Supplementary Table S1. Interventional and Observational Clinical Studies in Wine Metabolomics Research

Goal of the study	Study type	Intervantion period	Number of Parcitipants (gender)	Sexe	Health of participants	Control Group	Wine (mL)/day	Type of wine (variety of wine)	Matrix	Metabolomic Analysis	Analytical Technique	Sample Preparation	Statistical Analysis	Ref.
Association of previously-defined metabotypes with specific microbiological patterns and its response to a moderate wine intake, by combining metataxonomic and metabolomics data.	parallel interventional	4 weeks	19	male, female	healthy	abstention (5)	250	RW (not mentioned)	feces	Targeted	UPLC-ESI-MS/MS (-), SPME-GCMS	DVB/CAR/PDMS SPME	Shapiro–Wilk test, Levene test, One-way ANOVA, Tukey's test, Student's t-test	[22]
Assess the changes in Fecal Water cytotoxicity after a wine intervention and establish a possible relationship between FW cytotoxicity and fecal microbial-derived metabolites	parallel interventional	4 weeks	8	male, female	healthy	abstention (4)	250	RW (Pinot Noir)	feces	Targeted	UPLC-ESI-MS/MS (-), SPME-GCMS	**phenolic metabolites**: dilution with NaCl and again with ACN/water **short-chain fatty acids:** SPE	Student's t-test	[23]

Aim	Study design	Duration	N	Sex	Health	Control	Dose (mL)	Intervention	Sample	Approach	Technique	Sample preparation	Statistics	Ref
Evaluate the potential biological effects of moderate wine consumption on human microbiota and the fecal metabolome of healthy subjects.	parallel interventional	4 weeks	41	male, female	healthy	abstention (8)	250	RW (Pinot Noir)	feces	Targeted and Non targeted	UPLC-ESI-MS/MS (-), UPLC-TOF/MS (-)	dilution with NaCl and again with ACN	Shapiro-Wilk test, Student's t-test, Mann-Whitney test, Wilcoxon matched-pairs test, PCA	[26]
Assess the changes in the profile of phenolic metabolites in feces after wine consumption, and stratify the population based on their ability to metabolize wine polyphenols.	parallel interventional	4 weeks	41	male, female	healthy		250	RW (Pinot Noir)	feces	Targeted	UPLC-ESI-MS/MS (-)	dilution with NaCl and again with ACN	Student's t-test, Mann-Whitney test, Wilcoxon matched-pairs test, one-way ANOVA, Shapiro-Wilk test	[28]
Delve deeper into biological effects exerted by phenolic compounds by studying the metabolomic fingerprint of faecal samples.	parallel interventional	4 weeks	41	male, female	healthy	abstention (8)	250	RW (Pinot Noir)	feces	Non targeted	UPLC-TOF-MS (-)	dilution (x2) with NaCl, filtered with polyvinylidene difluoride (PVDF) membrane	Shapiro-Wilk test, Student's t-test, Wilcoxon matched-pairs test, PCA	[27]

Aim	Study design	Duration	N	Sex	Health	Abstention	Dose	Wine	Matrix	Analysis	Technique	Sample preparation	Statistical analysis	Ref
Examine the relationship between the plasma levels of polyphenols and the antioxidant activity of red and white wine	parallel interventional	15 days	20	male, female	healthy	Abstention (10)	300	RW, WW (not mentioned)	plasma	Targeted	HPLC-ESA	hydrolysis with glucuronidase/sulfatase, acetate buffer (pH 5.0), LLE with ethyl acetate	one-way ANOVA, non-parametric tests (Wilcoxon test, Kolmogorov-Smirnov test), Levene test, MANOVA, Bonferroni test, correlation analysis using Pearson's test	[29]
Verify the bioavailability of free and glucuronide t-RESV and association with or without a meal and its composition	parallel interventional	1 day	25 (10,5, 5 all m)	m	healthy	-	300, 600, 600	RW (Lambrusco, Cabernet Franc, Agliatico)	serum	Targeted	HPLC-UV-Vis (+), HPLC-MS (-), HPLC-MS/MS (-)	LLE with ethyl acetate	-	[30]
Examine the changes in the overall urinary metabolome after moderate wine consumption.	parallel interventional	4 weeks	41	male, female	healthy	abstention (8)	250	RW (Pinot Noir)	urine	Non targeted	UPLC-TOF-MS (-)	centrifugation and direct analysis	PCA, Student's t-test, OPLS-DA	[25]

