The Phytoestrogen Congeners of Alcoholic Beverages: Current Status (43839)

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Abstract. **The idea that alcoholic beverages might contain biologically active phytoestrogenic congeners stemmed from findings of overt feminization observed in alcoholic men with alcohol-induced cirrhosis. Specifically, in addition to being hypogonadal, these chronically alcohol-abusing men with cirrhosis frequently manifest gynecomastia, palmar erythema, spider angiomata, and a female escutcheon. These physical signs of exposure to active estrogen occur in the presence of normal or only minimally elevated levels of endogenous steroid estrogens. Because levels of circulating steroid hormones failed to provide a satisfactory explanation for the feminization observed, alternate explanations were considered.**

If the estrogenization observed was not entirely a function of tissue expose to steroid estrogens produced *endogenously*, then perhaps tissues were being exposed to *exogenous* estrogenic substances from dietary sources. Given the degree of alcohol abuse in the population in which hypotheses for feminization were being formed, alcoholic beverages became a prime candidate as a dietary source of exogenous estrogenic substances. [P.S.E.B.M. 1995, Vol 208]

The Rationale

Based on the estrogenic activity of a variety of plants and plant substances, the compounds causing this activity were termed phytoestrogens. Biologically active nonsteroidal phytoestrogens, as well as classical steroid estrogens and the sterol β-sitosterol, were ultimately isolated and identified from the plants, derivative oils, and mill by-products which had been known to possess estrogenic activity, (1–18). Major classes of phytoestrogens include the isoflavones and their isoflavonoid metabolites, as well as the lignans, mycotoxins, and the coumestans. The metabolism of the isoflavones and lignans has been studied extensively in animals (19–23) and more recently in humans (24–27).

Alcoholic beverages are made from a variety of plants sources. The isolation and identification of phytoestrogens, and the demonstration of their metabolism in humans provided the necessary linking data for the hypothesis that constituents of alcoholic beverages might play a role in the feminization of alcohol-abusing men with cirrhosis. Reports that hops, rice, and corn, the respective constituents of beer, saki, and bourbon, are phytoestrogenically active spurred experimental evaluation of the estrogenicity of alcoholic beverages (7, 28–30).

Bourbon Estrogenicity

The first alcoholic beverage to be systematically examined for estrogenicity was bourbon. Because bourbon is made from corn, the probability of phytoestrogens being present was high. Because bourbon had been tabulated as having the highest overall congener content (31), it was thought to be likely that phytoestrogens might also be present.

***In Vivo* Animal Studies.** The first tactic used to evaluate whether or not bourbon could produce estrogenic effects was simple: congener concentrates of bourbon were administered to appropriate experimental animals (32). The animal model used was one of endogenous estrogen deprivation: ovariectomized sexually mature rats. In such animals, the uterus is maximally atrophied, and levels of gonadotropins are maximally elevated. By using such a model, the likelihood

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is maximized of detecting a decrease in gonadotropin levels and/or an increase in the mass of the uterus, responses which would be indicative of exposure to exogenous estrogenically active substances.

The bourbon congener concentrates were prepared by rotoevaporation of bourbon to dryness and reconstitution in 1.8% or 3.6% ethanol containing 0.05 *M* sodium bicarbonate; the bicarbonate was required to achieve a neutral pH, and the ethanol was required to solubilize all the rotoevaporated nonvolatile congeners (33). The congener concentrates were administered as drinking water; 100 ml of drinking water contained the congener equivalent of either 1 jigger (44 ml [1.5 ounces] of 80 proof bourbon) in 1.8% ETOH, or of 2 jiggers of bourbon in 3.6% ETOH. Experimental animals consumed approximately 40 ml drinking water daily.

The effects of the administration of the two doses of bourbon congener concentrates for 30 days to ovariectomized wistar rats are shown in Figure 1. The weight of the uterus corrected for body weight increased significantly in a dose-dependent manner in the animals administered the bourbon congener concentrate ($r = 0.532$; $P < 0.01$); no such changes were observed among the ethanol controls. These changes in uterus mass reflected differences in histology. A columnar epithelial lining showing evidence of secretory activity with mucus-containing vacuoles was seen in the bourbon animals, while a low, flat cuboidal epithelial lining with no evidence of secretory activity was seen in the water and ETOH controls.

