Effects of food components and the ratio of epitestosterone to testosterone on steroid glucuronidation.

C. Jenkinson, J Barker, A Petroczi, D Naughton.
School of Life Sciences, Kingston University, Kingston Upon Thames, Surrey KT1 2EE

Introduction

-Endogenous testosterone has been known to be used by athletes as a method of enhancing performance in sporting competition.

-The use of testosterone in sport is currently banned and listed on the WADA prohibited substances list.

-UGT2B17 is the most effective UGT enzyme in the glucuronidation of testosterone to testosterone glucuronide (TG), for excretion in urine.

-The excreted TG serves as a marker for testosterone abuse in sport, by measuring the ratio of TG over epitestosterone glucuronide.

-Previous studies have shown pharmaceuticals such as ibuprofen and diclofenac are competitive inhibitors of UGT2B17, however little is known about the effect common foods could have on the metabolism of testosterone.

-The aim of the project was to screen common dietary substances for inhibition of UGT2B17 mediated testosterone glucuronidation as well as determining the interaction of epitestosterone on testosterone glucuronidation at ng/ml levels and in gender-different microsomes.

Methods

Enzyme assays where performed with 0.1-0.2mg/ml enzyme protein, with an enzyme substrate-only preparation as controls, to compare the effects of dietary components TG formation . Reactions where run for 30-90 minutes with a range of initial testosterone concentrations for analysis. The tea samples where dissolved in boiling water with equal weight/volume and wine samples were diluted in H_2O.
An HPLC method was developed to analyse the remaining testosterone to monitor UGT activity. The HPLC method was: methanol/water (80/20) with testosterone dissolved in DMSO, acetonitrile/water (39/61) when testosterone was dissolved in acetonitrile. Measurement was at 246nm. A reported HPLC method was also used to analyse the key compounds in the tea samples. HPLC method – methanol/water/ortho-phosphoric acid (20/79.9/0.1). The flow rate for all HPLC analyses was 1ml/min. LC-MS/MS analysis was used for detection of testosterone at ng/mL level for analysis of epitestosterone interaction.

RESULTS - Green and white teas and catechins

Samples	Testosterone glucuronidation Rate (ng/ml/min/mg prot.)
Control (enzyme substrate)	682.09 ±30.73
Green tea	179.56 ±22.64
White tea beard	249.83 ±18.87
White tea Leaf	246.22 ±16.61
White tea powder	69.57 ±11.04
Cacao beans	666.22 ±23.55
Cacao block	572.89 ±20.14

Table 1. Effect of green and white tea samples and cacao on testosterone glucuronidation. Reaction time 30 minutes. Protein concentration 0.2mg/ml

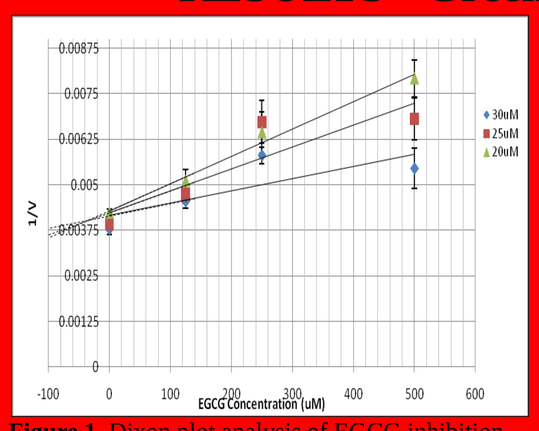


Figure 1. Dixon plot analysis of EGCG inhibition of testosterone glucuronidation. IC50 = 64µM

