THE EFFECT OF RED WINE ON PLASMA LEPTIN LEVELS AND VASOACTIVE FACTORS FROM ADIPOSE TISSUE: A RANDOMIZED CROSSOVER TRIAL

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Abstract — Aims: It has been reported that alcohol has multiple effects on appetite. To elucidate potential mechanisms we measured the levels of plasma leptin and the vasoactive factors after red wine intake. Methods: We conducted a randomized crossover trial to study the effect of red wine on the levels of leptin, TNF-α, TGF-β1, IL-6, ICAM-1, and VCAM-1 in healthy, non-smoking individuals. The subjects were randomized to drinking one glass of red wine (150 ml, 15 g alcohol) every day (’wine period’) or to undergo a period of total abstinence from alcohol (’abstention period’). After 3 weeks they switched the intervention group. Eighty-seven volunteers completed the study (mean age 50 years). Results: After 3 weeks’ daily intake of red wine, plasma leptin was significantly increased (from 6308 pg/ml to 7402 pg/ml, P = 0.05). There was a marked gender difference, as leptin levels increased only in females (P = 0.012). When calculated as leptin/body mass index (BMI) ratio, the trend and results were similar. Red wine consumption had no significant effect on other vasoactive factors measured in this study. Conclusion: Red wine increases levels of the appetite-regulating hormone leptin in females, but not in males. Whether red wine has an effect on appetite-regulation in its own right, remains to be solved.

INTRODUCTION

Adipose tissue is now widely recognized as an important endocrine organ. Several hormones, growth factors and cytokines are produced and secreted from fat cells, including tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6), and their respective soluble receptors, as well as leptin, estrogen, angiotensinogen, plasminogen activator inhibitor-1 (PAI-1), tissue factor and transforming growth factor-beta (TGF-β) (Fruhbeck, 2004).

Leptin is an adipocyte-derived polypeptide hormone that controls body weight through central regulation of food intake (Ahima and Flier, 2000). Abundance of leptin mRNA in adipose tissue and concentrations of leptin in the circulation are strongly and positively correlated with body weight and obesity in man (Considine et al., 1996). Both leptin and its receptor share structural and functional similarities with the IL-6 family of cytokines (Fantuzzi and Faggioni, 2000). Further evidence from in vitro and animal studies suggests that leptin is also involved in regulation of the humoral inflammatory response (Lord et al., 1998; Bouloumie et al., 1999; Fantuzzi and Faggioni, 2000; Caldefie-Chezet et al., 2001; Faggioni et al., 2001; Yamagishi et al., 2001; Zarkesh-Esfahani et al., 2001; Farooqui et al., 2002). Increased leptin levels in the metabolic syndrome indicates its relevance to many aspects of cardiovascular and vascular regulation, including angiogenesis, thrombosis, hemodynamics, and cardiac hypertrophy, among others (Sierra-Honigmann et al., 1998; Narkiewicz et al., 2001; Bodary et al., 2002; Wolk et al., 2003).

Recent studies have reported increased appetite after alcohol consumption, and it has been suggested that alcohol may have multiple effects on appetite; it suppresses fatty acid oxidation; increases short-term thermogenesis; and affects a number of neurochemical and peripheral systems involved in appetite control (Yeomans et al., 2003). Therefore, the purpose of this study was to investigate the effect of red wine intake in a randomized crossover trial, on the levels of leptin and the vasoactive factors such as: TNF-α; TGF-β1; IL-6; as well as adhesion molecules ICAM-1 and VCAM-1, in order to elucidate potential mechanisms underlying increase of food intake after administration of alcohol.

Subjects and methods

The participants were recruited through an advertisement placed in a widely circulated newspaper in Oslo. According to the protocol, all the participants were 35–70-year-old healthy individuals who had not smoked tobacco daily for at least 3 months. The subjects were randomized to start with a period of drinking one glass of red wine (150 ml of Cabernet Sauvignon, 15 g alcohol) every day (’wine period’), or to undergo a period of total abstinence from alcohol (’abstention period’). After 3 weeks, the participants switched the intervention group with no wash-out period.

