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Oral Presentations

Epidemiology

OC1

Associations between dietary intake of total, classes, and subclasses of polyphenols and all-cause mortality in the Mexican Teachers' Cohort.

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Background: Several observational studies have shown that a polyphenol-rich diet may play a beneficial role in preventing chronic disease and some cancers, but its association with mortality, including all-cause mortality and specific causes of death, remains unclear. We evaluated the associations between the intake of polyphenols (total, classes, and subclasses) and all-cause mortality in a Mexican population-based cohort study.

Methods: A total of 95,313 women from the Mexican Teachers' Cohort were included. Polyphenol intakes were estimated at baseline (2006-2008) through validated dietary questionnaires and Phenol-Explorer. Deaths were identified through employers' databases and next-of-kin reports. Associations were evaluated by Cox proportional hazard modeling.

Results: A total of 1,689 deaths occurred during the follow-up period (mean 6.8 years). After multivariable adjustment, comparing the highest vs. the lowest quintile of total polyphenol intake showed a lower all-cause mortality hazard ratio (HRQ5vsQ1: 0.82, 95% CI: 0.70–0.97, p trend = 0.028). Likewise, phenolic acid intake was inversely associated with a lower risk of all-cause mortality (HRQ5vsQ1: 0.76, 95% CI: 0.65–0.97). Similarly, the intake of some minor polyphenol subclasses – alkylmethoxyphenols, hydroxycoumarins, and tyrosols – were inversely associated with all-cause mortality (HRQ5vsQ1: 0.79, 95% CI: 0.67–0.91), (HRQ5vsQ1: 0.71, 95% CI: 0.61–0.83), and (HRQ5vsQ1: 0.75, 95% CI: 0.65–0.88), respectively. No statistically significant associations were observed for flavonoids, flavanols, and their subclasses.

Conclusion: This study suggests that a high intake of total polyphenols, especially phenolic acids, and some minor subclasses, is associated with a reduction in all-cause mortality in Mexican women.

Keywords

Polyphenol intake, Mortality, Diet, cohort study

Cardiometabolic diseases and mechanisms

OC2

Interindividual variability in response to aronia berry (poly)phenol consumption in middle-aged men and women: Multi-omic exploration of the relationships between vascular response, metabolome and gut microbiome.

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Background: Recent research underlined the high interindividual variability of response to (poly)phenol-rich interventions and its influence on the biological response.

Objectives: To investigate the interindividual variability following aronia (poly)phenol intake in prehypertensive middle-aged males and females.

Design: A total of 102 participants were included in a 12-week parallel double-blind, randomized controlled trial involving the consumption of a (poly)phenol-rich aronia berry extract or matched placebo daily for 12 weeks. Cardiometabolic health parameters as well as gut microbiome richness, function, and composition were assessed at baseline and after 12 weeks. Interindividual variability in response to treatment was assessed for the main vascular parameters and a multi-omic approach was led to explore the influence of the gut microbiome on the biological response among subjects following the intake of aronia extract.

Results: A high variability in response to aronia berry (poly)phenols was found with coefficients of variation ranging from ≈200% for 24h SBP and DBP to ≈2560% for total cholesterol and triglycerides. An interindividual variability analysis based on k-means clustering showed that responders to treatment for blood pressure presented a lower gene count at baseline compared with non-responders, and baseline abundance of the beneficial bacterium *B. adolescentis* was significantly correlated with improvement in 24h DBP. This analysis of responsiveness also highlighted that responders presented significant decreases in Δ24h and Δawake SBPbr and DBPbr compared with Control and non-responders.

Conclusions: A high variability in response to aronia berry (poly)phenols was observed among our population of prehypertensive middle-aged subjects. Importantly, gut microbiota seems to have an important role in explaining the variability in response, as we demonstrated for the first time the strong correlation between some beneficial butyrate-producing bacterial species and blood pressure as well as arterial stiffness following chronic intake of aronia berry extract.

Keywords

aronia, (poly)phenols, interindividual variability, vascular function, gut microbiome

OC3

Polyphenol enriched tomatoes protect against atherosclerotic plaque development in ApoE-/- mice through the modification of cholesterol efflux and inflammation

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Background and Objectives: Polyphenols are known to protect against cardiometabolic diseases. However, while there is a vast literature describing putative beneficial effects of isolated polyphenols and plant extracts, there are relatively few studies of polyphenols in the context of a food matrix. We aimed to investigate the effects of tomatoes expressing different types of polyphenols vs standard red tomatoes (low polyphenols) in atherosclerotic prone ApoE-/- mice.

Methods: Here we fed ApoE-/- mice diets supplemented with standard red tomatoes (low polyphenols) or tomatoes expressing different polyphenols. Effects of the diets on aortic sinus plaque size and macrophage infiltration were determined using immunohistochemistry and gene expression was measured using qRT-PCR. THP1 macrophages were used to ascertain the effects of anthocyanin metabolites, resveratrol and resveratrol phase II conjugates on the expression of inflammatory and reverse cholesterol transport (RCT) genes.

Results: Aortic sinuses of mice fed tomatoes expressing flavonols + anthocyanins or resveratrol had significantly reduced plaque sizes compared to those fed the red tomato control diet. Mice on the flavonol + anthocyanin but not the resveratrol diets had reduced aortic sinus macrophage infiltration. Tomato diets containing flavonols or isoflavones alone had no effect on plaque size and macrophage infiltration. In THP1 macrophages, anthocyanin metabolites significantly increased ABCA1 and TNF-α gene expression while resveratrol increased ABCG1 gene expression and reduced expression of inflammatory genes; TNF-α and JAM-B.

Conclusions: The data suggest that flavonol + anthocyanin tomato-matrix-matched diet reduced plaque size through a reduction in macrophage infiltration and increased RCT. In contrast, resveratrol-rich tomato diet reduced plaque size by attenuating inflammation but did not affect macrophage infiltration although it also increased RCT. Thus, anthocyanins and resveratrol offer promising therapeutic targets for promoting health ageing.

Keywords

Polyphenols, atherosclerosis, Inflammation, Reverse cholesterol transport, cardiometabolic diseases

Bioavailability, absorption and metabolism

OC4

Circulating (poly)phenol metabolites blood-brain barrier transport and brain availability

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Objectives/Background: (Poly)phenols have been extensively studied considering their beneficial brain-health effects, particularly regarding neurodegenerative disorders. Circulating metabolites resultant from colonic metabolism of dietary (poly)phenols are highly abundant in the bloodstream, though still marginally underexplored, particularly regarding their brain accessibility. Our goal is to disclose circulating (poly)phenol metabolites' capability of reaching the brain, *in silico*, *in vitro*, and *in vivo*.

Materials/Methods: For three selected (poly)phenol metabolites, in silico relevant molecular descriptors were obtained using Qikprop software. Metabolites' blood-brain barrier (BBB) transport and further metabolism were assessed in human brain microvascular endothelial cells (HBMEC) in transwells. Their fate towards brain, liver, kidney, urine, and blood, was also assessed in Wistar rats upon injection. Both UPLC-MS/MS and untargeted analysis were employed.

Results/Findings: The results from computational analysis indicate that all the studied metabolites can passively cross the BBB. Transport kinetics along time using HBMEC highlighted different BBB permeability rates of the (poly)phenol metabolites, with novel end-route metabolites appearing at the brain site. From *in vivo* experiments, we found that all the injected metabolites can almost immediately cross the BBB and reach the brain, though at distinct extents, presenting different tissue distribution rates.

Conclusion: Overall, we proved the ability of three circulating (poly)phenol metabolites to reach the brain, in circulating concentrations, with the ultimate potential to tackle neurodegeneration.

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Keywords

blood-brain barrier, circulating (poly)phenol metabolites, brain uptake

OC5

Flavanol metabolite 5-(3',4'-dihydroxyphenyl)-γ-valerolactone is a substrate for human paraoxonase in vivo: a novel flavanol metabolism pathway

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Objective/Background: Currently, 5-(3',4'-dihydroxyphenyl)-γ-valerolactone (gVL) metabolites are used as a biomarker of flavanol intake. Although gVLs are further metabolized by the gut microbiome, it is thought that once gVL metabolites reach systemic circulation, they do not undergo any further metabolism. However, there is a family of genes, paraoxonase (PON), with lactonase activity in humans that could potentially hydrolyze gVLM into the corresponding 5-(3',4'-dihydroxyphenyl)-γ-hydroxyvaleric acid (gVA) metabolites. Given the implications that such novel metabolic pathway could have on the significance of gVLM as a biomarker, this study aimed to determine if gVLs are substrates of PON, and if PON contributes to the overall profile of gVLs detected in humans.

Materials/Methods: PON activities in human sera and vascular endothelial cells were measured using gVL and gVLMs as substrates in vitro, using an UPLC-HRMS method. In addition, a flavanol-intake absorption study was conducted to assess if conversion of gVAs and gVLs takes place in vivo.

Results/Findings: gVL was converted into gVA forms by PON1 and PON3, the isoforms associated to lipoproteins in serum. gVL was a poor substrate for PON2, the isoform found in vascular endothelial cells. PON's reactivity with gVL metabolites depended on the type and position of the conjugation in the phenyl ring. Consistently, the profile of conjugated gVAM detected in urine following a flavanol intake corresponded to those gVLM preferably hydrolyzed by serum PON.

Conclusion: Flavanol-derived gVLs are metabolized into gVAs by PON and it plays a significant role in the profile and amount of gVLs and gVAs detected in humans. In addition, our novel finding suggests that the majority of gVAs detected in humans are derived from PON activity rather than from gut metabolism. Further studies should evaluate the impact of PON activity on the use of gVLs as biomarkers of flavanol intake.

Keywords

flavanols, gamma-valerolactone, ADME, biomarker, paraoxonase

Mode of action

OC6

Ferulic acid metabolites attenuate LPS-induced inflammatory response in enterocyte-like cells: insight into the mechanism of action.

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Objectives and Background: Ferulic acid (FA) is a polyphenol pertaining to the class of hydroxycinnamic acids present in numerous foods of a plant origin. Its dietary consumption leads to the formation of several phase I and II metabolites in vivo, which represent the largest amount of ferulates in the circulation and in the intestine in comparison with FA itself. In this work, we evaluated their efficacy against the proinflammatory effects induced by the Gram negative endotoxin lipopolysaccharide (LPS) in intestinal Caco-2 cell monolayers, as well as the mechanisms underlying their protective action.

Material and methods: Human colon adenocarcinoma cells (Caco-2), differentiated as normal enterocytes, were treated with LPS (1 μg/mL) following pretreatment with the metabolites at concentration of 1 μM, which can be easily achieved in vivo. The modulation of MAPKs p38 and ERK1/2, were assessed by Western blotting and immunofluorescence assays. Moreover, intestinal nitric oxide (NO) release consequently to iNOS expression, the expression of Nrf-2 and modulation of Akt/IκBα/NF-κB pathway were also tested.

Results: LPS-induced overexpression of proinflammatory enzymes such as inducible nitric oxide synthase (iNOS) and the consequent hyperproduction of NO and cyclic guanosine monophosphate (cGMP) were limited by physiological relevant concentrations of FA, its derivatives isoferulic acid (IFA) and dihydroferulic acid (DHFA), and their glucuronidated and sulfated metabolites, which acted upstream by limiting the phosphorylation of MAPK p38 and ERK and of Akt kinase, thus decreasing the NF-κB translocation into the nucleus. Furthermore, the compounds were found to promote the expression of Nrf2, which may have contributed to the downregulation of NF-κB activity.

Conclusion: The overall data show that phase I/II metabolites retain the efficacy of their dietary free form in contrasting inflammatory response by decreasing Akt and MAPK activity and promoting Nrf-2 expression, leading up to a significant decrease in NF-κB activity.

Keywords

metabolites, inflammation, polyphenols, intestinal cells, intracellular signaling

OC7

Cranberry proanthocyanidin and its microbial metabolite 5-(3',4'-dihydroxyphenyl)-ϒ-valerolactone modulate intestinal epithelial function and differentiation in mouse intestinal organoids.

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Cranberry proanthocyanidins (PACs) exhibit a unique polyphenol profile associated with health benefits, including antidiabetic and antioxidant effects. Due to their high degree of polymerization, PACs are poorly absorbed and reach the colon where the microbiota catabolizes them into 5-(3',4'-dihydroxyphenyl)-ϒ-valerolactone (DHPVLT) and other metabolites. After ingestion and prior to DHPVLT absorption, PACs interact directly with the intestinal mucosa and its specialized epithelial cells such as enterocytes, goblet cells, enteroendocrine cells, Paneth cells and stem cells. The objective of this work was to investigate the effect of cranberry PACs and its microbial metabolite, DHPVLT, on the intestinal epithelial function such as metabolism, mucosal barrier protection and organogenesis.

Methodology: Intestinal organoids, derived from C57BL/6 mouse duodenum, were incubated 3h and 24h with different concentrations of PAC polymers, purified from ethanolic extract of cranberry, or its synthesized microbial metabolite DHPVLT. The expression of genes involved in gut barrier function, metabolism and organogenesis were analyzed by qPCR and protein levels were measured by Western blot.

Results: After incubation with PACs and DHPVLT, we observed an increase mRNA expression of the transcription factors Hes1 and Atho1, which regulate the differentiation of intestinal absorptive and secretory epithelial cells, respectively. Furthermore, the PACs increased mRNA expression of secretory cell-specific genes in the small intestine, i.e. *Muc2* (goblet) and *Chga* (enteroendocrine). Genes involved in glucose homeostasis (*Gip, Gcg*) and membrane receptor (*Ahr, Gpr55*) were also increased by PACs, no change in tight junction complex genes was observed. Finally, Mucine 2 protein levels were increased in response to PACs, which mirrors mRNA expression.

Conclusions: This study shows that cranberry PACs and DHPVLT enhance intestinal barrier function by increasing intestinal stem cell differentiation and by promoting the cellular development of secretory and absorptive cells. Potential effects on enteroendocrine function were also found. Further mechanisms on epithelial function remain to be investigated

Keywords

Organoids, Cranberry PACs, Intestinal cell differentiation

Gut (Microbiome)

OC8

Upper gastrointestinal digestion is required for coffee and cocoa beverages to inhibit trimethylamine formation by gut microbiota

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Objectives: The production of pro-atherogenic trimethylamine N-oxide (TMAO) is dependent on the gut microbiota metabolism of quaternary amines (i.e., choline) into trimethylamine (TMA). Nutritional strategies that target this conversion could mitigate cardiovascular disease and atherosclerosis risk and burden by reducing subsequent TMAO formation. We have identified chlorogenic acid, catechin and epicatechin as dietary phenolics able to inhibit gut microbiota TMA production. This study aims to evaluate if polyphenol-rich beverages can reduce TMA formation and evaluate the effect of upper gastrointestinal digestion on activity in the lower gut.

Materials and Methods: To do this, either raw or digested coffee, regular cocoa and dutched cocoa beverages were evaluated for their TMA-d9 production inhibition in an ex vivo-in vitro fermentation model with human fecal slurries (2% of total volume) and choline-d9 (100 µM) as a substrate for 24 h.

Results: At physiologically relevant doses of beverages, results showed that upper gastrointestinal digestion was required to achieve inhibition of choline-d9 use and TMA-d9 production by these beverages. Undigested beverages barely affected choline-d9 use, and only slightly inhibited TMA-d9 formation at later timepoints (12-20h; ~7-26%) The overall effect of both digested cocoa treatments was a delay of 2h in choline-d9 use and TMA-d9 production compared to control conditions. A similar, yet not as drastic effect was observed for digested coffee. At the timepoint of maximum inhibition (12h), digested regular cocoa reduced TMA-d9 formation ~88%, digested dutched cocoa a ~82 %, and digested coffee a ~52 %. These effects were not due to cytotoxicity (no reduction of cell respiration rate was observed).

Conclusion: This study suggests that the consumption of these beverages could be a nutritional strategy able to reduce TMAO levels. In vivo studies should be performed to confirm the TMAO production inhibition of these treatments.

Keywords

microbiome, atherosclerosis, cocoa, trimethylamine N-oxide, choline

OC9

Transformation of flavonoids by human gut microbiota – from *in silico* analyses to experimental confirmation

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Objectives/Background: Flavonoids are a diverse group of secondary plant metabolites abundant in vegetable- and fruit-rich diets. However, the bacterial metabolism of flavonoids in the human gut is still widely unknown, despite its potential importance to human health.

Materials/Methods: We screened the Unified Human Gastrointestinal Genome collection including nearly 300,000 bacterial genomes for potential flavonoid-modifying enzymes by using sequences from known flavonoid-transforming enzymes as queries in a protein sequence similarity search (BLAST). The potential flavonoid-modifying enzymes were assigned to bacterial species and quantified via in-house linux and R scripts. Bacteria were cultivated with flavonoids and the medium was analyzed for potential products with reversed-phase HPLC.

Results/Findings: Potential flavonoid-modifying enzymes were highly abundant in bacteria not yet considered as flavonoid-metabolising species. For example, enzymes responsible for daidzein-to-equol conversion, well studied in *Slackia isoflavoniconvertens*, were encoded rarely in *Slackia* genomes. Instead, corresponding genes were frequently detected in uncharacterized *Eggerthellaceae* species. Of all potential flavonoid modifications, *O*-deglycosylation (including de-rhamnosylation) was by far the most abundant. Putative *C*-deglycosylating enzymes were encoded less often; mainly in *Agathobacter faecis*. Enzymes likely involved in flavonoid degradation, such as potential ring-cleaving reductases or phloretin hydrolases were of intermediate abundance. We tested three of the newly identified bacteria for their ability to convert flavonoids, including the highly abundant *Bacteroides ovatus*, *Blautia wexlerae* and a novel *Catenibacillus* species, that encodes the highes number of putative flavonoid-modifying enzymes herein. All three species exhibited flavonoid modifying activities, such as de-rhamnosylation, C-deglycosylation and oxidation.

Conclusion: This first comprehensive insight into the black box of bacterial flavonoid metabolism in the human gut highlights many overlooked bacterial species as potential key organisms in flavonoid conversion in the human gut. This could further unravel the impact of gut bacteria on flavonoid-mediated health effects in humans.

Keywords

Isoflavones, Glucosides, Microbiome, Polyphenol degradation

Brain and cognition

OC10

The acute effects of wild blueberries on mood and cognition in healthy young adults

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BACKGROUND: Previous studies have suggested that acute interventions with berries improve mood and cognition, but these findings have not been consistently replicated. The primary aim of this study was to determine whether an acute intervention with wild blueberries can improve mood and cognitive performance in healthy young adults. As secondary objectives, we investigated whether any potential improvements are related to changes in serum biomarkers of neuroplasticity and neurotransmitter levels.

METHODS: Thirty-three university students (18-25 yo) completed a cross-over study consisting of two sessions, during which 22 g freeze-dried wild blueberries and 22 g flavour-matched placebo powder were consumed in counterbalanced order. Two hours post-ingestion, participants completed an auditory-verbal word-learning task, a task-switching task to measure cognitive performance, as well as the PANAS-X mood questionnaire. Blood samples were taken to analyse serum levels of brain-derived neurotrophic factor (BDNF) and prolactin. Linear mixed models with covariate adjustment for baseline performance, sex, session, and habitual fruit and vegetable (FV) intake were used.

RESULTS: The blueberry intervention resulted in small but statistically significant improvements in task-switching accuracy ($p = 0.01$) and reported global positive affect ($p = 0.046$). In addition, FV intake was associated with higher positive affect scores ($p = 0.02$) especially among males. Interestingly, trend-level interactions with FV intake and treatment predicting positive affect ($p = 0.06$) and accuracy ($p = 0.09$) indicate that participants with higher FV intake responded to the blueberry intervention more favourably. There were no significant changes in the other measures, nor in peripheral BDNF or prolactin levels.

CONCLUSIONS: A single serving of wild blueberries was shown to improve mood and executive function in healthy young adults, particularly in those with higher FV intake. However, this improvement was not linked to changes in serum BDNF or prolactin levels. These findings indicate that blueberries can help promote psychological health.

Keywords

blueberries, anthocyanins, mood, cognition, acute intervention

OC11

Daily mango (*Mangifera indica* L.) consumption supplemented with probiotics differentially modulates inflammation and cognitive function in lean and obese individuals

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Mango is rich in gallic acid and gallotannins, which have been proven to possess antioxidant, anti-inflammatory, and anti-obesity properties, and alleviate cognitive impairment. The effects of daily mango supplementation with probiotics for 8 weeks on inflammation and cognitive function were assessed in lean and obese individuals. Mango supplementation with probiotic consumption has been postulated to have a synergistic impact by increasing the bioavailability of their metabolites and their therapeutic activities. Healthy lean (n=50; BMI 18-23kg/m2) and obese (n=44; BMI 27-35kg/m2) volunteers participated in this double-blind, randomized, placebo-controlled pilot trial. Over the course of eight weeks, participants took 400g of mango pulp along with one placebo/probiotics tablet. Participants were given cognitive function assessments and blood-collections on days 1 and 54. In obese participants, mango consumption with probiotics for 8 weeks lowered plasma levels of TNF-α and IL-10 ($p = 0.037$ and 0.049, respectively) compared to the mango placebo group, but showed no effect in lean participants. Lean participants had better visual cognitive performance ($p=0.050$), but training responses were equivalent to obese participants. Mango consumption increased overall cognitive performance in lean participants on the trail making test (attention) and digit span test (memory), but only on the digit span backward test in obese participants. In the trail making test, simultaneous intake of mango and probiotics was not substantially different from mango with placebo, however it had a synergistic impact in the digit span test. BMI ($p=0.0176$, $r=-0.1753$, Spearman correlation) and IFN-γ ($p=0.0336$, $r=0.1572$, Spearman correlation) were found to be directly linked with plasma metabolites. Mango consumption improved cognitive performance in lean individuals, while combining mango and probiotics had a synergistic impact in attenuating inflammation and improving cognitive functioning in obese individuals, at least in part due to increased bioavailability of mango polyphenols.

Keywords

mango polyphenols, obesity, cognitive function, probiotics, clinical study

Early Career Symposium

OC12

Bioavailability of green coffee phenols in overweight humans after acute and chronic consumption alone or in combination with oat beta-glucans

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Background: Obesity and its comorbidities may benefit from the consumption of nutraceuticals rich in bioactive compounds, like (poly)phenols (PP) and soluble dietary fibre (SDF). However, SDF may affect PP's intestinal absorption and colonic biotransformation. On the other hand, most studies on the bioavailability and metabolism of PP-rich foods or nutraceuticals are acute, missing to address the effect of sustained consumption. We formulated two nutraceuticals containing a green coffee phenolic extract (GCPE) alone or combined with oat beta-glucans (BG) and assessed PP's bioavailability acutely and after long-term intake.

Methods: A randomized, blind, crossover trial was conducted with 9 obese/overweight volunteers who consumed the GCPE nutraceutical containing 300 mg phenolic acids or the same nutraceutical enriched with 2.5 g of BG (GCPE+BG) twice daily during 8 weeks. Plasma, urine and faecal samples were collected over a 24-hour interval at baseline and after each intervention (week-8). Phenolic metabolites were analysed by LC-MS-QToF and plasma and urinary nutrikinetics were measured, along with urinary and faecal excretion rates.

Results: Fifty-four phenolic metabolites were identified in both interventions, including high amounts of ferulic acid-4'-sulphate, sulphated derivatives of dihydrocaffeic and dihydrocoumaric acids, feruloylglycine or 3´-hydroxyhippuric acid, formed mostly in the colon. There were no significant differences between both nutraceuticals for most of the metabolites. However, total urinary recoveries of colonic metabolites changed after sustained consumption, increasing from 78.4% at baseline to 79.4% at week-8 with GCPE, and from 77.5% to 84.7% at week-8 after GCPE+BG intake.

Conclusion: Nutrikinetic and bioavailability rates after long-term consumption indicated an apparent increase in colonic catabolism and absorption, which was slightly higher in the presence of BG, probably associated to the effect of the SDF on colonic microbiota. Interestingly, regular intake of both nutraceuticals did not modify the metabolite profile in any biological sample.

Keywords

Bioavailability, Pharmacokinetic profiles, Phenolic acids, Green coffee, Beta-glucans

OC13

Biotransformation of camu camu galloylated ellagitannins by *Lactiplantibacillus plantarum* with extracellular tannase activity

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Lactiplantibacillus plantarum is a probiotic bacterium with an outstanding repertoire of tannases and other polyphenol-transforming enzymes. Most *L. plantarum* strains possess an intracellular tannase. Nevertheless, certain strains also exhibit a broad esterase with tannase activity (Est_1092), and others, an extracellular tannase (TanA), that hydrolyzes complex molecules such as tannic acid. These strain-specific features may give these strains the advantage of transforming esterified and polymeric polyphenols, such as ellagitannins; however, this remains poorly explored.

In this study, we explored the capacity of Est_1092 and TanA producing strains to transform ellagitannins from Myrciaria dubia (camu camu) fruit. For this, we screened 114 strains to identify those harboring *est_1092* and *tanA* genes. Est_1092 and TanA activities were confirmed by two visual tests. Each selected strain was inoculated in a minimal culture media supplemented with an aqueous extract of camu camu. After fermentation, supernatants were collected to semi-quantify the ellagitannins and their metabolites by mass spectrometry. For the analysis, strains were grouped according to their tannase type and compared with one lacking these abilities (WCFS1). Strain ATCC 8014 and ATCC 1055 were positive controls for Est_1092 activity and ATCC 14917 for TanA activity.

Out of 114 *L. plantarum* strains, three showed Est_1092 activity and six TanA activity. None transformed vescalagine and castalagine ellagitannins. Strains with TanA hydrolyzed three different isomers of Di-HHDP-galloyl-glucose ($p<0.01$), releasing Di-HHDP-glucose and gallic acid. TanA producing strains also transformed tri-galloyl-HHDP-glucose and different isomers of HHDP-galloyl-glucose ($p<0.05$), releasing HHDP-glucose and gallic acid. Strains with TanA activity released at least four times more gallic acid than non-producing strains ($p<0.0001$). Moreover, TanA+ strains exhibiting gallate decarboxylase activity produced at least three times more pyrogallol ($p<0.001$).

In conclusion, *L. plantarum* with TanA activity have a unique ability to transform a wide range of galloylated ellagitannins from camu camu.

Keywords

Probiotics, biotransformation, bioaccessibility, tannins, camu camu

OC14

A randomized, double-blind, placebo-controlled study of the effect of daily cranberry juice supplementation for 42 days on the gut microbiome and inflammatory markers in overweight/obese adults.

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Objectives: Cranberries are a rich source of phenolic compounds associated with antibacterial, anti-inflammatory, and antioxidant properties. Even though extensive research has focused on evaluating their antimicrobial potential, fewer studies have focused on their role in inflammatory-related conditions. Therefore, this study focused on assessing the effect of daily cranberry juice supplementation on the gut microbiome and inflammatory markers in overweight/obese individuals.

Methods: Overweight/obese (n=45; BMI=28-35kg/m^2; age=18-65 years) individuals, with a body fat percentage greater than 18% for males and 25% for females, consumed daily 16 oz of treatment juice (placebo or cranberry) for six weeks. Inflammatory cytokines, tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), IL-6, IL-8, IL-10, interferon γ (IFN-γ), were analyzed in serum before and after six weeks. Fecal samples and digestive wellness surveys were collected at the beginning and end of the study. DNA was extracted from fecal samples to perform a 16S-rRNA metagenomics analysis.

Results: After six weeks of cranberry consumption, the serum levels of TNFα were significantly decreased in the male group (p-value < 0.0317), and IL-8 levels were significantly increased in the female group (p-value < 0.0121). Nevertheless, no significant differences were found among treatment groups. Additionally, cranberry consumption improved constipation after six weeks, based on the AGACHAN score system. Microbiome data showed significant differences among groups on the class Coriobacteriia, attributed to an increment in the relative abundance of unidentified Coriobacteriaceae sp. after six weeks of cranberry consumption. Likewise, significant differences among groups on the Bilophila genus were detected after six weeks, attributed to an increase in the relative abundance of unidentified Bilophila sp. on the placebo group.

Conclusion: A low dose of cranberry juice supplementation showed a slight impact on the inflammatory markers and increased the abundance of some beneficial bacterial species. These findings help us understand the bioactive properties of cranberry polyphenols.

Keywords

Inflammatory cytokines, gut microbiome

OC15

Performance of urinary phenyl-γ-valerolactones as intake biomarkers of dietary flavan-3-ols: preliminary findings from two companion studies

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Background: Dietary flavan-3-ols are linked with benefits on human health and disease however their intake assessment is hampered by a lack of objective measures. Recent developments highlight phenyl-γ-valerolactones as potential intake biomarkers of dietary flavan-3-ols. Herein, we investigate the performance of phenyl-γ-valerolactones for quantifying dietary flavan-3-ol intake. Methods: We report results of two companion studies: a 5-way randomised cross-over trial (RCT) and an observational cross-sectional study. In the RCT, 16 healthy participants were assigned to 1-day flavan-3-ol rich interventions of apples, cocoa, black tea, green tea or a water placebo, while consuming a diet devoid of flavan-3-ols and collecting 24-hour urines. In the observational study, 85 healthy participants collected 24-hour urines and concurrent weighed food diaries (under ad libitum dietary conditions) from which flavan-3-ol consumption was estimated using Phenol-Explorer. A panel of >10 urinary phenyl-γ-valerolactones was quantified for both studies using liquid chromatography tandem mass spectrometry. Results: Analysis showed that 3 phenyl-γ-valerolactones [5-(3'-hyrdoxyphenyl)-γ-valerolactone-4'-sulfate, 5-(4'-hydroxyphenyl)-γ-valerolactone-3'-glucuronide and 5-(3',5'-dihydroxyphenyl)-γ-valerolactone] were the principal compounds excreted, constituting ≥80% of the measured metabolites in the observational study and following each RCT intervention alike (except green tea). Results of the RCT showed the sum of these phenyl-γ-valerolactones was significantly higher than the water placebo following each intervention (Bonferroni-adjusted P-values all <0.05); individually, the compounds tended to display different patterns of excretion across foods. In the observational study, the sum of the principal phenyl-γ-valerolactones correlated with the sum of dietary flavan-3-ol intake (Spearman's Rho = 0.39, P <0.0001), with similar associations seen for the compounds individually. Conclusion: We conclude that 24-hour urinary phenyl-γ-valerolactones specifically reflect intake of flavan-3-ol rich foods and that in populations with ad libitum dietary intake, phenyl-γ-valerolactone output reflects flavan-3-ol intake in a dose-dependent manner. Overall, these results indicate phenyl-γ-valerolactones are plausible biomarkers of dietary flavan-3-ol intake.

Keywords

flavan-3-ol, phenyl-γ-valerolactone, biomarker

Poster Presentations

Analytical sciences - measurement of polyphenols

P1

Fast and accurate quantification of proanthocyanidins metabolites by combining enzymatic hydrolysis and high-throughput mass spectrometry

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To study the inter-individual variability associated with the metabolism of proanthocyanidins (PACs), absolute quantification of phase II PACs metabolites in urine is crucial. However, the accurate quantification of these molecules requires expensive or non-commercially available conjugated standards. This issue can be overcome by the use of enzymatic hydrolysis associated with the quantification of unconjugated metabolites using commercially available standards. Although widely used, β-glucuronidase and arylsulfatase from *Helix pomatia* have limited efficacy for polyphenols hydrolysis. Additionally, the high volume of samples resulting from large cohorts requires high-throughput methods for metabolite quantification. We aim to demonstrate that conjugated PACs metabolites in urine can be fully hydrolyzed by enzymes from bacterial sources and that Luxon-MS/MS, a high-throughput MS ion source, is a fast and effective tool to quantify these metabolites.

To compare the performance of the two enzyme sources and to cross-validate the Luxon-MS/MS method, 24 urine samples from a clinical trial where 12 healthy subjects were given a cranberry PACs supplement for 4 days were used. Urine samples were collected at the end of each period. Samples were analysed by UPLC-QToF and Luxon-MS/MS to quantify PACs metabolites following enzymatic hydrolysis.

Bacterial enzymes fully hydrolyzed 12 conjugated PACs metabolites out of 14 measured, while enzymes from *Helix pomatia* fully hydrolyzed only 8 conjugated metabolites. In general, bacterial enzymes were more efficient than enzymes from *Helix pomatia*, and they greatly reduce the hydrolysis time from 360 to 30 minutes.

Luxon-MS/MS was used to quantify phenyl-γ-valerolactones in the 24 urine samples following enzymatic hydrolysis. Analysis time was reduced from 22 minutes (UPLC) to less than 10 seconds. Passing-Bablock regression was used to cross-validate quantification results of hydroxyphenyl-γ-valerolactone (r=0.992) and dihydroxyphenyl-γ-valerolactone (r=0.957).

In combination with bacterial enzymes, Luxon-MS/MS is a promising innovative method to study PACs inter-individual variability in large cohorts.

