Plenary 2: Human Nutrition: Clinical Trials and Population Health

# Effects of flavonoid-containing foods on endothelial function in CVD

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Epidemiological studies suggest decreased cardiovascular risk in individuals with higher intake of flavonoid-containing foods, including grapes. Several potential mechanisms have been proposed to explain such a benefit. One possibility is a favourable effect on the vascular endothelium. The vascular endothelium regulates vascular homeostasis by producing factors that act locally in the vessel wall and lumen, including nitric oxide, which has vasodilator, anti-inflammatory, and anti-thrombotic factors. In atherosclerosis, effective release of nitric oxide is reduced and this change in endothelial function is accompanied by other changes in endothelial phenotype that promotes atherosclerosis. Recent studies suggest that consumption of grape products improves the function of the endothelium, which might reduce cardiovascular risk. Reduced platelet aggregation might also account for a beneficial effect of grape products, and it is well established that anti-platelet drugs such as aspirin and clopidogrel have marked effects on cardiovascular risk. Several studies have demonstrated decreased platelet aggregation following consumption of grape juice and ex vivo studies have confirmed important biological effects of grape-derived flavonoids on platelet function. Other potential mechanisms include reduced blood pressure, protection of LDL against oxidation, and an anti-inflammatory effect. Collectively, these studies provide a strong rationale for recommending consumption of grapes and grape products as part of comprehensive strategy to improve vascular health and reduce the risk for cardiovascular disease. This recommendation also fits well with the overall guidelines of the American Heart Association to increase consumption of fruits and vegetables. Finally, these important mechanistic studies raise the interesting possibility that concentrated grape products or specific grape components might be developed as food supplements or drugs. Randomised clinical trials would be required to confirm the benefit of such interventions for the primary or secondary prevention of cardiovascular disease.

# Plenary 2: Human Nutrition: Clinical Trials and Population Health

## Salt and Iodine: reluctant partners

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### Background

It is estimated that 31% of the world's population has insufficient iodine intake, and are at risk of iodine deficiency. Deficiency occurs when the soil is poor in iodine, causing a low concentration in the food supply. Deficiency can cause a spectrum of effects, including goitre, cretinism, and thyroid abnormalities. Even mild states of iodine deficiency appear to adversely affect cognitive function, as one randomised controlled trial found that increasing iodine status in mildly iodine deficient children improved perceptual reasoning [1]. Cardiovascular disease (CVD) accounts for 30% of 58 million deaths globally [2]. Hypertension is a major risk factor for CVD, and although there have been enormous advances in antihypertensive-drug therapy; the number of people with uncontrolled hypertension has continued to rise. The key strategy for reducing blood pressure at the population level is reducing sodium intake (primarily salt). It has been estimated that a 30% (3g/d) reduction in salt intake would reduce CVD by at least 13% [3].

### Objective

To describe the current and future population health status of Australia and New Zealand, with respect to iodine sufficiency and optimal dietary sodium (salt) intake.

**Iodine:** New Zealand, Tasmania and Australian Capital Territory have traditionally had low iodine intakes. More recently mild-to-moderate iodine deficiency has been documented in New South Wales and Victoria, although Western Australia and Queensland appear to have adequate intakes, whilst South Australia appears to be of borderline status. It is thought that the current low iodine status may be related to decreased consumption of iodised salt and/or the reduced use of iodine-based cleaning products in the dairy industry, leading lower iodine intakes. Iodisation of salt is generally considered as the first-line public health measure for preventing and controlling endemic goitre, induced by iodine deficiency, as it is inexpensive, is one of the few food items consumed by all throughout the year, does not significantly affect colour, taste, odour and the quality iodised salt can be monitored at production, retail and household levels. The mandatory use of iodised salt in most breads (range 25-65mg iodine/kg/salt) was instituted in Australia and New Zealand in October 2009 to address the issue of iodine insufficiency. Now most breads sold contain added iodine (from the use of iodised salt). It is thought that this strategy will provide sufficient amounts of iodine to most people, except for pregnant and breastfeeding women who have higher requirements.

**Sodium/salt:** More than 75% of dietary sodium consumed comes from the food bought off the shelf and is added in processing. A significant reduction in dietary sodium intake can be achieved through changes in the food supply without the requirement for individual dietary changes. This has already been achieved in the UK, with the sodium content of most processed foods being reduced by 20-30%, between 1998 and 2008. This has led to an average 1g reduction in salt intake (from 9.5 grams of salt/day to 8.6 grams/day) [4]. The Australian division of the World Action on Salt and Health (AWASH) was formally launched in 2007 with a national campaign to lower the salt intake of the Australian population to 6g/day. The estimated average salt intake in Australia is approximately 9g salt (155 Na mmol/day). One of the main objectives of AWASH is to lower salt in the food supply by 25%, through collaboration with industry. This will ultimately lead to a significant reduction in the sodium content in a range of foods, including bread. Based on the UK Food Standards Agency model, the US and Canada have developed similar sodium reduction targets which specify the level of sodium (mg/100g) permitted across a range of processed foods. Although, Australia is lagging behind in the development of comprehensive sodium reduction targets, there has been some moves to reduce the salt content of the food supply, with the release of Australia's Food and Health Dialogue targets for bread (400mg/100g) and breakfast cereals.

