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TITLE

Ethanol induces hydroxytyrosol formation in humans

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Abstract

Previous studies in animals have shown an increase of hydroxytyrosol (OHTyr), a potent phenolic antioxidant and a minor metabolite of dopamine (also called 3,4-dihydroxyphenylethanol or DOPET), after ethanol intake. The interaction between ethanol and dopamine metabolism is the probable mechanism involved. The aim of the study was to establish the contribution of the dose of ethanol on OHTyr formation. 24 healthy male volunteers were included. Subjects were distributed in three different cohorts and each volunteer received two doses of ethanol or placebo. Doses of ethanol administered were 6, 12, 18, 24, 30 and 42g. Study design was double-blind, randomized, crossover and controlled. Hydroxytyrosol, tyrosol (Tyr), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) urinary excretion, ethanol plasma concentrations and drunkenness were evaluated along a 6-hour period. Urinary excretion of OHTyr and Tyr increased with ethanol administered dose. A reduction in the ratio DOPAC/OHTyr from placebo to the highest dose was observed, compatible with a shift in the dopamine metabolism to preferently produce OHTyr instead of DOPAC. Also a dose-dependent increase in plasma ethanol concentrations and subjective effects was observed. This study demonstrates an endogenous production of OHTyr and Tyr in relation to ethanol administered dose in humans. Biological effects of both phenols from this source should be investigated in future studies.

Keywords

Alcohol, dopamine, hydroxytyrosol, DOPET, tyrosol

Abbreviations

4-HPAA	4-hydroxyphenylacetic acid
AE	adverse events
$AUC_{0\text{-}6h\_c}$	area under the blood concentration curve from 0 to 6h
$AUC_{0\text{-}6h\_e}$	area under the curve for effects (drunkenness) from 0 to 6h
C_{max}	maximum blood alcohol concentration
DA	dopamine
DOPAC	3,4-dihydroxyphenylacetic acid
DOPAL	3,4-dihydroxyphenylacetaldehyde
DOPET	3,4-dihydroxyphenylethanol
EIA	enzyme immunoassay
E_{max}	maximum alcohol effect (drunkenness)
GCMS	gas chromatography mass spectrometry
HDL	high density lipoprotein
HVA	homovanillic acid
HPLC/MS/MS	liquid chromatography coupled to tandem mass spectrometry
LDL	low density lipoprotein
OHTyr	hydroxytyrosol
t_{max_c}	time to reach maximum blood alcohol concentration
t_{max_e}	time to reach maximum effect (drunkenness)
Tyr	tyrosol
VAS	visual analog scale

1. Introduction

Accumulating scientific evidence indicates that light to moderate drinking done on a daily basis may significantly reduce the risks of coronary heart disease (CHD) and all-cause mortality [1-3]. A J-shaped relationship describes the association between alcohol and total mortality. Ethanol doses higher than 4 drinks per day in men or 2 drinks per day in women are associated with increased risk of medical complications and death [1].

Moderate alcohol consumption is thought to be protective because improves insulin sensitivity, reduces several coagulation factors and inflammation, increases fibrinolytic capacity and also rises high density lipoprotein (HDL) cholesterol concentrations in a dose dependent manner [4,5]. However, mechanisms involved are poorly understood and controversy still exists regarding if beneficial effects are primarily attributable to ethanol [6,7], to polyphenols or to both components in some alcoholic beverages, like wine [8].

Hydroxytyrosol (OHTyr) is the main phenol present in olive oil and also in minor quantities in wine [9]. It is one of the most potent antioxidants present in the Mediterranean Diet. In the EUROLIVE study, oxidative stress markers including oxidized low-density lipoprotein levels, decreased linearly with the increasing phenolic content (including OHTyr) of olive oil [10]. According to these data a health claim was released by the European Food Safety Authority (EFSA) for the consumption of 5 mg per day of OHTyr and its derivatives in olive oil [11] as protective of LDL particles from oxidative damage. In terms of safety it has been shown in vitro that OHTyr is non-genotoxic and non-mutagenic at concentrations exceeding those attainable after intake [12].

Data from a bioavailability study of resveratrol after red wine administration in healthy volunteers showed a recovery of substantial amounts of OHTyr that could not be explained by the small quantities contained in wine. A 200% of the administered dose was recovered in urine suggesting OHTyr endogenous formation after wine intake [9].

Furthermore, in a subsample (n=1009) of a large intervention clinical trial, intended at demonstrating the effects of a Mediterranean-style Diet on primary prevention of cardiovascular disease, it was observed that baseline OHTyr urinary concentrations correlated with wine consumption , but also with ethanol ingestion [13].

Previous studies in animals have shown an increase of DOPET (3,4-dihydroxyphenylethanol, OHTyr) formation, a minor metabolite of dopamine (DA), due to the presence of ethanol [14,15]. In a study with liver slices the addition of ethanol changed the ratio DOPAC (3,4-dihydroxyphenylacetic acid)/OHTyr from 10 to 0.25, compatible with a shift in DA metabolism from the oxidative pathway to produce DOPAC to the reductive one to produce OHTyr [16]. Other routes for OHTyr production had also been described in animals through the conversion of DOPAC to OHTyr via DOPAC reductase [17] or through DOPAL oxidation via an aldehyde reductase (ADR) [18]. MOPET (4-hydroxy-3-methoxyphenylethanol or HVAL) is the methylated metabolite of OHTyr while homovanillic acid (HVA) is the main metabolite of DOPAC. While OHTyr and HVAL are present physiologically in low concentrations in biological matrices DOPAC and HVA are more abundant and the last one is a typical biomarker of dopamine turnover. See in **Figure 1** a general description of all components involved in dopamine metabolism.