Keywords

Enzymatic hydrolysis, Urinary metabolites, High-throughput, Proanthocyanidins, Cranberry

P2

Development of a (poly)phenol-rich diet score and relationships with urine and plasma phenolic metabolites

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Food frequency questionnaires (FFQs) are widely used to estimate (poly)phenol intake in epidemiological studies despite that they were mostly validated for measuring nutrient intake rather than (poly)phenols. We aim to develop a novel (poly)phenol-rich diet score based on food intake and explore the relationships with (poly)phenol metabolites in urine and plasma. This study involved 232 healthy adults aged 18 to 80 years who participated in several dietary intervention studies conducted at KCL. Dietary intakes were obtained with the EPIC-Norfolk FFQ. Fasting plasma samples and 24h-urine samples were collected at the baseline visit. In total 114 (poly)phenol metabolites representing the major classes present in the diet were quantified in urine and plasma using a validated high-throughput LCMS/MS method with authentic standards. The (poly)phenol-rich diet score (PPS) was developed based on relative intake (quintiles) of 21 selected (poly)phenol-rich food or food groups, including tea, coffee, red wine, whole grains, breakfast cereals, chocolate and cocoa products, berries, apples and juice, pears, grapes, plums, citrus fruits and juice, potatoes and carrots, onions, peppers, garlic, green vegetables, pulses, soy and products, nuts, and olive oil. Foods included in the PPS were selected based on the (poly)phenol content of foods, main sources of (poly)phenol intake, and frequencies of their intake in the UK population. Correlations between the PPS and circulating phenolic metabolites were evaluated. The total PPS ranged from 27 to 82, with a median of 54. Spearman correlations showed PPS is positively correlated with total urinary (poly)phenols (rho=0.154, p=0.02), flavanones (rho=0.136, p=0.04), phenolic acids (rho=0.152, p=0.02), lignans (rho=0.211, p=0.002), and tyrosols (rho=0.144, p=0.03). Plasma phenolic acids is correlated with PPS (rho=0.159, p=0.03). The PPS correlated well with urinary biomarkers and could be a good reflection of dietary (poly)phenol intake in the UK population.

Keywords

diet score, dietary intake, (poly)phenol metabolites, (poly)phenol-rich food, biomarkers

P3

Examining the world of legumes' phenolic composition

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Although recognized as staple foods for human population, especially to those dependent on vegetable protein, legumes are also an excellent source of bioactive compounds such as the phenolic compounds. These compounds can contribute for the prevention of chronic diseases, (e.g. cardiovascular diseases, metabolic syndrome related to obesity and colorectal cancer). In this study six different legume species (PV - *Phaseolus vulgaris* L., LS - *Lathyrus sativus* L., VF - *Vicia faba* L., PS - *Pisum sativum* L., CA - *Cicer arietinum* L. and LC - *Lens culinaris* L.) were analysed to cope with the lack of comparative studies regarding the phenolic composition of the different legume species.

The six different species were cropped at the same location in Córdoba, Spain, with a Autumn-Winter sowing for the cool season LS, VF, PS, CA and LC, and a Spring-Summer sowing for PV. The mature dried seeds were milled and the obtained whole flours extracted with conventional solvents. The final extracts were analysed for total phenolic content and phenolic compounds quantification by liquid chromatography coupled different detectors (HPLC-DAD, Orbitrap high-resolution mass spectrometry). Multivariate analysis was performed to associate specific phenolic compounds to the different species. When possible, standards were used for the phenolic compounds identification.

The different legume species were characterized by differences in the TPC, chromatographic profiles and identified compounds. Most of the phenolic compounds belonged to flavonoids and hydroxycinnamic acid classes.

The detected variability emphasizes the importance of having access to different legumes for intake as a way of enhancing the diversity of phenolic compounds on the diet.

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Keywords

Legumes, Phenolic compounds, Diversity, Chromatography , Mass spectrometry

P4

Extraction of phenolic compounds to apply as antifungal agents against vineyard pathogens

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Vitis vinifera L., which is most cultivated for wine production in Europe is highly extremely sensitive to the attack of fungal pathogens, namely downy mildew Plasmopora viticola (Berk. & M.A. Curtis) Berl. & De Toni) and powdery mildew Erysiphe necator ((Schwein) Burrill). These pathogens are responsible huge damages to the wine production, as it destroys the vineyards. Currently, the solution to combat these pathogens rely in the use of artificial fungicides, which cause harmful effects such as toxicity to the ecosystem. In this sense, the PreVineGrape project aims at developing natural alternatives to the use of artificial fungicides. The main objective is the obtaining of enriched extracts in antifungal molecules, namely phenolic compounds, strong inhibitors of fungal pathogens, and analyze their behavior in loco, in Portuguese vineyards.

Until the moment, several promising extracts have been obtained, highlighting the PreVineEuc, which revealed the presence of powerful phenolics after extraction with methanol:water (80:20, v/v) through the maceration technique at 25ºC.

According to the results, this hydromethanolic extract revealed the presence of gallotannins, ellagic acid glycosides and quercetin derivatives, highlighting the contents in digalloyl-glucoside (30.5 ± 1.2 mg/g extract), 5-O-caffeoylquinic acid (22.3 ± 0.3 mg/g extract), ellagic acid glucoside (21.6 ± 0.3 mg/g extract), with a content in total phenolics of 173 ± 4 mg/g extract.

This extract will be applied in Portuguese vineyards to combat downy and powdery mildews and compared to commercial fungicides. The PreVineGrape project intends to promote the ecosystem´s health, through innovative and natural alternatives to common approaches. Also, this project will respond to the wine industry demands, that seek for less toxic and safer agents to protect their production.

Acknowledgments: FEDER through the North 2020 Regional Operational Program to the R&D project “PreVineGrape: Desenvolvimento de um biofungicida para combate a doenças da videira” (POCI-01-0247-FEDER-049695 (NORTE-01-0247-FEDER- 113508).

Keywords

Phenolic compounds, Vineyards, Downy and powdery mildews, Natural fungicides, Ultrasound assisted extraction

P5

Apple polyphenols exert anti-diabetic activities *in vitro*

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Apples, as an easily accessible and nutritious fruit, contain five major classes of phenolic compounds, namely phenolic acids, flavonols, dihydrochalcones, flavan-3-ols/procyanidins, and anthocyanins. Apple polyphenols contribute to the prevention of many non-communicable diseases. Our objective was to investigate efficacy of apple polyphenols as a potential nutraceutical to manage type 2 diabetes. In our experimental method, we evaluated total phenolic content (TPC) in 476 apple accessions from Canada's Apple Biodiversity Collection using the Folin-Ciocalteu method and examined the phenolic compound composition and anti-diabetic activities of 30 selected apple accessions (10 high TPC group, 10 commercial cultivars, and 10 low TPC group) using liquid chromatography-quadruple time-of-flight-mass spectrometry (LC-qTOF-MS) and colorimetric/fluorometric enzyme assays, respectively. The results indicated that TPC of the high TPC group was significantly different ($p<0.05$) than that of both commercial cultivars and low TPC group, and there was no statistical significance ($p>0.05$) between the low TPC group and commercial cultivars. The high TPC group can be categorized into three groups: European cider apples, *Malus Sieversii* accessions, and an advanced breeding line that is a dessert apple. Overall, the high TPC group contains significantly higher amounts of procyanidin B2 and epicatechin compared with the other two groups ($p<0.05$). We also found apple polyphenols in the high TPC group inhibited carbohydrate-hydrolyzing enzymes, dipeptidyl-peptidase-4 enzyme, and advanced glycation end products formation in vitro. Particularly, these apple polyphenols exerted prominent α-glucosidase enzyme inhibition. Furthermore, chlorogenic acid ($r_s=-0.83$; $p=0.003$), phloridzin ($r_s=-0.73$; $p=0.016$), epicatechin ($r_s=-0.66$; $p=0.038$) and procyanidin B2 ($r_s=-0.65$; $p=0.043$) were negatively correlated with α-glucosidase enzyme IC50, suggesting efficacies in controlling sugar release into the bloodstream. In conclusion, these results demonstrated the potential use of apple polyphenols as a nutraceutical to manage glycemia in prediabetic and type 2 diabetic individuals.

Keywords

polyphenols, apple, type 2 diabetes, LC-qTOF MS, nutraceutical

P6

Abstract withdrawn

P7

Assessing the bioactive profile of new Ecuadorian fine-flavour cocoa cultivars

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Cocoa (*Theobroma cacao* L.) fruit is one of the most important perennial tree crops worldwide in terms of production, economic value, consumer acceptance and impact on health. Flavour quality of cocoa beans depends on the genotype and plant origin. From an industrial point of view, two main types of cocoa are commercialized in the market, bulk (90% of the global cacao production) and fine-flavour cocoa (5-10%). Despite the low world production of fine-flavour cocoa, the interest comes from its recognized aromatic quality, which makes it a highly sought-after type of cocoa in gourmet recipes. Therefore, due to the increasing consumption and price of cocoa, and the search for innovative flavours, new typicity cultivars are increasingly being sought to respond the market demand. In this sense, the objective of the present work was the study of the bioactive compounds from different Ecuadorian fine-flavour accessions to evaluate their healthy chemical composition, and compare the results with those obtained for bulk cocoa cultivars.

Cocoa beans from bulk (Forastero and CCN51) and fine-flavour cultivars (ETT103 and LR14) and accessions (L41H88, L46H73, L25H60, L45H11, L41H70, L46H71, L30H25, L46H70) were obtained from the germplasm bank Finca La Represa (State Technical University of Quevedo, Ecuador). Methanol/water (80%) extracts of dried cocoa were analysed by HPLC-HR-MS. The results showed the higher amount of phenolics in the new studied cultivars. Specifically, accessions L25H60, L30H25 and L41H88 presented the highest contents, among the main compounds found: epicatechin, and its oligomers, procyanidins B (isomers I and II) and C (isomers I and II), as well as chlorogenic acid. On the other hand, ETT103 and LR14 fine-flavour and CCN51 bulk cultivars were richer in the flavone luteolin. This study highlights the interest of these accessions as important sources of bioactive compounds to increase the functional properties of subsequent cocoa food preparations.

Keywords

Theobroma cacao L., fine-flavour cultivars, bioactive compounds, antioxidant capacity

P8

In vitro digested olive pomace as a source of bioactive compounds.

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State of art and aim of the study: Olive pomace (OP) represents a by-products of olive oil industry produced in large volume annually. Connected to OP production, there are problems of environmental pollution and operating costs to neutralize it. However, OP represents also a natural source of various nutrients and bioactives such as phenolic compounds, unsaturated fatty acids, antioxidant dietary fiber and minerals. The aim of this study was to assess how an in vitro simulation of gastrointestinal digestion of OP, followed by dialyses process to simulate the intestinal and blood absorption, influence its phenolic composition and bioactivities.

Materials and Methods: Three different lots of sun-dried OP have been characterized for their profile in phenolic compounds (phenolic acids, flavonoids, secoiridoids) through HPLC-DAD, and in vitro antioxidant properties (ABTS+, FRAP and DPPH assays). The same evaluations have been performed on OP samples that underwent to an in vitro digestion. The OP absorbable sample (mw <3.5 kDa) with the higher phenolic content and antioxidant activities was further separated in reverse-phase HPLC and a metabolomics analysis of the most bioactive fraction was done by LC-MS/ MS (6530 QTOF LC/MS Agilent system equipped with a nano-HPLC).

Results: Phenolic profiles showed significant differences among the three OP lots, and most compounds were still present in digested samples, with a different distribution among them, depending on single molecules. Accordingly, the three OP samples and their digested solutions showed different antioxidant activities. The most active absorbable fraction of digested OP still contained 4 hydroxyphenylethanol, homocysteine and tyrosol as most represented fractions.

Conclusions: Notwithstanding only a low amount of ingested phenolics can pass the gut barrier [1], it is noteworthy that valuable and health-promoter nutraceutical compounds are still present in digested absorbable samples of OP. Their properties will be subsequently tested on cells.

[1] Ribeiro et al., 2021, /10.1016/j.foodres.2020.110032

Keywords

Olive pomace, in vitro digestion, metabolomic, polyphenols

P9

A robust UHPLC-MS approach for the simultaneous detection of polar phenols in rat brain tissue after consuming a Mediterranean food

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The health benefits of consuming Mediterranean foods, i.e., dried fruits, that are rich in bioactive microconstituents, such as polar phenolics, is continuously highlighted by scientific research. In fact, multiple studies have established a link between polar phenol consumption and a potential in delaying the onset of neurodegeneration, inhibiting neuroinflammation and improving cognitive function despite their low bioavailability. It's obviously vital to obtain quantitative and qualitative data on polar phenolics' accumulation in the brain to further assess their modes of action. Thereby, in the present work, the brain deposition of a wide spectrum of polar phenols was investigated using a simple, rapid, and selective UPLC-Orbitrap MS technique. Satisfactory results for all validation criteria, were achieved with sufficient sensitivity and selectivity for simultaneous determination of polar phenolics in rat brain tissue. The developed method was successfully implemented to evaluate the accumulation of polar phenols in rat brain tissue after consuming the dried fruit. To the best of our knowledge, this is the first study which investigates the brain accumulation of polar phenolics in rats following supplementation with a dried fruit. The study provides an insight for further research of the bioavailability and the tissue specific distribution of bioactive phytochemicals, which are associated with the consumption of Mediterranean foods, i.e., dried fruits. The ultimate goal is to emphasize on the importance of consuming Mediterranean foods, such as dried fruits, for the promotion of human health and to highlight the tissue specific protection provided by the consumption of Mediterranean foods rich in bioactive microconstituents.

Keywords

polar phenolics, dried fruits, rat brain, LC-MS

P10

Analytical performance of a method for the measurement of flavan-3-ol intake biomarkers in a dietary intervention study

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In support of the Cocoa Supplement and Multivitamin Outcomes Study (COSMOS), approximately 11,500 human urine samples were analysed using Liquid Chromatography – Tandem Mass Spectrometry (LC-MS/MS) to determine their 5-(3',4'-dihydroxyphenyl)-4-valerolactone-3'-sulfate (gVL3'S) and 5-(3'4'-dihydroxyphenyl)-4-valerolactone-3'-β-D-glucuronide (gVL3'G) content. To complete this objective within a short timeline, a robust analytical method with sufficient controls had to be established to allow for parallel running on multiple LC-MS/MS instruments without compromising the quality of data.

Quantification of analytes was achieved using calibrators and isotopically labelled internal standards, with quality control samples (QCs) used to demonstrate continued performance on a batch-to-batch basis. Spiked QCs prepared at different concentrations within the calibration range were analysed in each batch to confirm suitable performance by comparison to their nominal (spiked) concentrations. Incurred urine samples collected after flavanol intake and containing quantifiable levels of gVL3'S and gVL3'G were also analysed in every batch to verify the continued assay performance throughout the study. Batch data was accepted based on the performance of the calibration line and QC samples, and samples re-analysed if suitable performance was not demonstrated. In this large-scale study employing five LC-MS/MS systems, only 12 of 147 sample batches required repeat analysis, demonstrating a batch success rate of approximately 92% over a 49-day period.

The data demonstrates that the analytical method applied to this large-scale sample study was robust, accurate and suitable for the purpose of measuring the selected biomarkers of flavan-3-ol intake. With suitable methodology and controls, accurate assessment of analyte concentrations was achieved within the required timelines.

Keywords

5-(3',4'-dihydroxyphenyl)-4-valerolactone, flavan-3-ols, quantification, urine, LC-MS/MS

P11

Antioxidant activity of eight varieties of *Camelia japonica* flowers

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In recent decades, the interest of compounds with antioxidant properties has increased due to their involvement in biological systems and their implicit functions in a variety of chronic disorders. The genus Camellia is a genus rich in antioxidant compounds, so products derived from camellias such as teas and oils have been used in a traditional way due to their beneficial properties for health. The bibliography shows that all species from this genus have a similar composition, so it would be expected that *Camellia japonica* (*C. japonica*), a specie whose current use is almost exclusively ornamental, could be used for more purposes. In this work, we have made a screening for the search of antioxidative agents from flowers of *C. japonica*. Eight varieties of the three ecotypes (red, pink and white) in order to ascertain their inhibitory effect against free radicals were studied. Antioxidant activity was measured using the DPPH, reducing power and TBARS assays. From the varieties analyzed, two of them (Elegans variegated and Grandiflora Superba) were characterized by having a high antioxidant capacity with measurements about the DPPH radical scavenging activity of 136.5 and 86.8 μg/mL respectively.

Keywords

antioxidant activity, DPPH, radical scavenging activities, Camellia japonica, flower extract

P12

Developing cocoa flavanol and procyanidins analytics, from reference material development to method accreditation and modeling of historical method bias.

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Cocoa flavanols and procyanidins (CF) are a group of polyphenols, of which the consumption has been shown to confer a range of health benefits. Assessing the biomedical and nutritional relevance of CF can be challenged by CF chemical diversity and complexity. A complete and reliable analytical solution has been created through a CF commercially available reference material, advanced scientific understanding of separation science of procyanidin by degree of polymerization, a robust and accessible HPLC-based methodology and, external accreditation from AOAC as an Official Method of Analysis in 2020.

The successful deployment of AOAC 2020.05 required ensuring the continuity with past HPLC-based methodologies by developing comparative models. Over the past two decades, mostly two other HPLC-based methods have been used to characterize CF in foods and research materials. To compare biases of these 3 methods, samples including wide ranges of concentration (5 to 500 mg/g) and matrices (chocolate to extract) were analyzed to construct datasets. These methods were evaluated using historical data and when possible, run side by side. Three linear statistical models were built and, when possible cross-validated. Resulting models were applied to published clinical studies investigating the beneficial effects of CF intake to convert reported values to the accredited method, thus enabling a direct comparison between studies.

The results of recent work highlight the ability to develop robust methods for botanical characterization and through modeling, develop connections between methods so that research from the past few decades can be effectively evaluated and compared with future studies, supporting proper systematic reviews and meta-analyses critical to advancing understandings in the flavanol field.

Keywords

flavanol and procyanidins, NIST RM 8403, Cocoa, HPLC, AOAC

P13

Obtaining high valuable bioactive compounds from natural matrices

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Since ancient times that humans have been dedicated to the application of natural matrices in foods, to provide health benefits, given their richness in phenolic compounds with reported antioxidant, antimicrobial, anti-inflammatory, among other properties.

The food industry is the main sector interested in exploiting these natural molecules, as the consumers are more and more interested in avoiding artificial additives, due to their association with harmful effects, namely allergenic issues. Given their richness in high added-value compounds, plants and mushrooms have been widely explored for this purpose and considerable advances have been reached concerning extraction methodologies, stabilization techniques, and application in the food cosmetic and pharmaceutic industries.

As examples of recent studies achievements, phenolic acids (rosmarinic acid), flavonoids (quercetin derivatives), and ellagitannins (sanguiin H-10, lambertianin) from mushrooms, wild strawberry, rosemary, mountain sandwort, and flowers of silva brava were incorporated in gelatin, yogurt, and cottage cheese. Given their antioxidant and antimicrobial properties, polyphenol extracts from strawberry-tree, basil, lemon balm, sweet chestnut flowers, fennel, and German chamomile were used for preservative purposes in loaf bread, cupcakes, yogurt, cheese, and cottage cheese, namely flavonoids (catechin, quercetin and luteolin derivatives), phenolic acids (rosmarinic, chicoric, lithospermic, caffeic, caffeoylquinic acids), and hydrolysable tannins (trigalloyl-HHDP-glucoside). Also, bioactive colouring molecules like betalains (gomphrenins, isogomphrenins) from purple globe amaranth and anthocyanins (cyanidin, delphinidin, and malvidin derivatives) from rose, dahlia, centaurea, strawberry-tree, roselle, blueberry, sweet cherry, fig peel, blackthorn epicarp, among others, were applied in yogurt, waffles, and donut topping, among other food products.

Overall, these achievements are extremely important because they provide real alternatives to the use of synthetic additives/drugs, in line with global issues in obtaining high valuable and natural molecules that can be applied in different industries.

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Keywords

Food Chemistry, Natural products, Phenolic compounds, Bio-based ingredients, Food industry

P14

Applying Response Surface Methodology to Phenolic Compounds from Arbutus unedo: Case Studies with Ultrasound and Dynamic Maceration Extraction

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Arbutus unedo L. is a small Mediterranean plant, being found mostly in southern Europe, north-eastern Africa, Ireland, Palestine, and the Canary Islands, and have been studied for its chemical composition. The objective of this work focuses on the identification and quantification of polyphenols of extracts of *A. unedo* through two different techniques, namely dynamic maceration (DM) and ultrasound assisted extraction (UAE). Three independent variables were tested: time (10-60 minutes), temperature (30-80 ºC) and solvent ratio (ethanol) (0-100%) for DM, and swapping temperature for ultrasonic power (50-500 W) in UAE. The analyzed responses for each extraction were the solid residue after extractions, the four most abundant phenolic compounds in both techniques, namely (+)-catechin, isorhamnetin-O-deoxyhexoside, quercetin-O-deoxyhexoside, luteolin-O-deoxyhexoside and the total amount of phenolic compounds, identified through HPLC-DAD-ESI/MS. The maximization function was used to determine the optimal conditions for each response, being set at 60 minutes, 73 ºC and 66% of ethanol for DM, and 13 minutes, 402 watts and 26% ethanol (for the solid residue). Although individual optimizations were performed for each phenolic compound, the desirability function, which considers all responses, was set at 60 minutes, 65 ºC and 32% ethanol for DM, and 30 minutes, 500 W and 0% ethanol for UAE. Overall, considering DM, while the dry residue was promoted by high temperatures and ethanol, the phenolics were better extracted at lower temperature and half the quantity of ethanol. For UAE, the polyphenols were better extracted with longer extractive time and power and no ethanol. These results help science and the industry design improved extractive conditions to maximize certain polyphenols and reduce the presence of unwanted molecules.

Keywords

A. unedo, Phenolic compounds, Response surface methodology, Dynamic maceration, Ultrasound assisted extraction

P15

Effect of boiling on polyphenol contents in Japanese root vegetables.

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Objectives/Background If we expect polyphenols to be functional for humans, we need to analyze the polyphenols contained in vegetables after cooking, because humans eat cooked vegetables as meals. In Japan, root vegetables are often boiled and eaten. Therefore, we measured the polyphenol contents of Radish, Burdock and Lotus root before and after boiling.

Materials/methods We used UPLC to determine the polyphenol content of radishes that had been pre-boiled in different ways, burdocks that had been cut in different ways and lotus roots that had been boiled in different types of water.

Results/Findings The amount of polyphenols in the root vegetables decreased with boiling. In radish, rutin and quercetin decreased to 44-49% and 2-3%, respectively. There was no significant difference in the amount of polyphenols in radishes after different pre-boiling methods. In burdock, chlorogenic acid was decreased to about 30% and 1,5-dicaffeoylquinic acid to 24-36%. Cutting the burdock so as to increase the surface area reduced the 1,5-dicaffeoylquinic acid even more. In lotus root, chlorogenic acid was decreased to 39-42% and rutin to 31-43%. Boiling in vinegar water inhibited the elution of chlorogenic acid and rutin.

Conclusion The larger the area exposed to the boiling water, the more polyphenols are eluted. Simmering in a boiling water with a pH of 4 reduces the tissue degradation of root vegetables and the elution of polyphenols.

Keywords

Root vegetable, Boiling, Meals

P16

Polyphenols from mandarin (Citrus reticulata) peels – a unique combination of phenolic acids and flavonoids including rare polymethoxylated flavones.

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Over 90% of the world's mandarins are grown in China and it is estimated the mandarin canning industry generates 10 million tonnes of waste peels every year. The objective of the study was to elucidate the polyphenols composition, evaluate antioxidant, antifungal properties with applications in skincare and beyond. Indeed, as part of the international consortium behind the Citrusafe Project we have worked with local partners collecting and pre-processing the peel biomass. This premium hand-picked and hand-peeled plant material was then evaluated as a renewable and sustainable source of interesting and valuable specialty chemicals for various applications ranging from hydrocolloidic gelling agents, film-forming agents for bio-based packaging or personal care multifunctional ingredients.

Of particular attention was the isolation of flavonoids and the rare polymethoxylated flavones sinensetin, nobiletin and tangeretin. The dried and micronized mandarin peel was investigated for extraction efficiency as well as profile using aqueous or ethanolic systems. Other parameters such as reproducibility, scalability, solvent-recyclability and cost-efficiency were carefully considered as we aimed at translating the extraction process to pilot- and industrial-scale. The phytochemical composition of the crude and refined extracts was evaluated using colorimetric methods such as Folin-Ciocalteu assay for the Total Phenolic Content TPC, aluminium chloride assay for the Total Flavonoid Content TFC, and finer quantitative analysis using HPLC and NMR techniques. DPPH assays were used for the evaluation of the antioxidant capacity or radical scavenging activity RSA. The potential anti-ageing benefits for skincare applications was studied and initial results of the collagenase and elastase matrix metalloproteinases inhibition capacity collected. As outstanding results were obtained proving the performances of flavonoid-rich mandarin peel extracts, a range of skincare products helping the maintenance of the skin barrier function were specially formulated to enhance the antioxidant and soothing properties of this key ingredient.

Keywords

mandarin peel waste, polyphenols, flavonoids, extraction, skincare application

P17

Levels of polyphenol content in Purslane

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Purslane, (Portulaca oleracea) is a green, leafy succulent plant that naturally grows as a weed in orchards, lawns, crop fields, and even in roadsides. It has been receiving considerable attention from consumers due its potential nutritional values and potential for health benefits to humans. In this study we have investigated the profile of polyphenolic and additional important nutritional compounds in purslane. Purslane contained fatty acids up to 191.83 mg/100 g DW, total phenolics 117.4 mg GAE/100g, and organic acids 8423.09 mg/ 100 g DW. LC-MS/MS could detect up to 184 compounds including phenolic acids, phenolic alkaloids (oleraceins A, B, C, D, O, U, and W), organic acids (oxalic acid, malic acid, citric acid) and their derivatives, and flavonoids (quercetin, kaempferol, isorhamnetin, naringenin) as major compounds. The phytochemical profile shows distinct differences between purslane, on the one hand, and well-known healthy greens such as spinach and kale on the other. Early indications would suggest that nutrient-dense purslane can be beneficial to human health.

Keywords

Polyphenols, purslane, flavonoids, LC-MS/MS, alkaloids

P18

Assessment of the differences in the phenolic content, antioxidant capacity and enzyme inhibition of monovarietal fig liqueurs elaborated with fruits and leaves

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Nowadays there is an increasing demand for exclusive high quality gourmet products carefully prepared. In this sense, the alcoholic beverage industry has been focused on seeking varieties that meet consumer expectations. However, there are no studies on liqueur production. In addition, besides the type of elaboration process, the plant part used in maceration also influences the quality of the final beverage, so that ingredients that help the development of value-added functional products are sought.

According to the above, the present work intends to show the influence of the fig variety and the part of the plant (fruits and leaves) used in maceration, on the antioxidant capacity (ORAC and TEAC), inhibition against acetylcholinesterase (AChE), tyrosinase (Tyr) and glucosidase (Glu) enzymes, and the phenolic content by liquid chromatography coupled to photodiode array detector of monovarietal fig liqueurs. To this end, characteristic Portuguese cultivars namely Côtea (C), Burjassote branco (B), Castelhana branca (Ca), Eucharia preta (E) and Lampa preta (L), were used to produce the varietal liqueurs.

The principal component analysis showed great variability according to the plant part used in the maceration step. Furanic compounds (furfural and 5-hydroxymethylfurfural) were the unique compounds identified in fruit liqueurs, while leaf liqueurs also presented furanocoumarins (psoralen and bergapten), flavonoids (catechin, rutin and luteolin 7-o-glucoside) and a hydroxycinnamic acid (chlorogenic acid). Leaf liqueurs E and B presented the highest phenolic contents, antioxidant capacity and AChE inhibition (enzyme linked to Alzheimer`s disease), while fruit liqueurs showed higher inhibition to Glu (enzyme involved in diabetes) and especially C, E and L, higher Tyr (enzyme associated to Parkinson`s disease) inhibition. Therefore, the use of leaves, or the combination of leaves and fruits, would help to produce liqueurs with greater bioactivity.

Keywords

Ficus carica L., bioactive compounds, Furanic compounds, maceration, Portuguese cultivars

P19

Identification and quantification of apple pomace's polyphenols and their analysis using FTIR spectroscopy

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Polyphenols are currently used in some dietary supplements and have the potential to be used as antioxidants or antimicrobial agents in food products. Apple pomace is a good source of polyphenols; however, the total content and profile of these compounds vary according to the variety. Polyphenols characterization is a time-consuming analysis that requires specialized analytical instrumentation; so, it is essential to develop an alternative cost-effective and rapid analytical method for the characterization of these compounds in apple pomace. Our objectives were to identify and quantify the phenolic compounds in apple pomace of six different varieties using a UPLC-MS/MS and to develop FT-IR models correlating with the reference method for rapid characterization of the polyphenols in apple pomace.

Polyphenols were extracted from apple pomace using the "homogenizer-assisted methanol extraction" method. After methanol evaporation, the extracts were collected in water and analyzed using a UPLC-MS/MS. Polyphenols were quantified as epicatechin equivalents (Epi-Eq), and the total quantities and their profiles were compared among the varieties. FT-IR spectra of the freeze-dried samples were collected, and results were analyzed and compared to the reference method using PCA and PLSR.

Polyphenol contents ranged between 11.2 to 13.6 mg (Epi-Eq)/g DM, with no significant differences among the varieties. Polyphenols profiles, however, varied significantly among the varieties. The major polyphenols tentatively identified were caffeoylquinic acid, quercetin-galactoside, quercetin-rhamnoside, B type-procyanidin, quercetin-arabinoside, epicatechin, quercetin-xyloside, caffeoylshikmic acid, and quercetin-glactoside based on their overall abundances. PCA analysis showed a clear separation of the varieties based on their polyphenol profiles. According to our PLSR results, seven phenolic compounds were correlated with the IR spectra. In conclusion, the major phenolic compounds in apple pomace were identified, the polyphenol profiles were characteristic of the variety, and PLSR applied on the IR spectra showed to be promising in predicting quantities of certain polyphenols.

Keywords

Apple Pomace, Polyphenols, UPLC-MS/MS, FT-IR

P20

Corinthian raisins polar phenol content as affected by baking

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Corinthian raisins (Vitis vinifera L., var. Apyrena) are dried vine products, produced almost exclusively in the Southern of Greece. Bread products containing raisins, that is “raisin-breads”, are consumed lavishly in the Mediterranean countries. In the present study the Corinthian raisin polar phenol content fate after baking raisin-breads was evaluated. Oven baked bread products containing Corinthian raisins were prepared. Total polar phenol content, total flavonoid content, individual anthocyanins (by RP-HPLC) and antioxidant capacity in vitro of Corinthian raisins before and after the baking procedure were assessed. Prior baking Corinthian raisin total phenolic content was 240±16 mg gallic acid equivalents (GAE)/100 g while post-baking it was found as 118±7 - 220±6 mg GAE/100 g raisins. Raisins’ total flavonoid content was 42±9 mg rutin equivalents (RE)/100 g before baking while the respective content after baking was in the range 20±2 to 38±2 mg RE/100 g. Up to two Corinthian raisins’ anthocyanidin glycosides were identified and quantified in almost all products post-baking. Antioxidant capacity in vitro was 21±4 mg ascorbic acid equivalents (AAE)/100 g before baking while post-baking it was in the range 13±1 - 27±2 mg AAE/100 g. A significant Corinthian raisin polar phenol content was retained after oven baking suggesting that these Mediterranean dried fruits may be used as part of bread making recipes for the concurrent enrichment of baking goods with bioactive polar phenolics and the substitution of sugar.

Keywords

Raisins, Polyphenols, Anthocyanins, Baking, Bread products

P21

Effect of bioprocessing and storage on the isoflavones profile and sensory attributes of soybean meal biscuits

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Soybean meal (SBM), a co-product of soybean oil industry, is rich in protein and bioactive compounds, such as isoflavones. Isoflavones consumption has been associated with several beneficial health effects. These effects are highly dependent on isoflavones bioavailability, which is influenced by many factors, such as chemical form, which may be affected by food processing. This study aimed to evaluate the chemical stability of biscuits prepared using SBM, fermented SBM (FSBM) and enzymatically-processed SBM (ESBM). SBM was bioprocessed by fermentation with baker's yeast (*Saccharomyces cerevisiae*) (FSBM) and by enzymatic hydrolysis with a commercial food grade cellulase (ESBM). SBM, FSBM, and ESBM were used to replace 95% of wheat flour in a standard biscuit formulation (AACC method 10-54). Biscuits were vacuum packed protected from light and stored at room temperature during 180 days. Water activity, moisture, isoflavones profile (by HPLC-DAD-MS) were evaluated every 30 days during storage. Biscuits were sensorially evaluated using an acceptance test before and after storage. The water activity of biscuits ranged from 0.254 to 0.468, considered to be adequate for inhibition of microbial growth. Moisture and water activity of biscuits did not change during storage. SBM, FSBM and ESBM biscuits showed a total isoflavone content in aglycones equivalent of 65.4, 74.7 and 61.9 mg/100 g respectively. FSBM and ESBM biscuits showed aglycone contents 6.5 and 5.1 times higher when compared to SBM biscuits, respectively. The isoflavones contents and profile of biscuits remained stable for 180 days. While FSBM biscuits had lower sensory scores than SBM biscuits, ESBM biscuits had equivalent scores. Storage led to an increase in the texture and aroma scores of ESBM biscuits, while other biscuits were not affected. In conclusion, isoflavones profile and sensory qualities of all biscuits remained stable during storage for 180 days at room temperature.