### Conclusion

Over the next decade, there will be increasing public health initiatives worldwide to reduce the salt content of the food supply and an alternative vehicle for iodine must be found to maintain adequate iodine status. As a population wide reduction in salt will reduce the risk of cardiovascular disease, government support is essential to ensure an effective gradual reduction of salt in the food supply. It does not make sense to tag an essential nutrient, iodine, to a substance that has long-term adverse health effects. The World Health Organisation has already convened a number of meetings to address the issue. An ongoing program of regional iodine surveys in a country as large and geographically diverse as Australia is essential. The effectiveness of mandatory use of iodised salt in bread to address iodine insufficiency must be carefully monitored in different population groups with an adequately funded monitoring program. Such a program needs to utilise urine samples (with 24hr collections required for sodium), to assess population changes in sodium and iodine intakes. This will ensure evaluation of the effectiveness of current strategies in achieving optimal intakes of iodine and sodium for health for all Australians.

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# Concurrent Session 4: Reproductive and Child Health

## What is it about breast feeding that might protect against later overweight and obesity?

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### Background
A number of meta analyses have suggested that breast feeding may protect against later overweight and obesity, with the degree of adiposity usually being determined by BMI. However, whether it is exclusive breast feeding, any breast feeding or the length of breast feeding is much less clear, and data are often inconclusive and contradictory in many reports.

### Objective
The objective of this analysis was to highlight some of the methodological issues that may be responsible for many of the inconsistencies in the literature as well as exploring the evidence that exclusive and/or any breast feeding is the major factor in the protective nature of breast feeding against later overweight and obesity. Moreover, we have also explored a relatively new measure of adiposity, the waist height ratio, as an outcome in the relationship between early infant feeding and later adiposity.

### Design
Literature that related early infant feeding and later overweight and obesity was accessed, scutinised and evaluated for data to support the relative contributions of exclusive breast feeding, the length of breast feeding or any breast feeding as being the protective factor against childhood obesity.

A retrospective analysis of a contemporary cohort of children was also analysed to assess the impact of early infant feeding practices on waist/height ratio at 5 to 8 years of age in 896 children.

### Outcomes
Several methodological issues were apparent in the existing literature, notably the interpretation of definitions of breast feeding and the inability to undertake a randomized trial in this type of study. Waist height ratio was found to be lower in girls and boys at 5-8 years of age who were breast fed compared to those who received no breast milk, reaching significance in girls ($p<0.05$), but not in boys. Also, waist/height ratio was lowest in children that received any breast milk for >12 months, again reaching significance when compared to children who received no breast milk in girls ($p<0.05$) but not in boys.

### Conclusion
Interpretation of the literature relating to the possible protective effects of breast feeding on later overweight and obesity is confounded by methodological issues. Nevertheless evidence suggests that breast feeding reduces the incidence of overweight and obesity as assessed using the waist height ratio at age 5-8 years, especially in girls.

### Source of funding
University of Queensland

## Does low dose prenatal ethanol exposure alter body composition in adult offspring?

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### Background
Pre-natal exposure of rats to high doses of ethanol (36% of energy intake) has been shown to elicit fasting hyperglycaemia, reduced insulin-stimulated glucose uptake, hypersinulinaemia and overweight in 3-month old offspring. The effects of nutritionally more realistic doses of ethanol are unknown.

### Objective
To determine, in a rat model, whether prenatal exposure to low doses (15% of energy intake) of ethanol alters body composition in the adult offspring.

### Design
Following mating, female Sprague Dawley rats were fed *ad libitum*, throughout gestation, either a nutritionally-replete liquid diet (n = 8) or an isocaloric diet containing 6% ethanol (v/v) (n = 9). From birth onward, dams and offspring were maintained on water and fed standard laboratory chow *ad libitum*. At 8 months 1 week (± 1 week) of age, body composition was measured using dual-energy X-ray absorptiometry (DXA, Norland XR36) using the small animal mode. Data were analysed by 2-way ANOVA with sex and treatment (± ethanol) as factors.

### Outcomes
The volume of diet consumed each day and maternal weight gain during gestation was similar in both groups. Time of delivery, average litter size and pup weight at birth for control and ethanol-fed dams were not significantly different (n = 11 ± 1 pups per litter) with equal numbers of male and female offspring, indicating no evidence of intrauterine growth restriction or pup loss. At 8 months, male offspring were significantly ($P < 0.001$) heavier than female offspring and had significantly ($P < 0.001$) greater fat, lean and bone mineral mass. Within each sex, there was no significant ($P > 0.05$) difference in weight or body composition (either as lean, fat and bone mass or expressed as 5 body-weight) between the control and ethanol-treated rats and no interactive effects of sex and ethanol treatment.