Objective	Design	Duration	N	Sex	Health	Intervention	Dose	Wine	Sample	Analysis	Method	Sample prep	Statistics	Ref
Investigate the differential responsiveness of healthy subjects to moderate wine consumption and explore the differences in their metabotypes.	parallel interventional	4 weeks	41	male, female	healthy	abstention (8)	250	RW (Pinot Noir)	urine	Non targeted	UPLC-QTOF-MS (-)	centrifugation and direct analysis	PCA, HCA, one-way ANOVA, OPLS-DA	[24]
Study the pharmacokinetics of resveratrol metabolites after moderate RW consumption and grape extract tablets	parallel interventional	1 day	10	male	healthy	grape EXT tablets (3)	375	RW (Merlot)	urine, plasma	Targeted	LC–ESI–MS/MS (-)	HLB SPE	Mann–Whitney U test, Wilcoxon test	[31]
Investigate the profile of microbial phenolic metabolites obtained from red wine intake and compare it to samples taken after consuming dealcoholized red wine and gin.	crossover interventional	20 days	8	male, female	healthy		272, 100 (gin)	RW, DRW, gin (Merlot)	feces	Targeted	UPLC-ESI-MS/MS (-)	dilution with NaCl and again with ACN	Student's t-test, nonparametric Wilcoxon matched-pairs test, PCA	[35]
Investigate the H-NMR applicability on the fecal metabolomics to dietary human intervention trials.	crossover interventional	4 weeks	53	male, female	mildly hypertensive			capsules with mix of RW and GJ EXT	feces	Targeted	H-NMR	alkalized with NaOH and acidified with formic acid, LLE with D_2O, CD_3OD both containing TSP	Student's t-test, PCA, PLS-DA	[36]

Aim of study	Type of study	Duration	N	Sex	Health status		Dose (mL)	Wine type	Matrix	Approach	Analytical technique	Sample preparation	Statistical analysis	Ref
Impact of red wine in men with heart coronary disease on plasma metabolome, gut microbiota, and trimethylamine N-oxide (TMAO)	crossover interventional	3 weeks	42	male	CAD	-	250	RW (Merlot)	plasma	Non targeted	LC-MS/MS, UPLC-ESI-MS/MS (±)	PPT with MeOH	Shapiro-Wilk test, Student's t-test, paired Wilcoxon test, Spearman rank correlation test, Bonferroni test	[34]
Create a sensitive, precise, and selective analytical method for the identification and quantification of resveratrol metabolites in human low-density lipoprotein (LDL) following moderate red wine intake	crossover interventional	1 day	11	male	healthy		250	RW (Merlot)	plasma	Targeted	LC-ESI-MS/MS (±)	3 different methods tested [HLB SPE (selected), PPT with acidified MeOH, LLE with ethyl acetate]	-	[37]
Determine the detectability of certain phenolic acids in plasma after red wine consumption and assess the potential effect on serum and LDL oxidation ex vivo.	crossover interventional	1 day	12	male	healthy			RW, DRW, Phenol-stripped RW, water (Cabernet Shiraz)	plasma	Targeted	GC-MS	lyophilization, acetate buffer (pH 4.5), hydrolysis with b-glucuronidase, sulfatase, b-glucosidase, LLE with ethyl acetate,	ANOVA, general linear modeling	[38]