Keywords

Fermentation, Enzymatic hydrolysis, Stability, Aglycones

P22

Polyphenol Content, Antioxidant Activity, and Enzyme Inhibitory Activities of Some Coffee Cherry Extracts

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Coffee cherry is one of the most ubiquitous agricultural commodities that provides nutritional, stimulant, and health beneficial properties to humans. Coffea arabica (Arabica) and Coffea canephora (Robusta) are two most widely used coffee cherries. However, Coffea arabica is receiving much attention from consumers due to its sensory properties and remaining in great demand in the global coffee market. In this study we have investigated the polyphenolic composition of two unique, commercial extracts WCCE1 (NeuroFactor®) and WCCE2 (Coffeeberry® Energy) produced by proprietary multistep extraction using 70% ethanol and water respectively. LC-MS/MS studies indicated the presence of several phytochemicals comprising organic acids, phenolic acids, chlorogenic acids, flavonoids, diterpenoids and hydroxytryptamides. WCCE1 is rich in chlorogenic acid compounds consisting of minimum 40% with major isomers of caffeoylquinic acids, dicaffeoylquinic acid, and ferroylquinic acid. WCCE2 is rich in caffeine with a minimum of 70%. Multiple antioxidant assays (DPPH, ABTS, FRAP, ORAC, HORAC, NORAC, and SO-RAC) demonstrated that WCCE1 has stronger antioxidant activity compared to WCC and WCCE2. WCCE1 inhibited the activities of α-amylase and α-glucosidase and WCCE2 inhibited activities of acetylcholinesterase in dose-dependent manners with their IC50 values of 1.74, 2.42, and 0.09 mg/mL respectively. In vitro antioxidant activities and inhibitory activities against α-amylase, α-glucosidase, and acetylcholinesterase demonstrate that chlorogenic acid and nutraceuticals with important health benefits.

Keywords

Coffee Cherry, Polyphenols, Antioxidant activity, Enzyme Inhibition, LC-MS/MS

Bioavailability, absorption and metabolism

P23

Identification of aggregate metabolic phenotypes for dietary (poly)phenols and assessment of the factors associated with their formation: development of an oral (poly)phenol challenge test (OPCT)

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Background: (Poly)phenols are plant bioactives playing a role in cardiometabolic health. However, the inter-individual variability existing in their bioavailability and physiological response can impact their true efficacy. Individual (poly)phenol bioavailability is influenced by genetic and demographic background, gut microbiota, lifestyle, and health status. Individuals showing similar metabolic profiles for specific (poly)phenols can be clustered into phenolic metabotypes, while comprehensive phenolic metabolic profiles derived from main dietary (poly)phenols could be referred to as "aggregate phenolic metabotypes". This study aims at identifying aggregate phenolic metabotypes and the determinants related to their formation.

Methods: An intervention study is being carried out on 300 healthy volunteers (18-74 y) which provide information about sex, age, ethnicity, diet, smoking, physical activity, sleeping, anthropometric measures, health status, and biological samples. Subjects undergo a standardised oral (poly)phenol challenge test consisting in an acute supplementation of several classes of dietary (poly)phenols. Urine samples are collected for 24-h and analysed through UPLC-IMS-HRMS to assess the individual urinary excretion of phenolic metabolites, allowing clustering according to aggregate metabotypes. Blood samples are analysed to determine common cardiometabolic health biomarkers, and buffy coat processed to isolate PBMCs used for whole-genome genotyping. Transcriptomic signatures in PBMCs are also assessed. Gut microbiota composition will be profiled by shallow shotgun metagenomics. Cardiometabolic risk scores are also computed. Predictive models will be used to assess the determinants of inter-individual variation in (poly)phenol metabolism, providing indications in the cardiometabolic health status of each individual.

Conclusion: Metabotyping according to the metabolism of the whole set of dietary (poly)phenols may thus represent a promising attempt for cardiometabolic health promotion through personalised nutrition initiatives.

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Keywords

(poly)phenols, metabotypes, cardiometabolic health, intervention study, bioavailability

P24

Challenges in metabolite identification by mass spectrometry

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Dietary polyphenols are ubiquitous in plant derived foods, converted to new structures by food processing, gut microbial metabolism and finally human hepatic metabolism leading to myriad of chemical entities, summarized as metabolites, in human body fluids such as plasma and urine. Mass spectrometry has emerged as the gold standards analytical method for investigating human body fluids combining high sensitivity with unsurpassed resolution allowing identification of metabolites along quantification of metabolites.

A practitioner is typically faced with mass spectral data from human body fluids in intervention studies, revealing tens of thousands of analytes detectable, mostly originating from the diet. When attempting annotation of metabolites detected existing databases are prone to misassignments, however, the large majority of analytes are unknown.

Two novel strategies from our recent research will be presented to identify unknown metabolites in urine using multivariate statistics in combination with an adjusted study design1 and secondly tandem mass spectrometry molecular networks,2 relating unknown metabolites to structurally closely related structures.

Finally we will propose general measures to improve identification and quantification of human dietary metabolites among the global scientific community.

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Keywords

mass spectrometry, human metabolite, compound identification, network science, multivariate statistics

P25

Last updates of the PhytoHub database for better knowledge about food phytochemicals

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PhytoHub (https://phytohub.eu/) is an expert-curated database on food phytochemicals containing their chemical structures, food sources, physicochemical properties, spectral data and metabolites. In the framework of the JPI-FoodPhyt project, we aim to implement more comprehensive information on the metabolism/pharmacokinetics of these phytochemicals, and add new contents such as cardiometabolic health effects demonstrated in randomized controlled studies.

The evolution of PhytoHub structure and webpages was discussed through brainstorming sessions for making knowledge accessible for various purposes and end-users. Standard Operation Protocols and data collection templates were designed for literature survey by FoodPhyt partners and collaborators.

About 2000 food phytochemicals/metabolites are described in PhytoHub with common name, synonyms, several unambiguous identifiers and classification. The new KayCliffordCrozier nomenclature of polyphenol metabolites was added (AJCN 2020). Under guidance of University of Parma, > 10 groups collaborated to collect known metabolites of food phytochemicals, their host and/or microbial origin, pharmacokinetic parameters, and determinants of interindividual variation. Under guidance of MRI, FoodPhyt partners identified which compounds are candidate biomarkers of food intake, considering the criteria defined by the JPI-FoodBAll project. Under guidance of IDIBAPS/INRAE the new health module of PhytoHub is being developed. A search protocol for systematic reviews related to cardiometabolic diseases, risk factors, and biomarkers in RCTs is being applied for a list of >50 priority food/phytochemicals. A user-friendly web interface allows continuous update of PhytoHub by >20 invited expert groups (see website). A PhytoHub blog was built to publish articles summarizing knowledge on food phytochemicals for non-scientists. Other developments on analytics are coming.

PhytoHub is a reference database for food phytochemicals, which is regularly updated and upgraded by a network of collaborators. Feedback and new contributions are welcome to further improve its usefulness for a large community.

Keywords

PhytoHub, food phytochemicals, database, health effects, metabolism

P26

Cocoa (poly)phenolic catabolism study using an ex vivo digestion model.

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Background: Cocoa is a rich source of flavan-3-ols, whose beneficial effects on human health are due to their metabolites. This study investigated the bioavailability of (poly)phenols from a cocoa-based drink in the upper GIT tract in vivo and the of lower GIT using ex vivo modelling of (poly)phenol enriched ileal fluid.

Methods: Ileal fluids collected from 5 volunteers before and after (0-8 hr) intake of a flavanol-containing cocoa-based drink, underwent an anaerobic temperature and pH controlled faecal batch fermentation (24 hr). Samples collected after 0, 5, 10 and 24 hr were analysed using 16S rRNA gene sequencing for the microbiome composition and UHPLC-HR-MS for identification/quantification of (poly)phenolic compounds. The ileal samples were obtained from a randomised, double-blinded, two-way crossover acute feeding study (NCT03765606). Ten ileostomates consumed a flavanol-containing cocoa-based drink and a de-xanthinated cocoa-based drink (566 mg, 583 mg of flavan-3-ols, respectively), ileal fluids were collected 0, 4, 8, and 24 hours after consumption and their (poly)phenolic content characterised via UHPLC-MS/MS.

Results: Characterisation of the ileal fluids (poly)phenolic fraction showed that a significant proportion of the ingested flavan-3-ols reached the colon as methoxy- and/or sulphated-metabolites. Ex vivo fermentation of the ileal fluids led to significant ($p<0.05$) conversions of parent compounds into phenyl-γ-valerolactones, valeric acids, and simple phenolic catabolites. Significant changes in the microbiota community richness (alpha diversity) were observed for the inulin and two fermented ileal fluids (S04, and S11) after 10 hr of fermentation ($p<0.05$). Beta-diversity significantly discriminated based on faecal donors at each timepoint (Bray-Curtis, $p<0.01$). Significant ($p<0.05$) differences in the Operational Taxonomic Units' relative abundance compared to the controls were observed at the phylum and genus levels.

Conclusion: The 24 hour ex vivo faecal fermentation of ileal fluids enriched in flavan-3-ols significantly affected the microbiota and (poly)phenolic composition.

Keywords

ileostomy patients, flavan-3-ols, gut microbiota

P27

Trans-ε-viniferin metabolism: from evidence to its strong *in vivo* glucuronidation after oral administration on rat; to the measurement of its glucuronides anti-inflammatory properties

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Trans-ε-viniferin (εVin) is a resveratrol dimer found in *Vitis vinifera* shoot extract. This stilbene has shown many biological properties, often governed by its antioxidant and anti-inflammatory activities. The very low bioavailability of εVin, due to its poor absorption and high metabolism, raised the question about the role of metabolites for its *in vivo* biological activities. In rats, the predominant metabolites are represented by 4 isomers of εVin glucuronides (εVG). As εVG were not commercially available, the aim of this study was to synthesize εVG in order to first determine their pharmacokinetic parameters after an oral administration of εVin in rats and second to study their potential anti-inflammatory properties.

The 4 εVG were produced by hemi-synthesis reaction, purified by semi-preparative HPLC and used to validate their method of extraction and quantification by LC-HRMS. An *in vivo* study was conducted on Wistar rats that received εVin (20 mg/kg) by oral route. Blood samples were collected at different times (from 0 to 4 h) for kinetic monitoring. The anti-inflammatory properties of εVin and its εVG were evaluated on LPS-activated RAW264.7 macrophages by measuring NO production using Griess reagent.

εVG reached concentrations 100 times higher than the aglycone form in plasma. In addition, kinetics showed two absorption peaks indicating a potential enterohepatic circulation of the glucuronide forms that could increase the organism's exposure time to εVG. The 4 εVG dose-dependently inhibited NO production showed anti-inflammatory activities weaker than the native form.

For the first time, the glucuronidation of εVin has been demonstrated in rats after oral administration, indicating a significant intestinal and hepatic metabolism. The predominance of glucuronide forms and the demonstration of their anti-inflammatory activities could explain the *in vivo* biological activity of εVin. These results again highlighted the importance of metabolism in the study of the biological activities of natural compounds.

Keywords

Trans-ε-viniferin, glucuronidation, pharmacokinetic, anti-inflammatory properties, macrophages

P28

Study of the bioaccessibility of phenolic compounds from olive oil using the SHIME® procedure: An *in vitro* study of the digestion process throughout the gastrointestinal tract

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Phenolic compounds are minor components of virgin olive oil (VOO) including phenolic acids, phenolic alcohols (e.g.: hydroxytyrosol), flavonoids, lignans, and secoiridoids. Following our previous studies on bioavailability of phenolic compounds after VOO intake [1] we used the Simulator of Human Intestinal Microbial Ecosystem (SHIME®) to study the phenolic bioaccessibility of hydroxytyrosol and their metabolites during the digestion process. This approach simulated the digestion process throughout the gastrointestinal (GI) tract, including the colonic fermentation of the non-digestive fraction.

Products of digestion in the different sections of the GI tract were collected at different time points. The samples were analysed by LC coupled with several detectors as diode array, electrochemical and fluorescence and also by Orbitrap high-resolution mass spectrometry (HRMS). Identification of metabolites in HRMS was done by comparison with mzCloud and ChemSpider libraries. Multivariate analysis was performed to correlate the metabolites with the different groups of samples established according to the collection section in the GI tract. In this scope partial least square – discriminant analysis (PLS-DA) was applied.

PLS-DA analysis showed differences between the chromatographic profiles of samples classified according to the GI collection place. The hydroxytyrosol, tyrosol and homovanillic acid were present in the VOO. The hydroxytyrosol and homovanillic acid were also identified in the stomach after 2h but not in the remaining GI tract. At the colonic level, the test products of digestion stimulated cross-feeding interactions that benefit the propionate-producing micro-organisms (e.g. Bacteroides, Veillonella or Megamonas spp.).

This approach allowed us to identify phenolic compounds metabolites that might have a local interaction in the GI tract. Further studies will confirm the identification of these metabolites by using the corresponding standards. The role of the identified metabolites in beneficial health effects will require further investigation.

[1] Silva, S., et al. (2018). Molecular Nutrition and Food Research, 62(2), 1700065. (doi:10.1002/mnfr.201700065).

Keywords

Olive oil, Bioaccessibility, Hydroxytyrosol, Gastrointestinal tract, Colonic fermentation

P29

Influence of the flavan-3-ol structure on the production of phenolic metabolites after consumption of different flavan-3-ol sources by healthy subjects

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Flavan-3-ols are the most consumed flavonoids in Western diets and have been associated with beneficial effects on cardiometabolic health. When ingested, they are poorly absorbed in the small intestine and reach the colon, where they are metabolized by the gut microbiota being converted to low molecular weight catabolites. These catabolites or their phase II conjugates may act as mediators of diet-induced effects on health. Phenyl-γ-valerolactones and phenylvaleric acids are characteristic flavan-3-ol metabolites occurring together with other circulating metabolites, as phenylpropanoic, phenylacetic, cinnamic, and benzoic acids. The structural properties of parent flavan-3-ols may affect their microbial catabolism. The degree of polymerization, the type of subunit linkages, the extent of B-ring hydroxylation, and the presence of galloyl moieties can influence flavan-3-ol bioavailability and metabolism.
This study aims to evaluate flavan-3-ol bioavailability and metabolite profile in a double-blind, randomized, cross-over intervention trial in humans, through the assessment of the urinary excretion of phenolic metabolites after the consumption of 3 different extracts: green tea, grape seed with high monomeric content, and grape seed with high procyanidin content. Volunteers consumed 1000 µmol of total flavan-3-ols. Urine samples, collected 24 and 48 hours after flavan-3-ol consumption, have been analyzed by uHPLC-MS/MS.
The metabolic pathway of flavan-3-ol monomers and oligomers has been targeted. Differences in the quali-quantitative production of phenolic metabolites have been observed, based on the flavan-3-ol source. Green tea flavan-3-ols were metabolized to trihydroxylated and 3',5'-dihydroxylated derivatives, while grape seed flavan-3-ols gave origin to 3',4'-dihydroxylated derivatives. Oligomers had the lowest bioavailability.
The study of flavan-3-ol metabolism is key to develop biomarkers of intake to be used in intervention trials, and to study their mechanism of action in bioactivity studies. The assessment of the influence of flavan-3-ol structure on the production of phenolic metabolites is crucial to understand the beneficial properties associated with these phytochemicals.

Keywords

flavan-3-ol, bioavailability, metabolism

P30

Does metformin affect polyphenol metabolic fate? A supplementation study with a cocoa-carob blend in Zucker diabetic rats.

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Background and objectives: Metabolic transformations play a key role in the generation of bioactive metabolites from dietary polyphenols. Diverse factors, such as high fat diets, have shown an impact on the polyphenol metabolic fate through modifications on the microbiota profile. However, drugs can also affect polyphenol transformation, ultimately compromising their effectiveness as dietary adjuvants. The present study investigates the effects of metformin, the first-line drug used in type 2 diabetes management, on the polyphenol metabolic fate in the context of a cocoa-carob blend (CCB) diet rich in flavonoids.

Methodology: Zucker diabetic fatty rats (ZDF) were fed with a CCB diet (10%) or a control diet with or without metformin during 12 weeks. Fresh faeces were collected one week before the sacrifice and, targeted metabolomics was applied for identifying phenolic metabolites by using liquid chromatography coupled to a mass spectrometer with electrospray ionisation and a quadrupole/time-of-flight mass analyser (HPLC-ESI-QTOF MS).

Results: A total of 13 phenolic metabolites (valerolactones, phenylvaleric acids, phenylpropionic acids, phenylacetic acids and hydroxybenzoic acids) were found in CCB groups, but not in the control group. The main metabolite identified in ZDF rats fed with the CCB diet, treated with or without metformin, was gallic acid, probably derived from the transformation of gallotannins present in carob. Both cocoa and carob were sources for the rest of phenolic metabolites detected. Metformin consumption significantly increased the concentration of glucuronidated dihydroxyphenylvalerolactone, whilst it decreased that of 3 or 4-hydroxyphenylpropionic acid in rats fed with the CCB diet as compared to the animals not receiving metformin. No significant modifications were observed in the concentrations of all other metabolites.

Conclusions: This study with CCB suggest that, overall, metformin does not interfere with the metabolism of flavonoids, although the concentrations of some specific metabolites can be affected by the consumption of this drug.

Keywords

type 2 diabetes, metformin, cocoa, carob, polyphenol metabolites

P31

Cherry consumption in season decreases the proportion of saturated fatty acids in the liver and increases them in the muscle.

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Background:

The distinctive signature of phytochemicals, mainly polyphenols, could serve as adaptive metabolic signal from the environment for heterotrophs. It has recently been described that consumption of cherries from different origins and in different seasons had a differential effect on serum metabolic parameters and on the expression of lipogenic hepatic enzymes. Thus, the objective of this work was to evaluate the effect on the hepatic and muscular lipid profile of the consumption of cherries from different geographic origins and in different photoperiods.

Methods: 72 Fischer 344 rats were exposed to different photoperiods: short (L6), standard (L12) or long (L18), with 6, 12, and 18 hours of light, respectively. After 4 weeks, animals were divided into 3 groups (n = 8) according to supplementation with local cherry (LC), non-local cherry (nLC) or vehicle (VH) during 7 weeks. Analysis of the liver and muscle fatty acid (FA) profile was performed by gas chromatography. The gene expression of key enzymes in the biosynthesis of hepatic unsaturated FA, and muscle oxidative enzymes were analyzed by PCR.

Results: In-season consumption of cherry decreased hepatic saturated FA (SFA) and boosted a greater FA desaturation, due to greater expression of the desaturase Scd1. In addition, LC intake in-season tends to a higher expression of FAT/cd36, and higher muscle SFA and monounsaturated FA (MUFAs). In contrast, out-of-season is associated with a higher proportion of muscle stearic acid, lower MUFAs, and higher arachidonic acid content.

Conclusions: Beneficial effects of in-season cherry consumption, L18, on liver and muscle SFA, were observed, possibly due to increased uptake, desaturation, and oxidation.

Keywords

Polyphenols, fatty acids, seasonality, lipid metabolism

P32

Mango (Mangifera indica L.) carotenoids: Comparison of in Vivo and in Vitro Studies

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Background: Mango (Mangifera indica L.), is one of the most consumed tropical varieties in the world due to its sensorial attractiveness, nutritional composition and high level of health-beneficial compounds, among which carotenoids stand out. To exert bioactive effects, carotenoids have to be both bioaccessible and bioavailable. This study aims to evaluate the stability of mango carotenoids under gastrointestinal digestion using an in vitro digestion model contrasted to in vivo digestion (ileostomy feeding study).

Materials: Mango puree underwent a three stage in vitro gastrointestinal digestion model; oral, gastric and intestinal digestion. While in vivo, an acute ileostomy feeding study (REC/19/0097) required 10 participants to ingest 300 g of mango puree. Carotenoids were analysed by HPLC-DAD in samples from the in vitro digestion (24h) and ileal fluid 24h post mango consumption.

Results: Ten carotenoids were identified in mango puree with 13-cis-β-carotene predominating. In vitro, all mango carotenoids remained quite stable after oral phase and were partially degraded after gastric phase, and significantly affected by small intestinal conditions. The overall bioaccessibility of mango carotenoids was 30.23%, with α-carotene and 13-cis-β-carotene most sensitive to in vitro digestion. In contrast, the in vivo study reported lower recoveries of carotenoids in ileal fluid 24 h post consumption, with an overall average of 1.3%. These results highlighted that carotenoids are mainly absorbed in the upper gastrointestinal tract with only small quantities of carotenoids available to enter the large intestine and interact with the resident gut microbiota.

Conclusions: Carotenoid stability is a key factor which may greatly influence its bioavailability in humans, being the results from the present study of particular interest to provide insight into the carotenoids fate through gastrointestinal tract.

Keywords

carotenoids, mango, in vitro digestion, ileostomy patients

P33

Bioavailability of mango polyphenols in subjets with and without a colon.

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Background: Mango (Mangifera indica L.) is a tropical fruit that attracts much interest from a phytochemical perspective, due mainly to its (poly)phenolic composition. A growing body of evidence suggests that phenolic derivatives may be responsible for the beneficial effects associated with mango consumption. This study evaluated the absorption, metabolism and excretion of the mango (poly)phenols in volunteers with an intact gastrointestinal tract and ileostomists who have had their colon remove surgically.

Methods: An acute feeding study (REC/19/0097) was carried out at Ulster University where 10 healthy participants and 10 ileostomists ingested 300 g of mango. Urine, plasma and faeces/ileal fluid samples were collected over a 0-24 h period and analysed for parent compounds and metabolites by liquid chromatography coupled to a high-resolution mass spectrometer (UHPLC- HR-MS).

Results: Analysis of the ileal fluid revealed the presence of mango polyphenols and their metabolites accounting for 35% of intake. Of the compounds recovered, 67% were the parent compounds, gallic acid and gallotannins present in mango. In total 102 µmol of phenolics was excreted in urine by ileostomists, which corresponds to 52% of the 193 µmol (poly)phenol intake, in the form 3-methoxygallic acid-4-sulfate and two isomers of methoxygallic acid-glucuronide. Gallic acid catabolite excretion in subjects with a functioning colon decreased significantly from 102 µmol to 67 µmol, which is a 35% of recovery. Conjugated phase II metabolites of benzenetriols appeared in plasma of subjects with a colon after mango consumption, but not in ileostomists, suggesting that mango gallotannin absorption occurred principally in the lower gastrointestinal tract following degradation by the colonic microflora.

Conclusions: Following consumption of mango phase II metabolites of gallic acid are absorbed principally in upper GI tact while catabolites of gallic acid and gallotannins are absorbed in the lower bowel following the action of the resident microbiota.

Keywords

bioavailability, human metabolism, ileostomy patient, mango polyphenols

P34

Separation of isomeric forms of urolithin conjugates using supercritical fluid chromatography: a possible step to improve urolithin metabotype assignment?

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Background: Urolithins are gut microbiota metabolites produced from ellagic acid and ellagitannins and seem to be responsible of their health effects. In the systemic circulation urolithins occur mainly as glucuronide conjugates and different metabotypes have been described depending on the final urolithins produced (Urolithin A (Uro-A), isourolithin A (isoUro-A) and urolithin B (Uro-B). Glucuronides of these urolitihins are not commercially available and their biosynthesis leads to mixture of regional isomers. These isomers could be produced in diverse quantities in different individuals due to enzyme polymorphisms and may exert dissimilar effects. However, the lack of an appropriate methodology to separate these isomers hinders their analysis in biological samples.

Material and Methods: Standards of Uro-A 3-glucuronide, Uro-A 8-glucuronide, isoUro-A 3-glucuronide, isoUroA-9 glucuronide and Uro-B glucuronide were synthesized by VillaPharma Research S.L (Murcia, Spain). Ten volunteers consumed 30 g of walnuts/day for three days and urine sample was collected. Samples were analyzed by high performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC), both coupled with UV-Vis detection.

Results: Uro-A 3- and 8-glucuronide and isoUro-A- 9-glucuronide eluted together in the HPLC chromatogram and could not be resolved on reversed-phase columns. Only isoUro- A 3-glucuronide and Uro-B glucuronide were sufficiently resolved. However, the use of SFC allowed the separation of all the metabolites and to determine each isomer separately. In general, both isomers of Uro-A glucuronide were present in all the volunteers with similar proportions. In volunteers with metabotype B the most common isomer was isoUroA-3 glucuronide although in some cases isoUro-A 9-glucuronide was also detected.

Conclusions: Chromatographic separation of the different isomers of urolithin glucuronides was achieved. This could be an important step to improve the urolithin metabotype assignment and to explore the glucuronyl transferase polymorphisms that can also affect inter-individual variations in ellagitannin metabolism and their effects in human health.

Keywords

urolithins, isomers, supercritical fluid chromatography, inter-individual variation

P35

Interindividual variation in the metabolism of cocoa flavan-3-ols explored with untargeted metabolomics

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The metabolism of flavan-3-ols has been extensively described. However, exploration of the interindividual variation has been limited, mainly because studies enrolled small numbers of subjects, or did not quantify all relevant metabolites or analyzed samples over 24h only, while important microbial metabolites are excreted later. Our objective was to investigate the interindividual variation for flavan-3-ols metabolism using untargeted metabolomic in a large controlled intervention study.

128 healthy volunteers enrolled in the COB study collected urine in different fractions over 48h after consuming in controlled conditions a polyphenol-rich breakfast composed of cocoa, orange juice, and blackberry. Subjects were divided in 4 groups: young (18-30y) female, young male, old (65-77 y) female, and old men. Urine samples were analyzed by untargeted metabolomics (UPLC-QToF-MS, pos&neg). A target-screening approach was first adopted to identify expected flavan-3-ols metabolites. Correlation and clustering analyses were applied to the whole metabolomic datasets to identify possible profiles that may distinguish specific metabolic capacity of some subgroups in our study population. Metabolites of interest were annotated using spectral data and analysis of standards.

Twenty-nine metabolites were identified as epicatechin (EC), phenyl-g-valerolactones (VL), phenyl-g-valeric acids (VA) and their conjugated derivatives. Thirteen ions highly correlated with these flavan-3-ol metabolites were identified as small phenolics or metabolites of cocoa compounds. Hierarchical Clustering analysis on flavan-3-ols metabolites revealed a high interindividual variation and different metabotypes were distinguished with K-Means analysis. Application of V-test on the whole metabolomic profiles allowed to characterize the metabolites that are specifically associated with the various metabotypes. Associations between age, gender, BMI, and metabotypes were also determined.

Our untargeted metabolomic approach applied to the COB study samples revealed a large interindividual variation in the metabolism of flavan-3-ols and identified new metabotypes. Further work is deserved to demonstrate their relevance regarding the health effects of flavan-3-ols.

Keywords

flavan-3-ols, interindividual variation, metabotypes, intervention study, untargeted metabolomics

P36

In vitro faecal fermentation of monomeric and oligomeric flavan-3-ols: Catabolic pathways and stoichiometry

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Objectives/Background: Flavan-3-ols are largely consumed in the Western diet, due to their presence in foods and beverages consumed daily. This (poly)phenol class accounts for compounds which largely differ from each other, because of their chemical structure, which ranges from monomers, mainly (epi)catechins and (epi)gallocatechins, to complex high molecular weight compounds, namely proanthocyanidins. Their human microbial catabolism leads to specific bioactive catabolites, among which phenyl-γ-valerolactones (PVLs) and phenylvaleric acids (PVAs) represent the most important catabolites. The aim of this study was to evaluate the influence of flavan-3-ol structure, including the degree of polymerization, subunit linkages (A-/B-type), and the presence of galloyl moieties, on the production of PVLs and PVAs.

Materials/Methods: Flavan-3-ols, including (+)-catechin, (−)-epicatechin, dimer A2, B2, trimer AA, AB, BB, tetramer ABA, BBB, one pentamer, (−)-epigallocatechin gallate and theaflavin-3'-*O*-gallate were fermented *in vitro* for 24 h using human faecal microbiota. Microbial derived catabolites were analysed by UHPLC-ESI-MS/MS.

Results/Findings: A total of 32 catabolites, strictly related to microbial catabolism of parent compounds, were detected. After the incubation of 75 μmol/L of native compounds, (+)-catechin and (−)-epicatechin displayed the highest molar mass recoveries, expressed as a percentage with respect to the incubated concentration, for total PVLs and PVAs. Among A-type procyanidins, only dimer A2 was catabolized, undergoing the microbial ring fission step, whereas all B-type procyanidins underwent ring fission, although no differences in total PVL and PVA production were reported despite the different degree of polymerization.

Conclusion: This study sheds light on how the structural heterogeneity of flavan-3-ols can affect colonic microbial catabolism *in vitro*, influencing the production of PVLs and PVAs. Further studies are needed to fully clarify the catabolic fate of heterogeneous flavan-3-ols and their ability to produce key bioactive catabolites, as well as other possible catabolites not yet identified.

Keywords

Microbial catabolism, Flavan-3-ols, Phenyl-γ-valerolactones, Phenylvaleric acids

P37

Oral and gastrointestinal bioaccessibility of anthocyanins in fresh, frozen, and blended blueberries using the INFOGEST protocol

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Background: The structure of the food matrix is an important regulator of the bioaccessibility of plant bioactives. Little evidence exists on the oral and gastrointestinal bioaccessibility of anthocyanins in blueberries.

Methods: Anthocyanin bioaccessibility was evaluated in blueberries prepared in three commonly consumed forms: fresh, thawed and as a smoothie. In vivo mastication of both fresh and thawed blueberries performed by 3 healthy participants was compared to the in vitro oral phase of the INFOGEST protocol. Fresh human chewed (FHC), fresh simulated chewed (FSC), frozen-thawed human chewed (FTHC), frozen-thawed simulated chewed (FTSC) and blueberry smoothie (BS) samples were further digested using the INFOGEST gastric and duodenal phases. Anthocyanin bioaccessibility was determined by HPLC-MS.

Results: Overall, oral bioaccessibility accounted for <10% of total anthocyanins in all samples. Anthocyanin release was significantly higher in simulated mastication (5.96±0.48% for FSC and 7.97±0.19% for FTSC) than in vivo mastication (1.95±0.17% for FHC and 5.03±0.09% for FTHC, $p<0.05$). Frozen-thawed samples had significantly higher anthocyanin oral bioaccessibility than fresh samples ($p<0.05$). Compared with mastication, blending released 8.44±0.31% anthocyanins (BS sample), significantly higher than the other samples ($p<0.05$). Anthocyanin bioaccessibility following gastric digestion was higher than the oral phase for all samples ($p<0.05$), with BS 54.79±1.10%, followed by FTSC (52.41±2.20%), FTHC (37.45±0.73%), FSC (28.71±0.60%) and FHC (19.63±0.43%). After duodenal digestion, anthocyanin recoveries were reduced for all samples (4.67±0.01% for FHC, 6.19±0.13% for FSC, 4.98±0.14% for FTHC, 5.63±0.04% for FTSC and 9.05±0.13% for BS), likely due to the high pH environment.

Conclusions: Blueberry anthocyanins consumed as a smoothie had higher bioaccessibility than frozen blueberries, which were higher than fresh blueberries. Importantly, simulated mastication leads to higher release of anthocyanins than in vivo mastication, so results from in vitro mastication should be interpreted with caution.

Keywords

Anthocyanins, Blueberries, Bioaccessibility, Gastrointestinal digestion, INFOGEST

P38

Sulfated silymarin flavonolignans: identification as human metabolites and properties

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Objectives/Background: Silymarin, an extract from the fruits of milk thistle (*Silybum marianum*), contains flavonolignans and flavonoids. Although widely used as a hepatoprotective agent, its application remains somewhat controversial, particularly because of the low oral bioavailability and rapid conjugation of the flavonolignans.[1] Our objective was to investigate how sulfate conjugation affects the bioactivity of silymarin flavonolignans.

Materials/Methods: Sulfated metabolites were prepared in our laboratory in excellent yields using arylsulfotransferase from *Desulfitobacterium hafniense*[2], used as authentic standards in metabolic studies, and tested for antioxidant and reducing activities, interactions with human albumin and cytochromes P450 (CYPs), for vasodilator and anti-aggregant properties.

Results/Findings: The standards allowed the unequivocal identification of silybin-A-20-*O*-sulfate, silybin-B-20-*O*-sulfate, 2,3-dehydrosilybin-20-*O*-sulfate, silychristin-19-*O*-sulfate, and isosilybin A-20-*O*-sulfate as metabolites formed in isolated human hepatocytes[3], by human liver and intestinal cytosols, or recombinant human sulfotransferases[4]. Sulfated 2,3-dehydroderivatives were more active than the parent compounds in Folin–Ciocalteu reagent and ferric reducing activity assays, whereas the remaining sulfates were less active chemoprotectants.[2] Both parent compounds and sulfates formed stable complexes with human albumin. Sulfation abolished the inhibition of CYP enzymes by flavonolignans, but 2,3-dehydrosilychristin-19-*O*-sulfate showed strong inhibitory effect on CYP3A4.[5] Sulfated flavonolignans also showed vasodilatory activitiy in isolated rat aorta; silychristin-19-O-sulfate was the most efficient with detectable effects at hundreds of nM and EC_{50} = 19 μM. On the other hand, the effect of flavonolignans on human platelets was low or negligible.[6]

Conclusion: Sulfated flavonolignans are human metabolites of these important polyphenols that exert significant bioactivities.