### Conclusion
In adult rat offspring, pre-natal exposure to low doses of ethanol, equivalent to 15% of energy intake, does not elicit changes in body weight or composition in contrast to observations in rats exposed to high doses (36% of energy intake) of ethanol. This suggests that pre-natal exposure to low dose ethanol does not predispose offspring to an increased risk of obesity.

### Source of funding
NH&MRC, Diabetes Australia Research Trust.

# Concurrent Session 4: Reproductive and Child Health

## Does enteral feeding compare with orally fed children with cerebral palsy or typically developing children?

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**Background**
There is a paucity of literature on micronutrient and protein intakes in children with Cerebral Palsy (CP), despite well documented issues in undernutrition and osteopenia.

**Objective**
Assess micronutrition in children with marked CP according to feeding method and compare data to typically developing children.

**Design**
Twenty children 4-12 yr with marked CP fed orally (O, n=12) or enterally (E, n=9) were recruited, as well as age-matched typically developing children (C, n=16). Parents collected three consecutive days food replica of their child's actual intake. Composite samples were directly analysed for mineral, trace element and protein content. Similar data were derived in E from product information. Values were calculated as percentage (%) of estimated average requirement (EAR) or average intake (AI) where EAR values were not available.

**Outcomes**
Intakes varied widely between and within groups. Significant differences were found between E versus both O and C for Zinc: P<0.001 and for Iron: P=0.001, while Copper AI was found suboptimal in O and C groups: mean (±SD%) E=158 (±75); O=51 (±39); C=78 (±23) P<0.001. Protein consumption was significantly lower in children with CP than controls P<0.01, while Potassium was less than AI in all groups O=52 (±25), E=50 (±21) and C=72 (±34). Many O and C consumed insufficient Iodine 7/12 and 7/16 or Calcium 5/12 and 6/16 respectively, whereas many O=7/12 and E=5/9 had lowered Manganese intake. Sodium intakes were in excess of upper safety limits in C=6/16 and excess Zinc was documented in E=4/9.

**Conclusion**
Micronutrient balance may be a critical factor relating to health rather than deficiencies alone, particularly in children who rely solely on supplemental nutrition. Copper and manganese deficiencies may lead to skeletal problems, while their absorption is hindered by excessive intakes of zinc and iron. All groups had reduced potassium intake. If this is coupled with higher levels of sodium, calcium status and hence bone strength may be at risk. These data have important implications for bone health and general nutrition regardless of their study group.

**Source of funding**
Not applicable.

## Fatty acid composition in breastmilk of term mothers

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**Background**
Breastmilk contains up to 214 different fatty acids (FA). Medium chain fatty acids (MCFA) are synthesised *de novo* within the lactocyte, whilst long chain fatty acids (LCFA) are incorporated from the maternal bloodstream. These free fatty acids are then esterified to form triacylglycerols and secreted as a fat globule in breastmilk. FA composition of breastmilk has been shown to vary with stage of lactation, gestational age and diet.

**Objective**
To investigate the inter-relationship between the MCFA and LCFA in breastmilk of term mothers at 3 and 6 months of lactation

**Design**
Atopic mothers of term infants were recruited for the Infant Fish Oil Study. Milk samples, collected at 3 and 6 months of lactation, were analysed from a subgroup of mothers (n=82). FA were extracted, trans-esterified and then separated by gas chromatography.

**Outcomes**
The composition of FA in breastmilk varied greatly between mothers. Principle component analysis indicated that 10:0, 12:0 and 14:0 FA were negatively associated with 18:1cis9. While 8.0 and 16:1cis9 FA as well as majority of long chain fatty acids were positively associated with fatty acids (16:0, 18:0 and 18:2cis9.11).

**Conclusion**
The result suggests that the levels of LCFA in the maternal blood stream could influence the level of both LCFA and MCFA in the lactocyte, which in turns could result in an overall variation of the FA composition of breastmilk. It is important to realize that altering the concentration of one FA in milk results in subsequent and specific changes in another FA in breastmilk. This is important to further elucidate the variation observed over the course of lactation and between mothers.

**Source of funding**
National Health and Medical Research Council (NHMRC) of Australia (Grant ID 458502) and Medela AG, Switzerland.

