cortisol was observed at 45-min in all trials the effect of consuming commercially available alcohol on the level of DHEAS was variable with differences between alcoholic beverages being noted. Furthermore, consuming white wine and beer (only) prior to a meal can significantly alter the effect of food on cortisol and DHEAS concentration when food is finally consumed.

The data showing that consuming red wine, high alcohol beer, low alcohol beer, white wine and placebo prior to a meal can significantly alter salivary cortisol across time points does not support the claim that alcohol promotes little (Jenkins and Connolly, 1968), or no change (Davis and Jeffcoate, 1983; Joffe et al., 1984; Ida et al., 1992; Waltman et al., 1993; Gianoulakis et al., 1997), in cortisol concentration. In the present investigation, the assessment of cortisol and DHEAS in saliva was performed using the same saliva sample. Therefore, as a significant increase in DHEAS was only noted with red wine and high alcohol beer at various time points, it is unlikely that the significant change in cortisol reported here is due to an alcohol-induced dilution effect (Guazzo et al., 1996).

Similar to that previously reported by others it was shown that the level of DHEAS and cortisol is elevated under fasting conditions

(Tegelman et al., 1986; Vance and Thorner, 1989; Akanji et al., 1990; Lane et al., 1997; Kokavec and Crowe, 2001). Moreover, the intake of all alcoholic beverages and placebo promoted an initial decrease in the level of salivary cortisol and all beverages except for high alcohol beer promoted an immediate decrease in DHEAS, similar to that which can occur following food intake (Dallman et al., 1995; Nestler et al., 1995).

A significant increase in salivary DHEAS was observed in the high alcohol beer trial, which does not lend support to the claim that alcohol consumption significantly reduces DHEAS concentration (Andersson et al., 1986) under fasting conditions (Kokavec and Crowe, 2001). However, of interest is that an alcohol-induced increase in DHEAS has also been demonstrated when white wine is consumed alone after food (Kokavec and Crowe, 2001). Therefore, the DHEAS data obtained in the high alcohol beer trial appears to be more consistent with results obtained when assessing the effects of white wine on the HPA axis following a high-carbohydrate meal, despite participants not having eaten for several hours.

An elevation in cortisol can occur in response to food intake (Dallman et al., 1993, 1995). The role of cortisol in this instance is to modulate the effects of insulin on glucose utilization when glycogen stores are depleted to ensure that energy stores are replenished (Goldstein et al., 1993). The cortisol data presented here supports these claims with the level of cortisol in both placebo trials noted to increase following food intake. However, the level of cortisol at 180-min after food was finally consumed in the white wine trials was significantly lower after alcohol compared to placebo. Thus, the data by showing a significantly reduced level of salivary cortisol following food intake may suggest that consuming white wine prior to food may alter glycogen metabolism. In contrast, the effects of red wine, high alcohol beer, low alcohol beer and placebo on glycogen synthesis appear to be similar.

Food intake in the high alcohol beer, low alcohol beer and white wine trials resulted in the level of DHEAS becoming significantly reduced, which was at odds with the trend noted in both placebo trials. There is an antagonistic relationship between DHEAS and insulin (Kaplan and Pesce, 1996) and laboratory investigations have demonstrated that ethanol administration prior to ingestion of a glucose load (McMonagle and Felig, 1975) or carbohydrate rich food (Kokavec, 2000) can potentiate the glucose stimulating properties of insulin and promote a rapid release of insulin (O'Keefe and Marks, 1977). Moreover, when alcohol ingestion is combined with carbohydrates (Marks, 1978) the consumed alcohol can potentiate the insulin-stimulating properties of glucose (O'Keefe and Marks, 1977) and promote a rapid elevation in plasma insulin. Thus, it may be that the decrease in DHEAS following food intake in the white wine trial, high alcohol beer, and low alcohol beer trials is due to an abnormally large release of insulin. However, further investigation is required to confirm whether this is the case.

The pentose phosphate pathway is an energy pathway related to lipid synthesis and glucose-6-phosphate dehydrogenase (G6PDH) is the rate-limiting enzyme of the oxidative branch of the pentose phosphate pathway (Ayene et al., 2002). A direct effect of DHEAS is the inhibition of G6PDH (Ursini et al., 1997). Thus, a significant elevation in DHEAS can indirectly inhibit lipogenesis, which supports the suggestion that a low level of DHEAS is associated with obesity (Jakubowicz et al., 1995). The release of G6PDH is subject to nutritional regulation (Fritz et al., 1986). Insulin is a known inducer of G6PDH activity (Stapleton et al., 1993) and transcription of the *G6PDH* gene is usually elevated following ingestion of a high-carbohydrate diet (Prostko et al., 1989). During fasting, when carbohydrate intake is low, glucocorticoids alone have little effect on the rate of G6PDH activity (Stumpo and Kletzien, 1985). The role of glucocorticoids on G6PDH mRNA is a permissive one with glucocorticoids amplifying the insulin effect on G6PDH synthesis (Manos et al., 1991; Stumpo and Kletzien, 1985). Therefore, the amount of G6PDH mRNA may be modulated by these two hormones (Fritz et al., 1986).