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Keywords

silymarin, flavonolignans, sulfate, metabolite, milk thistle

P39

Analytical approach for a more accurate quantitation of oleuropein metabolites after ingestion of olive leaf extracts: application to a pilot pharmacokinetic study in humans

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Background: Oleuropein (OLP), a phenolic compound mainly found in olive leaf extracts (OLE), has been related to important biological activities that, as for the rest of polyphenols, depend on its bioavailability. However, the accurate quantification of its metabolites in biological samples is challenging due to the absence of authentic standards and alternatives such as the use of other available standards or enzymatic hydrolysis presents some limitations. The aim of the present study was to develop and alternative methodology for the accurate quantification of OLP metabolites in plasma and urine, that could be extensible to other families of polyphenols.

Material and Methods: Concentrated urine sample of one volunteer taken during the first three hours after consuming two capsules of OLE was quantified by HPLC-DAD and UPLC-ESI-QTOF MS using the available standards (hydroxytyrosol glucuronide and oleuropein aglycone) to establish the MS response factors for each identified metabolite. The developed methodology was applied to plasma and urine samples from a pilot 24h pharmacokinetic study with 15 healthy volunteers that ingested one OLE capsule (100 mg OLP)

Results: Large differences in response were observed between the compounds even when structurally related to the same parent molecule. This response factor was applied to quantify conjugated forms of hydroxytyrosol, homovanillyl alcohol and, for the first time of oleuropein aglycone in the pharmacokinetic study. Rapid absorption of these compounds in plasma (Tmax 2.4h and Cmax 30.8-570 nM) and high recovery in urine were observed.

Conclusion: For the first time oleuropein aglycone conjugates and its hydroxylated and hydrogenated derivatives were quantified after consumption of olive products. The small intestine was shown as the major site for OLP metabolites absorption. This analytical approach could also be used for other phenolic metabolites analyses when authentic standards are not available, opening a valuable method for pharmacokinetic and bioavailability studies.

Keywords

oleuropein, bioavailability, response factor , pharmacokinetic

P40

Dietary polyglycosylated anthocyanins, the smart option? Towards their stability and bioavailability

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Background: Anthocyanins have emerged as one of the most enthralling groups of natural phenolic compounds. The interest raised around anthocyanins goes way beyond their visually appealing colors and their acknowledged structural and biological properties have fueled intensive research towards their application in different contexts. However, the high susceptibility of monoglycosylated anthocyanins (MGA) to degradation under certain external conditions might compromise their applications and health properties. In that regard, polyglycosylated anthocyanins (PGA) might offer an alternative to overcome this issue.

Methods: PGA from different food sources (purple sweet potato, Chinese red wine, and Edible Flowers) were isolated and structurally characterized. The stability at different pH was evaluated. Thermal stability was also evaluated. Furthermore, their stability to the digestion processes was performed in vitro at the oral, gastric and intestinal level. Transepithelial transport assays were performed using gastric and intestinal celular models to evaluate the absorption and role of food matrices. Also, a nano-gene-silencing approach allowed the evaluation of the molecular mechanism of absorption of these anthocyanins. Confocal Microscopy was used to track PGA in gastric and intestinal cells.

Findings: The results suggested a higher stability at a broader range of pH values when compared to the already published kinetic and equilibrium parameters of MGA, and a higher thermal stability up to 60 celsium degrees. The digestions studies revealed a higher resistance of acylated PGA at the different levels and the transport studiesrevealed a structure-absorption efficiency relation in the presence and absence of food matrix. The involvement of GLUT1 and GLUT3 on the transport mechanism of PGA was observed. Upon incubation, PGA were concentrated in specific cell areas, suggesting localized bioactive actions.

Conclusion: These results elucidate new insights on PGA stability and bioavailability and suggest that this subclass on anthocyanins may be more appealing for both nutritional, health and technological applications.

Keywords

Anthocyanins, Bioavailability, Stability, Polyglycosylated

P41

The link between microbial composition and phenyl-γ-valerolactone metabolite production in young healthy males following acute pure (-)-epicatechin supplementation.

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Objective/Background: Large interindividual differences in clinical effects following flavan-3-ol (rich foods) consumption have been reported, which might partially be caused by large interindividual differences in bioavailable metabolite concentrations.

Flavan-3-ol metabolization in the colon results in phenyl-γ-valerolactone and valeric acid metabolites, that have been suggested to be bioactive. As the colonic microbiota are responsible for the metabolization of ~70% of for example total (-)-epicatechin intake, it is hypothesized that the large interindividual variation in microbial gut composition could be responsible for the heterogeneity in metabolite concentration and subsequently for the variation in clinical effects.

The aim of this single-arm study is to investigate if the microbial composition in the gut determines the inter-individual variation in of phenyl-γ-valerolactone and valeric acid metabolites. This will be investigated following the acute consumption of pure (-)-epicatechin.

Materials/Methods: A homogenous cohort of young males (n = 17; aged 23±3y, estimated resting metabolic rate: 1834 ± 141 kcal) was recruited. Following a 2-day low flavanol diet and standardized flavanol-free dinner, participants arrived fasted in the lab and received 150 mg (-)-epicatechin. After insertion of a catheter participants received standardized low-flavanol meals throughout the day while blood samples were drawn hourly up to 14 hours after intake of the supplement. A fasting blood sample was also obtained 24h and 48h after intake of the supplement. A stool sample was obtained the day before the experimental test. Phenyl-γ-valerolactone and valeric acid metabolite profiles in plasma will be determined by QToF-MS (MSe). A quantitative microbiome profiling is foreseen (16S rRNA).

Currently, samples are being analyzed, however by the time of the conference we will be able to present the preliminary data of this investigation.

Keywords

Bioavailability, absorption and metabolism, Gut microbiota, metabotype

P42

Metabotypes of flavan-3-ol colonic metabolites after cranberry intake: elucidation and statistical approaches

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Objectives: Extensive inter-individual variability exists in the production of flavan-3-ol metabolites. Preliminary metabolic phenotypes (metabotypes) have been defined, but there is no consensus on the existence of metabotypes associated with the catabolism of catechins and proanthocyanidins. This study aims at elucidating the presence of different metabotypes in the urinary excretion of main flavan-3-ol colonic metabolites after consumption of cranberry products and at assessing the impact of the statistical technique used for metabotyping.

Methods: Data on urinary concentrations of phenyl-γ-valerolactones and 3-(hydroxyphenyl)propanoic acid derivatives from two human interventions has been used. Different multivariate statistics, principal component analysis (PCA), cluster analysis, and partial least square-discriminant analysis (PLS-DA), have been considered.

Results: Data pre-treatment plays a major role on resulting PCA models. Cluster analysis based on k-means and a final consensus algorithm lead to quantitative-based models, while the expectation-maximization algorithm and clustering according to principal component scores yield metabotypes characterized by quali-quantitative differences in the excretion of colonic metabolites. PLS-DA, together with univariate analyses, has served to validate the urinary metabotypes in the production of flavan-3-ol metabolites and to confirm the robustness of the methodological approach.

Conclusions: This work proposes a methodological workflow for metabotype definition and highlights the importance of data pre-treatment and clustering methods on the final outcomes for a given dataset. It represents an additional step toward the understanding of the inter-individual variability in flavan-3-ol metabolism.

Keywords

Metabotypes, flavan-3-ols, inter-individual variation, phenolic metabolites, phenyl-γ-valerolactones

P43

Seasonal dependent effects of Grape Seed Proanthocyanidin Extract (GSPE) on hepatic metabolism of healthy F344 rats

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Intrinsically synchronized with the natural year, circannual rhythms are responsible for scheduling seasonal activities in relation to the external environment. These rhythms function as a clock for organisms to adjust their physiology and behavior to periodically changing conditions of their environment. Polyphenols, including GSPE, are bioactive compounds found in plants and have benefits for human health. Thus, we wondered if there is a differential impact on GSPE effects due to seasonal changes. To achieve this F344 rats were chronically exposed for 9 weeks to three photoperiods to mimic the day length of seasons: L6 (winter), L12 (autumn and spring), and L18 (summer), and were treated with GSPE 25 mg/kg or vehicle (VH) for 4 weeks. Results demonstrate not only changes in hormonal and hepatic metabolism due to photoperiods, but also the influences of specific photoperiods on GSPE effects. Interestingly, an increased phosphorylation of AMPK was observed in L18 and L6 GSPE rats, but not in L12. Moreover, on the one hand, L18-GSPE animals showed a reduction in LDL cholesterol levels (p=0.01) compared to its VH, a decreased in the expression of lipogenic genes, lower levels of corticosterone (p=0.002) and increased levels of melatonin (p=0.01) than L6-GSPE; on the other hand, L6-GSPE rats have a 150 % of increase in corticosterone levels, higher levels of blood glucose (p=0.05) and total cholesterol (p=0.006), as well as an increased in the expression of lipogenic and ER stress genes compared to its VH or to L12/L18-GSPE groups. These results make it plausible to suggest that exposure to L6 photoperiod could be inducing the seasonal expression of the thrifty genotype to prepare for periods of food shortage, which is commonly winter. Interestingly, it appears that GSPE is reinforcing this action when it is consumed under short photoperiod, preparing the organism for this specific season.

Keywords

Photoperiod, GSPE, Liver metabolism, Circannual rhythm

P44

Quercetin ingested by maternal mice may be transferred to newborn mice via breast milk

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Objectives: Quercetin (QUE) is converted into various metabolites by drug metabolism mechanisms. Although blood and urine have been used to evaluate the bioavailability of QUE, it has recently been reported that QUE and other flavonoids are also present in human breast milk. However, these previous studies have not quantified QUE aglycone and metabolites in neonates. Therefore, this study aimed to determine whether QUE ingested by mothers is transferred to newborns via breast milk in mice.

Methods: ICR mice were fed 1% QUE supplemented diet ad libitum. After mating and delivery, breast milk was collected once or twice from maternal mice until 13 days of the postnatal period. Blood and urine samples were then collected from maternal and neonatal mice immediately after the final milking. Each biological sample was prepared with or without deconjugation treatment. QUE and its metabolites in the samples were analyzed by LC-QTOF-MS/MS.

Results: QUE and its metabolites (isorhamnetin, quercetin-3-glucuronide and quercetin-3'-sulfate) were detected in breast milk, blood and urine of QUE-fed maternal mice. Interestingly, the total QUE concentrations in breast milk were dramatically higher than that in the blood. Furthermore, QUE and its metabolites were detected in the blood and urine of newborn mice raised by mothers fed QUE.

Conclusion: It is the first report that QUE aglycone and metabolites were detected in the breast milk of QUE-fed mother mice. These were also found in the blood and urine of newborn mice, suggesting that breast milk-derived QUE may exert biological activity in newborn mice.

Keywords

quercetin, breast milk, newborn, LC-QTOF-MS/MS

P45

Differential bioavailability of tomato (poly)phenols from distinct geographic origins: local vs. non-local

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Background and objectives: There is considerable epidemiological evidence indicating that consumption of diets rich in fruit and vegetables such as tomatoes is associated with health benefits, in part due to their content in (poly)phenols. However, environmental factors, such as geographical origin, have an important impact on (poly)phenol content. Therefore, characterizing the phenolic compounds present in tomatoes is of great interest to predict their bioactivity. Hence, our aim was to address whether geographical origin of tomatoes generates a differentiated pattern of (poly)phenolic bioavailability.

Methods: Tomatoes (Lycopersicon esculentum cv. Ekstasis) conventionally grown in two locations in Spain: in the northeast (local tomatoes, LT) and in the southeast (non-local tomatoes, NLT) were obtained from a local market at commercial maturity and phenolic compounds were determined by uHPLC-MSn. Wistar rats were acutely administered, by intragastric intubation, a dose of 3 g of LT or NLT per kg body weight. Blood samples were obtained at basal (0) and 2, 4, 7, 24 and 48 h after administration, and pharmacokinetics were obtained by uHPLC-MSn analyses.

Results: Each tomato had a particular phenolic and nutritional signature, which could be attributed to its diverse cultivation origin. In particular, these differences resulted in diversed kinetic profiles of the seven phase II and colonic phenolic metabolites, identified after acute administration of LT or NLT. These metabolites were mainly sulfate conjugates and reached their maximum concentrations in the first 4 hours.

Conclusions: Our findings advance the concept of local and seasonal fruit and vegetable consumption. They have the potential to inform more effective and accurate dietary approaches to promote health.

Keywords

bioavailability, pharmacokinetics, metabolomics, tomato

P46

The effect of chitosan on the bioaccessibility of anthocyanins from jussara (*Euterpe edulis* Martius) pulp alginate beads

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Considering the potential functional properties of jussara (*Euterpe edulis* M.), the present study aimed to evaluate the effect of chitosan on the bioaccessibility of anthocyanins from jussara pulp alginate beads. Beads were produced by ionic gelation using two distinct processes. Jussara pulp with 1.5% sodium alginate was dropped either into a single solution of 0.1 M $CaCl_2$ and 0.2% chitosan (chitosan mixed with the beads) or into 0.1 M $CaCl_2$ and 0.2% chitosan solutions separately (chitosan used to coat the beads). Digestion was performed to simulate the oral (2 min), gastric (2 h) and intestinal (15 to 120 min) phases. The contents of anthocyanins (cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside) in the beads and their release at each digestion step were determined by HPLC-DAD. Beads prepared with chitosan as coating had higher encapsulation efficiency, retaining 100% of anthocyanins present in jussara pulp, while the beads with chitosan mixed in the matrix only retained 68%. The use of chitosan to coat the beads promoted a greater encapsulation efficiency possibly due to the reduction of their porosity, as observed by scanning electron microscopy, reducing lixiviation of anthocyanins to the $CaCl_2$ solution. After the oral and gastric phases, beads coated with chitosan released lower quantities of anthocyanins (29% and 56%, respectively) compared to those in which chitosan was mixed with the matrix (45% and 78%, respectively). During the intestinal phase, both beads showed a progressive decrease in the release of anthocyanins, with a lower rate for those coated with chitosan. Therefore, the use of chitosan as coating promoted a more controlled release of anthocyanins throughout the digestion and led to their stability against alkaline intestinal conditions. In conclusion, jussara pulp alginate beads should be produced using chitosan as coating to ensure high encapsulation efficiency and stability during digestion.

Keywords

In vitro digestion, Ionic gelation, Cyanidin-3-O-glucoside, Cyanidin-3-O-rutinoside, Encapsulation

Brain and cognition

P47

Long term supplementation with anthocyanin-rich or -poor *Rubus idaeus* berries does not influence microvascular architecture nor cognitive outcome in the APP/PS-1 mouse model of Alzheimer's disease.

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Background: Disruption of microvascular architecture is a common pathogenic mechanism in the progression of numerous chronic diseases including Alzheimer's disease (AD). While dietary supplementation with berries is associated with improved cognition in animal models of AD, the mechanism is not fully elucidated. Given the anti-angiogenic activity of berry (poly)phenols, we sought to determine if long-term feeding of *Rubus idaeus* (raspberries) could ameliorate cerebral microvascular pathology and improve cognition in the APP/PS-1 mouse model of AD.

Methods: Male C57Bl/6J mice (50 wild type, 50 APP/PS-1) aged 4 months were fed for 24 weeks, with a normal diet enriched with either 100 mg/day glucose (control diet) or supplemented with glucose and freeze-dried anthocyanin-rich (red) or -poor (yellow) *Rubus idaeus* berries (100 mg/ day). Cerebral microvascular architecture was assessed by microvascular vascular corrosion casting and subsequent imaging by scanning electron microscopy (month 10). Behavioral assessment was conducted pre and post intervention.

Results: Wild-type cerebral microvascular mice was characterised by regularly spaced capillaries with uniform diameters, unlike APP/PS-1 transgenic mice which showed dysregulated microvascular architecture. Consumption of *Rubus idaeus* berries did not significantly alter the range of cerebral microvascular architectural pathology when compared the control diet. Non-targeted LC-MSn analysis of brain and plasma samples identified several metabolites that increased post raspberry intervention including endogenous metabolites (amino acid derivatives and lipids) and various components related to the raspberry consumption. After 24 weeks feeding, few significant changes were evident in cognitive behaviour of wild type or APP/PS-1 mice irrespective of dietary regime consumed. Limited modulation of the gut microbiota was observed as a result of the feeding regimes.

Conclusions: Long-term feeding of *Rubus idaeus* berries had no substantive effect on microvascular architecture or cognition in either the wild type or APP/PS1 mice although changes were evident in endogenous cerebral and plasmatic metabolites.

Keywords

cerebral microvasculature, Alzheimer's disease, Raspberries, Cognition, Microbiota

P48

The association between (poly)phenols and mood in healthy adults: evidence from dietary assessment and biomarkers

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Background: Increasing research indicates that dietary (poly)phenols may have protective effects on mental health. However, to our knowledge, the relationship between (poly)phenol biomarkers and mood has not been explored. In this study, we investigated the association between dietary (poly)phenol intake, circulating (poly)phenol metabolites, and mood in a cohort of healthy adults.

Methods: We included 314 healthy adults who had previously participated in dietary intervention studies, aged 18-79 years. Mood was measured with the Positive and Negative Affect Schedule (PANAS) questionnaire, standardised by Z scores. Dietary (poly)phenol intake was calculated by matching food consumption data from the European Prospective Investigation into Cancer (EPIC) food frequency questionnaires (FFQ) with the database of food (poly)phenols (Phenol-Explorer 3.0). A total of 114 (poly)phenol metabolites in fasting plasma and 24 h urine samples were quantified by liquid chromatography-mass spectrometry (LC-MS) using a validated method and authentic standards.

Results: Higher dietary intake of flavan-3-ols, flavanones, flavonols and hydroxybenzoic acids were significantly correlated with higher positive mood, but not with negative mood. When taking the sources of (poly)phenols into consideration, higher consumption of citrus fruits, grapes and tea were significantly associated with better positive mood. Linear regression models indicated that only dietary intake of flavonols remained significant (B=0.011, p=0.045) for positive mood after adjusting for potential confounders (age, sex, ethnicity). Total urinary (poly)phenols, flavonoids, phenolic acids demonstrated a moderate correlation with total dietary (poly)phenol intakes (r_s=0.29-0.39, p<0.01). Higher levels of urinary flavones, tyrosols and hydroxyphenylacetic acids were correlated with less negative mood (p<0.05). However, no correlation was found between plasma (poly)phenols and mood.

Conclusion: Urinary (poly)phenols, rather than plasma (poly)phenols may be a promising biomarker for dietary (poly)phenol intake. Higher intake of flavonoids and phenolic acids may be associated with better mood.

Keywords

(Poly)phenols, Metabolites, Mood

P49

Gut microbiota derived low molecular weight (poly)phenol metabolites attenuate microglia inflammatory response

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Background: Neurodegeneration reside on multifactorial changes with complex mechanisms and no existing cure. Neuroinflammation is a key mechanism on the development of neurodegenerative diseases. Prevention and treatment will require multi-targeted therapeutics with a focus on their anti-inflammatory properties. Studies with (poly)phenols have proven their pleiotropic ability. However, for the low molecular weight (poly)phenol metabolites, much is still unknown. Absorption of some of these metabolites leads to high blood concentrations and studies have shown their ability reach the brain.

Methods: In this work we have identified more than sixty low molecular weight (poly)phenol metabolites based upon a literature search of nutritional studies and tested them for their ability to impair cytokine release by microglia cells upon an inflammatory stimulus. Meanwhile the mechanisms by which they can impact the release of inflammatory cytokines are being elucidated, in this respect we conducted a multiplex kinase activity profiling for serine-threonine kinases.

Results: Our findings show the ability of the metabolites to reduce several cytokines e.g. TNF, IL-6, IL-1, through the reduction of more than 70 of the 144 kinases evaluated from NF-kB, MAPK and JAK-STAT pathways. In conclusion, we are deciphering the role of low molecular weight (poly)phenol metabolites at physiological conditions, showing their anti-inflammatory properties, and exploring the mechanism in microglia cells. Altogether we hope to these metabolites a useful tool to modulate neuroinflammation and limit neurodegeneration.

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Keywords

Microglia, Microbiota, Metabolites, Inflammation, Neurodegeneration

P50

Effects of treatment with coconut oil and epigallocatechin gallate on lipid profile, manual dexterity and disability in patients with multiple sclerosis. A pilot study.

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Introduction: Multiple sclerosis (MS) is an inflammatory and neurodegenerative disease, which causes demyelination of the nerve fibres in the central nervous system. These alterations can reduce the functional capacity and psychomotor skills that are characteristic of patients suffering from MS. Therefore, lipoprotein levels related to myelin composition could be linked to both variables.

Objectives: The aim of the study is to evaluate the impact of coconut oil intake (source of ketone bodies with neuroprotective capacity) and the antioxidant epigallocatechin gallate (EGCG) on disability status and manual dexterity, through changes in lipid profiles.

Material and methods: Applying the selection criteria, a sample of 51 patients with MS were randomly distributed between an intervention group (IG) receiving 800 mg of EGCG and 60 ml of coconut oil daily, and a control group (CG) receiving placebo for 4 months; with an isocaloric base diet of a Mediterranean nature for both groups. Before and after the intervention, total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL) and Apolipoprotein A1 (ApoA1) levels were quantified for all study participants from blood samples. The Nine-Hole Peg Test (NHPT) assessing fine motor skills and the Expanded Disability Status Scale (EDSS) assessing functional ability were administered.

Results: The results obtained showed significant improvements in functional and fine motor skills tests for the intervention group as well as a decrease in triglyceride levels, demonstrating an inversely proportional improvement and significant correlation in both tests.

Conclusions: In conclusion, this study shows that the administration of EGCG and coconut oil decreased blood triglyceride values, potentially resulting in improvements in fine motor skills and functional capacity in MS patients.

Keywords

Multiple sclerosis, Epigallocatechin gallate, Coconut oil, Triglycerides, EDSS

P51

Neuroprotective effects of circulating (poly)phenol metabolites in MPP(+)-stimulated dopaminergic neurons

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Objectives/Background: Diets rich in (poly)phenols have been associated with positive effects on neurodegenerative disorders, such as Parkinson's disease (PD). Several low-molecular weight (poly)phenol metabolites (LMWPM) are found in the plasma after consumption of (poly)phenols-rich food [1]. Then, it is expected that these LMWPM, upon reaching the brain, may have beneficial effects against oxidative stress and neuroinflammation, possibly attenuating cell death mechanisms related to dopaminergic neurons loss in PD.

Materials/Methods: The present study investigates neuroprotective potential of two blood-brain barrier permeant LMWPM [2], catechol-O-sulfate and pyrogallol-O-sulfate, in a more physiologically relevant human 3D cell model of PD. Neurospheroids were generated from LUHMES neuronal precursor cells and challenged by 1-methyl-4-phenylpyridinium (MPP(+)) to induce neuronal stress and dopaminergic cell loss.

Results/Findings: LMWPM pre-treatments were differently neuroprotective towards MPP(+) insult, in both cell viability and ATP release, presenting distinct effects on the neuronal transcriptome. Particularly, catechol-O-sulfate pre-treatment appeared to boost counter-regulatory defense mechanisms, as a pre-conditioning effect. Bioinformatic analysis pointed to proteins, transcription factors and miRNAs as significantly modified by LMWPM in LUHMES. When MPP(+) is applied, both LMWPM positively modulated glutathione and apoptotic-related proteins.

Conclusion: Our findings point to the potential of LMWPM to trigger molecular mechanisms that help dopaminergic neurons to cope with a subsequent toxic insult. They have emerged as promising molecules to be further explored in the context of preventing and attenuating parkinsonian neurodegeneration.

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Keywords

gene expression, transcriptomics, dopaminergic neurons, preconditioning, neurodegeneration

P52

Blueberry polyphenols activity on microglial cells

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Polyphenols exhibit pleiotropic activities, including anti-inflammatory and anti-oxidant activities, fighting neuroinflammation underlying most neurodegenerative diseases. They target astroglia and microglia activated cells switching their polarization state towards an anti-inflammatory phenotype. Blueberry (BB) is a well-known example of a food rich in polyphenols, with proven effects on cognitive impairment. Our results show that a hydroalcoholic BB extract, where chlorogenic acid was the most represented among the polyphenolic acids, plays an homeostatic role in the presence of an inflammatory stimulus. We assessed the activity of BB extract on cytoskeletal remodelling induced by LPS. LPS stimulation induced a round ameboid shape in BV-2 cells, which is a sign of M1 polarization. BB extract in combined treatment with LPS was able to partially reverse this phenotype affecting the dynamic rearrangement of the actin cytoskeleton that is also the recognized first step in the microglial cells migration. BB extract in LPS-BB-combined treatment significantly reduced microglial migration, both in scratch wound and Boyden chambers assays. We then observed an increase in activated Rac1 proteins in the sample treated with LPS and a decrease in the sample treated with BB in the presence or absence of LPS. Several studies demonstrated that the small GTPase Rac1 is a downstream effector for phosphatidylinositol 3-kinases which affects the rearrangement of the actin cytoskeleton, regulating actin polymerization in the lamellipodial protrusion. The BB extract inhibited the expression of pro-inflammatory markers in BV-2 microglia cells stimulated by LPS. A decreased protein expression of iNOS, and a contemporary increased expression of Arg-1, were detected by immunofluorescence when cells were supplemented with BB in the presence of LPS, as well as a significant decrease of mRNAs for IL-1beta, IL-6, TNF-alpha and an IL-10 mRNA increase. Our data suggest the involvement of BB in actin cytoskeleton rearrangement, affecting migration and functional microglia polarization.

Keywords

Blueberry, Microglia, Neuroinflammation, Cytoskeleton, Cytokines

P53

Potential neuroprotective effect of spent coffee grounds extracts against neurodegeneration.

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State of art and aim of the study: Spent Coffee Grounds (SCG), a waste product of the coffee industry, are a renewable source of a wide range of nutraceutical compounds. Coffee wastes are particularly rich in phenols like phenolic acids such as chlorogenic, ellagic, caffeic, gallic, trans-ferulic, p-coumaric, p-hydroxybenzoic, tannic, and protocatechuic acids; flavonoids such as rutin, catechin, epicatechin, and quercetin. The positive effects of polyphenols in counteracting neurodegeneration have been highlighted by several studies. Although neurodegenerative diseases have a multifactorial aetiology, oxidative stress and neuroinflammation play a key role in their onset.

The aim of this study was to evaluate the potential neuroprotective effect of 4 different SCG extracts in a *in vitro* model of oxidative stress (differentiated neuron-like SH-SY5Y cell line exposed to H_2O_2) and in an *in vitro* model of neuroinflammation (BV-2 microglial cell line activated with LPS).

Materials and Methods: The extracts were obtained using H_2O, MeOH, MeOH:H_2O (50 : 50, v/v), EtOH:H_2O (30 : 70, v/v). Cell viability was assessed by MTT assay, reactive oxygen species by DCFH-DA fluorescent assay, protein levels by immunoblotting and immunofluorescence assays, gene expression by RT-PCR.

Results: The methanol extract was the most effective in protecting neuronal cells from H_2O_2-induced oxidative stress via up-regulation of the main endogenous antioxidant enzymes (HO1, NQO1, GR and TRX). The aqueous extract significantly reduced the expression of proinflammatory mediators (IL-1β, TNF-α, iNOS and COX-2) through modulation of the TLR4/NF-kB pathway, demonstrating to be the most effective in counteracting LPS-induced inflammation on the microglial cell line. Interestingly, although EtOH:H_2O and MeOH:H_2O extracts were the richest in terms of phenols content, they were less effective in counteracting oxidative stress and inflammation.

Conclusions: SCGs, due to their antioxidant and anti-inflammatory activities, can be considered a valuable and renewable source of nutraceutical compounds to prevent/counteract neurodegeneration.

Keywords

Polyphenols, Neuroprotection, Oxidative Stress, Neuroinflammation, Coffee

P54

The Impact of Coffee-Derived Chlorogenic Acid on Cognition – A Systematic Review and Meta-Analysis

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INTRODUCTION: Coffee consumption has been associated with some beneficial effects on cognitive function. These effects have been associated with the polyphenol chlorogenic acid (CGA).

OBJECTIVES: The aim of this review was to evaluate and synthesise evidence of the relationship between coffee-derived CGA and cognitive function.

METHODS: Scopus, PubMed, Web of Science, ScienceDirect and PsycINFO databases were searched in April 2021 yielding 396 references after duplicates were eliminated. Six randomized control trials (RCTs) and 15 longitudinal studies which identified that complied with the inclusion criteria: human studies in which CGA dose was stated and a standardised test of cognitive function. RCTs without control groups or placebos were excluded. A meta-analysis was performed with the RCT data, including a sub-group analysis of cognitive domain and the longitudinal data was subject to a descriptive analysis.

RESULTS: The RCTs showed consumption of 300-1106 mg CGA was beneficial for executive function, attention, motor activity, and mood. However, the overall meta-analysis did not show a significant effect of CGA on cognition. Prospective cohort studies showed an association between moderate coffee drinking (3-5 cups daily) and lower risk of dementia and Alzheimer's, while low coffee consumption (0-2 cups daily) was associated with greater risk of cognitive impairment.

CONCLUSION: Based on these findings, there is some evidence that consumption of CGA from coffee may improve aspects of cognitive function and mood and potentially lower risk of dementia and Alzheimer's. However, the non-significant meta-analysis indicated that the evidence base from good quality RCTs is limited. Further research is required to explore cognitive effects of CGA from coffee.

Keywords

coffee, polyphenol, cognition

P55

Effects of resveratrol supplementation on cognitive function, cerebral blood flow and gastrointestinal microbiota in healthy, overweight adults.

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Objectives: Dysbiosis of the gut microbiome is linked to sustained intestinal inflammation and contributes to the development of chronic diseases and cognitive impairment. The 'Western diet' is associated with reductions in microbial diversity, alongside increased risk of obesity and chronic low-grade systemic inflammation. Conversely consumption of phenolic phytochemicals, including resveratrol, counteracts systemic inflammation (induced by a high fat diet) and promotes microbial diversity. The current study investigates the interrelationships between an individual's gut microbiome, levels of systemic inflammation, brain function and the potential to modulate these through resveratrol supplementation.

Materials and Methods: This randomized, placebo-controlled, parallel groups study investigated the effects of 500mg resveratrol in N = 110 overweight adults (aged 35-60 years). Cognitive function, mood and systemic inflammation effects were assessed following acute consumption (Day 1) and a 12-week consumption period (Day 84). A subsample (N = 55) also provided cerebral blood flow data using quantitative near-infrared spectroscopy (qNIRS) and N = 80 provided stool samples to assess gut microbiota composition.

Results: Consistent with previous findings, resveratrol modulated the haemodynamic response (during rest and task performance) as indexed by increases in total, oxygenated and deoxygenated haemoglobin. Reductions in plasma triglycerides and total cholesterol levels were also observed following resveratrol. No statistical difference was observed in alpha or beta diversity between treatment groups, however one differentially abundant taxa was observed following resveratrol. No clear effects on cognitive performance were observed, nor were any effects observed on mood outcomes, blood pressure, BMI or additional blood biomarkers.

Conclusions: These results confirm the lack of cognitive performance findings, despite clear CBF effects of resveratrol supplementation in overweight, older adults. There is limited evidence to suggest modulation to health parameters and as such, additional work in a similar cohort should be conducted.

Keywords

Resveratrol, Cognitive performance, Cerebral blood flow, Gut microbiota

Cancer

P56

Polyphenol curcumin targets colorectal cancer stem cells (Nanog+) in human colorectal tissues

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Objectives/ Background: Curcumin is a polyphenol compound, found in turmeric, a common spice. Curcumin has low systemic bioavailability but an oral dose coats the colonic epithelium, yielding high concentrations in colonic tissue, rendering it a good candidate for the prevention of colorectal cancer (CRC). The transcription factor Nanog is crucial for the self-renewal of colorectal cancer stem-like cells (CSCs) which are key drivers in the development of CRC. Nanog expression in CRC tissue correlates with lymph node metastasis and poor prognosis.

Methods: A range of colorectal cancer tissues (n=46) were profiled for Nanog expression and correlated with progression free survival of patients. Additionally, explant cultures were performed with CRC and adenoma tissue from 20 patients. Tissues were incubated with curcumin (0-10 µM) for 24h and effects on CSCs (defined by expression of Nanog) and CSC proliferation ($Nanog^+Ki67^+$) assessed. In addition, curcumin activity across consensus molecular subtypes (CMSs) of CRC was evaluated

Results: Nanog expression was significantly higher in adenoma and CRC samples compared to normal tissues. Following *ex-vivo* exposure to curcumin, the majority of samples (18/20) across all CMSs demonstrated a response, defined as a reduction in the $Nanog^+Ki67^+$ population, with a significant reduction observed at 0.1 and 1 µM curcumin. To assess the potential clinical relevance of this reduction, the top 25% $Nanog^+Ki67^+$ expressers were compared with the bottom 25%; median progression free survival for the bottom quartile was 1111 days compared to 379 days for the top quartile ($p < 0.001$).