On the other hand, ethanol is converted to acetaldehyde by hepatic oxidative metabolism in a reaction regulated by alcohol dehydrogenase (ADH). In turn acetaldehyde is converted in acetic acid (acetate) by acetaldehyde dehydrogenase (ALDH). Both reactions produce reduced nicotinamide adenine dinucleotide (NADH). The reductive environment created is thought to be responsible for the change in the aldehyde (DOPAL) metabolism enhancing the formation of the alcohol derivative (OHTyr or DOPET) instead of the acid one (DOPAC) [16]. A similar shift was also observed for serotonin, where the alcohol metabolite 5-hydroxytryptophol was preferably produced after ethanol intake instead of 5-hydroxyindolacetic acid [19].

Taking into account the studies with ethanol conducted in animals and preliminary data obtained with wine in humans it was hypothesized that the interaction of ethanol (also present in wine) with the metabolism of DA to produce OHTyr, could explain, at least in part, the human beneficial health effects of low doses of ethanol [9].

Tyrosol (Tyr or 2-(4-hydroxyphenyl)ethanol) is also a well-known phenolic compound that is mainly present in extra-virgin olive oil and wine. It has also anti-inflammatory and antioxidant properties [20,21]. However, in comparison with OHTyr, Tyr has lower antioxidant activity because it lacks of the hydroxyl group in position 3 of the phenolic ring [22]. In animals Tyr excretion increased after ethanol administration due to an alteration of tyramine metabolism [23]. In our study Tyr excretion was measured as a secondary outcome.

The aim of the study was to establish the contribution of the dose of ethanol on OHTyr formation.

2. Materials and methods

2.1 Participants

The study was conducted in accordance with the Declaration of Helsinki and approved by the local Ethics Committee (CEIC Parc de Salut Mar). Informed consent was obtained from all volunteers previously to any study related procedure and they were paid for their participation. The study was registered in clinicaltrials.gov (NCT01788670).

Eligibility criteria required social ethanol consumption. Subjects with daily alcohol consumption higher than 30g or meeting criteria of ethanol abuse or dependence were excluded. To confirm health status, volunteers were interviewed by a physician and underwent a general physical examination, routine laboratory tests, urinalysis, and a 12-lead electrocardiogram.

2.2 Study design, procedures and outcomes

The study design was double-blind, randomized, crossover, and controlled with placebo. Participants were distributed in three different cohorts. In cohort 1, doses of 18 and 30g of ethanol were administered to 12 subjects. In cohort 2, doses of 6 and 12g of ethanol were administered to 6 subjects. Finally in cohort 3 doses of 24 and 42g of ethanol were administered to 6 subjects. Thus each participant received two doses of ethanol and placebo in three different experimental sessions (6h duration per session) with a minimum wash out period of three days between them. Participants were randomly assigned to each treatment sequence using a balanced 3 x 3 Latin square design.

Subjects were requested to abstain from ethanol ingestion three days before each session. Olive oil and olives were also prohibited due to its high OHTyr content. Beverages containing xanthines were not allowed in the previous 24 h

and during the experimental sessions. Subjects were also requested to abstain for any drug of abuse during the study. Breath alcohol tests and drug of abuse tests in urine (Instant-View®, Alpha Scientific Designs, Inc, Poway, CA, USA) were conducted along the study to confirm abstinence.

On session day, participants arrived at the clinical trials unit at 08:00 AM. An intravenous catheter was inserted into a subcutaneous vein to obtain blood samples. Treatments were administered at 8:30 AM in fasting conditions and a light meal (half of a cheese sandwich) was provided 2 and 6h after treatment administration. Additional water was given to volunteers at 2h (300 ml) and 4h (100ml) after administration in order to assure urine generation in each time interval. Participants left the unit 6 hours after administration once verified that the breath alcohol test was negative. Tobacco smoking was prohibited during the experimental sessions.

The main outcome of the study was total OHTyr urinary concentrations from 0 to 6h after administration. Secondary outcomes included ethanol plasma concentrations and subjective effects (drunkenness feelings). DOPAC, HVA and total tyrosol (Tyr) urinary concentrations from 0 to 6 h after administration were also assessed. Total OHTyr was calculated as the sum of: OHTyr-3-*O*-glucuronide, OHTyr-4-*O*-glucuronide, OHTyr-3-*O*-sulfate, free OHTyr and total 4-hydroxy-3-methoxyphenylethanol (HVAL). Total HVAL in turn was the sum of free HVAL + HVAL-4-*O*-glucuronide. Total Tyr in urine was calculated as the sum of free Tyr and Tyr-4-*O*-glucuronide.

Ethanol in plasma was determined at pre-dose and at 15, 30, 45 minutes, and 1, 1.5, 2, 3, 4, 6 hours after administration. Subjective effects were measured by

means of a visual analogue scale (VAS) of drunkenness from 0 to 100 mm at pre-dose and 30 minutes and at 1, 2, 4 and 6h after administration [24].

Urine samples were collected just before administration (spot sample) and at different interval periods after treatment administration (0-2h, 2-4h, 4-6h). Urine 0-6h was the sum of the three collection intervals.

Heart rate, blood pressure and oral temperature were measured with CarescapeTM V100 monitor (GE Healthcare. Milwaukee, WI) across the sessions (baseline, and at 1 and 6h after administration) and adverse events during the study were also recorded.

2.3 Treatments

Ethanol conditions were obtained mixing ethanol (pharmaceutical grade) and lemon flavored water (Fontvella, Barcelona, Spain). Placebo consisted in lemon flavored water. The total volume of the beverages ingested was 150 ml. Beverages were administered in opaque recipients, served cold and ingested along 5 minutes.

2.4 Samples preparation and analysis

Blood samples were collected in lithium heparin tubes for alcohol analysis. After centrifugation at 3000 rpm for 10 minutes at 4° C, plasma was transferred to tubes sealed with a plastic paraffin film and frozen immediately to avoid alcohol evaporation. Blood ethanol concentrations were determined with the DRI® Ethyl Alcohol Assay (Thermo Fisher, Fremont, CA, USA).