# Concurrent Session 4: Reproductive and Child Health

## Comparison of breastmilk composition during repeated breast expression sessions

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**Background**
The estimated nutrient requirements of infants are based on the composition of breastmilk. Several factors including short-term (weekly) variations can affect the breastmilk composition. This variation has the potential to affect milk consumption by breastfed infants. Investigating variation in breastmilk composition is important for understanding the regulation of food intake by infants.
**Objective**
To investigate variation in breastmilk composition between the left and right breasts of mothers and over a 3 week period within the first 7 months of lactation.
**Design**
The left and right breasts of healthy mothers (n=23) of term infants were simultaneously expressed with an electric breast pump for 15 minutes, on three occasions, within three weeks. Milk samples (5 mL) were collected from the total expression volume of each breast at each session. A Mid-Infrared human milk analyzer was used to determine the macronutrient concentration (protein, fat and lactose), total solids and energy of the breastmilk. All samples were analysed in duplicate.
**Outcomes**
Milk composition varied markedly between mothers. The macronutrient concentration was similar for the left and right breasts, however, some (n=5) individuals were found to have consistently different protein concentration between breasts. Over the three weekly sessions, no apparent changes were found in either the protein or lactose concentration, however, a decreasing trend was observed in fat concentration. Total solids and energy significantly ($P<0.05$) decreased by week three on average from 13.5 to 12.7 g/100mL and 82.4 to 75.7 Kcal/100mL respectively, whereas the total expression volume significantly ($P<0.05$) increased from 68.4 mL to 78.73 mL.
**Conclusion**
While protein and lactose remained consistent, our preliminary findings indicate that there may be time dependent variations in the fat, total solids and energy content of milk and this requires further investigation.
**Source of funding**
Higher Education Commission (Pakistan) and Medela AG (Switzerland)

## Ultrasound pasteurisation and the quality of breastmilk: Variation of power and time of exposure

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**Background**
Donor human milk is pasteurised to prevent the potential risk of the transmission of pathogens to preterm infants. Currently, Holder pasteurisation (breastmilk held at 62.5 °C for 30 min) is used in most human milk banks, but has the disadvantage that it results in excessive inactivation of important bioactive components. Power-ultrasound (20-100 kHz) is an emerging technology for pasteurizing foods and could be an alternative method for the pasteurisation of breastmilk.
**Objective**
The aim of this study was to investigate the effect of different ultrasound settings on the viability of *E. coli* and the retention of bile salt stimulated lipase (BSSL) activity.
**Design**
First, different ultrasound-energies were created by holding ultrasound-power constant at 26 W and varying the exposure times. Secondly, ultrasound-energy was held constant at 1000 J but the ultrasound-power and exposure times were varied, that is, high ultrasound-power for a short exposure time compared to low ultrasound-power for a long exposure time.
**Outcomes**
Ultrasound with a constant power decreased *E. coli* viability exponentially over time until the temperature increased to between 51.4 °C and 58.5 °C, then a more rapid decrease was observed. BSSL activity decreased to 91% until a temperature of 51.4 °C and then it decreased to 8% between 51.4 °C and 64.9 °C. Ultrasound with a constant energy and various power and exposure times showed the highest temperature (53.7 °C) when treated with the longest exposure time and lowest ultrasound-power (276 s by 3.62 W) compared to 37.6 °C at 39 s by 25.64 W.
**Conclusion**
The findings predict that the viability of *E. coli* could be reduced by log 5 with a minimal loss of activity of BSSL by applying 13.8 kJ of energy in 12 mL of breastmilk using high ultrasound power over a short exposure time to ensure that the temperature remains below the critical level for protein denaturation. Alternatively, the use of lower power settings such as the 26 W used in the present studies would require a sophisticated cooling system to ensure the breastmilk BSSL was protected against temperature denaturation. However a longer exposure time would be required because of the loss of the synergistic interaction been temperature and ultrasound.
**Source of funding**
This study was supported by Medela AG (Switzerland)

# Concurrent Session 4: Reproductive and Child Health

## Infant sucking dynamics during the establishment of breastfeeding

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**Background**
Infant sucking and attachment problems during breastfeeding are common reasons for early weaning. There is little data describing sucking patterns during the initiation of lactation particularly at the point of secretory activation (Day3, range Day 2 to 5).

**Objective**
This study aimed to determine how sucking patterns, milk intake and breastfeeding patterns change during and after the initiation of lactation.

**Design**
Fifteen breastfeeding mother-infant dyads were assessed on day 3 postpartum and followed up between day 10-42 (n=eleven) post-partum. Sucking dynamics were assessed using submental ultrasound scans of the infant's oral anatomy for the duration of a breastfeed. Ultrasound measurements were performed when the tongue was up against the palate and down. Measurements included nipple diameter at 2,5,10 and 15 mm from the nipple tip, depth of the tongue and nipple to hard-soft palate junction distance during nutritive sucking (NS) and non-nutritive sucking (NNS). Milk intake was determined by test-weighing infants before and after a feed during the study visits. In addition the LATCH-R tool was used to assess latch, swallow, nipple type, comfort and position during breastfeeding on day 3 (D3) and at follow up (FU).