Conclusion: These data suggest Nanog is targeted by curcumin in colorectal tissues and a reduction in the $Nanog^+Ki67^+$population may have clinical benefit. Nanog may serve as a biomarker in clinical trials to identify those most amenable to benefit from curcumin to reduce CRC risk.

Keywords

Curcumin, Adenoma, Colorectal cancer, Cancer stem cells, Nanog

P57

Antiproliferative, antiangiogenic, and antimetastatic therapy response by mangiferin in a syngeneic immunocompetent colorectal cancer mouse model involves changes in mitochondrial energy metabolism

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Objectives/Background: In spite of the current advances and achievements in cancer treatments, colorectal cancer (CRC) persists as one of the most prevalent and deadly tumor types in both men and women worldwide. Drug resistance, adverse side effects and high rate of angiogenesis, metastasis and tumor relapse remain one of the greatest challenges in long-term management of CRC and urges need for new leads of anticancer drugs.

Materials/Methods: We evaluated antitumor effects of mangiferin (MGF), a glucosylxanthone polyphenol present in Mango tree stem bark and leaves (*Mangifera Indica L.*), in vitro and in vivo in a syngeneic immunocompetent allograft mouse model of murine CT26 colon carcinoma and performed integrative transcriptomic analysis to characterize a molecular mechanism of action.

Results/Findings: MGF induced dose-dependent tumor regression and decreased lung metastasis, which increased overall survival of syngeneic CT26 allografted mice. Antimetastatic and antiangiogenic MGF effects could be further validated in a wound healing in vitro model. Corresponding transcriptome pathway enrichment analysis demonstrated that MGF inhibits tumor growth, metastasis and angiogenesis by multitargeting of mitochondrial oxidoreductase and fatty acid β-oxidation metabolism, as well as PPAR, SIRT, NFκB, Stat3, HIF, Wnt and GP6 signaling pathways.

Conclusion: Antitumor, antiangiogenic and antimetastatic effects of MGF treatment hold promise to reduce adverse toxicity and to mitigate therapeutic outcome of colorectal cancer treatment by targeting mitochondrial energy metabolism in the tumor microenvironment.

Keywords

Mangiferin, antiangiogenesis, antimetastasic, colon carcinoma, metabolism

P58

(−)-Epigallocatechin-3-gallate (EGCG) attenuates cyclophosphamide-induced gut injury in mice, by modulating inflammation, the tight junctions, and dysbiosis

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Objective/Background: Although cyclophosphamide (CTX) is an anticancer drug commonly used to treat multiple tumor types, its toxic side effects, including gastrointestinal (GI) toxicity, affect treatment compliance. Thus, there is a critical need of evaluating strategies that may improve the associated GI toxicity induced by anticancer agents. In this work, we evaluated the capacity of epigallocatechin-3-gallate (EGCG), a major constituent of green tea, to improve CTX-induced GI toxicity in mice.

Materials/Methods: Six-week old mice were (i.p) injected with CTX (50 mg/kg), once daily for 5 days, to induce damage of the intestinal barrier. The next day, mice receive either vehicle control or EGCG (20 or 40 mg/kg) was orally administered for an additional 25 days. Following euthanasia, the intestine tissues were collected for analysis. 16S rRNA sequencing and GC-MS/MS analysis were performed to test feces' microbiome and short chain fatty acids (SCFAs) levels.

Results/Findings: Treatment with CTX for 5 days severely damaged the intestinal structure, increased immune-related cytokines (TNFα, IL-10 and IL-21), reduced the expression levels of tight junction proteins (ZO-1, occludin, claudin-1), induced reactive oxygen species, altered the composition of gut microbiota, and reduced short chain fatty acid levels. EGCG treatment, starting one day after the last CTX dose, significantly improved the intestinal structure, ameliorated gut permeability, and restored ZO-1, occludin and claudin 1 levels. Moreover, EGCG reduced TNFα, IL-10 and IL-21 levels and decreased oxidative stress by regulating the activities of the antioxidant enzymes catalase, superoxide dismutase and glutathione peroxidase. Finally, EGCG treatment restored the composition of gut microbiota, and the levels of the SCFAs.

Conclusion: These findings suggest that EGCG is an effective bioactive compound to minimize CTX-induced GI tract toxicity.

Keywords

EGCG, cyclophosphamide, intestinal inflammation, tight junctions, dysbiosis

P59

MANGO KERNEL EXTRACT INDUCES OXIDATIVE-STRESS-MEDIATED REACTIVE GLIOSIS AND APOPTOSIS IN GLIOMA CELLS

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Objectives: Mango (Mangifera indica) by-products demonstrated to be a valuable source of bioactive compounds with antiproliferative activities against several cancer cell lines. Glioblastoma multiforme is a malignant tumour for which limited treatments currently exist. Current approaches of intervention consist of tumor surgical resection, followed by radiotherapy and concomitant chemotherapy. However, it shows a poor prognosis due to its high recurrence rate. Therefore, there is an urgent need to uncover novel compounds able to extent patient's life expectancy. In this research, mango by-product extracts have been tested on in vitro glioblastoma-derived cells to evaluate their potential as antitumor agents.

Materials: Mango peel, kernel and pulp extracts were tested in vitro on T98 and A172 cell lines. MTT assays were performed to evaluate the impact on the different extracts and to optimise the best treatment conditions. Then, flow cytometry, immunofluorescence and western-blot assays were used to further elucidate the mechanisms of action of the extracts.

Results: Treatment with mango kernel extract (10 to 50 µg/ml at 72 h) was the most effective inhibiting cell proliferation in both lines tested, reaching up to 70 % of growth inhibition. Oxidative stress through ROS generation was clearly observed at the highest dose used. Assessment of apoptosis revealed an increase in cell death after treatment. Consequently, western-blot assays suggested variations in the components of apoptosis and DNA damage. In addition, fluorescent microscopy showed dose-dependent deterioration of certain cellular structures, such as the nucleus or the activation of astrogliosis processes (GFAP dye).

Conclussion: Our results suggests that Mango kernel extract might be a promising agent for the treatment of glioblastoma multiforme. Overall, our data lays the groundwork for future research in which in vivo studies together with biodisponibility assays should be carried out to evaluate the translational performance of this fruit by-product extract.

Keywords

Glioblastoma multiforme, ROS, Astrogliosis, By-product extracts, Cancer

P60

Anthocyanin-rich haskap berry: a dietary source for cancer prevention

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In this Canadian first study of assessing the nutritional and nutraceutical quality of Canadian haskap berry (*Lonicera caerulea* L.) cultivars, it was found that the antioxidant capacity of haskap was significantly greater compared to that of other commonly consumed fruits. Polyphenols extracted from haskap berry exerted suppression of the release of pro-inflammatory cytokines by LPS-induced macrophages in vitro, suggesting the anti-inflammatory properties. Independent of cultivars, cyanidin-3-O-glucoside (C3G) was found as the predominant bioactive presence in haskap berries. We have demonstrated for the first time that anthocyanin-rich haskap berry extracts could reduce carcinogen-induced DNA damage in cultured lung epithelial cells as well as in carcinogen-induced tumorigenesis in A/Jcr mice. Pre-treatment of cultured human lung epithelial BEAS-2B cells with the anthocyanin-rich haskap berry extracts significantly reduced carcinogen-induced DNA damage, DNA fragmentation, and intracellular reactive oxygen species and upregulated the ATM-dependent DNA damage repair cascade compared to non-treated BEAS-2B cells. Dietary supplementation of anthocyanin-rich haskap berry powder (262 mg C3G/kg body weight/day) for 22 weeks significantly reduced the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, (NNK)-induced lung tumor multiplicity and tumor area. Immunohistochemical analysis showed reduced expression of proliferative cell nuclear antigen (PCNA) and Ki67 in lung tissues. Therefore, haskap berry has the potential to be developed as a dietary supplement or natural health product for use in reducing the risk of various cancers among high-risk populations.

Keywords

Anthocyanins, Haskap berry, Cancer, Prevention, DNA damage

P61

Marine polyphenol thalassiolin B extract of thalassia testudinum arrests colorectal tumor growth, motility and angiogenesis by autophagic stress and immunogenic cell death pathways

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Objectives/Background: Drug resistance, adverse side effects and tumor relapse remain one of the greatest challenges in long-term management of colorectal cancer and urges need for new leads of anticancer drugs. Marine plants have become an inexhaustible reservoir of new phytopharmaceuticals for cancer treatment. The aim of the present work was to evaluate the anti-tumor activity of a Caribbean marine plant Thalassia testudinum against human colorectal cancer.

Materials/Methods: Potential antitumor effects of a polyphenolic fraction obtained from Thalassia testudinum and its constituent thalassiolin B were evaluated in in vitro and in vivo colon carcinoma models and integrative transcriptomic analysis was performed to characterize a molecular mechanism of action.

Results/Findings: A standardized thalassiolin containing polyphenol extract from the marine angiosperm Thalassia testudinum (TTE) revealed a dose-dependent decrease of cell viability of RKO, CT26, and SW480 colon cancer cells. Furthermore, TTE significantly prevented basal and bFGF-induced angiogenesis in a chicken chorioallantoic membrane angiogenesis assay. In addition, TTE suppressed bFGF-induced migration of endothelial cells in a wound closure assay. Finally, TTE treatment abrogated CT26 colorectal cancer growth and increased overall organism survival in a syngeneic murine allograft model. Corresponding transcriptome profiling and pathway analysis allowed for the identification of the mechanism of action for the antitumor effects of TTE. In line with our in vitro/in vivo results, TTE treatment triggers ATF4-P53-NFκB specific gene expression and autophagy stress pathways. This results in suppression of colon cancer cell growth, cell motility, and angiogenesis pathways in vitro and in addition promotes antitumor immunogenic cell death in vivo.

Conclusion: Here we demonstrated for the first time the antitumor activity of a marine *Thalassia testudinum* polyphenol extract in a syngeneic colon cancer mouse model. These results suggest the potential use of *Thalassia testudinum* marine plant polypenols as adjuvant treatment in cancer therapy.

Keywords

Thalassia testudinum, Thalassiolin B, marine seaweed, colorectal cancer, immunogenic cell death

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Combining advanced 3D cell models with omics methodologies to unveil the protective role of phenolic metabolites towards colorectal cancer

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Phenolic compounds present in fruits and vegetables have been highlighted to present anticancer potential in in vitro and in vivo models of colorectal cancer (CRC). However, there are few information regarding the effect of their main colonic metabolites on CRC cells to better elucidate their role in cancer prevention. Here we combined advanced 3D cell models with omics methodologies to investigate the effect of colonic phenolic metabolites derived from different food matrices on CRC. Experiments were carried out in HT29 cell spheroids generated in stirred culture systems that presents characteristics of in vivo solid tumors.

i) virgin olive oil

The effect of hydroxytyrosol (HT) and its major colonic metabolites - phenylaceticacid and hydroxyphenyl propionic acid- in inhibiting cell proliferation and targeting cancer stemness was evaluated. Results showed that HT presents the highest antiproliferative effect and was the only compound capable of targeting cancer stemness by reducing ALDH+ cells and inhibiting colony formation. Gene expression analysis indicated that HT at 200µM reduced the expression of markers related to stemness (NANOG, OCT4),EMT(VIM,SHH,TGFβ1), Sonic Hedgehog Pathway (GLI1,PTCH1) and proliferation (CCNA2), suggesting that HT have an anti-metastatic effect at this dosage. Metabolomics (nanoLC-ESI-TOF-MS) and transcriptomics (RNAseq) are being applied to identify metabolic processes and signal pathways modulated by HT on cancer cells.

ii) Jaboticaba peel powder(JPP)

JPP samples were fermented with human feces during over 48h and were incubated with spheroids. Results showed an increase of the antiproliferative response between 8h and 24h of fermentation, and this effect was associated with HHDP-digalloylglucose isomer and dihydroxyphenyl-γ-valerolactone. At 48h of fermentation, the antiproliferative effect was negligible, indicating that the presence of urolithins did not improve the bioactivity of JPP.

These findings provide relevant knowledge on the role of colonic microbiota fermentation to generate active/non active phenolic metabolites from different food sources towards CRC.

Keywords

colorectal cancer, HT29 cell spheroids, colonic metabolites, olive oil, jaboticaba fruit

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Isorhamnetin Inhibits Pancreatic Cancer-Associated Fibroblasts Growth

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Objectives/Background: Pancreatic ductal adenocarcinoma (PDAC) is extremely resistant to the conventional chemo- and radiotherapies due to the intense desmoplastic stroma (fibrosis) mediated by cancer-associated fibroblasts (CAFs). Isorhamnetin - natural flavonoid, shown by our previous studies to have anti-fibrotic effect by inhibiting fibrotic gene expressions and activation of stellate cells, is hypothesized in this study to effectively inhibit the stromal barrier source of PDAC to facilitate chemotherapeutic agents to reach its target.

Materials/Methods: CAFs isolated from PDAC patient were used *in vitro* to evaluate the effect of isorhamnetin on cell proliferation by colorimetric assay, apoptosis and cell cycle by flow cytometry, mitochondrial function by mito stress test and gene expression by qPCR specific to CAF subpopulations of PDAC environment – myofibroblast and inflammatory CAFs (named myCAFs and iCAFs).

Results/Findings: Distinct inhibition after 24 h could be observed in 50 µM isorhamnetin -treated cells ($p=0.0002$) while 20 µM reduced the viability by about 40% after 96 h of treatment showing isorhamnetin effectively inhibits CAFs viability in dose- and time-dependent manner. Flow cytometry analysis using Annexin V-PE/7-AAD staining after 24 h treatment of 20 µM isorhamnetin showed increased population in early and late apoptosis. Cell cycle was altered resulting in reduced number of cells in G1 phase ($p<0.0001$), while those in S and G2/M phases were increased ($p<0.0001$ and $p<0.001$ respectively). Oxygen consumption rate measured by mito stress test revealed that basal respiration and ATP production were significantly reduced by isorhamnetin treatment. Moreover, myCAFs marker gene expressions - *TGFB1*, *COL1A1* and iCAFs markers such as *IL1A*, *LIF* and *CXCL1* were drastically inhibited upon isorhamnetin treatment.

Conclusion: Anti-proliferation and pro-apoptotic effect of isorhamnetin on CAFs; and down regulation of iCAFs and myCAFs marker gene expressions collectively suggest that isorhamnetin can be proposed as complementary agent to enhance the conventional therapies efficiency in PDAC treatment.

Keywords

isorhamnetin, PDAC, pancreatic cancer, CAF, cancer associated fibroblasts

P64

Resveratrol effects on the gut microbiome of *BRAF$^{V600E/+}$* mice fed a high fat diet

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Background

Polyphenol resveratrol, widely known for its cancer protective activities has been shown to protect against the pro-tumorigenic effects induced by high-fat diets (HFD) in a mice model of colorectal cancer. Although, the mechanisms of actions of this compound still remain elusive due to its low bioavailability, recent studies report that its effects may be mediated partly through its interaction with gut microbiota. Here we sought to determine how HFD affects the intestinal microbiome and the impact of resveratrol on these alternations to better understand the chemopreventive effects of this compound.

Methods

BRAF$^{V600E/+}$; BRAF$^{V600E+/+}$(wild-type) mice were randomised into eight groups and fed either a standard diet or HFD supplemented with low or high dose of resveratrol (0.7mg/kg/day and 14/kg/day respectively) for 6-weeks. The mice faecal microbial profile was then assessed by performing 16S rRNA sequencing.

Results

HFD had significant alternations in the gut microbiome composition in both *BRAF$^{V600E/+}$* and *BRAF$^{V600E+/+}$* mice based on β-diversity analysis *(P_{ADONIS}<0.05)*. However, HFD effects were more prominent in *BRAF$^{V600E/+}$* mice with alternations at different taxonomic levels characterised by an enrichment in the relative abundance of *Faecalibaculum (P<0.02)* and growth inhibition of *Lactobacillus johnsonhii (P<0.05)* compared to mice fed a standard diet. Resveratrol administration did not counteract the HF-induced taxonomic changes in *BRAF$^{V600E/+}$* mice, although high dose resveratrol increased the proportion of genus *Muribaculum* (*P<0.001*) compared to HF-treated mice. Moreover, mice treated with high dose resveratrol had significant differences in the microbial composition compared to mice maintained on HFD only (β-diversity analysis; *P_{ADONIS}<0.05*).

Conclusion

Resveratrol administration modified the gut microbiota composition of *BRAF$^{V600E/+}$* mice but not to completely reverse the HFD-induced changes indicating that alternative mechanisms may also be involved in the cancer protective efficacy of this compound, which are currently under investigation.

Keywords

Gut microbiome, high fat diet, resveratrol, colorectal cancer

Cardiovascular disease

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Coffee chlorogenic acids for cardioprotection: sub-analysis of a systematic review

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Background: Consumption of coffee has been repeatedly associated with reduction of cardiovascular disease (CVD) risk; but association does not prove causation. We reviewed evidence from randomised controlled trials (RCTs) testing the impact of coffee on CVD risk parameters to try and understand this relationship.

Methods: A systematic review was performed. MEDLINE and Web of Science were searched from inception to December 2022. Inclusion criteria: RCTs, reporting haemodynamic measures of CVD risk (e.g. blood pressure; BP, arterial stiffness, endothelial function), conducted in adults, testing coffee (or coffee extract) versus an appropriate control. Exclusion criteria: cohort or observational studies, trials testing pure compounds. Here we present a sub-analysis of chlorogenic acid (CGA)-focused trials with a narrative analysis.

Results: Twenty-six papers were deemed eligible for inclusion in the full review (n=13 acute, n=13 chronic). Of those, CGAs were the focus of 9 RCTs (n=2 acute, n=7 chronic). The mean baseline BP of participants in n=6 trials was >140/90. Other than high BP, no trials were performed in participants with health conditions. All trials measured ΔBP, n=4 Δendothelial function (flow mediated dilatation; FMD), n=2 Δarterial stiffness (n=1 pulse wave velocity; PWV, n=1 augmentation index; AIx).

The data suggest modest improvements in systolic BP reduction with chronic coffee CGA consumption versus control, but no acute change. Modest acute improvements in endothelial function were also observed; chronic data is inconclusive. No changes are noted with arterial stiffness.

Conclusion: This data suggests that coffee-CGAs may be contributing to reductions in CVD risk seen in observational trials, however, more well designed RCTs are required to build a robust evidence base. Coffee is chemically complex and contains many other bioactive substances that may also play a key role; our full review with meta-analysis will provide a more detailed insight and will determine any clinical relevance of these data.

Keywords

Coffee, Chlorogenic acids, Cardiovascular

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Can plant-flavanols protect human vascular function from mental stress in a black male population?

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Background: Mental stress has been shown to induce acute endothelial dysfunction. Black ethnicities have a higher incidence of cardiovascular diseases, and vascular responses to stress have been implicated. Flavonoid-rich foods and specifically cocoa flavanols can attenuate the decline in stress-induced endothelial function, but little is known about this in higher-risk populations. This study aims to examine the effect of cocoa flavanols on stress-induced vascular responses in healthy black volunteers.

Methods: A randomised, double-blind, within-subject study was used to assess the effects of cocoa flavanols on vascular responses to mental stress. Healthy black participants (n=9) completed two sessions (order counterbalanced) and consumed either high-flavanol cocoa (150 mg (-)-epicatechin) or low-flavanol cocoa (< 4 mg (-)-epicatechin), prior to mental stress (8-minute paced auditory serial addition task: PASAT). Vasodilatory responses (forearm blood flow, FBF), systolic and diastolic blood pressure (BP), heart rate (HR), heart rate variability (HRV), and pre-ejection period (PEP), were assessed pre-flavanol consumption (B1), post-flavanol consumption at rest (B2) and during mental stress (S). Endothelial function (brachial artery flow-mediated dilatation, FMD) was assessed at B1 and 30-and-90 minutes post-mental stress (i.e., 2-3-hours post-flavanol intake).

Results: Preliminary results (n=9) indicate that stress induced significant increases in FBF, systolic and diastolic BP, HR, HRV, and PEP in comparison to baseline (B1/B2, p's<.05). FBF was significantly higher following high-flavanol cocoa compared to low-flavanol cocoa (p<.001). There was no significant decline in FMD following mental stress, yet FMD was significantly higher 30 minutes post-stress following high-flavanol cocoa compared to low-flavanol cocoa (p=.016) and compared to B1 (p=.029). No differences in PASAT performance were observed between dietary interventions.

Conclusion: The mental stress task evoked the expected cardiovascular responses, and this was consistent across dietary interventions. High-flavanol cocoa improved forearm vasodilatory ability, and improved endothelial function following mental stress, counteracting the expected decline in endothelial function post-stress.

Keywords

mental stress, cardiovascular diseases, endothelial function, cocoa flavanols, ethnicity

P67

Hibiscus Sabdariffa lowers blood pressure and positively impacts cardiovascular disease risk factors: a systematic review and meta-analysis

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Hibiscus sabdariffa has been proposed to impact cardiovascular disease (CVD) risk markers. However, the outcomes from individual human studies are not conclusive.

Our aim was to systematically review the evidence for use of hibiscus interventions on blood pressure, blood lipids and blood glucose. The search followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) protocol targeting randomized controlled intervention trials. The protocol was registered at PROSPERO (Registration number CRD42020167295).

Data from 17 studies comparing hibiscus to a placebo, a diet, tea beverage or a pharmaceutical medication was extracted to permit a meta-analysis, meta-regression, and trial sequential analysis (TSA).

The results demonstrated that hibiscus significantly lowered systolic blood pressure (-7.10 mmHg, 95% CI [-13.00, -1.20], I2 = 95%, p = 0.02) density lipoprotein (-6.76 mg/dL, 95% CI [-13.45, -0.07], I2 = 64%, p = 0.05). A non-significant trend for reduction in diastolic blood pressure was also observed (p = 0.09). Meta regression demonstrated that individuals characterised as stage I and II hypertensive had greatest lowering of blood pressure following hibiscus treatment. The blood pressure lowering effects of hibiscus were not discernible from blood pressure medication. Finally, TSA indicated that the results for systolic and diastolic blood pressure reached the required information size (total sample size for 80% power) to accept the anticipated effects with certainty.

The results of this meta-analysis indicate that regular consumption of hibiscus could confer reduced cardiovascular disease risk. Notably, the magnitude of blood pressure lowering is similar to pharmaceutical treatment. Hibiscus consumption should therefore be encouraged; however, further studies need to establish relationships between dose and duration of intake.

Keywords

Hibiscus, Blood pressure, Lipids, Cardiovascular disease

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(Poly)phenol intake, plant-rich dietary patterns and cardiometabolic health: a cross-sectional study

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Healthy plant-based dietary patterns have been associated with reduced cardiometabolic risk in epidemiological studies. However, no study has considered (poly)phenols as mediating factor in the relationship. Baseline data from 525 healthy participants were included for cross-sectional analysis with cardiometabolic markers measured: flow-mediated dilation (FMD), augmentation index (AIx), peripheral/central systolic/diastolic blood pressure (SBP/DBP/CSBP/CDBP), Fasting Plasma Glucose (FPG), Total, LDL, HDL, Non-HDL-Cholesterol (TC/LDL-C/HDL-C/Non-HDL-C), Triglycerides (TG). Diet data was collected with EPIC Norfolk Food Frequency Questionnaire (FFQs). A priori dietary scores were calculated: Dietary Approaches to Stop Hypertension (DASH), Amended/Original Mediterranean Diet (A-Med/O-Med), healthy/unhealthy Plant-based Diet Index (hPDI/uPDI), Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND). (Poly)phenol intake was estimated with Phenol-Explorer-database. Validated UHPLC-MS method was used to measure 114 (poly)phenol metabolites (fasting plasma/24h urine). Associations were analyzed with linear regression. Significant positive associations were found between estimated total (poly)phenols and all dietary scores, except for uPDI which had an inverse correlation ($r=-0.25$~0.30, $p<0.01$). Correlations were particularly strong for lignan intake and DASH ($r=0.48$, $p<0.01$), as well as SBP, DBP, TC, TG, LDL-C, non-HDL-C ($\beta=-0.13$~-0.09, $p<0.05$). DASH and hPDI were highly correlated with urine and plasma metabolites, including total (poly)phenols, benzene diols and triols, lignans, benzoic acids, phenylacetic acid, phenolic acids, propionic acids, tyrosols ($\beta=0.16$~0.33, $p<0.05$). Urinary flavonoids correlated with DASH and A-Med, flavanols correlated with DBP, FPG, HDL-C, while total (poly)phenols were negatively associated with TG ($\beta=-0.47$~0.35, $p<0.05$). Plasma cinnamic acids correlated with SBP, DBP, CSBP, CDBP, FPG, HDL-C, while flavanols correlated with DBP, CSBP, CDBP, FPG, HDL-C ($\beta=-0.52$~0.33, $p<0.05$). Higher (poly)phenols measured with FFQs and biomarkers is associated with higher adherence to plant-rich dietary patterns, the favourable cardiometabolic biomarkers indicating (poly)phenols may be mediating factors in beneficial effects.

Keywords

(poly)phenol intake, (poly)phenol metabolite, dietary score, cardiometabolic health

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Oregonin from Alnus incana preserves from atherosclerosis by preventing endothelial dysfunction through nutrigenomic and epigenetic regulations

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Background: Scientific evidence from experimental to clinical studies support a beneficial effect of polyphenols on cardiometabolic health. Oregonin is a polyphenol present in different parts of plants from genus Alnus that exhibits anti-inflammatory and anti-adipogenic properties. However, the underlying mechanisms of its effects are still not known. This research aimed to analyse pathways of oregonin effects on endothelial cells and its ability to preserve endothelial function under an inflammatory stress.

Methods: Human endothelial cells (HUVECs) were exposed to oregonin extract from bark of Alnus incana or control medium for 48 hours and then stimulated with tumor necrosis factor alpha (TNF-α) for 4 hours. Expression of adhesion (ICAM-1, VCAM-1) as well as monocyte adhesion were investigated. Methylation specific PCR for analysing ICAM-1 and VCAM1 methylation indexes was applied. The expression of total DNA-methyltransferase-1 (DNMT1), and mitochondrial DNA-methyltransferase-1 (mtDNMT1) and mitochondrial transcription factor (TFAM) by RT-PCR was analysed.

Results: The increased expression of vCAM-1 was inhibited by 50% with a pre-incubation of HUVECs with 7.5 µM oregonin while iCAM-1 expression did not change. Oregonin exposition significantly decreased the adhesion of monocytes to endothelial cell surface. A strong, and negatively dependent to oregonin concentration, correlation between monocyte adhesion and the expression of CAM proteins was observed. No differences were defined between methylation indexes of iCAM-1 and vCAM-1 in control and oregonin-exposed cells despite a significant decrease of DNMT1 in oregonin pre-incubated cells. The expression of DNMT1 was not changed after TNF-α cell activation. However, a significant decrease of TFAM and mtDNMT1 mRNA expression was established in oregonin exposed cells.

Conclusion: All together these results show the ability of oregonin to counteract endothelial inflammation by alleviating pro-inflammatory genes expression and monocyte adhesion, and by regulating of DNMT1 and mtDNMT1 expression, that make this polyphenol an interesting candidate to prevent cell-specific epigenetic change in atherosclerosis.

Keywords

oregonin, endothelial function, inflammation, epigenitic, prevention

P70

Wine pomace product attenuates intestinal oxidative stress in obese rats

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Obesity is characterized as a low-grade inflammation and oxidative stress that impairs physiological functions, including intestinal functioning. Dietary polyphenols can be a strategy for obesity management, collaborating to modulate intestinal dysfunction through antioxidant and anti-inflammatory actions. Our previous studies showed that wine pomace product (WPP), exhibited anti-inflammatory and antioxidant properties, due to its ability to neutralize ROS or to regulate signaling pathways.

This study investigated the effect of WPP against intestinal disorders induced by high –fat diet in obese rats and the molecular mechanism involved. Wistar rats fed with high-fat diet for 14 weeks were used as model of obesity and the diet was supplemented with WPP: from the first week or from the seven week, in order to determine whether the WP prevents the obesity development or ameliorates the disorders of an established obesity. Antioxidant status and Sirt-1, NF-KB expression were also analyzed at 14th week.

The diet supplementation with WPP (100 mg/Kg/day) prevents intestinal oxidative stress increasing the antioxidant status, decreasing of 8OHdG levels and restoring the redox status. Moreover, the results showed that WPP should up-regulate the expression of Sirt-1 and induce a significant decrease in NF-κB, with consequent change of gene expression involved in the protection against oxidative stress.

Therefore, wine pomace product attenuated obesity-related disorders by amelioration of intestinal inflammation and oxidative stress, suggesting their potential preventive clinical benefits.

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Keywords

wine pomace, oxidative stress, obesity

P71

Cocoa flavanol intervention improves lower extremity endothelial function in healthy individuals and people with type 2 diabetes (T2D)

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Objectives/Background: Diabetes and age are major risk factors for the development of lower extremity peripheral artery disease (PAD). Cocoa flavanol (CF) consumption is associated with lower risk for PAD and can improve brachial artery (BA) endothelial function. We assessed if femoral artery (FA) endothelial function is impaired in people with T2D and evaluate the acute effect of CF intervention on it.

Materials/Methods: In a randomised, controlled, double blind, cross-over study, 11 healthy and 11 individuals with T2D without cardiovascular disease were recruited. Participants received either 1,350 mg CF or placebo capsules on 2 separate days in random order. Endothelial function was measured as flow-mediated dilation (FMD) using ultrasound of the common FA and the BA before and at 2 hours after interventions.

Results/Findings: Both FA-FMD and BA-FMD were significantly lower in T2D (FA:3.1±1.1%, BA: 4.8±0.8%) as compared to healthy (FA:5.6±0.8%, BA: 6.0±0.7%; each $p<0.001$). While in both groups FA-FMD was significantly lower than BA-FMD, FA-FMD was relatively lower in T2DM (68±22%) as compared to healthy (94±14%, $p=0.005$). The baseline BA blood flow did not differ between healthy and T2DM. FA blood flow was significantly lower in T2D (424±238 vs 732±347 ml/min, $p=0.037$). Analysis of covariance showed that CF consumption led to a significant acute improvement of both FA and BA FMD at 2 hours as compared to placebo in both healthy and T2D (3.4%, 95%CI: 2.7%, 4.2%; $p_{intervention}<0.001$, $p_{interaction\ interventionxdiabetes}=0.086$, $p_{interaction\ interventionxlocation}=0.109$). Baseline BA and FA blood flow significantly increased after CF in both groups.

Conclusion: The data indicate that CFs not only have the potential to increase endothelial function of the BA but also of the FA that supplies the legs both in healthy people and people with T2D who are at increased risk of developing PAD.

Keywords

Endothelial function, Diabetes mellitus, Cocoa flavanols, Femoral artery, Blood flow

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Assessing variability in vascular response to cocoa flavanols using n-of-1-trials and personal devices

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Objectives/Background: Controlled clinical intervention studies have demonstrated that cocoa flavanols (CF) can decrease blood pressure and arterial stiffness in healthy humans, although a large variability in the effect size across trials has been reported. Here, we evaluated intra- and inter-individual variability of responses to CF in everyday life using a series of n-of-1 trials in healthy free-living humans with personal devices.

Materials/Methods: Eleven healthy young humans completed a repeated cross-over randomized controlled double-blind n-of-1 trial. On eight days, each volunteer consumed 6 CF capsules (750 mg CF/day) for four days and 6 matched placebo capsules (0 mg CF/day) for another 4 days in a randomized alternating sequence. On each day the capsules were taken at the same time in the morning with breakfast after baseline measurements. Each subject was provided with an upper arm blood pressure monitor and a finger clip that measures pulse wave velocity (PWV). Upper arm blood pressure and PWV were measured hourly over 12 hours.

Results/Findings: The overall mixed model analysis showed that CF significantly decreased systolic and diastolic blood pressure and PWV by 1.4±0.3 mmHg, 0.5±0.3 mmHg, 0.11±0.03 m/s with large inter-individual variation in responses (1-intra-cluster correlation [1 - ICC]: 0.59, 0.76, 0.59). Peak effects were observed within the first 3 hours for blood pressure and PWV while PWV decreased again after 8 h post ingestion. There was also considerable intra-individual variation in responses that varied greatly between subjects (ICC: 0-0.30, 0-0.22, 0-0.45). Interestingly, the effect sizes inversely correlated with baseline blood pressure values both when comparing between and within-subjects.

Conclusion: The data confirm that CF can decrease blood pressure and arterial stiffness during real life. The large inter- and intra-individual variation in responses call for more personalized nutritional intervention strategies.