Urine samples were collected in different containers and the total amount of urine generated in each time interval was registered. Three aliquots were saved from each time interval for the assessment of phenolic compounds and DA metabolites' concentrations. Urines were treated with hydrochloric acid to

acidify the sample. Phenolic compounds and its metabolites were determined by liquid chromatography coupled to tandem mass spectrometry (HPLC/MS/MS), as previously described [25,26]. DOPAC and HVA were measured by GCMS [27,28].

2.5 Statistical analysis

Differences from baseline were calculated for both subjective and physiological outcomes. Regarding plasma concentrations of ethanol and subjective effects the following pharmacokinetic parameters were calculated: maximum concentration (C_{max}), or maximum effect (E_{max}), the time to reach the maximum concentration ($t_{max\_c)}$ or effect (t_{max_e}), and area under the curve from 0 to 6 hours for concentrations ($AUC_{0\text{-}6h\_c}$) and effects ($AUC_{0\text{-}6h\_e}$). The AUC were calculated using the trapezoidal rule. The same parameters were calculated for physiological outcomes. Total urinary excretion of OHTyr and Tyr as well as the ratio between DOPAC and OHTyr excretion were calculated from 0 to 6h. For each of the outcomes of interest, a linear mixed model with a random intercept and ethanol dose as independent variable was fitted. These models account for the correlation between the repeated measures within study participants. In the case of phenols' excretion, DOPAC/OHTyr ratio, and the $AUC_{0\text{-}6h}$ of the ethanol concentrations, the relationship between the outcomes and ethanol dose was not always linear. For that reason, log-transformations of only the outcomes and of both the outcomes and the ethanol dose were also considered and those models that showed the most adequate model fit based on graphical inspection of the corresponding residual plots were used for the corresponding analysis. In addition, the analyses for the main outcomes were also performed with the weight-adjusted ethanol dose as independent variable. Pearson's correlation

coefficient was used to quantify the association between ethanol concentrations, subjective effects, total OHTyr, total Tyr and DOPAC excretion (0-6h). Statistical significance was set at 0.05 and the statistical software package R, version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria) was used for the analyses.

3. Results

3.1 Participants

Twenty four male healthy volunteers were included in the study. All were non-smokers but eight (27.8%). Their average consumption of alcohol was 7g a day (5 units per week; 1 unit=10g of ethanol). The mean age, body weight and body mass index were 25.8 ± 4.5 years, 79.2 ± 6.5kg and of 24.3 ± 2.2kg/m^2, respectively.

3.2 Ethanol concentrations

Baseline samples were all negative for ethanol. Ethanol pharmacokinetic parameters calculated from 0 to 6h after administration increased with the ethanol administered dose. C_{max} increased in a dose linear manner (each gram of ethanol increased the C_{max} on average in 0.43 nmol/ml (95%-CI: [0.4, 0.5]; p<0.001). The logarithm of the $AUC_{0\text{-}6h\_c}$ increased linearly as a function of the logarithm of the ethanol dose (log-dose): on average, augmenting the log-dose by one unit, the log-$AUC_{0\text{-}6h\_c}$ increased by 1.47 units per gram of alcohol (95%-CI: [1.3, 1.7]; p<0.001), which is equivalent to an increase of the $AUC_{0\text{-}6h\_c}$ by factor 1.47. Maximum blood alcohol concentrations were reached at 23 minutes (6g), 30 minutes (12g, 18g), 37 minutes (30g) and 45 minutes (24g,

42g) after administration. Model-based mean estimations of the C_{max} and $AUC_{0\text{-}6h\_c}$ and the corresponding 95% prediction intervals are shown in **Table 1.**

Ethanol could be detected longer for higher doses. Some correlative doses (24-30g and 12-18g) showed similar concentrations probably because they were obtained in different subjects (see **Figure 2**).

3.3 Phenols and DA metabolites excretion

Baseline OHTyr concentrations were low and not different between treatment conditions (n=24: 0.4 ± 0.5 nmol/ml).

OHTyr total urinary excretion from 0 to 6h increased with ethanol dose (each gram of ethanol increased the log-OHTyr on average in 0.026 units (95%-CI: [0.02, 0.04]; p<0.001), which is equivalent to an increase by factor 1.03 per gram of alcohol). High variability was found between subjects as the coefficient of variation of the different doses ranged from 49% to 78%. A clear dose relationship was found when high and low dose of ethanol given to the same subjects were compared (18 vs. 30g or 24 vs. 42g). However excretion with 24g was higher than with 30g probably due to intersubject variability. Observed values of total OHTyr excretion and its metabolites are presented in **Figures 2** and **3.**

Model-based mean estimations for total OHTyr and the corresponding 95% prediction intervals are shown in **Table 2**.

OHTyr was excreted mainly in its conjugated form with sulfate (**Figure 4**). The sulfate metabolite and HVAL increased with ethanol administered dose while this relationship was not found with glucuronides. Amounts of free OHTyr excreted were very low and apparently unrelated to ethanol dose.