**Outcomes**
For both NS and NNS, as the tongue lowered during a suck cycle all nipple diameters significantly increased ($P<0.001$) and the nipple moved closer to the HSPJ ($P<0.001$) at D3 and FU. There were no differences in parameters within an infant between D3 and FU, however there was a trend towards larger nipple diameters at 10 mm ($P=0.07$) and 15 mm ($P=0.049$) at FU. During NS nipple diameters and the depth of the tongue were significantly larger than that of NNS ($P<0.001$). The LATCH-R tool showed a trend towards improvement (in nipple comfort state) from D3 to FU ($P=0.05$). In addition, milk intake during the study visit increased from D3 to FU ($P=0.003$).

**Conclusion**
Preliminary analysis show infant sucking dynamics are similar between D3 and FU, suggesting babies are born with a mature sucking reflex. Key differences between NS and NNS suggest when milk flow is absent sucking action is different. Furthermore, mothers may experience less nipple discomfort as lactation progresses.

**Source of funding**
Medela A.G, Switzerland

# Concurrent Session 5: Obesity/Insulin Resistance

## Do diets matter in the battle against obesity?

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### Background

The management of obesity is associated with a very high long-term failure rate. There have been many studies and much controversy as to which is the best diet to use to lose weight. Many have been tested, generally in short-term studies. Low fat, high protein and low carbohydrate diets have all been championed at different times. However a recent randomised study published in the New England Journal of Medicine in 2009 concluded that; "reduced-calorie diets result in clinically meaningful weight loss regardless of which macronutrients they emphasise." In other words, provided energy intake is less than energy expenditure weight loss will follow. In the same year, we showed that the composition of the diet (either high protein or high complex carbohydrate) had no impact on the maintenance of weight loss at 1 year after weight loss using a very low energy diet.

### Objective

The object of this talk is to explore reasons for the irrelevance of diet composition and the very high failure rates of diets in the management of obesity.

### Outcomes

Fat (triglyceride) is the body's preferred method for storing excess energy. Carbohydrate can be stored in liver and muscle, but we have evolved with the capacity to store carbohydrate for only 2 days worth of energy needs. Energy expenditure is made up of different components; basal metabolic rate, post-prandial thermogenesis, spontaneous activity and voluntary physical activity. There is no evidence that obesity is associated with or caused by a reduction in basal metabolic rate. This is not surprising as the chemical reactions that generate basal metabolism (such as the sodium/potassium ATPase) are essential for survival. There is very good evidence that basal metabolic rate is proportional to lean body mass. Since obese subjects have elevated lean body mass, they have elevated basal metabolic rate. Post-prandial thermogenesis is considered to be due to the energy needed to digest and store food and thus is similar between individuals. Spontaneous activity is almost certainly genetic. This leaves only voluntary activity as our only discretionary influence on daily energy expenditure. To substantially increase energy expenditure requires a lot of activity. Most obese subjects do not have the inclination, stamina or fitness to undertake vigorous long bouts of physical activity. Reducing energy intake below expenditure will result in stored energy to be mobilised in order to supply the body's obligatory needs. It does not matter which food group is restricted, provided total energy intake is reduced. Hence the recent results.

So now let's ask, why is long-term weight loss so difficult to achieve? Weight is regulated and there is emerging evidence that it is vigorously physiologically defended. The central regulator is found in the hypothalamus. The process begins in the arcuate nucleus. In this area of the hypothalamus, there are neurons that produce NPY and AGRP and that stimulate hunger and the desire to eat. Nearby there are other neurons that produce CART and αMSH that powerfully inhibit food intake. These "first order" neurons project to other areas of the hypothalamus that mediate the conscious desire to eat or not eat. The arcuate nucleus is one area of the brain that sits outside the blood brain barrier. The major regulators of these first order neurons are circulating hormones made and secreted by peripheral organs. There are a surprising number of circulating factors that can influence food intake. These include Ghrelin (made in the stomach); CCK, PYY, GLP-1, Oxyntomodulin (made in the small bowel); leptin (made in fat cells) and insulin, amylin and pancreatic polypeptide (made in the pancreas). It is interesting to note that of these only 1 (ghrelin) stimulates hunger while all the rest inhibit food intake.

Following weight loss, many changes occur that are designed to encourage weight regain. Firstly there is a mild decrease in energy expenditure (maximally about 300 Kcal/day). Ghrelin levels increase while leptin, CCK, insulin, amylin, PYY all decrease. The net effect is for hunger to increase. The weight-reduced obese individual is then in the difficult situation of being hungrier than normal while being surrounded by freely available food. It is not surprising then that gradual weight regain is almost inevitable.