Keywords

blood pressure, pulse wave velocity, cocoa flavanols, n-of-1, variation of responses

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(–)-Epicatechin (EC) attenuated high fructose (HF)-induced modifications in perivascular adipose tissue (PVAT) in rats

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PVAT can release a variety of factors with paracrine effects, e.g. cytokines and oxidants, which influence vascular tone. PVAT dysfunction can aggravate hypertension, and the use of dietary compounds to attenuate these effects is a valuable tool. The aim of this work was to study the impact of EC administration on PVAT modifications induced by a HF diet in rats in terms of redox and inflammatory aspects. Male Sprague-Dawley rats were divided into 4 four groups: C, control diet and water as beverage; CEC, EC (20 mg/kg BW/d) in the diet and water; HF, control diet and 10% (w/v) fructose in the water; and HFEC, EC in the diet and fructose in the water. After 8 w, blood pressure (BP) was determined, animals were euthanized and blood (plasma), aorta, and PVAT were obtained. EC supplementation attenuated BP increase induced by HF diet in parallel with changes observed in PVAT expansion. In addition, adipocyte size was not modified for the treatments, suggesting that the expansion was due to adipocytes hyperplasia. NADPH oxidase (NOX) activity, evaluated in PVAT as superoxide anion production, was C=0.6±0.1, CEC=1.2±0.2, HF=4.0±0.5*, and HFEC=2.0±0.1 AU (*p<0.05 vs all, ANOVA, Tukey's test). NLRP3 inflammasome pathway was studied at systemic and tissue levels. No differences in plasma IL-1β were detected among the 4 experimental groups. IL-1β expression in PVAT was C=100±4, CEC=130±4, HF=174±10*, and HFEC=170±10* AU (*p<0.05 vs C and CEC, ANOVA, Tukey's test). As conclusion, in terms of PVAT modifications, EC could contribute to the antihypertensive effect attenuating the pro-oxidant environment, without effects on NLRP3 inflammatory pathway. Further studies will be necessary to identify the NOX isoforms involved in this effect. PIP-CONICET 11220170100585CO, PICT 2018-03052, and UBACyT 20020170100586BA and 20020190100157BA.

Keywords

hypertension, NOX, inflammasome, flavanols

P74

(–)-Epicatechin (EC) prevents claudin-2 modifications induced by high-fat (HF) diet in kidney mice.

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Obesity and high-fat diets can affect kidney by disturbing the tubular epithelium tight junction complex (TJ) that control selective paracellular pathway and cell polarity. Claudin-2 (cldn2) is preferentially expressed in TJs of epithelia, where it forms cation-selective and water permeable paracellular channels. The objective of this work was to study, in a high-fat diet (HFD) fed model in mice, the changes in kidney cldn2, and the associated mechanisms affected by (–)-epicatechin (EC) supplementation. C57BL/6J male mice were divided into 4 groups: control (C); control + 20 mg EC/kg body weight (CE); HFD (60% fat from lard) (HF); and HFD + EC (HFE). At the end of 14-d treatment, mice were euthanized, and blood and kidney were obtained for chemical, biochemical, and histochemical analysis. Cldn2 and IL-6 abundance were evaluated by histochemistry. Cldn-2 was significantly higher in HF group respect to the others (% of positive area, C:29±1, CE:29±1, HF:38±1*, and HFE:19± 2** AU (*p<0.05 vs C and CE; **p<0.05 vs all the others, Kruskal–Wallis, Dunn's test)). Results for IL-6 detection were C:2.9±0.6; CE:3.2± 0.9; HF:51± 4*; HFE:2.1± 0.5 AU, (*p<0.05 vs all the others, Kruskal–Wallis, Dunn's test). NFκB and AP-1 pathways were also studied. AP-1-DNA binding was C:1.0±0.1, CE:0.9±0.1, HF:1.8±0.2*, and HFE:1.3± 0.1 AU (*p<0.05 vs all the other groups, ANOVA, Tukey test), and no significant differences were found in NFκB-DNA binding. These results suggests that changes in cldn-2 abundance induced by HF diet in kidney are susceptible to be modified by dietary EC, associated with modifications in IL-6 via the AP-1 pathway. Further experiments will be necessary to understand the functional impact of cldn-2 modulation in terms of ion transport, and tubular epithelium permeability. PIP-CONICET 11220170100585CO, PICT 2018-03052, and UBACyT 20020170100586BA and 20020190100157BA.

Keywords

Obesity, Renal, Inflammation, AP-1, Permeability Barrier

Clinical trials

P75

ABSTRACT WITHDRAWN

P76

CurrantCraft® blackcurrant extract promotes visual health: A randomized, double blinded, placebo controlled clinical trial

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Background / Objectives

Anthocyanin-rich blackcurrant (Ribes nigrum) berries are known to exhibit antioxidant and anti-inflammatory activity. This activity provides a wide range of health benefits, including ocular protection, which is a timely need. Eye health has worsened during the pandemic as education and business activities transitioned to online formats. This increased screen time has resulted in blurry vision, dry eyes, and eye strain.

Materials / Methods

To examine the visual health potential of blackcurrant supplementation, we conducted a randomized, double blind, placebo controlled clinical trial in the United States. The purpose of this study was to examine the potential ocular protective benefits of ten weeks of CurrantCraft® supplementation. Sixty-one otherwise healthy female participants aged 30-59 years old who spent 6+ hours per day in front of a digital screen participated in the study, which was powered to detect a large effect size. This would allow identification of the specific domains of visual health most impacted by CurrantCraft® supplementation.

Results / Findings

Baseline scores on all metrics of eye health were similar. An analysis of covariance found that participants who supplemented their diet with CurrantCraft® blackcurrant extract experienced a significant reduction on the blurry vision domain which included symptoms such as blurred vision, double vision, and difficulty refocusing, as compared to the placebo group. The effect size of these benefits was extremely large. Smaller, nonsignificant reduction effects were also noted on the domains of dry eye and eye strain.

Conclusion

These findings provide evidence that CurrantCraft® improves eye health by targeting symptoms of blurred and double vision. As the transition to digital screens is expected to continue, supplementation that protects from adverse effects caused by these new habits are of critical and timely importance.

Keywords

blackcurrant, cassis, Ribes nigrum, eye health , visual fatigue

P77

Polyphenol supplementation inhibits angiogenesis in adipose tissue during an experimental overfeeding in healthy volunteers.

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The development of adipose tissue during the course of obesity is associated to deleterious events leading to complications (diabetes, cardio-vascular diseases…). Many strategies are proposed to help counteract obesity and associated diseases. Among these, polyphenols are found to be a promising approach. We hence decided to investigate the effects of polyphenol supplementation on adipose tissue development in humans using an experimental overfeeding trial.

42 healthy male subjects were submitted to 31 days of overfeeding, supplemented with either 2 g of polyphenols (procyanidins, anthocyanins and resveratrol) or a placebo. Adipose tissue biopsies were collected before and after the intervention and RNA-sequencing was performed in order to measure gene expression and immunohistochemistry to confirm the changes at the protein level. Polyphenols were measured in the plasma of the volunteers and the most abundant ones in the polyphenol group were investigated in vitro using wound assays on endothelial cells.

Overfeeding increased fat mass and body weight similarly in both groups. RNA-sequencing confirmed the effect of overfeeding on lipid storage and adipose tissue development and revealed a preventive effect of polyphenols on the angiogenic response in the subcutaneous fat. Immunohistochemistry using anti-CD31 antibodies reinforced these observations as its quantification decreased in the polyphenol (-0.09%; pvalue<0.05) compared to the placebo group (+0.05%; pvalue>0.05). Among the polyphenols under investigation, quercetin inhibited the most endothelial cell migration during wound assays (-11%; p value<0.05).

Polyphenol supplementation cannot protect against weight gain during overfeeding, but, inhibits angiogenesis in the subcutaneous adipose tissue. The next step will be to find the genes, proteins or pathways involved in this inhibition of angiogenesis in the subcutaneous adipose tissue.

Keywords

Polyphenols, Insulin resistance, Overfeeding, Angiogenesis, Adipose tissue

P78

Effects of a polyphenol-rich dietary supplement on anthropometric, biochemical, and inflammatory parameters in participants with morbid obesity: study protocol for a randomised, controlled trial.

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Background: Obesity is one of today's most visible public health problems. Morbid obesity, characterized by body mass index (BMI) ≥ 40 kg/m2, represents a severe health risk and implies the need of therapeutic action. Different strategies have been developed to address this issue, including dietary, lifestyle, pharmacological, and surgical interventions. Polyphenols may play a relevant role in the management of obesity. They have been shown to modulate physiological and molecular pathways involved in energy metabolism and adiposity. However, very few studies have assessed their effects on morbid obesity. The purpose of this double-blind, placebo-controlled, randomised trial is to determine if polyphenol supplementation, in combination with a dietary intervention, can promote weight loss and improve obesity markers in participants with morbid obesity.

Methods: Adults (n=40) with morbid obesity, mostly bariatric surgery candidates, will be recruited from the Bellvitge University Hospital in Barcelona, Spain, and randomised (stratified by sex) to intervention (polyphenol-rich supplement 1200 mg/day + hypocaloric diet) or control group (placebo + hypocaloric diet), for 12 weeks. The primary outcomes of the study are anthropometric markers, measured through standardized methods and a bioimpedance scan. Secondary outcomes are biochemical and inflammatory biomarkers, metabolic pathways, and gut microbiota. Anthropometric measures, dietary, physical activity and lifestyle questionnaires, and plasma and urine samples will be collected at baseline, 6 weeks, and 12 weeks. At baseline and at 12 weeks faecal samples will also be collected. Ethical approval and informed consent of the participants will be obtained before the start of the study.

Discussion: The present study is expected to provide evidence on the effects of a combination of polyphenols on several well-established obesity markers, and to unravel possible underlying mechanisms by metabolomic analyses and gut microbiota diversity. As such, the study may contribute to future strategies for prevention or treatment of obesity and related conditions.

Keywords

Morbid obesity, Polyphenol supplement, Anthropometry, Obesity biomarkers, Clinical trial

P79

Mental health in new parents: A randomised control trial investigating dietary flavonoid intake and mental health in the postnatal period

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Background During the postnatal period, parents face psychological challenges and resultingly, changes in mood and mood disorders such as postnatal depression (PND), have become increasingly prevalent following birth. Previous research demonstrates that consuming a higher proportion of flavonoids in the diet is beneficial for mental health outcomes in healthy populations. Considering this, flavonoid rich foods may offer protection against onset or severity of PND symptoms. Recent evidence has shown that a dietary flavonoid intervention can significantly reduce state anxiety and increase perceived quality of physical health in mothers with infants under one year (Barfoot et al, 2021). However, it is unclear whether a similar intervention would have comparable effects in both parents in the immediate, six-month postnatal phase when mood is thought to be more labile. **Methods** The aim of this study was to investigate whether a two-week dietary flavonoid intervention would improve parents' mental health in the postnatal period. The study employed a randomised, parallel groups, controlled design to explore the effects of a flavonoid intervention versus control group on several outcomes, including mood, postnatal depression, postnatal anxiety, food frequency and quality of life. Forty participants were randomised to either a 'flavonoid' or 'control' condition. The flavonoid group were asked to add two flavonoid rich foods from a pre-determined list into their daily diet whilst controls were asked to continue with their usual diet for two-weeks. **Results** Data analysis is on-going, and results will be presented at the conference. **Conclusion** We hypothesise that those in the flavonoid intervention will have a reduction in symptoms of anxiety and depression, improved perception of quality of life and improved mood. This will provide support for the utility of easy, self-administrable changes to the diet for improving mood outcomes and reducing risk of PND in parents during an especially challenging time.

Keywords

Mental health, Flavonoids, Postnatal period, Diet, Mood

P80

Acute and long-term effects on satiety and appetite of a green coffee phenolic extract alone or combination with oat beta-glucan in subjects with overweight and obesity

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Background: Several human studies suggest that green coffee phenolic extract (GCPE) has beneficial effects on body weight, as do beta-glucans (BG). However, the influence on satiety and appetite control of GCPE has been scarcely explored, whilst BG have shown increased perception of fullness and satiety mainly in acute studies. Considering the importance of appetite regulation to control weight, particularly in overweight/obese persons, it is relevant to study the effects of regularly consuming GCPE and GCPE+BG nutraceuticals as potential tools to reduce food and caloric intake.

Methods: In a randomized, blind, crossover study, 29 overweight/obese volunteers (BMI=30.09±0.64 kg/m^2) consumed a GCPE nutraceutical containing 300 mg hydroxycinnamates alone or combined with 2.5 g BG (GCPE+BG) twice a day for 8 weeks. The effects on postprandial appetite and satiety were analysed through visual analogue scales (VAS) and actual food intake on day=0 and after each 8-week-intervention (day=56). Moreover, in a subgroup of 9 volunteers, blood levels of cholecystokinin, peptide-YY, glucagon-like-peptide-1, ghrelin and leptin were analysed at different times (30, 60, 90, 150, and 210 min) after consuming the nutraceuticals.

Results: Long-term consumption of GCPE+BG induced higher reduction of hunger (p=0.029) and desire to eat (p=0.03) than GCPE according to VAS results; however, energy and food intake at breakfast and lunch did not show statistically significant differences. Regarding objective measurements, after acute consumption of GCPE+BG (day=0), leptin concentration was higher at 150 min compared to GCPE (p=0.025), and regular GCPE+BG intake (day=56) decreased maximum ghrelin levels compared to GCPE (p=0.03).

Conclusions: GCPE+BG nutraceutical appears to increase satiety and reduce appetite in overweight/obese subjects more efficiently than GCPE alone. Higher postprandial levels of leptin (acute-effect) and lower ghrelin concentrations (chronic-effect), together with a lower hunger sensation, support the additional benefits of incorporating BG to GCPE.

Keywords

Satiety, Hydroxycinnamic acids, Green coffee, Beta-glucans, Nutraceuticals

P81

Regular intake of green coffee phenols, oat β-glucans and green coffee phenols/oat β-glucan nutraceuticals is not enough to induce changes on body composition in adults with overweight or obesity

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Background: Both green coffee phenols (GCP) and beta-glucans (BG) are well known for their hypolipidaemic and hypoglycaemic effects, having potential as dietary tools or part of dietary strategies to combat cardiometabolic traits, including overweight/obesity and associated comorbidities such as diabetes or hypertension. However, evidences of their ability to reduce/manage weight are contradictory and more research is needed. In addition, there is no information on the potential effect of the combined consumption of both types of bioactive compounds. Therefore, we aimed to investigate the impact of the regular consumption of three nutraceuticals containing GCP, BG and their combination (GCP/BG) on body composition in overweight/obese subjects.

Methods: A randomised, cross-over, triple-arm, blind trial was performed in 29 obese/overweight volunteers who consumed a decaffeinated GCP extract (600 mg/day), BG (5 g/day) or GCP/BG (600 mg + 5 g/day) for 8 weeks, without changing their dietary habits and physical activity. At the beginning and end of each of the interventions, body weight, body mass index, body fat percentage, intracellular and extracellular water, skinfolds (tricipital, bicipital, subscapularis, supriliac, leg and thigh) and body circumferences (waist, hip, thigh, calf, branchial) were measured. Dietary intake was analysed with 72-hour dietary records and physical activity by using accelerometers during 7d in each intervention stage.

Results: Volunteers maintained their habitual dietary intake and physical activity throughout the study. The intervention resulted in no changes in any of the body composition parameters analysed with any of the three nutraceuticals.

Conclusions: The regular intake of GCP, BG and GCP/BG, without changes in dietary habits and exercise level, is not an efficient strategy to induce positive changes in weight and other body composition parameters. We do not discard that with a longer intervention or more volunteers the effects could have been improved.

Keywords

Body composition, Weight managemen, Hydroxycinnamic acids, Green coffee, Beta-glucans

P82

Effects of consuming green coffee phenols, oat β-glucans or green coffee phenols/oat β-glucan nutraceuticals on lipid and glucose metabolism biomarkers in obese/overweight subjects at moderate metabolic risk

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Background: Findings from clinical trials support the positive effects of green coffee phenols (GCP) on lipid and glucose metabolism. Similarly, EFSA issued favourable opinions on the effects of β-glucan (BG) maintaining normal blood cholesterol levels and reducing post-prandial glycaemic responses. Since both bioactive compounds may act through different metabolic pathways, greater health benefits might be achieved with their joint intake. Therefore, we aimed at investigating the potential effects of the regular consumption of three nutraceuticals containing a decaffeinated GCP extract (DGCPE), BG and their combination (DGCPE/BG) on different biomarkers of glucose and lipid metabolism in overweight/obese subjects with insulin resistance and/or dyslipemia.

Methods: A randomised, cross-over, blind trial was carried out in 29 overweight/obese volunteers who consumed DGCPE (600 mg/day), BG (5 g/day) or DGCPE/BG (600 mg + 5 g/day) for 8 weeks. Total cholesterol (TC), triglycerides, LDL-C, HDL-C, VLDL-C, adipsin, adiponectin, C-reactive protein, ALT, AST, glucose, glycosylated haemoglobin, insulin, glucagon, C-peptide, leptin, resistin and visfatin were analysed in blood samples taken after a 12-hour fast at the beginning and end of each intervention stage. Oral glucose tolerance tests were performed at 0, 30, 60, 90 and 120 min after the intake of each nutraceutical, alongside determining insulin concentration at 0 and 120 min.

Results: Volunteers maintained their dietary intake and physical activity unchanged during the intervention. Contrary to previous observations with DGCPE/BG in overweight/obese volunteers with normal blood cholesterol and fasting glucose levels, in the present study no significant differences were observed in any of studied biomarkers with none of the three nutraceuticals.

Conclusions: The regular intake of BG, DGCPE and DGCPE/BG is not enough to improve lipid and glucose status in subjects at moderate cardiometabolic risk. Results should be taken with caution as the study was slightly underpowered.

Keywords

Dyslipemia, Glucose homeostasis, Overweight/obesity, Decaffeinated green coffee phenolic extract, Beta-glucans

P83

Impact of polyphenol-rich fruit juice on lipid metabolism in humans

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Polyphenols are an important class of secondary metabolites, which possess antioxidant or anti-inflammatory properties and are associated with many health benefits. They occur in many fruits, especially in red fruits and their juices. In vitro studies have indicated that extracts of fruit juices can influence lipid metabolism by reduced lipid accumulation, phosphodiesterase (PDE) activity or increased lipolysis.

To assess the effects of polyphenol-rich fruit juice (containing chokeberry, cranberry, pomegranate juice) on the lipid metabolism a placebo-controlled intervention study with 36 male healthy subjects (age 20–40 years, body mass index 19–25 kg/m^2) was carried out. After one week wash-out period, the volunteers consumed 750 mL of a polyphenol-rich fruit juice or placebo drink for eight weeks on a daily base. After the wash-out period as well as after four and eight weeks of intervention, blood sampling was performed to analyse biomarkers of the lipid metabolism, such as blood lipids and cyclic adenosine monophosphate (cAMP)-phosphodiesterase (PDE) activity in platelets. In addition, the effects on body weight/composition and food intake were investigated.

Blood lipids were not significantly altered after polyphenol-rich juice consumption, whereas triglyceride levels increased ($p<0.01$) after placebo drink intake. In platelets, a significant ($p<0.01$) inhibition of cAMP-PDE activity was observed, more pronounced after juice consumption. During the intervention with juice increased fat-free mass ($p<0.05$) was shown after four weeks, while a significant ($p<0.05$) elevation in body weight was observed after placebo drink consumption. Only juice consumption was found to decrease fat and protein intakes significantly ($p<0.05/0.01$) compared to the wash-out period.

Taken together our findings are suggestive for an influence of polyphenol-rich fruit juice on lipid metabolism in humans by reduced nutrient intake and modulation of biomarkers.

Funding: This IGF-Project (20277N) was supported by the Federal Ministry of Economic Affairs and Climate Action (via AiF) and FEI.

Keywords

lipid metabolism, polyphenol-rich fruit juice, human intervention study

P84

Impact of dark sweet cherry (DSC) juice consumption in obesity-induced inflammation: a randomized controlled trial.

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Background: Cherries have been shown to be rich in polyphenols, more specifically anthocyanins, known to have antioxidant and anti-inflammatory properties that might improve obesity-related metabolic syndrome.

Objectives: To assess the effects of DSC juice on biomarkers of inflammation in adults with obesity.

Methods: Eligible participants (>18 years, body-mass index (BMI) = 30-40 kg/m2, no history of chronic disease and/or antibiotics), were allocated to cherry or placebo groups following a single-blind randomized design after a 2-week run-in period. Participants were asked to drink 200 mL of DSC juice supplemented with 3g of DSC powder (n = 19) or placebo drink (n = 21) twice a day for 30 days. Fasting blood samples were collected on study days 1 and 30 and levels of inflammatory markers were assessed using the multiplex bead-based immunoassay Luminex system using the Milliplex ® MAP Human Cytokine/Chemokine/Growth Factor panel (EMD Millipore; Billerica, MA) following the manufacturer's protocol. Results were expressed as mean ± SE.

Results

After the 4-week intervention, no significant differences in tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), interleukin-18 (IL-18) were detected among treatments. However, participants in the DSC group had decreased levels of monocyte chemoattractant protein 1 (MCP-1) compared to the placebo group (changes of -34.62 in DSC group vs. changes of + 37.95 in placebo) but did not attain significance. Since MCP-1 is critical for the development of cardiovascular diseases, its downregulation may contribute to reducing obesity-associated heart disease.

Conclusions:

These findings suggest that consumption of DSC juice over 4 weeks may decrease obesity-associated metabolic syndrome by exerting anti-inflammatory effects.

Keywords

sweet cherries, anthocyanins, obesity, inflammation

P85

Nutrimetabolomic study to identify biomarkers of anthocyanins: Targeting the gut microbiota activity through a randomized, controlled, cross-over trial in healthy individuals.

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Objectives/Background:

Anthocyanins (ACN) are polyphenols present in fruits like blueberries and grapes. The majority of the ingested ACN can reach the large intestine where they are extensively metabolized by gut microbiota. The study aimed to identify biomarkers of ACN intake by a nutrimetabolomics approach in plasma, urine and feces using an UHPLC-MS/MS method.

Materials/Methods:

A randomised, double-blind, placebo-controlled, cross-over, 28-day dietary intervention comparing the effects of ACN-rich juice vs. placebo (n=35). After an overnight fast, blood, 24h uine and feces samples were collected at day 0 and day 28 of each intervention phase. A targeted UHPLC-MS/MS was used to identify biomarkers of ACN intake. Log2 fold-change was used to calculate the change between “end of dietary intervention -baseline” measurement, and linear mixed models were used to compare the effects of the juice vs. placebo. Statistical analysis were adjusted for multiple comparisons using the Benjamini-Hochberg procedure.

Results/Findings:

The participants were young (age: 24.5±2.5 years), most of them female (77%) and normal-weight (mean body mass index: 21.7±2.3 Kg/m2). Compared to placebo, out of the 1,232 metabolites quantified in the nutrimetabolomics method, 22 (including 10 parent ACN, 1 organic acid and 11 phenolic metabolites) were significantly associated with the ACN intake, three in plasma (2,4,6-trihydroxybenzaldehyde (THBAld), 4’-hydroxy-3’-methoxyphenyl-γ-valerolactone glucuronide (MHPV-G) and 3’,4’-dihydroxyphenyl-γ-valerolactone (3,4-DHPV)), twelve in urine (apart from parent ACN: o-coummaric acid (oCOU), 4-hydroxybenzophenone (4-HPB), 3’-methylepicatechin sulfate (3-MeEC-S), 2-hydroxybenzoic acid sulfate (2-HBA-S), 3’,4’-dihydroxyphenyl-γ-valerolactone 3'-glucuronide (3,4-DHPV-3G) and 3’,4’-dihydroxyphenyl-γ-valerolactone 4'-glucuronide (3,4-DHPV-4G)), and seven in feces (apart from parent ACN: glutaric acid, vanillic acid (VA), homovanillyl alcohol (HVAlc)).

Conclusion:

By using a nutrimetabolomics approach we identified several biomarkers of ACN intake. Most of them were gut microbiota phenolic metabolites, which could also be responsible for some of the health benefits associated with ACN consumption, such as decreasing cardiovascular risks and anti-cancer activities.

Keywords

Anthocyanins, Polyphenols, Nutrimetabolomics, Gut microbiota, Valerolactones

P86

Adherence to a Mediterranean dietary pattern and response to an exercise program to prevent hospitalization-associated disability in oldest-old adults

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Objectives/Background: Hospitalization-associated disability (HAD) is associated with an increased institutionalization, long-term disability, and mortality in older subjects. Mediterranean diet (MedDiet) may play a key role on healthy ageing. In this study we aimed to investigate the relationship between adherence to the MedDiet pattern and the response to an exercise program to prevent HAD in acutely hospitalized oldest-old patients.

Materials/Methods: The mean (SD) age of participants (n = 102) was 87.5 (4.6) and 42% were women. Adherence to a MedDiet pattern was measured with MEDAS questionnaire. Urinary total polyphenols (UTP) were analyzed using the Folin-Ciocalteu assay. After admission, participants were randomized into the control group (n = 42, usual care) or the intervention group (n = 60, supervised exercise, i.e. walking and rising from a chair [1–3 sessions/day]).

Results/Findings: At admission, no differences in functional status were observed between different levels of MedDiet adherence or UTP. At discharge, patients in the intervention group who had low levels of MedDiet or UTP showed a significant increase in functional status as measured by the Barthel Index [adjusted mean (95% CI) = 78.9 (71.5–86.2) points, p-value = 0.006, and adjusted mean (95% CI) = 77.4 (67.6–87.2) points, p-value = 0.03, respectively].

Conclusion: Oldest-old individuals with low adherence to the MedDiet may benefit more from a physical exercise intervention. Therefore, admission assessment of oldest-old patients should include MedDiet adherence as a variable to design strategies to prevent HAD.

Keywords

Mediterranean diet, phenolic compounds, elderly, hospital-associated disability, functional ability

Epidemiology

P87

Change in habitual intakes of flavonoid-rich foods and all-cause mortality in US men and women

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Background: Higher intakes of flavonoid-rich foods and beverages are associated with a lower risk of mortality in observational studies. However, associations with changes in intakes remain unclear and will contribute to our understanding of whether increasing intakes of specific foods and beverages will offer benefits in optimizing long-term health.

Methods: In 56,253 women from the Nurses' Health Study (NHS) and 30,182 men from the Health Professionals Follow-up Study (HPSF), we examined associations of 8-year changes in intakes of flavonoid-rich foods and beverages with subsequent 2-year lagged 6-year risk of all-cause mortality. We also examined associations of 8-year changes in an a priori defined flavodiet score, with 6-year risk of all-cause and cause-specific mortality. Multivariable-adjusted Cox proportional hazard models were used to estimate hazard ratios (HRs) and 95% CIs and data were pooled using fixed-effects meta-analyses.

Results: We documented 14,557 deaths in the NHS and 8,988 deaths in HPFS from 1986-2018. Compared with participants with stable intakes, those with the greatest increase (≥7 servings/week) in intakes of tea, red wine and peppers had a 5% [Pooled HR (95% CI); 0.95 (0.92, 0.98)], 11% [0.89 (0.84, 0.95)], and 6% [0.89 (0.84, 0.95)] lower risk of total mortality, respectively. Conversely, an increase in intakes of onions and citrus fruits/juices was associated with a higher risk in both cohorts. A 3 servings/day increase in the flavodiet score was associated with a 9%, 13% and 14% lower risk of total mortality [0.91 (0.88, 0.95)], respiratory mortality [0.87 (0.76, 0.99)] and neurological mortality [0.86 (0.78, 0.95)], respectively, after multivariable adjustments.

Conclusion: Encouraging an increased intake of specific flavonoid-rich foods and beverages, namely tea, red wine, and peppers, even in middle-age, may lower early mortality risk, particularly related to respiratory and neurological causes.

Keywords

Flavonoids, Mortality, Prospective cohort, Tea, Red wine

P88

Assessment of urinary flavanoid concentrations as biomarkers of dietary flavanoids intakes within the European Prospective Investigation into Cancer and Nutrition (EPIC) study

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Background: Traditional methods for estimating flavanoid intake (i.e. self-reported questionnaires & food composition tables) have several limitations that could be overcome using nutritional biomarkers. Objective: To assess the correlation between the acute and habitual dietary intake of a selected group of flavonoids, their main food sources, and their 24-h urinary concentrations in the European Prospective Investigation into Cancer and Nutrition study (EPIC). Participants and Methods: A convenience sample of participants (N=419), belonging to three EPIC countries (France, Italy, and Germany), provided a 24-h urine sample and completed a 24-h dietary recall (24-HDR) on the same day. Acute and habitual dietary data were captured through a standardised 24-HDR and a country/centre-specific validated dietary questionnaire. Intake of flavanones, flavanols and flavonols was estimated using the Phenol-Explorer database. Urinary concentrations (µmol/24 h) of flavonoids were determined using a liquid chromatography system coupled to tandem mass spectrometry, with previous enzymatic hydrolysis. Simple and partial (adjusted by BMI, age at recruitment, sex, centre, and smoking status) spearman correlations were conducted to assess the correlations between variables. Results: Acute and habitual intake of individual and subtotal flavonoid groups were weakly to moderately ($r_{partial}$~0.10-0.50; P~<0.05-0.001) correlated with their corresponding urinary concentrations. Similarly, urinary concentrations of flavonoids were weakly to moderately correlated with both acute and habitual intake ($r_{partial}$~0.10-0.60; P~<0.05-0.001) of selected flavonoid-rich foods, including fruits, citrus fruits and juices, onion, garlic, tea, apple and pear, berries, cocoa products and wine, among others. Correlations between urinary and dietary variables were found stronger when using acute than habitual intakes data. Conclusion: Twenty-four-hour urinary concentrations of a selected flavanones, flavanols and flavonols can be considered from weak to modest biomarkers of their acute and habitual intake. Further controlled clinical studies are needed to discern if they are useful biomarkers or if dietary assessment is accurate enough.

Keywords

Flavonoids, Biomarkers, Intake, Urine, EPIC

P89

Descriptive analysis of dietary polyphenol intake in the subcohort MAX from DCN-NG: "Diet, Cancer, and Health – Next-Generations Cohort"

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Objectives/Background: Polyphenols are bioactive compounds widely distributed in plant-based foods. Currently, limited data exist on the intake distribution of polyphenols across meals. This study aimed to estimate dietary intakes of all individual polyphenols and total intake per class and subclass by meal event, and to identify their main food sources in the subcohort MAX from the Diet, Cancer, and Health – Next-Generations cohort (DCN-NG).

Materials/Methods: Dietary data were collected using three web-based 24-hour dietary recalls over one year. In total, 676 participants completed at least one recall. The dietary data were linked to Phenol-Explorer database using standardized procedures and an in-house software. We categorized foods/drinks into five options of meal events selected by the participant: 'Breakfast', 'Lunch', 'Evening', 'Snack', and 'Drink'. Dietary polyphenol were estimated using general linear models.

Results/Findings: Adjusted total polyphenols mean intake by meal was the highest in the drink event (563 mg/day in men and 423 mg/day in women) and the lowest in the evening event (146 mg/day in men and 137 mg/day in women). The main overall polyphenol class contributor was phenolic acids (55.7-79.0 %), except for evening and snack events where it was flavonoids (45.5-60 %). The most consumed polyphenol subclasses were hydroxycinnamic acids and proanthocyanidins. Nonalcoholic beverages (coffee accounted for 66.4%), cocoa products, and cereals were the main food sources of total polyphenols.

Conclusion: This study provides data on the variability in the intake of classes and subclasses of polyphenols and their main food sources by meal event according to lifestyle data, age and gender in a Danish population.

Keywords

Phenolic compounds, Meal Times, Food Intake, Denmark

P90

Comparison of flavonoid intake assessment methods using USDA and Phenol Explorer databases: Subcohort Diet Cancer Health-Next Generation - MAX study.

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Objectives/Background: Flavonoids are bioactive plant compounds that are widely present in the human diet. Estimating flavonoid intake with a high degree of certainty is challenging due to the inherent limitations of dietary questionnaires and food composition databases. This study aimed to evaluate the degree of reliability among flavonoid intakes estimated using four different approaches based on the two most comprehensive flavonoid databases: United States Department of Agriculture (USDA) and Phenol Explorer (PE).

Materials/Methods: In 678 individuals from the MAX study, a subcohort of the Diet, Cancer and Health-Next Generations cohort, dietary data were collected using three 24-hour diet recalls over one year. Estimates of flavonoid intake were compared using flavonoid food content from PE as 1) aglycones (chromatography with hydrolysis), 2) aglycones transformed (converted from glycosides by chromatography without hydrolysis), 3) as they are in nature (glycosides, aglycones and esters), and 4) using flavonoid content from USDA as aglycones (converted). Spearman, intra-class correlation (ICC) and weighted kappa (K) coefficients were calculated for the reliability analysis.

Results/Findings: When comparing PE total aglycones to USDA total aglycones, there was a moderate reliability when a continuous variable was used (ICC: 0.73, 95% confidence interval (CI): 0.70-0.76) and an excellent reliability when flavonoid intake was modeled as a categorical variable (K: 0.89, 95% CI: 0.88-0.90). The degree of reliability among all methods of estimated flavonoid intakes was very similar, especially between database pairs, especially for the flavanol subclass. While larger differences were observed for flavone, flavonol and isoflavone subclasses.

Conclusion: Our findings indicate that caution should be taken when comparing the results of the associations between flavonoid intakes and health outcomes from studies, when flavonoid intakes were estimated using different methods, particularly for some subclasses.