298 Baseline tyrosol concentrations were also not different among conditions (n=24:
299 0.1 ± 0.1 nmol/ml). Total Tyr excretion also increased with ethanol dose (each
300 gram of ethanol increased the log-Tyr on average in 0.051 units (95%-CI:[0.04,
301 0.07]; p<0.001), which is equivalent to an increase by factor 1.05 per gram of
302 ethanol). See **Table 2** for model-based estimations of the mean.
303 DOPAC and HVA excretion did not show any statistically significant relationship
304 with ethanol administered dose (p=0.286 and p=0.498). DOPAC excretion
305 mean values for 0, 6, 12, 18, 24, 30 and 42g of ethanol were 3239, 3484, 4688,
306 2024, 2559, 2190, 3393 nmol, respectively. HVA excretion for all doses was
307 higher than DOPAC excretion (1.6-3.7 times).
308 A statistically significant association was observed between both ethanol C_{max}
309 and $AUC_{0\text{-}6h\_c}$ with the logarithm of total OHTyr excretion (r= 0.53; p=0.005 and
310 r=0.45; p=0.02, respectively). DOPAC/OHTyr ratio decreased with the ethanol
311 content of the beverage (p<0.001). The ratios observed were 14.0 ± 14.7 (0g),
312 10.1 ± 5.6 (6g), 11.7 ± 8.7 (12g), 3.9 ± 2.6 (18g), 3.8 ± 2.8 (24g), 4.0 ± 2.5 (30g)
313 and 3.6 ± 2.0 (42g) and estimated values ranged from 8.4 (95% CI: 6.0-12.0) for
314 placebo to 2.4 (95% CI:1.5-3.7) with the highest dose.
315 *3.4 Subjective effects*
316 Drunkenness increased with ethanol dose (except for doses of 12-18g, obtained
317 in different subjects). High variability in subjective effects was found between
318 subjects as coefficient of variation of different doses ranged from 63% to 150%.
319 The median t_{max_e} value was 30 minutes for all ethanol containing beverages
320 except 1 hour for the dose of 42g. Subjective effects time-curves are presented
321 in **Figure 5.**

The highest drunkenness-E_{max} (38 of 100) and $AUC_{0\text{-}6h\_e}$ (96 mm x h) were obtained with the highest dose of ethanol. $AUC_{0\text{-}6h\_e}$ and C_{max} showed a slight correlation (r=0.35; p=0.01) similar to the correlation between E_{max} and C_{max} (r=0.31, p=0.055).

3. 5 Physiological outcomes and adverse events

No differences were found in heart rate, blood pressure and temperature between the different doses of ethanol administered.

No serious adverse events (AE) were reported during the study. 14 subjects reported a total of 26 AE. Those considered to be related with treatment (13) were mainly headaches (9). One subject reported nausea, unsteadiness and dizziness with 42g of ethanol.

3. 6 Alcohol dose adjusted to weight

No overlap between doses was observed when the dose of ethanol was adjusted to weight (6g: 79 ± 4 mg/kg, 12g: 157 ± 7 mg/kg, 18g: 226 ± 23 mg/kg, 24g: 305 ± 25 mg/kg, 30g: 376 ± 38 mg/kg, 42g: 533 ± 43 mg/kg). Results obtained with ethanol adjusted doses for the different outcomes showed the same trends previously described (data not shown).

4. Discussion

In this study we report for the first time in healthy volunteers and in a controlled setting the endogenous generation of OHTyr after the ingestion of ethanol. OHTyr formation was ethanol dose dependent. Doses tested (except 42g) are in the range of daily doses associated with a reduction of all-cause mortality [1] and recent consensus recommendations about moderate alcohol use [29].

As previously mentioned, in animal studies it has been shown that ethanol can induce a shift in the metabolism of DA from a predominantly oxidative to a reductive pathway with formation of OHTyr (DOPET) instead of DOPAC [14, 16] In our study a reduction in the ratio DOPAC/OHTyr from placebo to 42g of ethanol was observed (from 14 to 3.6), compatible with the occurrence of a shift in the oxidative metabolism of DA with ethanol. OHTyr excretion with 42g of ethanol triplicated the values obtained with placebo (1296 vs 427 nmol). However OHTyr excretion was at least 3 times lower (for the dose of 24g) in comparison with the administration of the same amount of ethanol contained in wine in a previous study [9]. Therefore the endogenous generation of OHTyr via ethanol interaction with DA oxidative metabolism only explains a relatively small portion of the recoveries of OHTyr after wine ingestion for the same alcohol dose.

To explain biological activities when free forms of phenols are almost undetectable it has been postulated that conjugates could act as depot forms and be hydrolyzed intracellularly releasing free OHTyr [30,31].

The demonstration in humans that ethanol ingestion can endogenously produce a potent phenolic antioxidant is in contrast with the fact that ethanol is typically considered a pro-oxidant substance [32]. In a previous observational study a relationship between circulating levels of oxidized LDL and ethanol consumption was reported [33]. Future experiments should evaluate whether the antioxidant effects of OHTyr generated *in vivo* can be overshadowed by the pro-oxidant influence of ethanol. The balance between wine phenolic compounds and ethanol concentrations has been already suggested may be critical in the protection of LDL oxidation [34]. In addition to the OHTyr ability of protecting

LDL against oxidation it also displays anti-inflammatory and antiaggregant activities [8,35] that could be also contributing in ethanol cardioprotective effects.

The increase in Tyr excretion with ethanol dose is reported for the first time in humans. Its formation is also ethanol dose dependent. Amounts recovered are about 40% of those observed for OHTyr at the higher ethanol doses. The mechanism involved could be a shift in tyramine oxidative metabolism to preferably produce Tyr instead of 4-hydroxyphenylacetic acid (4-HPAA), also described in animals [23]. Globally Tyr recovery increased 10 fold in the range of doses tested.

No relationship was found between DOPAC or HVA excretion and ethanol administered dose. As these compounds are found in very high concentrations in body fluids in comparison with OHTyr, it is plausible that small changes due to OHTyr formation could be not detected.

Ethanol concentrations and time to reach maximum concentration increased with the administered dose. Furthermore a linear relationship was described for C_{max} while for $AUC_{0\text{-}6h\_c}$ the linearity was lost at higher doses. Delayed t_{max} can be explained due to a reduction in gastric emptying with more concentrated beverages and $AUC_{0\text{-}6h\_c}$ disproportionate increase was related to the limited capacity of alcohol elimination by ADH [36,37].