Animal studies have shown that ethanol administration can promote a significant reduction in G6PDH (Buyukokuroglu et al., 2002), under fasting and feeding conditions (Oh et al., 1998). Insulin is a known inducer of G6PDH activity (Stapleton et al., 1993) and consuming white wine (Kokavec and Crowe, 2006) and red wine (Halloran and Kokavec, unpublished data) before food does not promote insulin synthesis. Furthermore, the significant wine-induced decrease in cortisol reported here under fasting conditions could support the suggestion that G6PDH activity (Oh et al., 1998; Buyukokuroglu et al., 2002) and as a result lipogenesis, is not promoted when wine is consumed prior to a meal.

However, it was also shown that the consumption of food after white wine and high alcohol beer can significantly decrease DHEAS concentration, while red wine prior to food promotes no change in DHEAS following food consumption. When these findings are considered together with the suggestion that ethanol prior to glucose (McMonagle and Felig, 1975) and white wine before food (Kokavec, 2000) can promote a rapid release of insulin (O'Keefe and Marks, 1977), it is possible that consuming alcoholic products such as white wine, high alcohol beer and red wine before food may promote lipogenesis when food is finally consumed. This may be particularly true for red wine given that the level of cortisol following food intake, rather than being reduced as occurred in the high alcohol beer and white wine trials, was similarly elevated when compared to placebo.

Alcohol administration by selectively impairing functioning of excitatory NMDA glutamate receptors (Lustig et al., 1989), in hippocampal neurons (Lovinger et al., 1989), potentiates the function of inhibitory $GABA_A$ receptors (Mehta and Ticku, 1988; Givens and Breese, 1990). However, while glucocorticoids inhibit GABA at higher concentrations, they may also potentiate GABA at lower concentrations (Majewska et al., 1985). The cortisol findings presented here to some extent support the suggestion that alcohol may impair NMDA receptor function via potentiation of GABA (Mehta and Ticku, 1988; Givens and Breese, 1990). However, the significant increase in DHEAS, a known GABA antagonist (Majewska et al., 1990; Demirgoren et al., 1991) that was noted at 45-min in the heavy beer trial is more likely to suggest that consuming some forms of commercially available alcohol products can also potentiate the function of excitatory NMDA receptors (Baulieu, 1996).

Cortisol and DHEAS differentially influence the function of the hippocampus (Diamond et al., 1996; Tombaugh et al., 1992), an area thought to be associated with spatial learning (Morris et al., 1982; Olton et al., 1982), and memory formation (Rickard et al., 1994). Prolonged elimination of glucocorticoids can decrease glucocorticoid receptor activity in the hippocampus and activation of glucocorticoid receptors is necessary for the development of behavioural strategies and storage of spatial information (Morris et al., 1982; Oitzl and De Kloet, 1992; Olton et al., 1982). Impairments in visuo-spatial (Molina et al., 1994), and problem-solving tasks (Brandt et al., 1983), is well documented in the alcohol literature, and the data presented here could suggest that the deficit in organization, planning and spatial learning often observed in alcoholics is due to an alcohol-induced inactivation of glucocorticoid receptors in the hippocampus (Morris et al., 1982; Olton et al., 1982). The cortisol data in the present study suggests that consuming commercially available alcohol can significantly decrease the level of fasting cortisol and in some instances post-meal cortisol, which could have some affect on glucocorticoid receptor activity in the hippocampus. This could possibly lead to specific cognitive impairment if alcohol is consumed on a regular basis.

The results of this study highlight the importance of design methodology when assessing the effect of alcohol on the HPA axis. It was shown that while all alcohol products can significantly reduce the level of salivary cortisol, the effect of alcohol on DHEAS is variable and may be dependent on the nutritional content of the beverage being consumed. Therefore, it is recommended that the nutritional content of the test beverage, in particular the carbohydrate content, be

carefully controlled when assessing the effect of alcohol on the HPA axis.

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