Keywords

food composition, polyphenol, aglycone, glycoside, concordance

Infectious diseases

P91

Inhibition of *Listeria* invasion by white wine pomace polyphenols

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Wine pomace products (WPP), with rich contents of the bioactive compounds' polyphenols, are known to exhibit antimicrobial activity against a variety of microorganism such as *Listeria monocytogenes*. Effects ranging from growth inhibition to reduction of *L. monocytogenes* cell invasion. Our previous reports showed antilisterial activity by red wine polyphenols in epithelial cells associated with the WPPs' ability to protect the integrity of the intestinal barrier by modification of the adherens and tight junction proteins expression.

To elucidate the molecular mechanism of white Wine Pomace Product (wWPP) against *L. monocytogenes* epithelial invasion, human strains of *L. monocytogenes* and *L. innocua* were incubated with Caco2 intestinal monolayers pre-treated with wWPP bioaccessible fractions. Listeria invasion was evaluated by *in vitro* virulence assay, and cell-cell junction`s proteins and Nrf2/NF-κB expression were analyzed by qPCR.

The phenolic content of wWPP was characterized by high levels of phenolic acids including homoprotocatechuic and caffeic acids, and flavanols catechin, epicatechin and procyanidins. Wine pomace product inhibits *L. monocytogenes* invasion of epithelial cells, and the level of this inhibition is dependent of the strain. This effect is associated to gene expression modulation of both Nrf2/NF-κB transcriptional factors and adherens and tight junction proteins by wWPP in the epithelial cells.

These results indicate that white Wine Pomace Product can reduce human *L. monocytogenes* infection by strengthen cell junctions and modulation of Nrf2/NF-κB gene expression.

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Keywords

Polyphenols, Listeria, Nrf2/NF-κB, Wine Pomace, E-cadherin

P92

The role of wine pomace product as antimicrobial agent against *Campylobacter jejuni* in C57BL/6 mice

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Background: *Campylobacter jejuni* is a Gram-negative bacterium that causes the gastrointestinal disease Campylobacteriosis, being by far the food zoonosis, which produces more cases of disease in developed countries. *C. jejuni* infection occurs by oral entry into the human intestine, where it colonizes the mucosal layer of the small intestine or binds to intestinal epithelial cells. Adhesion and subsequent invasion of *C. jejuni* provokes a proinflammatory response and triggers a reaction of the host's innate immune system. The selection of a natural antimicrobial product from winemaking residues rich in phenolic compounds can be an alternative to the use of antibiotics in the fight against *Campylobacter*.

Objective: The aim of this work is to study the administration of a wine pomace product on the infectivity of *C. jejuni* strains in mice C57BL/6 as animal model.

Methods: Three-week-old male wild-type C57BL/6 mice were inoculated with *Campylobacter* at a concentration of 10^8 CFUs. The study was terminated at 16 days when the *Campylobacter* content in faeces was not detected. The mice were supplemented with a product derived from winemaking residues and troponin-I levels in plasma and proinflammatory/anti-inflammatory cytokines in tissues were analyzed.

Results: The product derived from wine pomace rich in proanthocyanidins, catechins and anthocyanins administered to infected mice exerts a reduction in serum troponin levels as well as a decrease in interleukin IL-6, IL-4 and MCP-1 levels in intestine.

Conclusions: Supplementation with a wine pomace product reduces the inflammatory effect of *Campylobacter* infection in mice, thus allowing to elucidate a possible antimicrobial mechanism of the phenolic compounds it contains.

This work was supported by Autonomous Government of Castilla y León/FEDER (Project BU064P20).

Keywords

wine pomace product, Campylobacter jejuni, antimicrobial, cytokines, inflammation

Gut microbiota

P93

Microbial metabolites from proanthocyanidins-rich cranberry blunt UPEC colonic-virulence and urovirulence in a bipartite model of gut microbiota and a 3D tissue-engineered urothelium

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Objectives/Background: Uropathogenic *Escherichia coli* (UPEC) are the primary cause of recurrent urinary tract infections (UTI). The poor understanding of UPEC ecology-pathophysiology from its reservoir – the gut, to its infection site – the urothelium, partly explains the equivocal benefits of cranberry against urinary tract infections. We assessed the effects of proanthocyanidins (PAC) rich cranberry extract microbial metabolites on UTI-89 virulence and fitness in contrasting ecological UPEC's environments.

Materials/Methods: We developed an original bipartite model combining a colonic fermentation system with a dialysis cassette enclosing UPEC and a 3D tissue-engineered urothelium. The dialysis device allows gut microbial metabolites to interact with UPEC without direct competition with the microbiota. Two healthy fecal donors inoculated the colons. Dialysis cassettes containing 7log10 CFU/ml UTI-89 were immersed 2h in the colons to assess the effect of untreated (7-day control diet) / treated (14-day PAC-rich extract) metabolomes on UPEC behavior. Engineered urothelium were then infected with dialysates containing UPEC for 6 hours. UPEC genes expression were measured by RT-qPCR. PAC metabolites were quantified on UPLC-Q-ToF. Friedman test with post-hoc Wilcoxon was used to compare conditions.

Results/Findings: PAC microbial-derived cranberry metabolites displayed a remarkable propensity to blunt activation of genes encoding toxin (*hlyA, cnf1*), adhesin/invasins (*papG, fimH, Sf*a) at early-stage in the gut ($p \leq 0.05$), and in the urothelium (not significant), in a donor-dependent manner. A differential production of PAC metabolites was seen between the two donors. Donor B, producing 5-(3',4'-dihydroxyphenyl)-γ-valerolactone, exhibited an overall better inhibitory response to the PAC treatment against UPEC.

Conclusion: Specific PAC metabolizing gut microbiota may attenuate UPEC virulence, thereby explaining the preventative, yet contentious properties of cranberry against UTI. Variability in subjects' gut microbiota and ensuing contrasting cranberry PAC metabolism affects UPEC virulence and should be taken into consideration when designing cranberry efficacy clinical trials.

Keywords

PAC, Cranberry, Gut metabolome, Urinary tract infections, Urothelium

P94

The metabolic pathways of anthocyanin degradation by the human gut microbiota

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Anthocyanins (ANCs) are a major subclass of polyphenols and give the natural colour to many plant foods which there is considerable interest in their putative health benefits. However, human and animal studies of ANC bioavailability have shown that ANC derived phenolics are the main forms found in the circulation, while intact ANCs are very poorly bioavailable. The availability of several human metabolites reported previously suggest that there are additional ANC degradation routes, presumably involving the gut microbiota. This work aimed to investigate the role of the gut microbiota on ANC metabolism and to identify microbial metabolites that may be responsible for delivering the beneficial effects to human health.

An ANC extract from black rice was incubated with human live-faecal, as well as autoclaved-faecal inocula, using a batch colon model over 24 hours. Control vessels were prepared by incubating live-faecal inoculum without the ANCs. HPLC was used to quantify the rate of loss of anthocyanins over time, and UPLC-MS/MS methods was used to identify and quantify the ANC metabolites that appeared. The study investigated the inter- and intra-individual differences.

Loss of ANCs occurred over 24 hours. However, the rate of reduction in ANC concentration was considerably faster in the presence of live faecal inoculum compared to autoclaved faecal inoculum (16.8 ± 7.8 µM/h compared to 12.7 ± 5.2 µM/h for the first 2 h; $P<0.05$). The most abundant initial ANC metabolites produced in the presence of gut microbiota were 3,4-dihydroxyphenylacetic acid, protocatechuic acid, phloroglucinaldehyde, dihydroferulic acid, catechol, and phloroglucinol carboxylic acid. The production of the most abundant metabolite, catechol, was completely microbiota-dependent, providing strong evidence that the gut microbiota is important for the metabolism of ANCs. It is possible that catechol may be responsible for delivering the health benefits of ANC-rich foods.

Keywords

Polyphenols, anthocyanins, gut microbiota, bioavailability, metabolites

P95

Alleviation of collagen induced arthritis in mice is correlated with restoration of the gut microbiome in mice

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Objective: This study aimed to investigate the effect of muscadine grape polyphenols (MGP) and muscadine wine polyphenols (MWP) on collagen induced arthritis (CIA) and associated gut dysbiosis in mice.

Methods: MGP and MWP were concentrated using Amberlite XAD16N resin. Male DBA/1 mice were divided into four treatment groups (1) healthy control, (2) CIA, (3) CIA + MWP, and (4) CIA+ MGP. Arthritis was induced by intradermal injection of type II collagen on days 0 and day 21. Mice in groups 3 and 4 were gavaged with MWP and MGP using a dose of 400 mg/kg body weight for a total of 21 days. After 42 days, histological analysis on the hind paw assessed pannus formation, cartilage destruction, bone erosion, and inflammation. 16S ribosomal RNA extracted from feces collected on day 42 was analyzed for gut microbial composition.

Results: MWP and MGP significantly decreased pannus formation ($p \leq 0.001$), cartilage destruction ($p \leq 0.001$), and bone erosion ($p \leq 0.01$) but did not significantly decrease histological score for inflammation. PCoA analysis of Bray Curtis and weighted UniFrac revealed that mice in healthy control and CIA+MWP groups had similar gut microbiome composition, but were distinct from mice in the CIA+MGP and CIA groups ($p \leq 0.001$ Bray Curtis and $p \leq 0.01$ UniFrac). CIA mice had a decreased abundance of the family Muribaculaceae but increased abundance of Lachnospiraceae, Erysipelotrichaceae, and Prevotellaceae compared to the healthy control. MWP restored their levels ($p \leq 0.05$), while MGP was able to decrease the levels of Prevotellaceae ($p \leq 0,05$). Spearman correlation analysis revealed that microbial genera were correlated with inflammatory markers and severity of CIA.

Conclusion: Muscadine grape and wine protected the joint against damage caused by arthritis in mice and restored the gut microbiome. As a result, they may offer a promising dietary approach to manage arthritic symptoms.

Keywords

Polyphenols, Gut microbiome, Arthritis, Inflammation

P96

Characterization of microbial metabotypes of proanthocyanidins metabolism in a simulator of the human intestinal microbial ecosystem (TWIN-SHIME)

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Several studies and meta-analysis have pointed out the interindividual variability in polyphenols physiological response. Proanthocyanidins (PACs) interindividual variability is being investigated and metabotypes have been proposed based on PAC metabolism and valerolactones production in the gut. A metabotype is a metabolic phenotype shared by a group of individuals harbouring a similar gut microbiota profile. Until now, characterizations of PAC metabotypes have relied on urine metabolite profiles. Here, we aim to demonstrate the microbiota modulation induced by PACs and identify consistent metabotypes for PACs metabolism, following their behaviour in the in vitro TWIN-SHIME model. This artificial digestive model allows to dynamically follow the metabolism simulating the human digestive system. To select eventual metabotypes, twelve healthy subjects were supplemented with a cranberry polyphenols extract (32mg/day of PACs) for three days. Urines samples were analysed through metabolomic (Q-IMS-ToF and Orbitrap) and six donors with contrasting metabolites production profiles were identified and provided faecal matter to inoculate the TWIN-SHIME. During the fermentation (14 days), the artificial colons were fed with the same cranberry extract at 86,8mg/day of PACs. The results show that microbial metabolism (SCFA) was strongly altered after addition of the extract. The 16s ribosomal DNA analysis of the microbiota confirmed the strong modulation of the microbiota exerted by the treatment. Preliminary results show a marked increase of the Simpson α-diversity index in the mucosal environment and several bacterial species modulation in all compartments. Based on preliminary metabolomic results, we show that PAC metabolites production is taking place in the transverse colon, where phenyl-γ-valerolactone is especially produced five hours following supplementation. Additionally, the microbial metabolite production between contrasting subjects shows significant variations, suggesting a potential metabotypes stratification. We are in the process of correlating metabolomics and metagenomics to pinpoint candidate bacterial species producing bioactive metabolites involved in the PACs metabolism.

Keywords

proanthocyanidins, gut microbiota, TWIN-SHIME, interindividual variability

P97

Cooking, Simulated Digestion and *In Vitro* Colonic Fermentation of Nigerian Wholegrains Affects Phenolic Acids Metabolism and Gut Microbiota Composition

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Objectives/Background:

Wholegrains are a source of both fibre and phenolic acids (PAs), and their gastrointestinal modifications are critical for their bioavailability and bioactivity. We evaluated the modifications on the PAs profile and gut microbiota composition in selected Nigerian wholegrains, following cooking and gastrointestinal digestion.

Materials/Methods:

Red fonio (RF), red millet (RM), red sorghum (RS) and white corn (WC) were cooked, digested and fermented *in vitro*. A total of 26 PAs derivatives were quantified in soluble and bound fractions by UPLC-MS/MS analysis. DNA samples were analysed by 16S rRNA gene-based next-generation sequencing to determine the microbiota composition.

Results/Findings:

In raw grains, soluble PA ranged from 1.42 ± 0.6 - 393.1 ± 0.1 ng/mg, bound PA ranged from 233.1 ± 5.3 - 2201.1 ± 34.4 ng/mg, and total PA ranged from 281.4 ± 10.8 - 2202.5 ± 35.0 ng/mg. Cooking and digestion significantly affected the levels of PAs in all grains ($p<0.05$).

Colonic fermentation led to an increase in total soluble PA over a 24 h period, with total soluble PA in RS and WC peaking at 4 h, and RM and RF peaking at 24h. The sequencing of samples yielded a total of 45 genera, with *Enterobacteriacea* been the most abundant genus at 24 h in all grains studied. 3-hydroxybenzaldehyde increased positively abundance of *Dorea* and the proportion of the mucus degrader *Akkermansia* ($p<0.05$), whereas hydroferulic and isoferulic acid levels decreased the *Oscillospira* and *Ruminococcaceae* members ($p<0.05$) respectively.

Conclusion:

Our data indicate that cooking, digestion and colonic fermentation affected the release of bound phenolic acids from wholegrains, and consequently their metabolic conversion. Thus, phenolic acid fermentation in the gut can lead to relevant improvements in the intestinal microbial ecosystem. This *in vitro* study lays the foundations for the design of an *in vivo* human intervention study that can confirm the promising trends herein observed.

Keywords

Wholegrains, Phenolic acids, Bio-accessibility, Gut microbiota

P98

Effect of a cranberry proanthocyanidins extract on an *in vitro* simplified intestinal microbiota.

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The positive effect exerted by proanthocyanidins (PACs) on human health is in part due to their transformation by the intestinal microbiota into beneficial metabolites. Conversely, PACs can modulate the composition of the microbiota through a *duplibiotic* effect: the dual antimicrobial and prebiotic mode of action of an unabsorbed substrate. PAC's influence on trophic and symbiotic relationships established between members of the microbiota as well as on the production of bacterial metabolites remains to be clarified. However, the high complexity and inter-individual variability associated with models based on complete intestinal microbiota affect our ability to elucidate these phenomena.

To circumvent these problems, we have designed a reproducible and simplified intestinal microbiota (SiIMi) composed of eight bacterial strains immobilized in polymer beads and maintained in a PolyfermS-type fermenter mimicking the colonic environment. A cranberry polyphenol PACs extract was added to this system to evaluate its impact on bacterial populations (qPCR) and metabolite production (GC, LC/Q-TOF).

The strategies developed have enabled the implementation of reproducible methods for the assembly and analysis of a stabilized and repeatable SiIMi. Preliminary iterations of the system demonstrated the ability of PACs to influence the composition and activity of our bacterial consortium and confirmed the ability of the latter to metabolize PACs. The addition of PACs to the system increased the population of bifidobacteria and decreased that of lactobacilli; increased the production of acetate and reduced that of butyrate; and generated metabolites resulting from the bacterial degradation of PACs (epicatechins and phenolic acids).

The SiIMi therefore constitutes a robust model to study the microbial ecology and the complex cross-feeding interactions occurring within the microbiota in the presence of PACs, and to deepen our knowledge of the prebiotic potential of these molecules.

Keywords

Proanthocyanidins, Microbiota, Prebiotics, Synthetic microbial ecology, Metabolomics

P99

The critical role of the digestion and fermentation processes in the microbiota-modulating effect of tannins.

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In the scientific literature it is reported that tannins can reach the intestine almost intact. However, our previous results showed that human digestion has a considerable influence on the metabolization of tannins.

The aim of the present study was to reveal whether the effect of tannin enzymatic digestion, prior to microbial fermentation, could alter the effect of tannins on the composition and functionality of the gut microbiota. In view of that, two tannin extracts obtained from chestnut and quebracho wood, representing hydrolysable and condensed tannins respectively, were subjected to in vitro digestion and fermentation or directly to fermentation. The antioxidant capacity was used as an indicator of the effect of tannin metabolization, while 16S rRNA sequencing was used to evaluate the changes in microbiota composition and the release of short chain fatty acids (SCFAs) was used as a measure of the microbiota fermentative function.

The antioxidant capacity exerted (evaluated with three different methods) and the SCFAs produced were greater in tannins submitted to both in vitro digestion and fermentation. From the study of microbiota composition, it emerged that tannins interact differently with the intestinal microbiota depending on whether they undergo prior digestion or not. The mixOmics network function (sPLS-based) provided valuable information showing a close match among the three antioxidant methods, showing a correlation with SCFA production and with the abundance of the same bacterial taxa. Moreover, the presence of tannins increased the relative abundance of some genera (i.e., Collinsella, Intestinimonas) which have been recently described as being involved in tannin catabolism.

Going through both digestion and fermentation steps is a fundamental requirement in order to maximize tannin bioactive effects, especially with regard to condensed tannins.

Keywords

Gut microbiota, In vitro digestion-fermentation, Tannins, Quebracho, Chestnut

P100

Prebiotic effects of an intervention with a polyphenol rich extract from black elderberry fruits - Results from the ELDERGUT trial

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Background: The intestinal microbiome is a major contributor to human health and disease. Influencing the microbiome potentially improves gastrointestinal symptoms. Prebiotics are one way to influence the microbiome and pre-existing microbiome configuration is influencing their effectivity. Potential prebiotic effects of polyphenols include increased production of short-chain fatty acids and higher abundance of bacterial target species with beneficial effects on the host. Better characterization of determinants for efficacy of prebiotics is needed. We aimed to characterize the interaction of a black elderberry extract (ElderCraft®) rich in polyphenols with the human intestinal microbiome and host physiology.

Methods: The ELDERGUT Trial was a longitudinal cohort trial in 30 healthy participants with 3 periods of 3 weeks. Prior to the intervention period (325 mg extract twice daily), patients were characterized for 3 weeks, and the intervention period was followed by a 3 week wash-out phase. Patients completed weekly symptoms questionnaires and provided a weekly sample set. 16S amplicon sequencing was applied to fecal DNA and metabolomics data were generated from urine samples by nuclear magnetic resonance spectroscopy (NMR).

Results: While no negative effects on clinical symptoms were observed, microbiome analysis revealed a sharp increase in alfa-diversity both at the beginning and after the end of the prebiotic intervention. A similar pattern was observed in an analysis of beta-diversity (unweighted unifrac index and Bray-Curtis Dissimilarity), indicating strong prebiotic-induced changes of intestinal microbiome composition. On the genus level, changes in multiple taxa including Lactobacillus and Akkermansia could be observed.

Conclusion: The ELDERGUT human clinical trial revealed a rapid prebiotic effect following an intervention with black elderberry extract. After initial perturbation of community structures, counterregulatory responses seem to establish a new stable equilibrium accompanied by changes in the taxonomic composition and metabolite output of the microbiome.

Keywords

Prebiotic, Elderberry, Polyphenols, Anthocyanins, Microbiome

P101

Wholegrain fermentation affects gut microbiota composition, phenolic acid metabolism and pancreatic beta cell function in a rodent model of type 2 diabetes.

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Objectives/Background:

Wholegrains are beneficial for diabetes prevention and management, and both fibre and phenolic acids (PAs) can play a role in glycemic control. We hypothesise that the fermentation of PAs-rich wholegrain affects the microbiota composition in diabetes compared to healthy controls, with a subsequent effect on pancreatic beta cell function.

Materials/Methods:

Using an in vitro gut fermentation model inoculated with faecal stool samples from control and high fat, high fructose (HF/HF) fed diabetic mice, we investigated the impact of a wholegrain wheat substrate on microbiota composition and phenolic acid profile. 16S rRNA sequencing and UPLC-MS/MS analysis were applied. The effect of fermentation supernatants on insulin secretion from MIN6 pancreatic beta cells was assessed by radioimmunoassay.

Results/Findings:

The phenolic acid profile was significantly altered in the HF/HF group vs. control ($P<0.001$: 6h), with lower levels of 3OH-benzoic acid ($P<0.001$) and isoferulic acid ($P<0.01$) reported after 6h. A dynamic modulation of the gut microbiota composition was observed, with a trend towards increased diversity at 6h on the control group, primarily reflecting a temporary increase in Bacteriodetes relative to Firmicutes, and a decrease in diversity of HF/HF (p value = 0.1; Wilcoxon), led by increases in Proteobacteria, more specifically, the Cupriavidus spp. In MIN6 cells, an inhibitory effect on stimulated hormone release after exposure to pre-fermentation samples containing slurry but no substrate was observed ($P<0.05$). This was further exacerbated in the control group with the addition of the wheat digest, whereas fermentation negated this effect with time.

Conclusion:

We conclude that HF/HF mice as a model of T2D are characterised by a dysbiotic microbiota, which can be modulated by the fermentation process. The differences in microbiota composition may have potential implications for phenolic acid profile dynamics and for the secretory capacity of pancreatic beta cells.

Keywords

wholegrain, phenolic acids, microbiota, type-2 diabetes, beta cell function

P102

A 24-hour simulated colonic fermentation study of a cocoa extract; microbiome and (poly)phenolic characterisation.

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Background: Intake of flavan-3-ols, including proanthocyanidins (PAC) from dietary sources such as cocoa, is associated with beneficial health effects. Significant amounts of these compounds reach the colon and interact with the resident gut microbiota, generating metabolites that are likely responsible for flavan-3-ol health effect. We conducted anaerobic faecal fermentations with PAC enriched cocoa extract (CE) to better elucidate this interaction.

Methods: An anaerobic temperature- and pH-controlled *in vitro* faecal batch fermentation model (37°C, pH 5.5-6.2) was used to investigate the 24-h colonic metabolism of a flavan-3-ol source (CE, 300 mg/L) inoculated in the presence of the gut-fermentable fibre inulin. The experiment was performed using freshly collected stools from four donors and run in parallel with a positive (inulin) and negative (no substrate) control. Samples were collected after 0, 5, 10, and 24 h. 16S rRNA gene sequencing, UHPLC-MS/MS and UHPLC-HR-MS were used for the microbiome and (poly)phenolic characterisation, respectively.

Results: No significant differences in Alpha-diversity indices were observed between substrates over the fermentation, while inulin induced a decrease in community richness after 10 hours of fermentation ($p<0.05$), consistent with enrichment of saccharolytic species. Beta-diversity significantly discriminated between faecal donors (Bray-Curtis, $R^2 \geq 0.77$, $p<0.01$), but not between fermentation substrates, at each timepoint. No significant ($p<0.05$) differences in the Operational Taxonomic Units' relative abundance of CE compared to controls were observed at either Phylum and Genus level. We measured a decrease in parent flavan-3-ols of the cocoa extract (i.e. unconjugated monomers and PAC) and a concomitant increase in microbially-derived metabolites, including (di)hydroxy-phenyl-γ-valerolactones and valeric acids.

Conclusion: 24-hours *in vitro* faecal fermentation of a flavan-3-ol source induced microbial transformation of proanthocyanidins into small phenolic compounds that were previously linked with *in vivo* health effects of flavanols.

Keywords

cocoa, gut microbiota, flavan-3-ols, faecal digestion

P103

The Effect of Polyphenols on the Gut Microbiota: A Systematic Review

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Background

With data suggesting that polyphenols are metabolized by the colonic microbiota and these are associated with protective effects against non-communicable diseases, it is feasible that this may be mediated by a modulatory effect on the gut microbiota.

Objectives

The aim of this systematic review was, therefore, to investigate whether polyphenols, either present naturally or enhanced in food or as extracts, can modulate the gut microbiota, through the analysis of human intervention trials.

Method

Web of Science and PubMed was searched for journal articles and double-blind, randomized controlled trials were included. Studies that analyzed the effect of supplementation with dietary polyphenol extracts from foods, or polyphenol-rich foods, on gut microbial composition were used. Additionally, trials were included if they used a control group who were given an appropriate placebo, or negative control. The number of papers reviewed at each stage in the study was recorded in a PRISMA flow diagram.

Results

Eleven double-blind, RCTs were found to be eligible for inclusion in a qualitative synthesis. There was a large variation in study characteristics, including the dose and type of polyphenols, and the food matrix they were administered in. All but two studies reported significant changes to microbial genera and/or phyla. Polyphenols found naturally occurring and enhanced in food and drinks, such as cranberry juice, olive oil, and cocoa had the greatest bifidogenic effect. In overweight subjects, polyphenol supplementation generally reduced the abundance of Firmicutes(p=0,028) and increased Bacteroidetes(p=0,008) but had mixed effects on bacterial genera.

Conclusion

The evidence collated in this systematic review suggests that polyphenols can modulate the gut microbiota through the stimulation of different genera and phyla, and potentially through subsequent bacterial cross-feeding. However, more double-blind clinical trials are needed with similar study characteristics in order to confirm this modulatory effect on the gut microbiota.

Keywords

gut microbiota, polyphenols, randomized controlled trials

Mechanisms in action

P104

Cocoa flavanols exert sex specific protection for pancreatic beta-cell function and other glycemic control markers in a rodent model of obesity and type-2 diabetes

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Objectives: Cocoa (Theobroma cacao) is a rich source of flavanols with the potential to prevent or ameliorate chronic metabolic diseases such as type 2 diabetes (T2D). We aimed to 1) determine whether various cocoa flavanols ameliorate T2D and preserve beta cell health, and 2) identify sex differences in response to flavanols in an obesity/T2D model.

Materials and Methods: Three cocoa fractions were prepared: flavanol-rich cocoa extract (CE), high molecular weight flavanol fraction (HMW), and purified (-)-epicatechin (EC). Flavanol mean degree of polymerization (mDP) was measured by thiolysis. Male and female C57BL/6J mice were fed the following (n=12/sex/group): low-fat (LF), high fat (HF), HF+CE, HF+HMW, HF+EC. Low-dose STZ was administered at week 7 to animals on HF diet to accelerate beta cell damage. Weight gain and food intake were measured weekly. Oral glucose and insulin tolerance tests (OGTT/ITT) were performed at baseline and endpoint. Colonic ZO-1, insulin secretion and serum triglycerides were quantified by kits.

Results: Mean mDPs for HMW, CE, and EC were 2.9, 2.1, and 1.0, respectively. CE significantly protected against weight gain in males, but only slightly in females, along with HMW. CE prevented against fasting hyperglycemia and slightly protected insulin sensitivity in males only. EC protected beta cell viability in males compared to CE/HMW. Compared to males, females had improved overall beta cell viability in HF/STZ animals, however HMW displayed the highest degree of beta cell dysfunction among female s. EC and HMW increased intestinal barrier function among HF/STZ females. CE and EC provided protection against hypertriglyceridemia compared to HMW in females only.

Conclusion: Cocoa flavanols exert different anti-diabetic activities in males and females. Specific fractions provide distinct activities such as protection against obesity, hyperglycemia, hyperlipidemia, and intestinal permeability. Further studies are needed to delineate the mechanisms behind these differences.

Keywords

cocoa, diabetes, beta cell, insulin, obesity

P105

Contribution of betalains to antidiabetic properties of beetroot

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Background - Consumption of red beetroot has been associated with a number of health benefits such as lowering of blood pressure and anti-diabetic properties, although the evidence underpinning some of these effects is limited. Attention has been drawn to betalain pigments, which are known for their strong tinctorial properties, yet the contribution of betalains to anti-diabetic properties and other mechanisms is unclear. Our previous research has demonstrated the hypoglycaemic effects of red beetroot juice, and we have evaluated the inhibitory potential of individual betalains and betalain/polyphenol-rich samples towards carbohydrate hydrolysing enzymes α-amylase and α-glucosidase.

Methods – Inhibition of α-amylase and α-glucosidase enzyme activities were determined using previously established absorbance based kinetic protocols in different extracts derived from rainbow beet varieties (dark red, pink, yellow, white) as well as previously isolated individual betalains. Further analyses were performed to identify and quantify betalain and polyphenols i.e. using HPLC coupled with mass spectrometry (MS) and to establish other biological activities i.e. antioxidant and anti-inflammatory properties.

Results – Data demonstrate dose-dependent inhibition of α-glucosidase in all samples up to around 50%, independent of betalain content (0.24-9.21 mg/g in pink, yellow and red samples, except white beet). When normalized to mg polyphenol/ml, enzyme activity was lowest in pink and yellow samples, emphasizing the lack of betalain contribution. Indeed, purified individual betalains (betanin, vulgaxanthin I, indicaxanthin) did not exert inhibition towards α-glucosidase, although all of these betalains demonstrated effective inflammation-inhibitory properties. In addition, none of the extracts or individual betalains were able to inhibit α-amylase activity.

Conclusion – In summary, despite marked differences in betalain pattern and content of the different beet varieties, they can be considered as comparable with regards to their α-glucosidase inhibitory properties indicating that betalains are not contributing to anti-diabetic effects associated with beet consumption via this mechanism.

Keywords

betalains, polyphenols, alpha-amylase, alpha-glucosidase, diabetes

P106

Red wine extract, a polyphenolic cocktail, could prevent age-related macular degeneration by inhibits VEGF secretion and its signaling pathway in human retinal cells.

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Age-related macular degeneration (AMD) is the most common reason of blindness in developed countries which is characterized by damages in the central part of the retina, the macula. This degenerative disease is mainly due to neoangiogenesis via molecular mechanism involving the production and secretion of vascular endothelial growth factor (VEGF). These past decades, therapy such as anti-VEGF have been mainly use to reduce neoangiogenesis. Nevertheless, the progression of the disease is often observed without reverse vision quality. In order to enhance AMD treatment, attention has been paid to prevention where diet seems to plays a preponderant role. Indeed, it has been shown that polyphenols such as resveratrol, can prevent VEGF secretion induced by stress from retinal cells. Polyphenols can not only reduce oxidative stress but also alter cellular and molecular signaling as well as physiological effects involved in ocular diseases such as AMD. In this context, we have investigated the potential effect of Red Wine Extract (RWE), a polyphenolic cocktail, on the secretion of VEGF and its signaling pathway in human retinal cells ARPE-19. The composition of RWE has been characterized quantitavely and qualitatively by High Performance Liquid Chromatography and tandem Mass Spectrometry. We have shown by ELISA and Western blotting that RWE are able to decrease, in a concentration-dependent manner, the protein expression of VEGF-A and its secretion in ARPE-19. This decrease is sustained by the reduction of VEGF-receptor 2 protein expression and its phosphorylation. RWE-induced alteration in kinase pathway activation by preventing the phosphorylation of MEK and ERK ½ in ARPE-19. To conclude, our study highlights the potential interest of polyphenolic cocktails in prevention strategy in AMD.

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Keywords

Red Wine extract, AMD, Retinal cells, degenerative diseases, ocular diseases

P107

Antifibrotic Effects of Isorhamnetin Explored in Human Amniotic Epithelial Cells Using Global Gene Expression Analysis of Microarray Data

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Fibrosis-related diseases are responsible for over 45% of all deaths in the developed world. Current fibrosis treatments typically target inflammatory response; however, the fibrogenic mechanism is distinct from the inflammatory mechanism. Therefore, development of effective and safe antifibrotic drugs is urgently needed.

In this regard, the antifibrotic potential of isorhamnetin, a natural flavonoid, has gained attention. We have previously reported isorhamnetin's preventive effects in the *in vivo* models of NASH and cardiac fibrosis, and in TGFβ-induced hepatic stellate cells (Ganbold *et al.*, 2019, Aonuma *et al.*, 2020).

In the present study, we aimed to explore the biological functions and molecular pathways underlying the antifibrotic activities of isorhamnetin in human amniotic epithelial cells (hAEC), a stem cell-based tool. The 3D hAEC spheroids were treated with 20 µM isorhamnetin for 10 days, total RNAs were extracted from three replicates of control and isorhamnetin-treated cells, and Affymetrix microarray gene expression profiling was performed.

Top biological process gene ontology included extracellular matrix organization (GO:0030198), wound healing (GO:0042060), cellular response to TGFβ stimulus (GO:0071560), collagen fibril organization (GO:0030199), inactivation of MAPK activity (GO:0000188), and epithelial to mesenchymal transition (GO:0001837). Top enriched KEGG pathways include PI3K-Akt, p53, Hippo, TGFβ, AMPK, Wnt, FoxO, MAPK, Jak-STAT, and prolactin signaling pathways. Curated gene-disease association analysis reveals that isorhamnetin could regulate 286 gene expressions associated with fibrosis (MeSH ID: D005355). K-means clustering and PPI network analysis identified a cluster of 139 proteins with functional importance in antifibrotic effects of isorhamnetin. Most importantly, tissue-specific expression analysis shows isorhamnetin could regulate several tissue-specific gene expressions, including adipose tissue, adrenal gland, esophagus, heart, kidney, lung, nerve, skin, stomach, and liver.

Altogether, our integrated transcriptome analysis of isorhamnetin-treated hAEC reveals its multiple tissue-specific antifibrotic potentials and involved molecular pathways. Therefore, antifibrotic effects of isorhamnetin in different tissue-specific models are worthy of further exploration.