Similar to the effect on the uterus, a significant dose-dependent decrease in plasma levels of luteinizing hormone (LH) ($r = -0.292$; $P < 0.05$) was seen only in the rats given the bourbon congener concentrates. These changes in both uterus weight and LH concentrations only among the two dose groups of rats administered the bourbon congener concentrates occurred in the absence of differences in levels of the endogenous steroid estrogen estradiol among the experimental groups (Fig. 1). These findings in ovariectomized rats clearly demonstrated that the bourbon congener concentrates were fully capable of eliciting a dose-dependent estrogenic response.

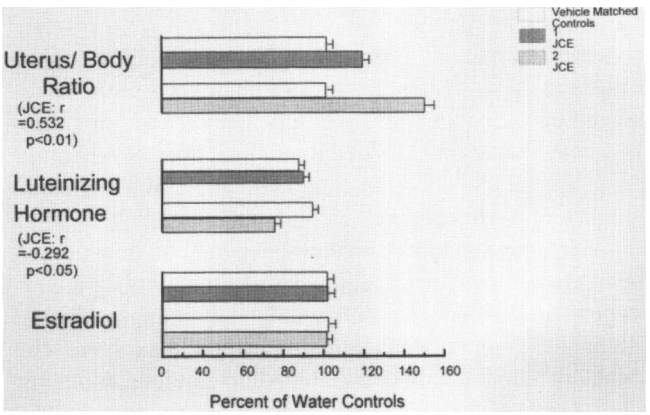

Figure 1. Effect of 4-week administration of bourbon jigger congener equivalents (JCE) to ovariectomized rats. Values for the uterus/body ratio, luteinizing hormone, and estradiol in the experimental animals are expressed as a percentage of the mean value observed in the control group (water alone). The length of the bar represents the mean, and the brackets represent the standard error of the mean. For the 1-JCE animals, vehicle controls received 1.8% ethanol, while for the 2-JCE animals, vehicle controls received 3.6% ethanol.

***In Vitro* Estrogen Receptor Studies of Bourbon.** The second tactic used to evaluate the estrogenicity of bourbon was an assessment of the ability of a bourbon extract to compete with radiolabeled estradiol for estrogen receptor binding sites. The extract was prepared by 24-hr constant choloform extraction, followed by rotoevaporation and resuspension of the sludge in 10 ml of absolute ETOH; 1 ml of the resultant extract contained the congener equivalent of 15 ml of bourbon. Rabbit uterine cytosolic estrogen receptor preparations were made and receptors binding assays were performed using previously published methods (33). Percentage tritiated estradiol binding remaining after competition with the undiluted extract was 37%, with a 1:4 dilution was 60%, and with a 1:10 dilution of the bourbon extract, equivalent to 0.375 ml of bourbon, was 96%. The ability of the bourbon extract to effectively compete for estrogen receptor binding sites not only provided a biological mechanism for the estrogen responses seen in the ovariectomized rats administered the bourbon congener concentrates, but also fit well with literature reports of the capability of phytoestrogens to interact with estrogen receptors (34–36).

Gas Chromatography/Mass Spectrometry Identification of Bourbon Phytoestrogen Congeners. Having demonstrated the estrogenicity of the bourbon preparations, the next tactic was to isolate and identify the phytoestrogens that might be present. Using previously reported standard methods, GC/MS analysis was performed in both the repetitive scan and selected ion monitoring modes (33, 37). Both biochanin A and β-sitosterol were identified on the basis of both retention time and scanned mass spectra. Quantitation studies in three different brands of bourbon found β-sitosterol to be present in amounts ranging from 7 to 21 μg/100 ml bourbon (38).