The study was approved by the regional ethics committee and all the participants gave their written informed consent. More details are given in a previously published report (Retterstol et al., 2005).

Measurement of leptin, ICAM-1, VCAM-1, IL-6, CRP, TNF-α, and TGF-β1. All the assays were performed with the investigators blinded to the group. Quantitative immunologic determinations of leptin, IL-6, TNF-α, and soluble ICAM-1 (sICAM-1) were performed in citrate plasma samples by enzyme-linked immunosorbent assay (ELISA) techniques with ELISA Quantikine (R&D Systems, Minneapolis, MN), and for VCAM-1 Quantitative ELISA (Bender Med Systems, Vienna, Austria). The procedural details recommended by the manufacturer were strictly followed. All determinations were done in duplicate. Intra-assay and inter-assay precisions were evaluated by calculating coefficients of variation with duplicates and triplicates of samples and control

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preparations. The intra-assay coefficients of variation were 4.4 and 5.9%, and the inter-assay coefficients of variation were 6.0 and 9.6%, respectively.

Determination of the serum concentration of the active form of TGF-β₁ was performed by an ELISA-technique using the ELISA-Quantikine kit (R&D Systems, Minneapolis, USA) as recommended by the manufacturer. Acid activation of the latent form of TGF-β₁ was achieved with 2.5 N acetic acid/10 M Urea followed by neutralization with 2.7 N NaOH/1M HEPES. Intra- and inter-assay coefficients of variation were 4.89 and 9.24%, respectively, and were considered to be satisfactory.

Statistical analysis. Since the distribution of most investigated parameters were skewed, non-parametric methods were applied to test for differences and correlations (Mann–Whitney U-test, Spearman’s correlation coefficient and Kruskal–Wallis test).

Out of the 92 subjects included, 87 participants (95%) completed the study. Mean age was 50 years (SD 9.6) and 57 out of 87 were females (66%).

RESULTS

Descriptive statistics for subjects and details regarding their use of alcohol at inclusion are displayed in Tables 1 and 2. Table 3 shows the values of measured parameters at baseline, total alcohol abstention, and after 3 weeks’ consumption of 150 ml of red wine per day. The results are presented for the whole group, and we observed a significant increase only in leptin levels, while no significant effects were observed on levels of ICAM-1, VCAM-1, IL-6, TGF-β₁.

TNF-α levels were below the lowest limit of detection for the ELISA (<3.5 pg/ml).

Furthermore, we divided the studied group into gender subgroups. Although an increase of leptin levels was observed in the whole group, it was only statistically significant in the female sub-group (Fig. 1). When baseline leptin levels at inclusion were analysed, we also found gender differences; females had considerably higher ($P < 0.002$) concentrations of leptin compared to males: 7574 versus 4452 pg/ml (median) (Fig. 1).

We also calculated leptin/body mass index (BMI) ratio, and the results were similar to the results given above: while increased ratio was observed in the whole group, it was statistically significant ($P = 0.017$; median levels 391.6 vs 302.4) only in the female sub-group. No significant changes were observed in the male sub-group (148.8 vs 146.5; $P = 0.09$).

In addition, the level of soluble form of VCAM-1 was significantly reduced among women: from 503.39 ng/ml in the abstention period to 470.80 ng/ml in the period of red wine drinking ($P < 0.018$) (results not shown).

Leptin correlated significantly only with CRP (rho = 0.26, $P = 0.02$) and fibrinogen (rho = 2.24, $P = 0.03$). Only gender and BMI were significant determinants for leptin.

DISCUSSION

In our study, the influence of moderate drinking of red wine on plasma leptin and vasoactive factors from adipose tissue was tested.

The clinical relevance of this finding is uncertain. Several explanations are possible, including the following: (i) red wine could interact with the regulation of appetite through leptin, and thereby, either increase or decrease food intake, (ii) red wine increases BMI, and thereby, leptin levels. The latter explanation could not be supported from our data.