### Conclusion

The answer to the question posed in the title is a clear and unambiguous NO. Diets do not matter. The best way to lose and keep weight off (if one wants to avoid surgery) is to lose weight by reducing calorie intake and then after weight loss is achieved to maintain weight loss with diet, exercise and appetite suppressants. We have recently shown that the best way to lose weight is rapidly using a safe properly formulated VLED. During the weight loss phase, external appetite suppressants are not required because the ketosis induced by the VLED suppresses hunger. Any appetite suppressant that is used must be safe long term. At present we have sibutramine, which is available in Australia for weight loss and topiramate that is available for epilepsy, migraine and nerve pain but not weight loss. As such, if used to control hunger, it needs to be used off label. However there is an extensive literature showing that it can cause weight loss.

### Source of funding

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# Concurrent Session 5: Obesity/Insulin Resistance

## The effects of weight loss versus weight loss maintenance on sympathetic nervous system activity and metabolic syndrome components

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**Background**
Sympathetic nervous system (SNS) over-activity participates in both the pathogenesis and adverse clinical complications of metabolic syndrome (MetS) obesity.
**Objective**
To compare the effects of active weight loss and weight loss maintenance on SNS function and MetS components.
**Design**
Eighteen (14 M, 4 F) untreated subjects, mean age 53 ± 1 yr, BMI 30.9 ± 0.9 kg/m$^2$, who fulfilled ATP III MetS criteria participated. A 12-week hypocaloric diet using a modified DASH diet (30% fat, 22% protein, 48% carbohydrate)(WL) was followed by a 4-month weight loss maintenance (WM) program. Measurements of muscle sympathetic nerve activity (MSNA) by microneurography, whole-body noradrenaline (NA) kinetics by isotope dilution, cardiac baroreflex sensitivity (BRS), plasma renin activity (PRA), substrate oxidation by indirect calorimetry, anthropometric, metabolic and cardiovascular parameters were performed at baseline and at the end of WL and WM phases.
**Outcomes**
Body weight changes averaged -9.3 ± 0.8 % at week 12 and -9.8 ± 1.0 % at the end of WM (P both <0.001). Respiratory quotient and carbohydrate oxidation decreased at week 12 and increased after WM (P all <0.05). NA spillover rate decreased by 23 ± 16% (P=0.006), MSNA by 25 ± 3 bursts per 100 heart-beats (P<0.001), supine PRA by 0.25 ± 0.09 ng/ml/h (P=0.01), clinic blood pressure by 13 ± 3/5 ± 2 mmHg (P<0.001), whilst BRS increased by 5.2 ± 2.2 msec/mmHg (P=0.004) and insulin sensitivity by 59 ± 12% (P<0.001) at week 12. At the end of WM, beneficial effects of weight loss on NA spillover rate, blood pressure, insulin sensitivity, plasma leptin, triglyceride and C-reactive protein concentrations were preserved. In contrast, there were significant attenuations in MSNA (by 20 ± 5 bursts/100 heartbeats, P=0.001 versus WL), BRS and PRA after WM.
**Conclusion**
Divergent effects of successful WM on whole-body NA spillover and MSNA suggest organ specific differentiation in sympathetic neural adaptation to weight loss under conditions of negative versus stable energy balance.
**Source of funding**
Supported by grants from Diabetes Australia and the National Health and Medical Research Council.

## Timing of protein ingestion relative to resistance exercise training does not influence body composition, energy expenditure, glycaemic control or cardiometabolic risk factors in a hypocaloric, high protein, low fat diet in patients with type 2 diabetes

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**Background**
Altering the timing of ingestion of protein relative to resistance exercise training (RT) within a high protein, low fat energy restricted diet may have implications for body composition, resting energy expenditure (REE), glycaemic control and cardiometabolic risk factors. The effect in patients with type 2 diabetes (T2DM) remains unknown.
**Objective**
To investigate timing of protein ingestion relative to RT during weight loss on a high protein, low fat diet in overweight and obese patients with T2DM.
**Design**
34 men/women with T2DM (age: 57±7 yrs, BMI: 34.9±4.2 kg.m$^2$) were randomly assigned to the ingestion of a high protein meal (860 kJ, 21 g Pro, 29.6 g Cho, 0.7 g Fat) either immediately prior to RT or at least 2 hours following RT. All participants followed a 16-week, energy restricted (6-7 MJ.day$^{-1}$), high protein, low fat diet (HP; Cho:Pro:Fat, 43:33:22) and participated in supervised RT (3d.wk$^{-1}$).
**Outcomes**
There was an overall reduction in bodyweight (-11.9±6.1 kg), fat mass (-10.0±4.4 kg), fat-free mass (-1.9±3.1 kg), waist circumference (-12.1±5.3 cm), REE (-742±624 kJ.day$^{-1}$), glucose (-1.9±1.7 mmol.L$^{-1}$), insulin (-6.1±6.7 mU.L$^{-1}$) and HBA1c (-1.1±0.1%), P≤ 0.01 time for all variables, with no difference between groups (P≥0.41 group effect). Strength improved and cardiometabolic risk factors were reduced similarly in both groups; single repetition maximum chest press 11.0±8.7 kg, single repetition maximum lat pull-down 9.9±6.0 kg, total-C -0.6±0.5 mmol.L$^{-1}$, HDL-C -0.1±0.2 mmol.L$^{-1}$, LDL-C -0.3±0.5 mmol.L$^{-1}$, triglycerides -0.6±0.7 mmol.L$^{-1}$, blood pressure [systolic/diastolic] -13±10/-7±7 mmHg (P≤0.04 time effect, P≥0.24 group effect).
**Conclusion**
A high protein, low fat, energy restricted diet with RT was effective in improving glycaemic control, body composition, strength and cardiometabolic risk factors in overweight/obese patients with T2DM irrespective of altering the timing of protein ingestion relative to RT.
**Source of funding**
Funding provided from the Heart Foundation of Australia, Diabetes Australia Research Trust and the Pork CRC. Foods for this study donated by George Weston Foods.