Drunkenness feelings reported were mild and increased with ethanol administered dose. High interindividual variability was found probably due to different degrees of tolerance to ethanol. No serious adverse events were reported although headaches that appeared after several hours of consumption with higher doses could correspond to hangover symptomatology.

The study has several strengths and limitations. The cross over design allowed the same subjects to be treated with at least two different doses of ethanol and the double blind procedure was optimal to study ethanol subjective effects. However, for practical issues not all subjects received all doses and some comparisons were indirect. We enrolled only male volunteers for avoiding potential sex differences in ethanol pharmacokinetics and subjective effects, mainly due to a lower volume of distribution and a reduced tolerance to ethanol in women [38,39]. The ethanol dose was not adjusted to weight however no overlap between doses was observed when doses were adjusted. The biological implications of the observations made still has to be investigated, most probably with one of the ethanol doses tested but with additional comparison groups other than placebo.

5. Conclusions

There is a dose-related increase of urinary excretion of OHTyr and Tyr after ethanol administration. Results can be explained by endogenous generation produced by shifts in dopamine and tyramine oxidative metabolism, respectively, in the presence of ethanol. The biological significance of these findings deserves further evaluation in future clinical trials.

6. Registration

The trial was registered in Clinicaltrials.gov (NCT01788670).

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Table 1. Model-based estimations of the mean (95% prediction intervals) of the pharmacokinetic parameters as a function of ethanol dose (from 0 to 42g).

Parameter	6g	12g	18g	24g	30g	42g
$AUC_{0\text{-}6h\_c}$ nmol x h/ml	2.5 (1.8-3.4)	6.8 (4.4-10.5)	12.3 (7.4-20.6)	18.9 (10.8-33.0)	26.2 (14.4-47.7)	43.0 (22.2-83.1)
C_{max} nmol/ml	2.8 (1.3-4.3)	5.4 (4.2-6.6)	7.9 (7.0-8.9)	10.5 (9.6-11.4)	13.0 (12.0-14.0)	18.1 (16.5-19.8)
$AUC_{0\text{-}6h\_e}$ mm x h	-3.1 (-18.1-12.0)	9.3 (-3.7-22.2)	21.6 (9.8-33.4)	33.9 (22.1-45.8)	46.3 (33.3-59.3)	58.6 (43.6-73.6)
E_{max} mm	1.3 (-4.9-7.4)	7.1 (1.7-12.5)	12.9 (7.9-17.9)	18.8 (14.0-23.8)	24.6 (19.2-30.0)	30.5 (24.3-36.6)

$AUC_{0\text{-}6h\_c}$, area under the blood concentration curve from 0 to 6h; $AUC_{0\text{-}6h\_e}$, area under the curve for effects (drunkenness) from 0 to 6h; C_{max}, maximum blood alcohol concentration; E_{max}, maximum alcohol effect (drunkenness)

602 Table 2. Total OHTyr and Tyr urinary excretion from 0 to 6h after administration.
603 Model-based estimations of the mean (95% prediction intervals) as a function of
604 ethanol dose (from 0 to 42g).

Urinary excretion	0g	6g	12g	18g	24g	30g	42g
Total OHTyr nmol	322 (235-441)	375 (281-500)	437 (333-574)	510 (389-668)	594 (448-788)	693 (509-942)	941 (640-1385)
Total Tyr nmol	56 (39-81)	76 (56-103)	103 (79-134)	139 (108-180)	189 (142-252)	256 (181-362)	470 (282-782)

605 OHTyr, hydroxytyrosol; Tyr,tyrosol

Figure(s)