Keywords

Isorhamnetin, Antifibrotic effect, Human Amniotic Epithelial Cells, Global gene expression analysis

P108

(-)-Epicatechin and the colonic metabolite 2,3-dihydroxybenzoic acid strengthen insulin signalling and regulate glucose uptake and lipid accumulation in cardiomyocytes.

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Background and objective: (-)-Epicatechin (EC) and derived phenolic metabolites generated by intestinal microbiota exert beneficial effects on health, although their mechanism of action remains largely unidentified. Heart damage constitutes a global health issue and under certain pathological situations seems to be connected with insulin resistance, which is frequently present in metabolic diseases. The present work studies the effect of EC and microbial-derived flavanol metabolites compounds on the energy metabolism and insulin signalling in H9c2 cardiomyocytes.

Methods: H9c2 cells were treated for 24h with realistic concentrations of EC, 3,4-dihydroxyphenylacetic acid (DHPAA), 2,3-dihydroxybenzoic acid (DHBA), or 3-hydroxyphenylpropionic acid (HPPA) (0.5-20µM). In the experiments with the inhibitors, cells were pre-incubated with 10µM LY294002 (AKT inhibitor) or 10µM Compound C (AMPK inhibitor) for 1h prior to EC or DHBA incubation for 24h. Glucose uptake and lipid accumulation were evaluated by 2-NBDG and Oil Red-O staining assays, respectively. Expression levels of proteins related to insulin signalling, glucose transport and lipid accumulation such as IR, AKT, GSK3, GS, GLUT-1, GLUT-4, SGLT-1, AMPK and CD36 were analysed by Western blot.

Results: None of the natural compounds damaged cell integrity during the incubation time. EC and DHBA reduced glucose uptake (1-20µM and 10µM, respectively), and decreased lipid accumulation (>0.5µM), whilst DHPAA and HPPA did not exert any effect on both parameters. EC and DHBA strengthened the insulin-signalling pathway by increasing p-(Tyr)- and total-IR levels, and stimulating the PI3K/AKT pathway. EC and DHBA upregulated GLUT-4 levels and decreased CD36 values without changing GLUT-1 and SGLT-1. Mechanistically, EC and DHBA modulated the energy metabolism via AKT and AMPK in H9c2 cells.

Conclusion: EC and DHBA increase the glucose uptake and diminish the lipid accumulation via AKT and AMPK, as well as strengthen the insulin-signalling route by activating crucial proteins of this pathway in H9c2 cardiomyocytes.

Keywords

Epicatechin and colonic metabolites, Glucose uptake, H9c2 cells, Insulin-signalling pathway, Lipid accumulation

P109

Interactions study between stilbenes for their anti-inflammatory and anti-oxidant activities.

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Stilbenes are polyphenols known for their anti-inflammatory and anti-oxidant properties, which are usually studied at a single molecule level. However, within a plant extract, these compounds exist in a complex mixture and therefore can interact with each other resulting in either additive, synergistic or antagonistic effects. Our aim was to study the interactions of three stilbenes: resveratrol (RSV) with two of its oligomers, Viniferin (VNF) and Vitisin B (VB), in murine macrophages (RAW 264.7) during inflammatory and oxidative stress induced by LPS.

RAW 264.7 cells, exposed to LPS (0.1 µg/mL, 24h), were co-treated with stilbenes alone or in equimolar combinations (1:1) RSV+VNF or RSV+VB from 0.5 to 25 µM (final concentration for the compound alone or the combination). Cytotoxicity was measured by the MTT test and the anti-inflammatory and anti-oxidant activities were evaluated by measuring the NO (Griess reagent) and intracellular ROS (DCFHDA) productions, respectively. The interactions between stilbenes were evaluated by calculating the combination index (IC) developed by Chou et al. using Compusyn® software. An IC<1 indicates a synergistic effect, an IC=1 an additive effect and an IC>1 an antagonistic effect.

Results showed that the 3 stilbenes alone reduced the intracellular NO and ROS productions in a dose-dependent manner. Their IC50 for the anti-inflammatory and anti-oxydant effects were reached at non-toxic concentrations. The combined treatments RSV+VB and RSV+VNF produced synergistic effects in the inhibition of NO with CI equal to 0.76 and 0.72, respectively. While for the inhibition of ROS production, the CI for RSV+VNF was equal to 1.03 suggesting an additive effect.

Overall, our results have indicated that the combinations RSV+VNF and RSV+VB inhibited synergistically inflammation and additively oxidative stress. This opens toward interesting strategies for the treatment of complex diseases whose pathophysiology both involves inflammation and oxidative stress.

Keywords

Stilbene, Inflammation, Oxidative stress, Synergy, Additivity

P110

Exploring the mechanisms of action of the beneficial effects of Montmorency tart cherry juice consumption on cardio-metabolic health in adults with Metabolic Syndrome

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Objectives/Background: Metabolic Syndrome (MetS) encompasses a cluster of different cardio-metabolic criteria and increases risk of developing cardiovascular disease and type 2 diabetes (Alberti et al., 2009). Tart cherries possess a high polyphenol content (Ou et al., 2012) and evidence suggests Montmorency tart cherry juice (MTCJ) consumption improves cardio-metabolic function in humans with MetS (Desai, Roberts and Bottoms, 2019, 2020; Johnson et al. 2020). Clinically relevant improvement in 24-hour ambulatory blood pressure was observed after 7 days supplementation (Desai, Roberts and Bottoms, 2020). Hence, this study aimed to delineate potential mechanisms centred around the Vascular Endothelial Growth Factor (VEGF) signalling pathway.

Methods: In a randomised, single-blind, placebo-controlled, crossover trial, twelve participants with MetS (50 ±10 y; 6M/6F), consumed MTCJ or placebo (PLA) for 7 days. Blood-based biomarkers VEGF-A, VEGF Receptor 2 (VEGFR2), endothelial nitric oxide synthase (eNOS) and prostacyclin (PGI2) were measured pre- and post-supplementation. Comparisons were made by two-way, repeated-measures ANOVA design.

Results: A significant interaction (P=0.011) was observed for VEGFR2 between PLA (pre-post supplementation, 13885±1584 vs. 14123±1415 pg.mL-1) and MTCJ (pre-post supplementation, 14437±1536 vs. 13948±1582 pg.mL-1). No significant differences were observed for changes in response for VEGF-A (P=0.826) or PGI2 (P=0.458). The change in response over time between conditions tended towards significance for eNOS (P=0.08), with concentrations increasing more after MTCJ (pre-post supplementation, 78.9±92.3 vs. 94.4±105.6 ng.mL-1) consumption compared to PLA (pre-post supplementation, 85.6±106.8 vs. 86.5±107.3 ng.mL-1).

Conclusion: Interestingly, 7 days MTCJ consumption significantly reduced soluble VEGFR2 concentrations compared to PLA, indicating MTCJ-induced responses to eNOS may be independent of the VEGFR2 signalling cascade. The hypotensive actions of MTCJ remain to be fully elucidated, however MTCJ-induced reductions in VEGFR2 may indicate MTCJ has anti-tumour properties, providing scope to assess its efficacy in cancer populations.

Keywords

Metabolic Syndrome, Polyphenols, Cardio-metabolic Health, Anthocyanins, Hypertension

P111

Grape seed proanthocyanidins modulate the hepatic molecular clock via microRNAs

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Circadian rhythm is an endogenous and self-sustained timing system. It is sensitive to external cues as light, food and phenolic compounds such as proanthocyanidins (PACs), which regulate the expression of clock genes. In addition, it has been demonstrated that PACs interact with miRNAs to exert some of their beneficial effects. In this study, we determined whether the activity of grape seed extract rich in PACs (GSPE), as a modulator of hepatic clock gene Bmal1 and clock-controlled gene Nampt, could be mediated by miR-27b-3p and miR-34a, respectively. To address this, an acute oral dose of 250 mg/Kg of GSPE was administered to male rats at zeitgeber time (ZT) 0 (light turned on) and ZT12 (light turned off). An additional group was induced to 6-hour jet lag (from ZT6 to ZT12) and the GSPE was administered at ZT6. All groups were sacrificed at different time points post-administration to study the circadian rhythms of hepatic clock gene and miRNA expressions. The results demonstrate that, GSPE adjusted the jetlag-disrupted expression of Bmal1 via miR-27b-3p, while Nampt expression was modulated by GSPE independently of miR-34a. Cosinor-based rhythmometry revealed significant rhythm of miR-27b-3p when rats were exposed to jet-lag and treated with GSPE, while the rhythmicity of miR-34a was not detected. GSPE restored the rhythmicity of Bmal1 after jet-lag disruption via miR-27b-3p.

Keywords

circadian rhythm, peripheral molecular clock, polyphenols, microRNA

Other

P112

Effects of anthocyanins on inflammatory and metabolic responses to a high-fat meal in healthy subjects: a randomized placebo-controlled study

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Background: Consumption of unhealthy diets can trigger inflammatory conditions that have been associated with a higher risk for cardiovascular disease and mortality, type 2 diabetes, and non-alcoholic fatty liver disease. Objective: to investigate in healthy individuals that consumed a high fat meal (HFM), the effects of the supplementation with a cyanidin- and delphinidin-rich extract (CDRE) on the postprandial changes of parameters of inflammation/endotoxemia, and of parameters of lipid and carbohydrate metabolism associated with changes in redox homeostasis and signaling. Materials and methods: participants (25) consumed a 1026-kcal high-fat meal (HFM) simultaneously with either the CDRE providing 320.4 mg of AC, including 166.4 mg cyanidin and 121.7 mg delphinidin, or a placebo free of AC. Diets were randomly assigned in a double blind, placebo-controlled crossover intervention (NCT03309982, www.clinicaltrials.gov). Each intervention (2 visits) lasted 5 h after consumption of the HFM and CDRE or placebo, was separated by a washout period between visits. Blood samples were collected for biochemical assays in plasma and serum, and to isolate peripheral blood mononuclear cells (PBMC). Results: the CDRE mitigated HFM-induced endotoxemia (primary end-point) reducing blood LPS from 0.68 ± 0.12 to 0.38 ± 0.15 EU/ml (5-h AUC, $p<0.04$) and LPS binding protein (LBP) from 16.4 ± 2.7 to 8.2 ± 3.3 to µg/ml (5-h AUC, $p<0.03$). The CDRE also reduced events associated with HFM-triggered postprandial dysmetabolism including: i) plasma glucose and triglycerides increases; ii) TNFα and NOX4 upregulation in PBMC; and iii) JNK1/2 activation in PBMC. No statistically significant changes were observed upon CDRE treatment in HFM-mediated increases in plasma insulin, GLP-1, GLP2, and cholesterol (total, LDL-, and HDL-cholesterol), and IKK phosphorylation in PBMC. Conclusion: Dietary AC, i.e. cyanidin and delphinidin, exerted beneficial actions against diets rich in fat by modulating the associated postprandial dysmetabolism, i.e. endotoxemia, oxidative stress, and altered glycemia and lipidemia.

Keywords

Anthocyanidins, Endotoxemia, Inflammation, High fat diet, Oxidative stress

P113

Extracellular Vesicles as mediators of flavonoid effects - Impact of flavanone metabolites on postprandial endothelial EVs and their miRNA content

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Objectives/background: Recurrent alteration of metabolism in the postprandial phase due to high food intake or unbalanced diet has been identified as a risk factor for cardiometabolic diseases. Among the physiological modifications occurring after a meal, the postprandial release of extracellular vesicles (EVs) has been poorly investigated. EVs are shed membrane particles of less than 1µm in diameter that convey proteins, nucleic acids and lipids. These structures constitute a hot topic in biology as potential health biomarkers and as actors of cell-to-cell communication. Some rare studies have demonstrated that dietary polyphenols lowered the release of EVs associated to vascular disorders. However, nothing is yet known about the impact of polyphenols on the secretion, the content and the biological function of postprandial EVs. The objective of this work was to investigate the impact of flavanone metabolites on postprandial endothelial EVs and their miRNA content.

Methods: EVs were isolated from medium of human aortic endothelial cells incubated in basal condition or post-prandial-like condition including or not a physiologically relevant mix of hesperidin metabolites. EVs were characterized (size, concentration) by a tunable resistive pulse sensing method and phenotyped by immuno-staining coupled with electronic microscopy. EV miRNA content was assessed by microarray.

Results: The physiologically relevant mix of hesperidin metabolites decreased the release of endothelial EVs associated to the postprandial stimulation, without any change of EV size distribution. EV miRNA content differed intensively between basal and post-prandial-like conditions. We observed that hesperidin metabolites mix reversed miRNA profile changes produced by the postprandial stimulation. The computational enrichment analysis of these EV miRNA profiles suggest huge potential biological functions for these vesicles.

Conclusion: These data demonstrate that flavanone metabolites modulate the secretion and the miRNA content of stimulated postprandial endothelial EVs. This support the capacity of flavanone metabolites to counteract EV-mediated detrimental effects of a postprandial.

Keywords

flavanone, extracellular vesicles, postprandial, endothelial function

P114

The acute effects of cocoa flavanols on upper and lower limb vascular haemodynamic during uninterrupted sitting

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BACKGROUND

Prolonged sitting affects endothelial function in peripheral conduit arteries. Nutritional strategies might play an important role in counteracting these negative effects. Consumption of cocoa flavanols enhances peripheral vascular function, as measured in the brachial artery via flow-mediated dilation (FMD), however, whether this positive outcome is also present in the superficial femoral artery is untested. The objective of this study is to investigate whether acute ingestion of cocoa flavanols can prevent sitting-induced endothelial dysfunction in both the upper and lower body vasculature of young healthy adults.

METHODS

In a randomized, double-blind, cross-over trial, 8 young healthy men (age: 23.2 ± 2.8 years) completed two, 2-hour sitting conditions with consumption of either a high or low flavanol cocoa beverage. FMD and shear rate of the femoral and brachial artery, and blood pressure, were collected before and after the 2-hour sitting intervention.

PRELIMINARY RESULTS

In these initial 8 participants (recruitment target n=16), no significant changes were observed in FMD for either the femoral or brachial artery. However, these preliminary data do show on average differences between the conditions following the sitting intervention. Specifically, femoral artery FMD was on average lower following sitting (pooled data: 3.9 vs. 3.2%, $p=0.11$), with observed changes potentially related to the cocoa intake (interaction: $p=0.11$). Similar changes were observed for brachial FMD (pooled data: 5.8 vs. 5.3%, $p=0.17$; interaction: $p=0.13$), In addition, positive shear rate declined in both the femoral ($p<0.01$) and brachial artery ($p<0.01$) following sitting, although this was not differentially affected by flavanol content (interactions: $p>0.23$). In both conditions ($p=0.77$), systolic blood pressure decreased ($p=0.02$), while diastolic blood pressure remained similar ($p>0.05$) following sitting.

CONCLUSION

Preliminary observations highlight the potential for uninterrupted prolonged sitting to alter vascular function in both the upper and lower limbs. This study is ongoing, therefore the cocoa drink content remains blinded.

Keywords

Cocoa flavanols, Prolonged sitting, Endothelial function, Flow-mediated dilation, Shear rate

P115

How drying processes affect the phenolic composition, bioactivity, and bioaccessibility of Salicornia ramosissima J. Woods

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Halophyte plants are salt-tolerant plants being commonly found in saltmarshes and coastal areas worldwide, representing at most 2% of terrestrial plant species. Among all, Salicornia species have been recognized as promising candidates for the food industry as a salt substitute. However, their applicability in several food matrices requires the use of drying processes, which may impact the nutritional and functional value of the plant.

This study aimed to investigate the effect of two drying processes- oven (ODry) and freeze drying (FDry)- on the phenolic content and bioactivity of Salicornia ramosissima. Several analytical methods (LC-DAD-ESI-MS/MS, GC-MS) and in vitro assays (Folin-Ciocalteu, ORAC, ACE inhibition and antiproliferative of HT29 cells) were used to compare the phenolic composition and bioactivity of plants.In vitro bioaccessibility of phenolic compounds was followed under simulated gastrointestinal conditions (oral, gastric, and intestinal) using INFOGEST protocol.

Results showed the phytochemical content and antioxidant capacity of plants were significantly reduced in ODry (TPC: 7.41±0.29 mg GAE/g; ORAC: 291±18 µmol TEAC/g), when compared to FDry (TPC: 9.74±0.88 mg GAE/g; ORAC: 419±54 µmol TEAC/g). The major phenolics affected by the ODry were the quercetin glycosides, whereas the chlorogenic acids and their derivatives were slightly reduced. Notwithstanding, both dried samples demonstrated similar antiproliferative (ODry- EC50 = 17.6±1.1 mg/mL; FDry- EC50 = 17.2±1.4 mg/mL) and antihypertensive (ODry- IC50 = 24.6±1.7 mg/ml; FDry- IC50= 19.0±0.7 mg/ml) activities. Considering the results, FDry samples proceeded to in vitro gastrointestinal digestion assay. Modifications in the phenolic profile were observed, with a considerable decrease in the total and individual phenolic compounds, being the highest percentage of bioaccessibility detected in the gastrointestinal fraction (21.4%). Moreover, the antioxidant activity also increased after the gastric phase when compared to the other digestive phases.

Keywords

Salicornia ramosissima, halophyte plants, bioacessibility, Infogest, ACE inhibition

P116

Maximising the content of extractable free (poly)phenols by bio-degradation of agricultural residues with *Phanerochaete chrysosporium* fungus

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The utilisation of agri-food residues as feedstocks for the production of useful compounds is one of the most promising solutions for sustainable development and circular economy.

(Poly)phenols constitute the biggest class of natural bioactives (ca. 8000 chemicals including tannins, flavonoids, flavanols, anthocyanins, phenolic acids, phenylpropanoids), and their health benefiting properties have been confirmed by numerous in vitro, in vivo, pre- and clinical studies.

However, the generally low content of free polyphenolic bioactives in biomass feedstocks reduces the economic attractiveness of the extractive biorefinery. Therefore, the main aim of the current project is to maximise the content of extractable free (poly)phenols by biomass degradation with the best known lignin degrader *Phanerochaete chrysosporium* fungus.

In the H2020 BBI JU project Phenolexa, several agri-food residues (onion, chicory, vineyard and olive grove leftovers) were subjected to bio-degradation by *Phanerochaete chrysosporium* fungus at 37 °C for 10-14 days followed by the extraction of bioactive compounds using aqueous ethanol as extractive solvent. The total polyphenols content in the obtained extracts was measured using the standard Folin-Ciolcateu method and the effect of the fungal pre-treatment on the polyphenols yield was compared to control samples with no fungal pre-treatment.

It was found, that the fungus growth was significantly affected by the type of the feedstock with chicory residues being the most preferable substrate while onion skins were the worst substrate for the fungus growth. The total polyphenols content increased by 73 % upon the degradation of chicory leaves (reaching 38.5 gGAE/gd.w.) and by 41 % for degraded vineyard prunings in comparison to controls without the biological pre-treatment.

In conclusion, it was shown that the fungal degradation of agri-food residues is a promising method to increase the content of free extractable (poly)phenols to be used as active ingredients in pharmaceutical, cosmeceutical and nutraceutical products.

Keywords

agri-food residue, polyphenols, fungal degradation, biorefinery

P117

Nutraceutical properties of wines from Negroamaro grapes through NMR-based metabolomic approach and biotoxicological analysis: preliminary data

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Background: Wine is a beverage produced by alcoholic fermentation of the fruit of the vine, and is a complex matrix consisting of water, ethyl alcohol and molecules such as polyphenols, organic acids, tannins and biologically active compounds, responsible for its beneficial properties. This multidisciplinary study aims to define the nutraceutical aspect of wines obtained from Negroamaro grapes, a native vine of Southern Italy, through the chemical characterization of the metabolic profile and the analysis of the biotoxicological activity. Methods: 41 Wine samples produced from Negroamaro grapes, grown in the same Apulian area were analyzed. The chemical analysis was carried out with Nuclear Magnetic Resonance spectroscopy on a Bruker Avance III spectrometer. A significant sample of wines was selected for the evaluation of the biotoxicological activity: the HepG2 cells were exposed to five scalar concentrations of lyophilized extract of the sample, and the cell count and viability were evaluated using the technique staining with Acridine Orange/DAPI. Results: The 5.5 - 8.5ppm region of the spectrum is characterized by the presence of signals attributable to the polyphenolic component; among these, the most intense ones have been attributed to gallic, caffeic, syringic acid and resveratrol. The evaluation of biotoxicity showed that all the red wine samples led to a marked concentration-dependent reduction in both cell viability and absolute number of live cells. In the rosés, the phenomenon is very attenuated, while the whites showed no activity. Conclusion: Through the analysis of the metabolomic profile, a specific fingerprint is obtained which allows us to distinguish the variety, geographical area of production and processing techniques. The results obtained show that the technological processes of winemaking, even starting from the same crop variety, lead to differences both in the profiles of the bioactive principles and in the biological activity against liver cancer cells.

Keywords

human health, nutraceutical, polyphenol, metabolomics, cytotoxicity

P118

Functional enrichment of poultry meat and eggs with polyphenols, vitamins, minerals, essential fatty acids

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Several nutrients affect poultry meat and egg nutritional value and have to be considered to enhance the quality of these products. Feeding chickens on high levels of vitamins can enrich the vitamin component of conventional chicken's tissues, as these tissues are linked to water-soluble vitamins and some trace elements. Certain aspects of minerals such as selenium, zinc, and iodine in the nutrition of laying hens are considered in relation to the production of functional eggs enriched with these trace elements. The favorable aspects of vitamins, carotenoids, and flavonoids in the poultry feed result in an increase to the maximum level of vitamins and carotenoids in the eggs and meat that makes them a significant source of vitamins D, E, B, carotenoid, and flavonoids for humans. Other functional fortification approaches involve the use of essential fatty acids in poultry meat and eggs. This paper sheds light on the enrichment of poultry meat and eggs with vitamins, minerals, essential fatty acids, carotenoids, and flavonoids.

Keywords

carotenoids, eggs, flavonoids, meat, minerals

P119

Grape seed extract supplementation may prevent intestinal barrier dysfunction in young-healthy Wistar rats according to quantitative proteomics

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Intestinal epithelial barrier has a crucial role in maintenance of the host homeostasis and alterations in barrier function may lead to inflammatory diseases, colorectal cancer, and metabolic disorders. Grape seed extract (GSE) outbreaks as rich source of flavonoids and diets rich in this class of polyphenols has been related with lower incidence of many non-infectious diseases[1]. However, the effects of GSE supplementation in gut homeostasis or its mechanism of action are not fully understood.

In this study we evaluate the ileal proteome of young-healthy Wistar rats that were supplemented with GSE (25 mg of GSE/kg body weigh/day) for 28 days, to gain insight into the molecular and cellular processes that were affected by these extract. Ileum proteomes of control and GSE treated rats were analysed by SWATH-MS quantitative proteomics. To facilitate the biological interpretation of the results, differentially represented proteins identified were searched against Metascape (https://metascape.org/gp/index.html#/main/step1) and Reactome Pathway Databases (https://reactome.org/).

A total of 236 proteins were significative modulated after GSE supplementation, from which 13 proteins were over-represented and 223 proteins under-represented. Attending to biological pathways, proteins implicated in the regulation of endocytosis, a process associated to tight junction (TJ) assembly and integrity were over-represented in GSE-treated rats. Moreover, proteins related to energy metabolism and innate immune system were under-represented indicating a reduction in the catabolic activity and inflammatory status of the ileum. Our proteomic study also reveals that base excision repair and package of telomere ends are among the processes upregulated by GSE supplementation whereas neutrophil degranulation, IL-12 signaling, and apoptosis are among those downregulated in GSE-treated rats, suggesting an anti-inflammatory and anti-cancer properties of GSE-extracts.

In summary, we conclude that GSE supplementation at low doses may improve the metabolic function and prevent aberrant over-activation of immune responses in the ileum.

1.Biochem.Pharmacol.2020.173:113719.

Keywords

grape seed extract, ileum, proteome, SWATH-MS

P120

Cyanidin and delphinidin restore colon physiology in high fat diet-fed mice: relevance of signaling downstream the TLR-4 receptor

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Background: Consumption of high fat diets (HFD) can impair intestinal barrier integrity, leading to endotoxemia and associated unhealthy conditions. We previously showed that 15-week consumption of a HFD together with a cyanidin- and delphinidin-rich extract (CDRE), prevented HFD-induced alterations in intestinal permeability, endotoxemia and dysbiosis in mice. **Objective:** To investigate if 4-week CDRE supplementation could reverse HFD-induced alterations of colonic physiology and the relevance of the Toll-like Receptor 4 (TLR-4) signaling pathway in these processes. **Materials and methods:** Six-week old C57BL/6J male mice were fed control or HFD diets for 4 weeks and then subdivided in two groups that either continued on the diets, or were supplemented with 50 mg CDRE/kg BW for the subsequent 4 weeks. Endotoxemia, colon structure, barrier integrity, and activation of TLR-4 and redox-signaling pathways were evaluated. Caco-2 cell monolayers were treated with lipopolysaccharide (LPS) with and without the addition of cyanidin, delphinidin, and their metabolites protocatechuic and gallic acid to assess mechanisms. **Results:** The endotoxemia triggered by 8-week HFD consumption was associated with colonic: i) increased TLR-4 and NADPH oxidase NOX1 expression; ii) activation of redox sensitive- and TLR-4-triggered pathways (NF-κB, MAPKs, PI3K/Akt), and iii) disruption of tight junction proteins (occludin, ZO-1, and claudin-1). All these events were prevented/reversed by CDRE. Supporting the relevance of CDRE-mediated downregulation of TLR-4, cyanidin, delphinidin, and their metabolites, mitigated LPS-induced Caco-2 cells monolayer permeabilization by restoring tight junction structure/dynamics. It was also observed that in the mice, the CDRE also mitigated HFD-mediated alterations in goblet cell differentiation and function including downregulation of markers of differentiation (Klf4), mucin production (Muc2) and intestinal mucosa healing (Tff3). **Conclusion:** 4-week supplementation with select anthocyanidins (cyanidin and delphinidin), mitigates and/or reverses HFD-induced alterations in colon physiology in part through the modulation of TLR-4-regulated signaling.

Keywords

High fat diet, Anthocyanidins, Endotoxemia, Colon physiology, Redox homeostasis

P121

Biological activities of the Italian ancient apple 'Mela Rosa dei Monti Sibillini' in a Drosophila melanogaster model.

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State of art and aim of the study: 'Mela Rosa dei Monti Sibillini (MR)' is an ancient apple variety which is cultivated in Central Italy. Apple is known as a source of bioactive compounds containing high amounts of polyphenols. Five classes of phenolics are usually found in apples: flavan-3-ols/procyanidins, flavonols, phenolic acids, dihydrochalcones and anthocyanins, with flavan-3-ols/procyanidins as the most represented class. On these bases, MR could be a promising source of nutraceuticals to prevent oxidative stress and inflammation, conditions that are closely connected to aging. The aim of the present study was to investigate the effects of a lifelong supplementation of two different MR extracts on both longevity and the endogenous antioxidant defence system of Drosophila melanogaster.

Materials and Methods: Flies (n.200/sex) were lifelong supplemented (0.5%) or not (CTR) with two MR extracts characterized for their phenol contents by HPLC-DAD. The extracts were obtained from the whole apples (A) or from their peels (B). Fly mortality was lifelong recorded. At 15 and 45 days (females) or 15 or 30 days (males), flies were collected to evaluate the expression, by RT-PCR, of genes related to antioxidant defences, such as: heme oxidase-1; thioredoxin reductase; superoxide dismutase and glutathione peroxidase.

Results: Longevity in supplemented female flies was significantly increased in respect to CTR group (by 10 and 20% in A and B groups, respectively) and B group presented a much higher increase of half-life vs A groups (A vs B; $p < 0.0001$). The supplementation did not influence longevity in male flies. Interestingly, antioxidant gene expression was modulated with a different time pattern in the two sexes.

Conclusions: The MR confirmed to be a source of important nutraceuticals and Drosophila melanogaster showed to be a reliable and flexible in vivo model to study the effect of dietary supplementations taking into consideration sex differences.

Keywords

Apple, Drosophila melanogaster, Polyphenols, aging

P122

Purification and LC-MS^n characterisation of bioactive phlorotannins from brown seaweeds

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Background: Brown seaweeds produce oligomeric polyphenols called phlorotannins which appear to function as anti-feedants and anti-microbial agents but also have been suggested to have biological effects in humans (1). Extracts from brown seaweeds native to the UK have been shown to have antiviral activities, including against Covid-19, that could produce biocides to contain the spread of these diseases. The objective of this work was to fractionate and identify the most active components and to optimise the extraction of natural antiviral agents.

Materials & Methods: Seaweed extracts from different brown seaweeds were supplied by Byotrol Ltd. These were fractionated using solid phase extraction (SPE) and absorption methods previously known to be selective for polyphenols and phlorotannins. The composition of the fractions was followed using LC-MS^n techniques (1).

Results: Fractionation using SPE and absorption to Sephadex LH-20 produced fractions highly enriched in oligomeric phlorotannins whilst removing non-polyphenolic components. LC-MS^n analysis noted novel polyphenol components but also significant variation in phlorotannin composition (i.e., in size range, inter-unit linkages, and isomers) between different seaweed species and between different seasons.

Conclusions: This study provides scope for structure-activity relationships to be assessed and more effective anti-viral components to be identified.

Reference: Allwood JW, Evans H, Austin C, McDougall GJ. Extraction, enrichment, and LC-MS^n-based characterization of phlorotannins and related phenolics from the brown seaweed, Ascophyllum nodosum. Mar Drugs. 2020 27;18(9):448. doi: 10.3390/md18090448.

Keywords

Phlorotannins, Diversity, Bioactivity, Anti-viral, Health-beneficial

P123

ABSTRACT WITHDRAWN

P124

Organic versus non-organic plant-based foods – a comparative study on phenolic compounds composition and antioxidant capacity

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Background and objectives: Phenolic compounds, as other phytochemicals, are produced by plants as response to stress. Therefore, farming systems have a deep impact in their content and distribution. In this sense, it has been described that their synthesis can be differentially modulated by the use of organic (ORG) or non-organic (NORG) farming systems. However, contradictory results have been observed. To shed light on this issue, the aim of this study was to evaluate the effects of ORG or NORG farming management systems on the phenolic compounds content and antioxidant capacity in plant-based foods grown in Tarragona, Spain.

Methods: Thirteen plant-based foods, including fruits (olive (cv. Arbequina), orange (cv. Navel), sweet cherry (cv. Burlat) and tomato (cv. Ekstasis and Tores)), vegetables (onion (cv. Figueres), sweet pepper (cv. Italia) and swiss chard (cv. Delta)), nuts (almond (cv. Marcona), hazelnut (cv. Castanyera and Negreta) and walnut (cv. Serra)) and legumes (carob pods (cv. Banya de cabra)), cultivated in ORG and NORG systems were compared in terms of antioxidant capacity, total content of phenolics, anthocyanins, flavan-3-ols and flavonols.

Results: NORG fruits tended to have higher phenolic content, whereas ORG fruits had more antioxidant capacity. NORG legume had higher values from all the parameters analyzed in comparison to its ORG equivalent. ORG nuts showed more flavan-3-ols and flavonols than their NORG counterparts, nonetheless, tended to be less antioxidant. ORG vegetables displayed higher phenolics and anthocyanins which reflected in higher antioxidant capacity than NORG ones.

Conclusions: These findings suggest that farming systems differentially modulate phenolic compound composition and antioxidant capacity based on the plant species studied.

Keywords

farming systems, organic system, antioxidant activity, fruits, vegetables

P125

Bioactive properties of macroalgae: *Undaria pinnatifida* and *Himanthalia elongata* aqueous extracts

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Throughout the centuries, humanity has always struggled against the need for different food sources to ashore quantity and quality of basic nutrition. Macroalgae have been included in the human diet especially in Asian countries. Nowadays, due to the continuous search for functional and nutraceutical food products and ingredients, macroalgae have gained growing interest in the scientific community [1]. Moreover, the scientific community has been focused on the evaluation of bioactive compounds that confer recognized bioactive properties to macroalgae such as antioxidant, anti-inflammatory, antimicrobial or cytotoxic capacity, etc.

Himanthalia elongata (Linnaeus) and *Undaria pinnatifida* (Harvey) are two common edible brown algae bellowing to the Phaeophyceae class and have both been described as showing antimicrobial, antioxidant, and free radical-scavenging capacities.

In this study, microwave-assisted extraction using water as solvent was employed as a green technology to obtain higher yields of macroalgae extract with antioxidant properties [2]. The obtained extracts were evaluated considering the following features: i) antimicrobial activity; ii) free radical-scavenging capacity, and iii) inhibition of the acetylcholinesterase, butyrylcholinesterase, and monoaminoxidase oxidase A and b, both enzymes related to neurological/psychiatric disorders.

Both algae extracts showed inhibitory activity towards Staphylococcus aureus, while Undaria pinnatifida extract was also active against Pseudomonas aeruginosa. Furthermore, the aqueous algae extracts presented promising bioactive characteristics especially free radical scavenging activity, being effective against reactive oxygen species (ROS), i.e., superoxide, hydrogen peroxide and hydroxyl radical in a concentration dependent manner.

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Keywords

algae, antioxidants, bioactivity

AUTHOR INDEX