relationships in the elderly are unknown. Our aim was to investigate the relationships between green leafy vegetables and CVD mortality in elderly women.
Methods: 1,456 women aged 70-85 years at baseline (mean ± SD: 75.2 ± 2.7 years), were followed-up for 15 years. Green leafy vegetable intake, including lettuce and other salad greens, celery, spinach and silverbeet, was measured at baseline using a validated food frequency questionnaire. Cause-specific deaths were examined using adjusted Cox regression modelling. The primary outcome was CVD death.
Results: Mean ± SD consumption of green leafy vegetables at baseline was 19 ± 12 g/d, which included lettuce and other salad greens (9 ± 7 g/d), celery (6 ± 5 g/d), and spinach and silverbeet (4 ± 6 g/d). During follow-up, CVD was the primary cause of death in 235 (16.1%) participants. In multivariable-adjusted analyses (adjusted for age and other variables related to CVD), the HR (95% CI) per SD increase was: (i) lettuce and other salad greens, 0.78 (0.66, 0.92), $p = 0.004$; (ii) celery, 0.93 (0.79, 1.09), $p = 0.367$; (iii) spinach and silverbeet, 1.01 (0.86, 1.19), $p = 0.885$; and (iv) total green leafy vegetables, 0.84 (0.72, 0.99), $p = 0.037$.
Conclusions: Higher intakes of green leafy vegetables, particularly lettuce and other salad greens, were associated with a substantially lower risk of CVD mortality in this cohort of elderly women.
Funding source(s): NHMRC and Healthway.

ADDED SUGAR INTAKE AND INCIDENCE OF METABOLIC SYNDROME IN OLDER AUSTRALIANS

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Background/Aims: The aim of this study was to assess the association between percentage of energy intake from added sugar (EAS%) and incidence of metabolic syndrome (MetS) in a cohort of older Australians with 10 years of follow-up.
Methods: Data from participants of the Blue Mountains Eye Study (aged ≥ 49 y at baseline, 1992-1994) were used. Dietary data were collected using a 145-item semi-quantitative food frequency questionnaire (FFQ). Added sugar content of the FFQ items was estimated using a stepwise systematic method. Participants without MetS symptoms at baseline who had MetS data at 5-year and 10-year follow-ups were included in the study ($n = 1319$). Logistic regression was used to assess the association between baseline EAS% intake and incidence of any MetS in 10 years. The analysis was adjusted for a range of confounding variables, including age, gender, smoking, physical activity, energy intake and other dietary variables, and pre-existing diseases.
Results: Incidence of any MetS was 11.7% throughout the 10-year follow-up. Median (IQR) intake of EAS% quartiles were 3.8% (0.1-5.8), 7.3% (5.8-8.6), 10.2% (8.6-12.3) and 14.9% (12.3-31.4), respectively. In preliminary analyses, participants in the highest quartile of EAS% at baseline were not more likely to develop MetS than participants in the lowest quartile of EAS % [OR: 0.82, 95%CI: 0.47-1.43, $p = 0.48$].
Conclusions: Baseline EAS% was not associated with the 10- year incidence of MetS in this cohort of older Australians.
Funding source(s): NHMRC.

CONCURRENT SESSION 17: GUT.
LARGE INTESTINAL BACTERIAL COMMUNITY AND THE EFFECT OF MANGO, PURIFIED PECTIN, AND LOW FIBRE DIETS

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Background/Aims: Plant cell walls (PCW) of ingested fruit, while resistant to digestive enzymes, are available for bacterial fermentation in the large intestine (LI). Bioactive nutrients are often metabolic end-products of fermented substrates. Thus, LI bacterial composition and function is key. Furthermore, understanding the influence of these dietary components, will aid in future recommendations for functional foods, which will beneficially change the LI microbiota. Our aim was to investigate shifts in the LI bacterial community after consumption of fruit pulp (mango) or a soluble fruit fibre (pectin).
Methods: Eighteen male pigs were fed one of three diets: low-fibre (S), 15% mango-pulp (M), or 10% pectin (P). The diets were fed for ~3 weeks, the pigs euthanised, and LI digesta collected from four sites. The bacterial 16S rRNA gene amplicon was sequenced from digesta, thus enabling us to investigate LI microbial community dynamics.
Results: Principal coordinates analysis showed separation between diets, though M & P were clustered more closely to each other, than the S diet ($p < 0.05$). Clustering of samples from all LI sites was tighter at the distal colon than at the caecal level.
Conclusions: Mango and pectin diets changed the LI bacterial population, both in terms of species and abundance. Such changes are relevant as they indicate that fruit consumption (with intact PCWs), can shift the population, though a detailed species characterization will provide more information. This study is novel in its characterisation of the *in vivo* response of the LI-associated bacterial community to a fruit pulp.
Funding source(s): ARC.