											derivatization with BSTFA		
Evaluate changes in plasma (+)-catechin concentrations after a single, moderate serving of dealcoholized red wine reconstituted with either water or water and alcohol	crossover interventional	1 day	9	male, female	healthy	120	water, DRW, water and alcohol (ARW) (Cabernet sauvignon)	plasma	Targeted	GC-MS	hydrolysis with b-glucuronidase, arylsulfatase, LLE with methylene chloride/water and ethyl acetate, derivatization with BSTFA	Student's t test, Wilcoxon signed-rank test, Fisher's exact test	[39]
Determine the levels of catechin and its metabolites in plasma after the consumption of red wine and de-alcoholized red wine.	crossover interventional	1 day	9	male, female	not mentioned	120	RW, DRW (Cabernet Sauvignon)	plasma	Targeted	GC-MS	hydrolysis with β-glucuronidase, sulfatase, LLE with methylene chloride/water and ethyl acetate, derivatization with BSTFA	Least-squares nonlinear regression, ANOVA, Student's t-test	[33]
Optimize a targeted metabolomics method for the analysis of diet-related metabolites in blood samples, ommiting an enzymatic hydrolysis step of phase II metabolites.	Crossover interventional	4 weeks	10	male, female	healthy	270	RW (not mentioned)	plasma	Targeted	UHPLC-MS/MS (±)	3 different methods tested [PPT with ACN/formic acid/ammonium formate, HLB SPE, hybrid PPT]	Student's t-test	[40]

Objective	Study type	Duration	N	Sex	Health status	Dose	Wine type	Sample	Approach	Method	Sample preparation	Statistics	Ref
Evaluate tartaric acid as a possible biomarker of wine intake.	crossover interventional	1 day	21	male	healthy	100, 200, 300	RW (Tempranillo (85 %), Graciano and Garnacha Tinta (15%))	urine	Targeted	LC–ESI-MS/MS (-)	dilution with formic acid/water	omnibus K2 D'Agostino-Pearson test, Shapiro-Wilk test, non-parametric Mann-Whitney U test, Wilcoxon test, Clopper-Pearson exact binomial method.	[41]
Divide a population into phenotypic groups based on biochemical features - utilize H-NMR-based urinary metabolomics to examine the differed metabolic reactions following red wine polyphenol intake.	crossover interventional	4 weeks	57	male, female	T2D or ≥3 CHD risk factors		DRW (Merlot)	urine	Non targeted	H-NMR	Mixed with TSP, NaN$_3$, KH$_2$PO$_4$ in D$_2$O - buffer KOD (pH=7)	OSC-PLS-DA	[44]
Evaluate the effectiveness of wine intake biomarkers in a wine intervention	crossover interventional	4 weeks	56	male, female	T2D or ≥3 CHD	272, 100 (gin)	RW, DRW, gin (not	urine	Non targeted	H-NMR	Mixed with TSP, NaN$_3$, KH$_2$PO$_4$ in D$_2$O	Mann-Whitney U test, Mann-	[45]

Aim	Study design	Duration	N	Sex	Health status	Dose	Intervention	Sample	Approach	Method	Sample preparation	Statistical test	Ref
study by using an NMR metabolomic approach.					risk factors		mentioned)					Whitney test, logistic regression model	
Determine the most comprehensive urinary metabolomic fingerprinting of phenolics and microbial-derived phenolic acids in males at high cardiovascular risk after regular consumption of dealcoholized red wine.	crossover interventional	4 weeks	36	male	healthy	272	DRW (Merlot)	urine	Targeted	UPLC–MS/MS (-)	MCX SPE	Student's t-test	[46]
Identify urinary metabolomic differences with H-NMR based metabolomic approach of moderate wine intake	crossover interventional	4 weeks	61	male	T2D or ≥3 CHD risk factors	272, 100 (gin)	RW, DRW, gin (Merlot)	urine	Non targeted	H-NMR	Mixed with TSP, NaN_3, KH_2PO_4 in D_2O - buffer KOD (pH=7)	ANOVA test, Fisher's LSD test	[47]
Develop a sensitive and optimized analytical assay in order to more accurately quantify all known resveratrol metabolites	crossover interventional	4 weeks	10	male	T2D or ≥3 CHD risk factors	272	RW (Merlot), DRW	urine	Targeted	UPLC–MS/MS (-)	HLB SPE	Kolmogorov test, Levene test, nonparametric Friedman test, paired Wilcoxon test	[48]