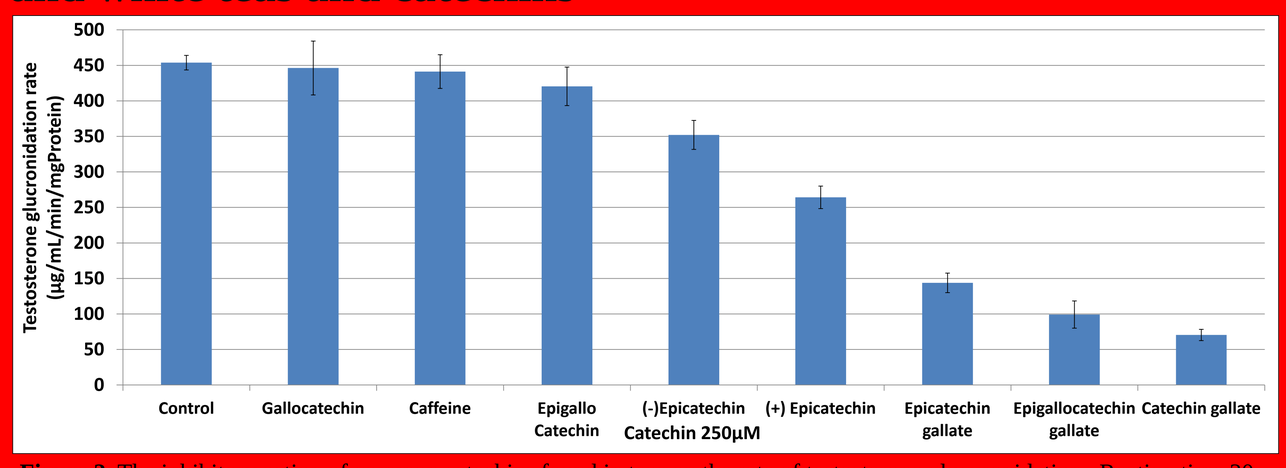
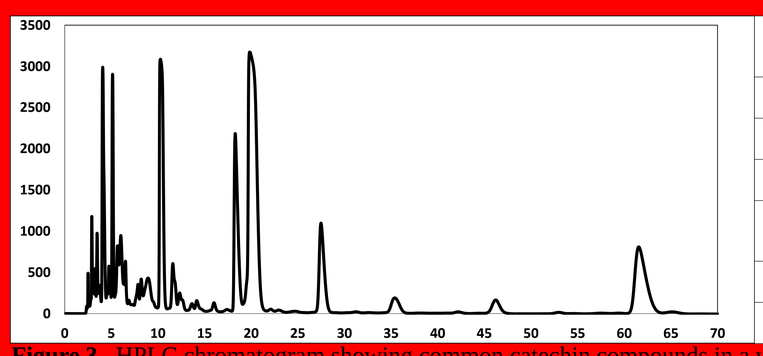


Figure 2. The inhibitory action of common catechins found in teas on the rate of testosterone glucuronidation. Reation time 30 minutes. UGT2B17 protein concentration 0.1mg/ml



Compound	Retention time (min)
Gallo catechin	5
Epigallocatechin	10.2
Caffeine	18.5
Epigallocatechin gallate	20.2
Epicatechin	28
Epicatechin gallate	62

Figure 3. HPLC chromatogram showing common catechin compounds in a white tea leaf sample used as an inhibitory sample in figure 1.

Red wine and phenolic compounds

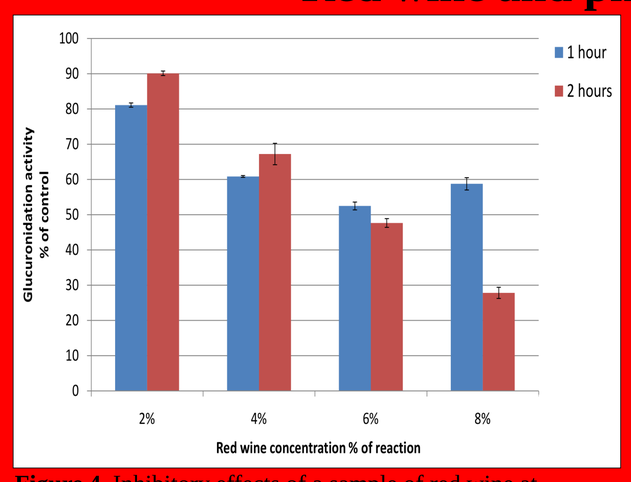


Figure 4. Inhibitory effects of a sample of red wine at increasing concentration on testosterone glucuronidation.