ADDING GLUCOSE TO FRUCTOSE REDUCES BREATH HYDROGEN BUT NOT SYMPTOMS IN FRUCTOSE MALABSORBERS WITH A FUNCTIONAL BOWEL DISORDER

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Background/Aims: Fructose absorption is enhanced by the addition of equal amounts of glucose in healthy volunteers. The success of this strategy in reducing abdominal symptoms when consuming free fructose or fructans in functional bowel disorders (FGID) is unknown. This randomised, double-blind, cross-over trial aimed to address these issues.
Methods: Breath hydrogen and symptom response to sugar solutions-glucose; sucrose; fructose; fructose + glucose; fructo-oligosaccharide (FOS); FOS + glucose – were assessed in patients with fructose malabsorption and a FGID. Following a 24h run-in period where participants consumed a diet low in fermentable carbohydrates (fibre and FODMAPs), participants collected breath samples at baseline and every 20 min for 4 hours after consuming the sugar solution. Breath hydrogen was calculated as area-under-the-curve. Symptom scores were recorded at the end of each day, using a 100mm visual analogue scale.
Results: In 26 participants (3 male, aged 22-65 y), breath hydrogen response to 25 g fructose [775 ± 904 ppm·4 hours (mean ± SD)] reduced following the addition of 25 g glucose (84 ± 99; $p = 0.012$, *t*-test), which was similar to that after glucose alone (133 ± 175). Breath hydrogen response to 10 g FOS (3089 ± 1688) was unchanged with glucose addition (2166 ± 1320; $p = 0.559$). Overall abdominal symptoms after fructose (median 15 mm, IQR 2-46) or FOS (19, 2-32) were not changed with glucose addition (5, 1-35; $p = 0.236$; 17, 2-46, $p = 0.926$, respectively). Glucose addition worsened abdominal pain with FOS (5, 1-16 *vs.* 13, 2-18; $p = 0.049$) and nausea with fructose (1, 0-2 *vs.* 2, 1-10; $p = 0.018$).
Conclusions: These results do not support the addition of glucose to free fructose or fructans as it does not reduce, and potentially worsens symptoms associated with consumption of these sugars in patients with FGID.
Funding source(s): N/A.

EMU OIL PREVENTS BODYWEIGHT LOSS IN A MOUSE MODEL OF CHRONIC ULCERATIVE COLITIS

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Background/Aims: Ulcerative colitis (UC), a type of inflammatory bowel disease, is characterised by colonic inflammation and ulceration. Orally-administered Emu Oil (EO), extracted from Emu fat, accelerated the intestinal repair process in a pre-clinical model of *acute* UC. We hypothesized that EO would reduce the severity of dextran sulphate sodium (DSS)-induced *chronic* UC in mice.
Methods: Female *C57BL/6* mice ($n = 10$/group) were gavaged with water or EO (80 μL or 160 μL) thrice weekly. Mice were subjected to two cycles each consisting of *ad libitum* access to water or DSS (2% w/v) for one week and two weeks water recovery. Followed by one week water or DSS and mice culled two days later. Bodyweight, blood profile, organ data and myeloperoxidase activity were assessed. $p < 0.05$ was considered significant.
Results: DSS decreased bodyweight (days 6-19 and 26-30; maximum of 24%), compared to normal controls ($p < 0.001$). In DSS-treated mice, high dose EO significantly increased bodyweight (days 6-12), compared to controls ($p < 0.05$). DSS decreased red blood cell count, compared to normal controls ($p < 0.05$); an effect not improved by EO. Compared to normal controls, DSS increased liver (16%), spleen (45%), lung (19%) and small intestine (20%) weights ($p < 0.05$), although EO had no significant effect ($p > 0.05$). DSS increased colon myeloperoxidase activity compared to normal controls ($p < 0.05$), however, EO was unable to significantly reduce these levels.
Conclusions: EO prevented bodyweight loss in this mouse model of chronic colitis, however, was unable to improve other preliminary parameters. Analyses currently underway include chronic inflammatory markers, histological morphometry and cell kinetics.
Funding source(s): N/A.