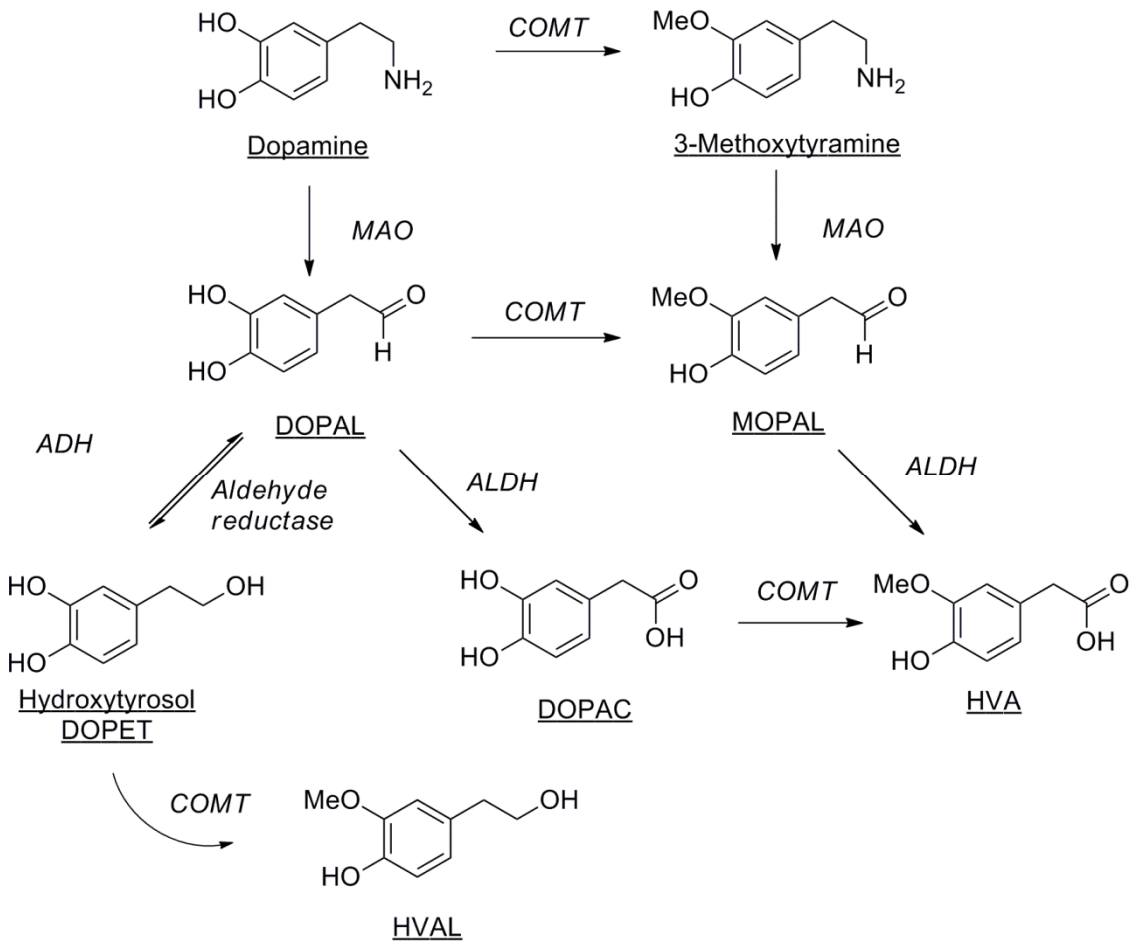


Figure 1. General diagram of dopamine metabolism. ADH: alcohol dehydrogenase.; ALDH: aldehyde dehydrogenase; COMT:catechol-O-methyl transferase; DOPA: 3,4-dihydroxyphenylalanine; DOPAL: 3,4-dihydroxyphenylacetaldehyde; DOPAC: 3,4-dihydroxyphenylacetic acid; DOPET: 3,4-dihydroxyphenylethanol; HVA: homovanillic acid; HVAL: homovanillyl alcohol; MAO: monoaminooxidase; MOPAL: 3-methoxy-4-hydroxyphenylacetaldehyde; MOPET: 4-hydroxy-3-methoxyphenylethanol.

Figure(s)

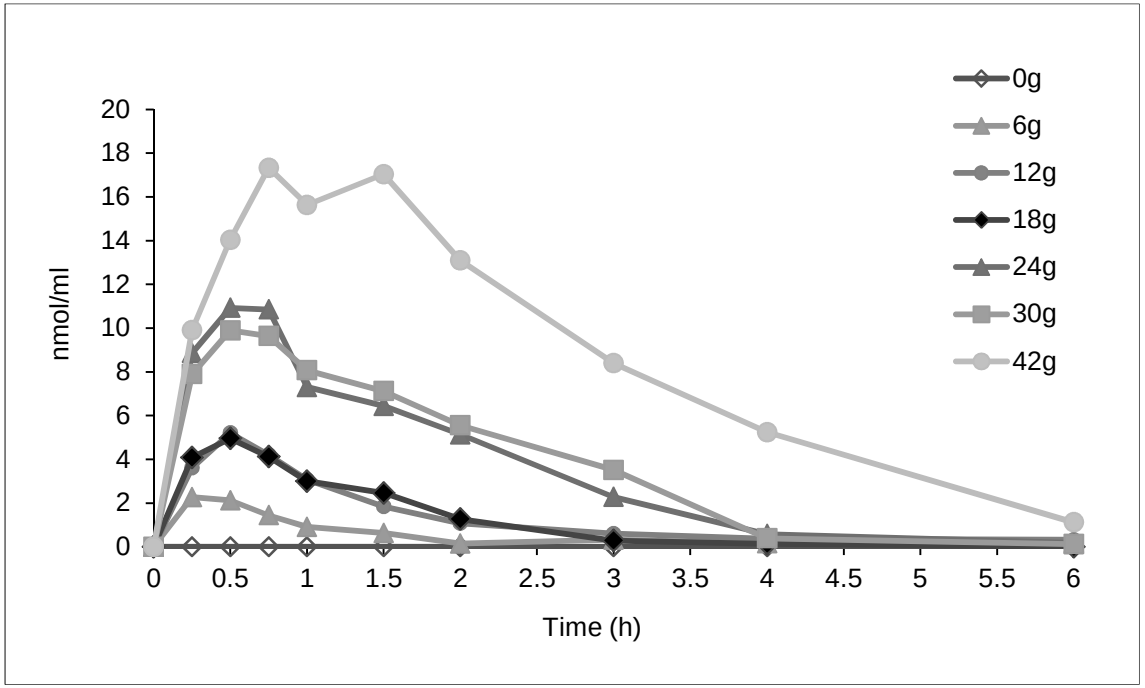


Figure 2. Plasma ethanol concentrations. Doses of 6 and 12g (n=6), doses of 18 and 30g (n=12), doses of 24 and 42g (n=6) and placebo (n=24).

Figure(s)