# Concurrent Session 5: Obesity/Insulin Resistance

## Learning to prefer an obesity protective diet: results of the Melbourne InFANT Program

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**Background**
During early childhood (0-5 yrs) obesity-promoting behaviours, including eating behaviors are established.
**Objective**
This paper describes the impact of The Melbourne Infant Feeding Activity & Nutrition Trial (InFANT) Program, an obesity prevention intervention delivered to first-time parents in existing social groups, on parents views regarding appropriate food and feeding and on young children's obesity promoting eating behavours.
**Design**
The Melbourne InFANT Program is a cluster-randomised controlled trial involving 542 first-time mothers (87% recruitment uptake, 9% attrition). The program focused on providing mothers with knowledge and skills to enable them to support the development of positive diet and physical activity behaviours and reduced sedentary behaviours in their infants. The program involved quarterly two hour sessions for intervention parents and ran for 15 months, from when infants were aged 3 to 18 months.
**Outcomes**
At intervention conclusion, compared to control group mothers, a significantly higher proportion of intervention group mothers believed parents should included fruit or vegetables in all children's meals and snacks (96% vs 91%, P=0.04), that TV should be turned off when children are eating meals (90% vs 81%, P=0.02), and that parents shouldn't fuss if their child doesn't eat their meal (94% vs 85%, P=0.004). Compared to control group mothers, a significantly lower proportion of intervention group mothers believed parents should offer other foods if their child doesn't eat their meal (20% vs 44%, P<0.001) and that parents should encourage children to finish all food on their plates (29% vs 42%, P=0.08). Overall, compared to control group children, children in the intervention group were significantly more likely to consume more than two serves of fruit per day (81% vs 70%, P=0.03); to consume more than two serves of vegetables per day (56% vs 42%, P=0.04) and to eat more than two serves of both fruit and vegetables per day (38.6% vs 25.6%, P<0.001).
**Conclusion**
The Melbourne InFANT Program, a low-dose, low-cost obesity prevention intervention, shows promising impact on parent attitudes to children's feeding and on children's consumption of fruits and vegetables.
**Source of funding**
This research was funded by a NHMRC project grant and supported by Heart Foundation Victoria.

## Normal insulin demand for dose adjustment: the NIDDA study

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**Background**
Carbohydrate counting assumes only carbohydrates influence the dose of insulin required in type 1 diabetes. A food insulin index (FII) has been developed and validated for predicting normal insulin demand generated by mixed meals in healthy adults. It may have clinical application in estimating mealtime exogenous insulin dose in type 1 diabetes.
**Objective**
To compare the novel algorithm based on normal insulin demand with conventional carbohydrate counting on measures of postprandial glycaemia in adults with type 1 diabetes on insulin pump therapy.
**Design**
Twenty eight participants consumed two different breakfast meals of equal energy, glycaemic index, fibre and insulin demand (FII = 60 for both meals) but two-fold difference in carbohydrate content in random order on three consecutive mornings. Meal A was consumed using CHO counting only. Meal B was consumed on two occasions, once using CHO counting as the basis for the insulin dose (ie half the dose of insulin) and once using the novel algorithm (same dose as Meal A). Continuous glucose monitoring system was used to assess blood glucose levels (BGL).
**Outcomes**
Compared with carbohydrate counting, the novel algorithm significantly decreased glucose incremental area under the curve over 3 h (by 56%, P < 0.01), peak glucose excursion (P = 0.001) and improved the percentage of time within the normal BGL range (4 – 10 mmol/L) (P < 0.001). The number of hypoglycaemic episodes was similar.
**Conclusion**
The current findings indicate that the use of a novel algorithm based on normal insulin demand in healthy subjects may improve glycaemic control without increasing the risk of hypoglycaemia in type 1 diabetes patients using insulin pump therapy.
**Source of funding**
Funded by internal revenue, the University of Sydney