Aim	Study design	Duration	N	Sex	Health	Dose	Product	Sample	Approach	Method	Sample preparation	Statistics	Ref
Investigate urinary excretion of microbial-origin phenolic acids after grape juice and wine extracts intake	crossover interventional	4 weeks	58	male, female	mildly hypertensive		capsules 2:1 polyphenol-rich mix of RW and GJ EXTs, capsules with GJ EXT	urine	**GC-TOF-MS**: target, H-NMR: Non targeted	H-NMR, GC-TOF-MS	**H-NRM**: phosphate buffer (pH 7, TSP) **GC-MS:** hydrolysis with β-glucuronidase, LLE with ethyl acetate, derivatization with BSTFA/trimethylchlorosilane	Wilcoxon test, ML-PLS-DA	[49]
Develop a fast, simple, and environmentally friendly method for analyzing major wine organic acids in human urine using LC−ESI-MS/MS	crossover interventional	1 day	5	male	healthy	200	RW (Tempranillo)	urine	Targeted	LC−ESI-MS/MS (-)	dilution with water/formic acid	Student's t-test	[42]
Investigate the urinary excretion of catechin and its metabolites in human subjects following the intake of red wine and dealcoholized red wine.	crossover interventional	1 day	9	male, female	healthy	120	RW, DRW (Cabernet Sauvignon)	urine	Targeted	GC-MS	hydrolysis with β-d-glucuronidase, sulfatase, LLE with ethyl acetate, derivatization with BSTFA	Student's t-test	[32]
Compare the alterations in plasma malvidin-3-glucoside and its urinary excretion after the ingestion of red wine, dealcoholized red wine, and red grape juice.	crossover interventional	1 day	6	male	healthy	500	RW, DRW, GJ (Lemberger)	urine	Targeted	HPLC-UV-Vis (+)	C18 SPE	ANOVA, Fischer's test, linear regression analysis	[43]

Objective	Study design	Duration	N	Sex	Health	Dose	Intervention	Matrix	Approach	Technique	Sample preparation	Statistics	Ref
1) Compare multilevel PLS-DA against the ordinary PLS-DA approach, and 2) select and analyze candidate biomarkers after treatment effect.	crossover interventional	4 weeks	29	male, female	mildly hypertensive		capsules with mix of RW EXT and GJ EXT	urine	Non targeted	H-NMR	buffer phosphate and sodium salt (TSP)) (pH 3)	PLS-DA	[50]
Assess the impact of nano-encapsulation of a phenol extract on human plasma pharmacokinetic parameters, examine phenolic metabolite in urine clearance and identify new metabolites.	crossover interventional	1 day	12	male, female	healthy	272, 100 (gin)	DRW with EXT, DRW with encapsulated EXT, gin	urine, plasma	Targeted	UPLC-ESI-MS/MS (±)	HLB SPE	ANOVA, Tukey's test	[51]
Develop a sensitive method about c-RESV, t-RESV and dihydroresveratrol in urine and plasma after red wine, grape juice and grape extracts (prepared as tablets) ingestion.	crossover interventional	1 day	11	male	healthy	250, 1000 (GJ), 10 tablets	RW, GJ, capsules with RW EXT (not mentioned)	urine, plasma	Targeted	GC-MS	**urine**: dilution with water, **plasma/urine**: acetate buffer (pH 5.2), hydrolysis with β-glucuronidase, LLE with ACN/ethyl acetate (in urine a solution of NaCl were added before extraction), derivatised with MSTFA: NH_4I: 2-	least-square regression analysis	[52]

												mercaptoethanol reaction mixture (ammonium iodide and 2-mercaptoethanol per litter of MSTFA)	
Discover and evaluate food intake biomarkers in urine and plasma phenolic and microbial profile by targeted metabolomics after wine intervention	crossover interventional	4 weeks	36	male	T2D or ≥3 of the CVRFs	272, 100 (gin)	RW, DRW (Merlot), gin	urine, plasma	Targeted	UPLC-ESI-MS/MS (-)	HLB SPE	PCA, HCA, ANOVA, Bonferroni test, Binary stepwise logistic regression analysis	[54]
Study metabolic effects of red wine and grape polyphenols in plasma and urine from healthy male adults consuming a mix of red wine and grape juice extracts.	crossover interventional	5 days	35	male	healthy	630	capsules 2:1 polyphenol-rich mix of RW and GJ EXTs	urine, plasma	**H-NMR:** target**, GC-MS, LC-MS/MS:** target and Non targeted	GC-MS, LC-MS/MS (±), H-NMR	**plasma:** PPT with ACN, LLE with water/ethanol/dichloromethane. **urine:** dilution **H-NMR urine**: see (van Dorsten et al., 2010) **GC-MS:** fatty acid esterification, derivatization	Mann-Whitney U test, Wilcoxon test	[55]