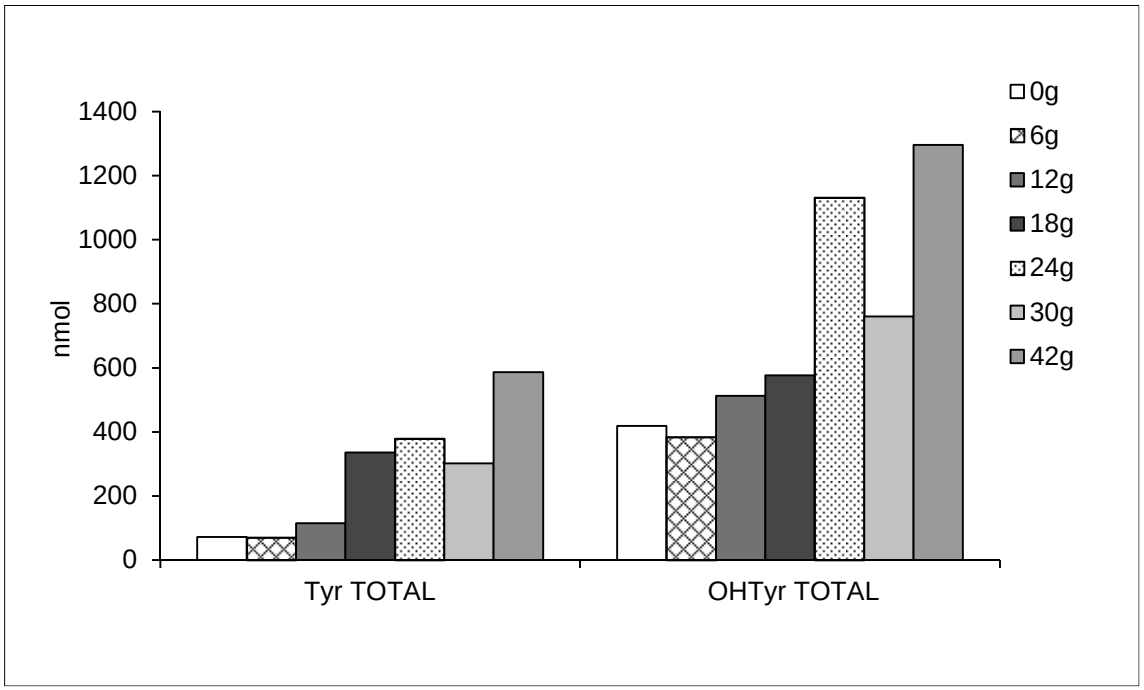


Figure 3. Urinary excretion of total hydroxytyrosol (OHTyr) and tyrosol (Tyr). Doses of 6 and 12g (n=4), doses of 18 and 30g (n=9), doses of 24 and 42g (n=6) and placebo (n=19).

Figure(s)

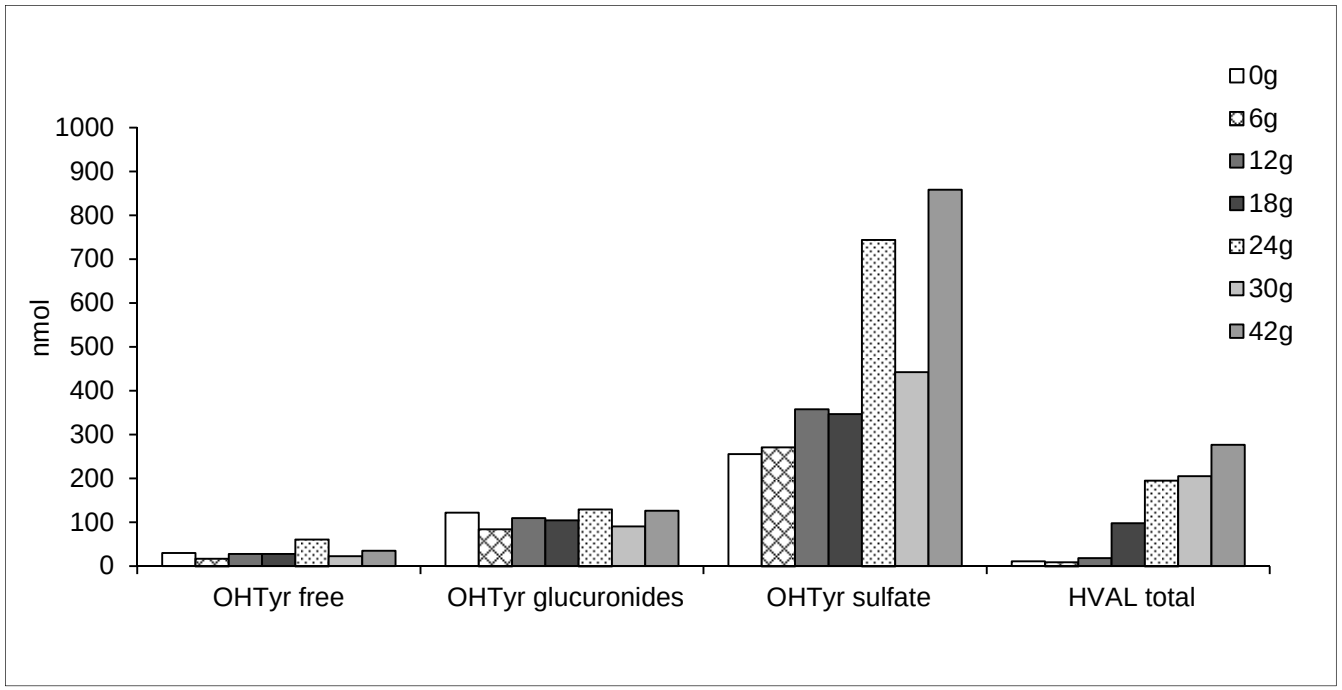


Figure 4. Urinary excretion of hydroxytyrosol (OHTyr) metabolites. Doses of 6 and 12g (n=4), doses of 18 and 30g (n=9), doses of 24 and 42g (n=6) and placebo (n=19).Urinary excretion of hydroxytyrosol metabolites.

Figure(s)