DIETARY ADVANCED GLYCATION END PRODUCTS (AGES) INDUCE CHRONIC KIDNEY DISEASE (CKD) AND CHANGES IN GUT HOMEOSTASIS

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Background/Aims: Over-consumption of dietary AGEs, which are formed by heat-treatment of foodstuffs, is thought to lead to CKD. Research suggests that AGEs may modulate gut microbiota. The aim of this study was to characterise the effects of dietary AGEs on gut homeostasis and CKD.
Methods: Male *C57BL/6* mice 6-8 weeks old were fed (*ad libitum*) a low AGE diet (unbaked rodent chow, LAGE) (n = 10-20) or a high AGE diet (baked rodent chow, 160 degrees C for 1h, 5-fold higher AGE content, HAGE) (n = 10-16) for 24 weeks. Urine albumin was measured by ELISA. Expression of the tight junction protein occludin was determined in jejunum by qPCR. Plasma endotoxin was measured using the Limulus Amebocyte Lysate assay. 16S rRNA sequencing of caecal extracts was used to profile the gut microbiome.
Results: Chronic consumption of dietary AGEs led to increased caecal bacterial diversity (LAGE *vs.* HAGE, mean ± SEM, 0.86 ± 0.02 *vs.* 0.95 ± 0, Simpson diversity index, $p = 0.0002$) and decreased occludin expression in the jejunum (1.17 ± 0.23 *vs.* 0.43 ± 0.08 fold change, $p = 0.003$). Plasma endotoxin was increased after high AGE feeding (1.45 ± 0.12 *vs.* 1.91 ± 0.14 EU/mL, $p = 0.028$). The HAGE diet increased urinary albumin excretion (36.04 ± 3.55 *vs.* 59.61 ± 4.05 μg/24 hours, $p = 0.0003$).
Conclusions: These data indicate that excess consumption of AGEs leads to albuminuria, which is associated with increased intestinal permeability and alterations in gut microbiome. This association remains to be fully defined. Further studies in this area are warranted.
Funding source(s): N/A.

REPRODUCIBILITY OF LACTULOSE AND FRUCTOSE BREATH HYDROGEN TESTING AND IMPACT ON CLINICAL UTILITY

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Background/Aims: Breath hydrogen testing is useful to assess colonic fermentation of malabsorbed carbohydrates and their role in symptom genesis. Specifically, lactulose and fructose breath responses can guide dietary restriction of FODMAPs (fermentable carbohydrates) in patients with irritable bowel syndrome (IBS). However, data regarding their reproducibility is limited. The aim was to investigate the reproducibility of lactulose and fructose breath tests.
Methods: A retrospective audit was conducted in 27 IBS patients completing a 15 g lactulose breath test and in 32 patients ingesting 35 g fructose. A repeat test was performed 6-8 weeks later for lactulose and ≥ 6 weeks for fructose. Changes in responses between test and retest were analysed qualitatively [positive response: 2 × ≥ 10 ppm hydrogen rise) and quantitatively as area-under-curve (AUC)] and oro-caecal transit time (OCTT). The effect of duration between testing and variability was also assessed.
Results: A positive lactulose response was maintained in 96% subjects, but 31% ($p = 0.0006$) lost a positive fructose response upon retest. Initial hydrogen AUC to lactulose and fructose were poorly correlated with hydrogen AUC values on repeat testing (lactulose: $r^2 = 0.08$, $p = 0.16$; fructose: $r^2 = 0.07$, $p = 0.18$; regression analysis). Such variations in fructose responses was independent of the duration between test and retest ($r^2 = 0.003$, $p = 0.82$. Lactulose OCTT ($r = 0.29$; $p = 0.15$; Spearman's correlation) or fructose ($r = 0.29$; $p = 0.31$) were not correlated between test-retest.
Conclusions: Poor reproducibility of lactulose and fructose breath testing was demonstrated. Making clinical decisions (e.g. malabsorptive diagnosis or to guide dietary fructose restriction) on the results of a single test cannot be justified.
Funding source(s): Fonterra™ & Yakult Australia.

CONCURRENT SESSION 18: ANTIOXIDANTS.

ANTI-INFLAMMATORY EFFECTS OF POLYPHENOL-RICH PROPOLIS EXTRACTS BY MODULATING UBIQUITINATION OF TRAF6 DURING NF-κB ACTIVATION

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Background/Aims: Propolis is a resinous product collected by honeybees from polyphenol-rich plants. It has documented antioxidant and anti-inflammatory properties although its mechanisms of action are understood poorly. In this study, the anti-inflammatory effects of polyphenol-rich propolis extracts (PPE) from China (CPPE) and Brazil (BPPE) were examined.
Methods: Folin–Ciocalteu's method and chromatographic analysis were used to compare their chemical compositions and *in vitro* antioxidant activities were measured using several different indices. The anti-inflammatory effects of PPE from China and Brazil were examined in murine endotoxin-induced inflammatory lung injury as well several cellular inflammation models.
Results: CPPE and BPPE showed differences in their polyphenolic composition and *in vitro* free radical scavenging activities. Oral administration of PPE to lipopolysaccharide (LPS)-challenged mice decreased serum proinflammatory cytokine concentrations and inhibited pulmonary nuclear factor (NF)-κB activation. Both PPE types modulated LPS-induced key inflammatory mediators and cytokine gene expression in RAW 264.7 macrophages. Reactive oxygen species (ROS) production and several inflammatory mediators were suppressed by both PPE types in a time and dose-dependent manner. In HeLa-T6RZC stable cells where NF-κB signalling is initiated at the level of TNF receptor-associated factor 6 (TRAF6), we found PPE suppressed NF-κB activation by delaying the ubiquitination of TRAF6. In an *in vitro* kinase assay system, both PPE types directly disrupted polyubiquitin synthesis by TRAF6.