Aim	Study design	Duration	n	Sex	Health	Dose (mg)	Intervention	Sample	Analysis	Technique	Extraction	Statistics	Ref
											with O-methylhydroxyamine hydrochloride, MSTFA		
Assess the bioavailability and bioactivity of anthocyanins of red grape juice versus red wine intake.	crossover interventional	1 day	9	male, female	healthy	400	RW, GJ (Lemberger)	urine, plasma	Targeted	HPLC-UV-Vis (+)	C18 SPE	Shapiro-Wilk test, Student's t-test	[53]
Develop a new method for measuring catechin, quercetin, and resveratrol, as well as their conjugates, in plasma and urine.	crossover interventional	1 day	10	not mentioned	not mentioned		WW, GJ, vegetable cocktail (Lindemans Chardonnay)	urine, serum	Targeted	GC–MS	LLE with ethyl acetate, derivatization with BSTFA	Pearson test	[57]
Evaluate resveratrol metabolites as potential biomarkers of wine consumption, in humans after moderate consumption of sparkling, white, or red wines.	crossover interventional	4 weeks	52	male, female	healthy	200 (RW, WW), 300 (sparkling wine), 100 (gin)	RW, WW, gin (not mentioned)	urine, serum	Targeted	LC-MS/MS (-)	HLB SPE	Kolmogorov test, Levene test, Wilcoxon test, Student's t-test, ANOVA	[58]

Objective	Study design	Duration	N	Sex	Population	Intervention	Sample	Targeted	Analytical method	Sample preparation	Statistical analysis	Ref.
Develop a GC–MS-based method for the profiling of phenolic microbial fermentation products in urine, plasma, and fecal water.	Crossover interventional	4 weeks	26	male, female	not mentioned	capsules with mix of RW EXT and GJ EXT	urine, plasma, feces	Non targeted	GC–TOF–MS	**urine, plasma:** hydrolysis with β-d-glucuronidase, acidification with HCl, LLE with ethyl acetate, derivatization: BSTFA/TMCS	PCA, OPLS-DA	[56]
Assess the relationships between short-term and long-term dietary intake of resveratrol and wine and urinary resveratrol excretion in a European population.	observational		475	male, female	general population		urine	Targeted	UHPLC-ESI-MS/MS (+)	hydrolysis with β-glucuronidase, sulfatase, LLE with ethyl acetate	one-way ANOVA, Spearman's rank correlations, partial Spearman's correlations	[66]
Determine whether resveratrol could serve as a reliable biomarker for accurately measuring wine consumption in a specific population, compared to	observational		25	not mentioned	healthy		plasma	Not targeted	HPLC-ESI-MS/MS (± tested but – preferred)	PPT with acetic acid, HLB SPE	not used a statistical analysis test rather than	[61]

										statistical parameters	
Develop a novel algorithm to identify urinary polyphenol metabolite patterns explaining the intake of polyphenol-rich foods and compare their performance with individual biomarkers in assessing dietary intake within the EPIC study.	observational	475	male, female	general population		urine	Targeted	UPLC-ESI-MS-MS (+)	hydrolysis with β-glucuronidase, sulfatase LLE with ethyl acetate	expectation-maximization (EM) algorithm, GLMs, RRR-VIP method, reduced rank regression, LASSO regression, RRR analysis, internal two-fold cross-validation	[67]
Compare polyphenol urine excretion in individuals from different European countries with a wide range of polyphenol intakes and lifestyle factors.	observational	1386	male, female	general population		urine	Targeted	UPLC-ESI-MS/MS	hydrolysis with β-glucuronidase, sulfatase LLE with ethyl acetate	PC-PR2, one-way ANOVA	[68]

self-reported dietary assessments.