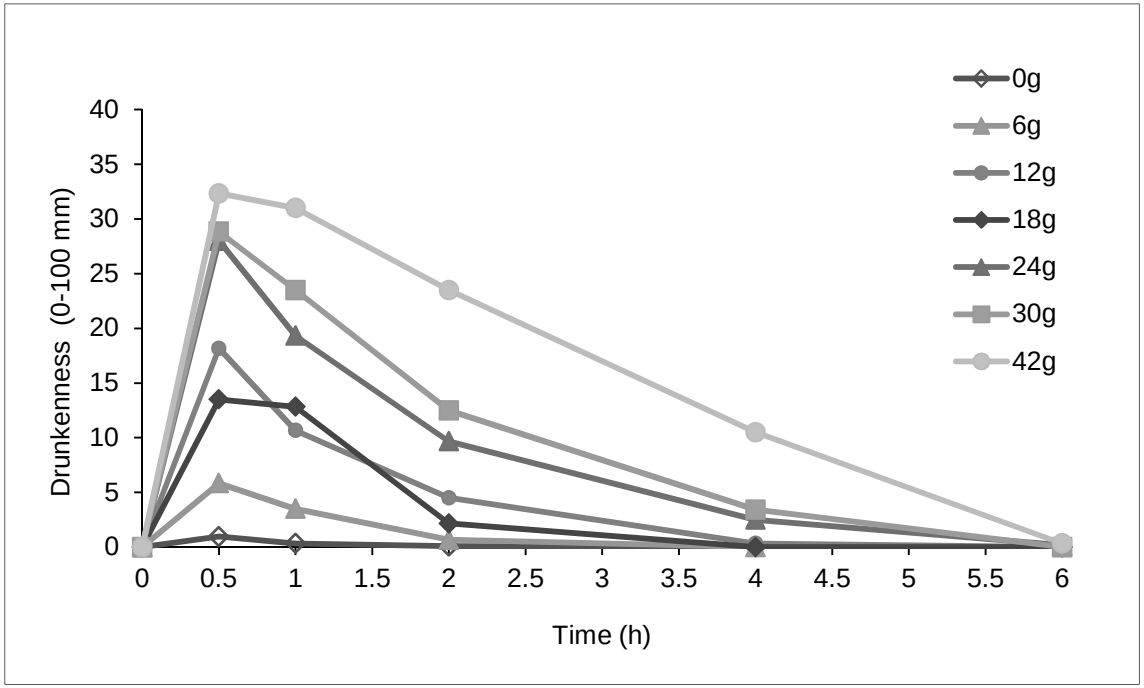
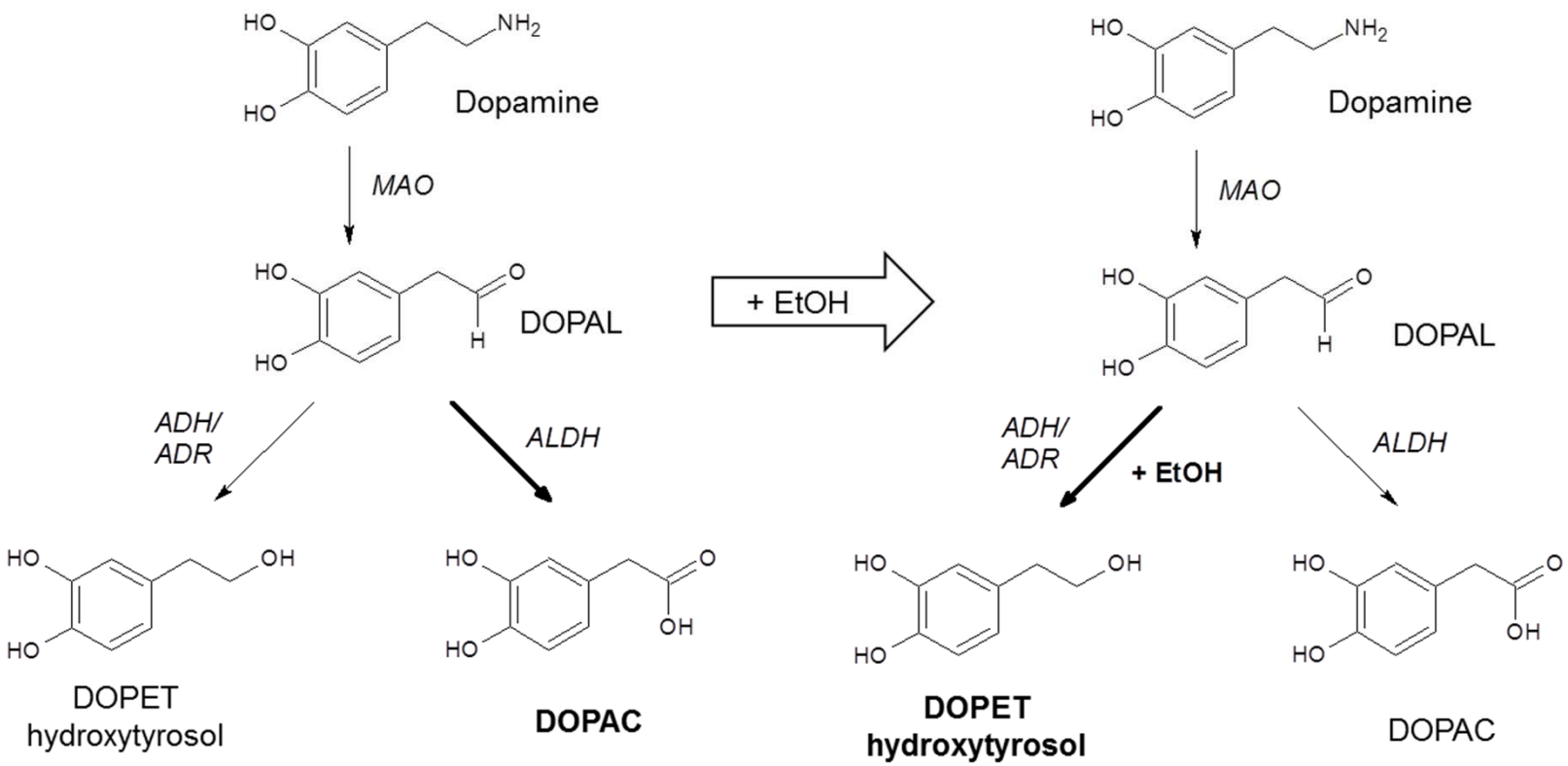


Figure 5. Ethanol-induced drunkenness. Doses of 6 and 12g (n=6), doses of 18 and 30g (n=12), doses of 24 and 42g (n=6) and placebo (n=24).

Graphical Abstract (for review)



Graphical abstract. Hydroxytyrosol generation due to the interaction of ethanol with dopamine metabolism.