It has previously been suggested (Szkudelski et al., 2004) that increase of blood leptin in ethanol-drinking rats may lead to restriction in food intake. Other reports, however, indicate that ethanol may enhance appetite due to leptin resistance transiently caused by ethanol (Fujita et al., 2003). With respect to studies in humans, it has been reported that acute moderate intake of alcohol (0.6 g/kg ethanol) had no effect on leptin levels in healthy male volunteers (Dammann et al., 2005). However, another controlled feeding and alcohol ingestion study (Roth et al., 2003) performed in post-menopausal women found increased leptin levels after moderate (15–30 g of alcohol per day) alcohol intake. Both studies are in agreement with our findings.

Nicolas et al. (2001) found elevated plasma leptin in active alcoholics and significantly related to lifetime ethanol consumption: the longer the period of alcohol consumption, the higher the leptin plasma levels. Nevertheless, a study on 122 Japanese workers (Yokoyama et al., 2004) reported no significant association between ethanol consumption and serum leptin level. This indicates the possibility of differences between ethnic groups.

In order to explore the question as to what might be the pathophysiological mechanism that links alcohol intake with increased leptin plasma levels, a study of whether TNF-α might represent the link was conducted on alcohol addicts.
Influences of red wine on leptin levels

Regarding gender differences, a recent clinical study (Valtuena et al., 2005) reported that serum concentrations of leptin and insulin were positively correlated in men independent of body composition, but not in post-menopausal women. In men, the steatogenic effect of hyperinsulinemia/insulin resistance in the context of low-to-moderate alcohol consumption appears to be mediated by high concentrations of serum leptin, whereas body fat alone could identify post-menopausal women at high risk for liver steatosis (Valtuena et al., 2005). Moreover, it has been shown that leptin correlates better with total body fat in females, as well as, the brains of females have been shown to be more sensitive to the catabolic actions of low doses of leptin (Woods et al., 2003). We found significantly higher baseline levels in females compared to male volunteers. The increased baseline levels of leptin in females may also explain why the increments were only significant among women.

Regarding correlations, we were able to confirm a report (Shamsuzzaman et al., 2004) that increased leptin is associated with increased CRP independent of gender, measures of adiposity, and other variables. This correlation can strengthen the possibility that leptin may be linked to cardiovascular pathophysiological processes, including a low-grade inflammation, and thereby, increased cardiovascular risk.

 Recently, acute effects of ingestion of red wine on markers of inflammation in men with coronary disease has been studied and no effect on VCAM-1 was observed (Hukshorn et al., 2004), which is in agreement with findings from our study. However, we observed gender differences with a significant decrease in VCAM-1 levels in females only. This could be explained by suggested gender difference in monocyte adhesion to endothelial cells, e.g. inhibition of monocyte adhesion by inhibiting expression of VCAM-1 by estradiol (Nathan et al., 1999).

The intake of red wine in the present study was modest (150 ml, 15 g alcohol), and this had no clinically relevant effect on the inflammatory markers such as IL-6 and adhesion molecules. Our study and a previously published report (Retterstol et al., 2005) did not support the hypothesis that moderate intake of wine reduces inflammation. These findings are apparently at variance with observational studies indicating that moderate intake of alcohol has a beneficial effect on cardiovascular diseases through an effect on inflammation. Perhaps there is a threshold effect of alcohol on the levels of inflammatory markers above the amount consumed in this study. If the red wine intake had been two or three glasses, we might have observed a greater effect on the levels of inflammatory markers.

Some epidemiological studies have found a lower risk of cardiovascular disease among wine drinkers than among drinkers of other types of ethanol. These findings should
be interpreted with caution, as these data were obtained from questionnaires, and combinations of different alcoholic beverages were frequently reported (Retterstol et al., 2005). The effect of red wine, non-alcoholic compounds of red wine and placebo on established cardiovascular risk factors have been studied in healthy volunteers (Hansen et al., 2005). Desirable changes in HDL-C and fibrinogen were found after moderate consumption of red wine for 4 weeks compared with drinking water with or without red grape extract. Thus, the impact of wine appeared primarily explained by an alcohol effect. Nevertheless, randomized prospective studies comparing wine and beer (or other types of alcohol) should be used to address this question.

CONCLUSION

Red wine raises the level of the appetite-regulating hormone leptin. The effect is gender dependent. Whether red wine has an effect on appetite-regulation on its own, remains to be solved.

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