Discover a set of metabolites to discriminate red wine consumers.	observational	1157	male, female	T2D or ≥3 major CVRFs	plasma	Non targeted	UPLC-MS/MS (-)	**amino acids and other polar metabolites**: LLE with ACN /MeOH/formic acid, **lipids:** LLE with isopropanol	cross-validation	[60]
Identify and evaluate metabolites reproducibility that are biomarkers of usual dietary intake.	observational	502	male, female	healthy	plasma	Non targeted	UPLC-ESI-MS/MS (±)	PPT with MeOH	2-sided statistical tests (chi-square test, Mann-Whitney U Test), FDRs, LASSO regression	[62]
Evaluate the relationship between more than 100 food groups/items and 1141 metabolites measured six months apart in racial and ethnically diverse men and women.	observational	671	male, female	general population	plasma	Targeted	UPLC-ESI-MS/MS (±)	PPT with MeOH	Pearson's partial correlation	[59]
Discover and assess the reliability of new biomarkers that can indicate habitual food intake	observational	3559	female	general population	plasma, serum	Targeted and Non targeted	UPLC-MS/MS (±), GC-MS	**UPLC**: PPT with MeOH, **GC**: PPT with MeOH, derivatization: BSTF	linear regression analysis	[63]

Aim	Study type	N	Sex	Population	Sample	Approach	Analysis	Extraction	Statistics	Ref
Verify previously reported food-metabolite connections and uncover novel metabolomics-based biomarkers.	observational	849	male, female	general population	serum	Non targeted	UPLC-ESI-MS/MS (-)	LLE with MeOH	Student's t-tests, chi-square test	[64]
Discover potential food biomarkers and assess their accuracy in predicting.	observational	1369	female	healthy postmenopausal women	serum, urine	Non targeted	UPLC-ESI-MS/MS (±)	PPT with MeOH	Pearson's partial correlation, linear Support Vector Machine multivariate classification model	[72]
Establish a correlation between urinary tartaric acid as a biomarker of wine consumption and cardiovascular risk factors in postmenopausal women at risk of developing cardiovascular disease.	observational	222	male, female	T2D or ≥3 major CVRFs	urine	Targeted	LC–ESI-MS/MS (-)	dilution with water/formic acid	Shapiro-Wilk test, multiple adjusted linear regression models	[65]
Validate urinary resveratrol metabolites as a biomarker of wine consumption in PREDIMED trial	observational	1000	male, female	T2D or ≥3 CHD risk factors	urine	Targeted	UPLC-MS/MS (-)	HLB SPE	chi-square tests, Kolmogorov test, Levene test, one way	[71]

Compare urine and serum metabolite profiles of habitual diet	observational	253	male, female	new or recurrent colorectal adenoma cases and adenoma-free controls		urine	Non targeted	UPLC-MS, UPLC-ESI-MS/MS (±), GC-MS	direct analysis	ANOVA, Mann-Whitney test Wilcoxon's signed rank test, chi-square test, Partial Pearson correlation, FDR calculation (Benjamini-Hochberg procedure), LASSO regression	[69]
Identify novel biomarkers of intake for a selection of polyphenol containing foods by metabolomics approach	observational	481	male, female	general population		urine	Non targeted	UPLC-QTOF-MS (-)	dilution with water	OPLS-DA, HCA	[70]

T2D: type 2 diabetes, CAD: coronary artery disease, CHD: coronary heart disease, CVRFs: cardiovascular risk factors PPT: protein precipitation, DVB/CAR/PDMS: Divinylbenzene/Carboxen/Polydimethylsiloxane, ACN: acetonitrile, MeOH: methanol, BSTFA: N,O-bis (trimethylsilyl)trifluoroacetamide, TSP: 3-(trimethylsilyl)-proprionate-2,2,3,3-d4, NaN3: sodium azide, D2O: deuterium water, KOD: potassium deuteroxide, CD3OD: deuterated methanol, LC: liquid chromatography, MS/MS: tandem mass spectrometry, UPLC: ultra-high performance liquid chromatography, SPME: solid phase microextraction, DDA: data dependent acquisition, MRM: multiple reaction monitoring, PCA: Principal component analysis, ANOVA: Analysis of Variance, MANOVA: Multivariate analysis of variance, PLS-DA: Partial Least Squares Discriminant Analysis OPLS-DA: Orthogonal Partial Least Squares Discriminant Analysis, OSC-PLS-DA: Orthogonal Signal Correction-Partial Least Squares Discriminant Analysis, HCA: Hierarchical Clustering Analysis, PC-PR2: principal component partial R-square analysis, GLMs: general linear models, FDR: false discovery rate, LASSO: least absolute shrinkage and selection operator, LSD: Least Significant Difference, nm: not mentioned