A Definitive Experimental Approach: Administration of Bourbon Congener Concentrates to a Clinical Sample. The fourth tactic used to address the question of whether or not alcoholic beverage phytoestrogen congeners are capable of eliciting a clinical estrogen response involved the logical extension of the experimental rat model: normal postmenopausal women. Four postmenopausal women were administered a bourbon congener concentrate equivalent to 4 oz of bourbon (2.7 jiggers; less than three standard

drinks) daily for 28 days (39, 40). Blood samples were obtained 1 week before the initiation of the experiment, weekly throughout the course of administration of the congener concentrate, and 1 week following the end of the experiment. Blood was assayed for levels of LH, follicle-stimulating hormone (FSH), and prolactin, as well as for sex hormone binding globulin (SHBG), total cholesterol, and high density lipoprotein cholesterol (HDL). Control data for hormone levels were available from a study in which the variability of hormone levels in normal postmenopausal women over the course of 4 weeks had been evaluated (41).

The hormone data are shown in Figure 2. Levels of both LH and FSH decreased during the period of administration among the women receiving the bourbon congener concentrate and then returned to baseline after cessation of exposure; no change in levels of either LH or FSH were observed among the normal controls over time. A similar pattern was seen for the increase in prolactin levels. As seen in Figure 3, levels of two hepatic estrogen-responsive proteins, SHBG and HDL, also increased during the period of bourbon congener concentrate administration and then returned to baseline 1 week following the end of the experiment.

The changes observed were the changes which would have been hypothesized to occur with exposure to biologically active estrogenic substances. Thus, the pilot study in normal postmenopausal women not only confirmed and extended the results obtained in the ovariectomized rat model, but also provided evidence of clinical relevance. With the demonstration of estrogenic responses in human subjects, these findings shifted from perhaps an interesting epi-phenomenon to a concept with important epidemiological implications.

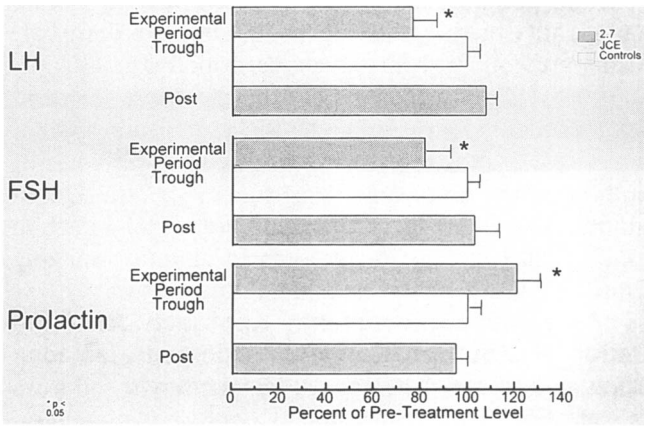

Figure 2. Effect of 4-week administration of bourbon jigger congener equivalents (JCE) to normal postmenopausal women: pituitary hormones. Values are expressed as a percentage of pretreatment levels in the experimental group, and a percentage of Week 1 levels in the control group. The length of the bar represents the mean, and the brackets represent the standard error of the mean. An asterix indicates values which are statistically different from pretreatment values.

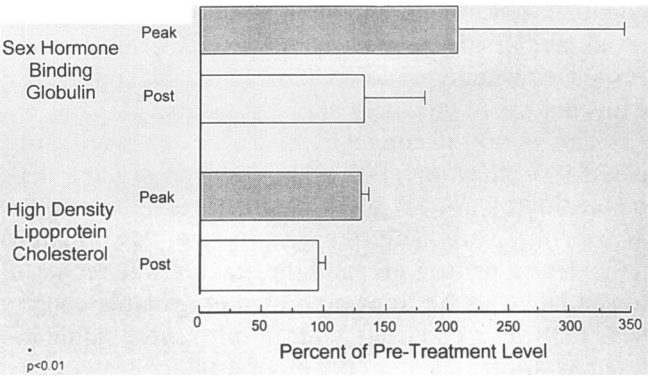

Figure 3. Effect of 4-week administration of bourbon jigger congener equivalents (JCE) to normal postmenopausal women: estrogen responsive hepatic proteins. Values are expressed as percent of pretreatment levels. The length of the bar represents the mean, and the brackets represent the standard error of the mean. An asterix indicates values which are statistically different from pretreatment values.