Initial testosterone concentration (µM)	Test sample	Test sample Concentration (µM)	Glucuronidation % of control (±SEM)
100	4-Ethylphenol	750	78.90 ±1.131
50	4-Ethylphenol	750	57.30 ±13.548
100	Gallic acid	250	91.01 ±10.946
100	Caffeic acid	250	78.65 ±5.685
100	p-Coumaric Acid	250	N/A
100	Chlorogenic Acid	250	N.A
100	Quercetin	250	28.01 ±2.800
20	Quercetin	20	65.62 ±14.298

Table 2. Reduction in UGT2B17 testosterone glucuronidation activity by phenolic compounds commonly found in red wine.

Epitestosterone

Epitestosterone (ng/ml)	T/E ratio	Testosterone glucuronidation rate (ng/ml/min/mg prot.)
0		8.27 ±0.129
3.125	16	8.30 ±0.099
6.25	8	8.28 ±0.057
12.5	4	8.16 ±0.149
25	2	7.97 ±0.240
50	1	7.85 ±0.064
100	0.5	7.64 ±0.085
200	0.25	7.63 ±0.084

Table 3. LC-MS/MS testosterone glucuronidation rate analysis with increasing epitestosterone.

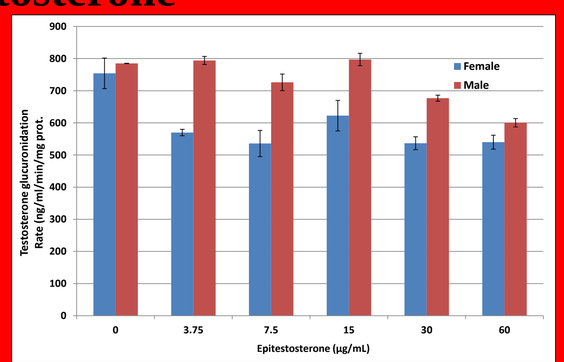


Figure 5. Testosterone glucuronidation rate with male and female microsomes and additional epitesosterone.

Conclusions

-Commonly consumed dietary teas and red wine interact with testosterone glucuronidation by inhibition of UGT2B17. Epitestosterone reduces the rate of testosterone glucurondation.

-These dietary compounds could have implications on the current method of detecting testosterone abuse in sport by having an impact on altering steroid metabolites in urine as well as potentially altering circulating levels.

-Current studies are analysing further dietary samples for inhibition of these UGT enzymes and analysing these samples at lower serum concentrations.

-References
1. Sten T *et al.* (2009). Steroids. 74: 971-977.
2. Jenkinson, C., Petroczi, A., Barker, J. and Naughton, D. P (2012a). "Dietary Green and White Teas Suppress UDP-Glucuronosyltransferase UGT2B17 Mediated Testosterone Glucuronidation," Steroids, 77 (6) 691-695.

Results and discussion

-The green tea samples caused a reduction in UGT2B17 testosterone glucuronidation activity over a 90 minute period, along with two catechin compounds commonly found in teas and other foods, epicatechin (EC) and epigallocatechin gallate (EGCG).

-The analysis of the tea samples by HPLC revealed the overall concentrations of catechins present in these samples, this appeared to show a correlation of increasing catechin concentration with increased inhibitory effect, this was particularly evident for the powdered white tea sample.

-Inhibition by the catechin compounds appeared greatest at lower initial concentrations of testosterone (7.5-40µM). The EGCG compound also showed competitive inhibition based on the intersecting trendline of the Dixon plot being above the X axis.

- Studies confirmed the interaction of epitestosterone on testosterone glucuronidation at lower ng/ml concentration, representing concentrations commonly found in the body.