Estrogenicity of Other Alcoholic Beverages

The studies of the biological activity of the phytoestrogen congeners of bourbon are seminal. However, because bourbon is neither the exclusive beverage nor the sole alcoholic beverage of choice of alcohol users, the question about phytoestrogenic congeners in other alcoholic beverages becomes insistent. Two approaches have been used to begin to answer the question.

First, because hops contained estrogenic activity and are a major constituent of beer, GC/MS methodology was used straightaway to identify the phytoestrogen congeners of beer. Two isoflavonoid phytoestrogens, daidzein and genistein (42), were identified. That different isoflavonoid phytoestrogens are present in bourbon as compared with beer is an important finding *ipso facto*. For disease risk and health benefit states such as osteoporosis and coronary heart disease in which estrogen exposure may be a factor which influences risk, difficulty in establishing a clear association between risk and alcoholic beverage consumption may be at least partially a function of different alcoholic beverages having different phytoestrogen congeners with varying biologic activity.

The second approach has used estrogen receptor methods to begin to screen a variety of alcoholic beverages for estrogenic activity (43, 44). Using cytosolic estrogen receptor preparations from male rat liver, the ability of wine and bourbon to compete detectably for binding sites was evaluated. Extracts of four wines and of bourbon were prepared, using sep pac extraction methods, and tested. As seen in Figure 4, the equivalent of 1.25 ml of each beverages tested competed effectively for estrogen receptor binding sites. Figure 4 also shows the relative potencies of the four phytoestrogens that have been identified by GC/MS in bourbon and beer.

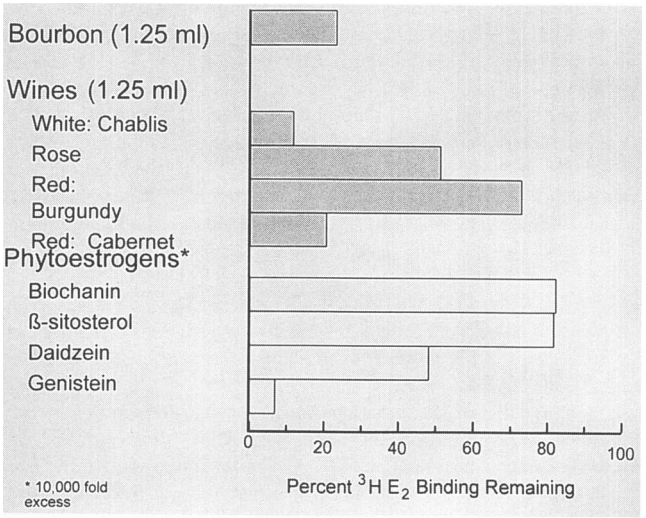

Figure 4. Estrogenic activity of selected alcoholic beverages. The results of the ability of 1.25 ml selected alcoholic beverages and phytoestrogen standards to compete with estradiol for cytosolic estrogen receptor binding sites. The length of the bar represents the percentage of labeled estradiol binding remaining; the shorter the bar, the higher the relative *in vitro* estrogenic activity.

Summary

The evidence obtained from *in vitro* estrogen receptor competitive binding studies that bourbon and red wine extracts, as well as the four identified phytoestrogens in bourbon and beer, are biologically active is clear. The evidence obtained using GC/MS that two types of alcoholic beverages, bourbon and beer, contain phytoestrogen congeners is unequivocal. The evidence obtained from *in vivo* studies in rats, and even more importantly in human volunteers, that the phytoestrogen mix contained in bourbon is fully capable of producing hormonal and biochemical estrogen-exposure responses is straightforward. Although a great deal of work remains to be done, it is clear that the diversity of phytoestrogen congeners contained in alcoholic beverages and the differences in relative potencies of these phytoestrogens congeners will dictate more careful scrutiny of specific types of alcoholic beverages in studies related to alcoholic beverage consumption and disease risk and protection.

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