# Concurrent Session 5: Obesity/Insulin Resistance

## Association of dietary and dieting behaviours with waist circumference change in Australian adults: a 15-yr longitudinal study

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**Background**
Abdominal obesity increases the risk of chronic disease, but there is little evidence of the determinants of abdominal fat assessed by waist circumference (WC), and patterns of change over time.
**Objective**
To examine the relationship between dietary and dieting behaviours and change in WC in Australian adults between 1992 to 2007.
**Design**
WC was measured in 1992 and 2007 in 1,317 participants of the Nambour Skin Cancer Study aged 25-75 yrs at baseline. Data on diet and lifestyle factors were reported using self-completed questionnaires. The associations between WC and its determinants over a 15-yr time interval were analyzed using generalised estimating equations (GEE). Multivariable models were adjusted for confounding factors and stratified by sex.
**Outcomes**
WC gain was higher in women than men (0.25 cm/yr vs. 0.12 cm/yr). Consumption of visible fat on meat was associated with WC gain over time in men (0.38 cm/yr in those who ate most of fat on meat vs. 0.07 cm/yr in those who cut it off, P=0.006). Also, men, who ate fried foods at home >=1/day had greater WC gain compared to those who consumed fried foods <1/week (multivar. adj: 0.43 cm/yr vs. 0.07 cm/yr). In women, consumption of visible fat on meat was also associated with WC gain (0.60 cm/yr in those who ate most of fat on meat vs. 0.23 cm/yr in those who cut it off, P=0.03). Attempts to lose weight was associated with WC gain in women, with the highest gain in those who "always" tried to lose weight compared with those who never tried (0.48 cm/yr vs. 0.17 cm/yr, P=0.002).
**Conclusion**
Consumption of visible fat on meat, high frequency of eating fried foods at home, and intentional weight loss were associated with gain in waist circumference in this Australian population.
**Source of funding**
National Health and Medical Research Council of Australia (data collection) for the Nambour Skin Cancer Study. S Arabshahi is supported by a University of Queensland Graduate School Research Scholarship and a Top-Up Scholarship from the Queensland Institute of Medical Research.

## The effect of overweight and obesity on blood pressure responses and recovery to acute psychological stress in men

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**Background**
Psychological stress is one of many mental health disorders which contribute to the global burden of disease. Overweight and obese individuals may have exaggerated blood pressure responses and delayed recovery from stress.
**Objective**
To determine the effect of overweight and obesity on blood pressure response and recovery to acute psychological stress.
**Design**
In a parallel design, lean (BMI 20-25 kg/m$^2$) and overweight/obese (BMI 27-35 kg/m$^2$) men aged 50-70 yr completed an acute psychological stress test (60 minutes resting, 30 minutes stress, 90 minutes recovery). Clinical blood pressure (BP) was measured (resting- 5 measurements, stress- 4 measurements, recovery- 6 measurements). The effect of body type on BP responses during resting, stress and recovery was assessed using repeated measures ANOVA (body type x time) with covariates of resting BP and age.
**Outcomes**
Forty-two men completed the study [lean (n=25), mean±SD BMI 23.5±1.2 kg/m$^2$ and overweight/obese (n=17), BMI 31.2±2.5 kg/m$^2$]. The overweight/obese compared to the lean men were younger (mean±SEM 59.6±1.2 yr versus 63.5±1.0 yr, P=0.015) and had a higher resting systolic BP (SBP) (125.8±2.6 mmHg versus 117.3±2.2 mmHg, P=0.015) and diastolic BP (DBP) (72.3±2.2 mmHg versus 65.8±1.2 mmHg, P=0.007). Overall, there was no effect of body size on BP response to stress (SBP, P=0.422; DBP, P=0.941). During stress for both groups, there was a significant increase in SBP [lean: +26.0±2.5 mmHg, overweight/obese: +20.6±2.8 mmHg, P<0.05 for both] and diastolic BP (DBP) [(lean: +16.1±1.8 mmHg, overweight/obese: +15.2±1.6 mmHg, P<0.05 for both]; however, there was no difference in the change in SBP and DBP during stress between the groups, P>0.05 for both. There was no significant difference in recovery time between groups for both SBP and DBP (data not shown, P>0.05).
**Conclusion**
Older men who were overweight/obese in comparison to lean men had a higher resting BP, but did not appear to have a more exaggerated BP response to stress and delayed recovery after stress.
**Source of funding**
Internal grant at Deakin University

# Concurrent Session 6: Food Factors 1

## Inhibition of 20-hydroxyeicosatetraenoic acid synthesis using plant lignans: *in vitro* and human studies

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**Background**
Sesamin, the major lignan found in sesame, has been shown to increase vitamin E levels, by inhibiting its metabolism via the Cytochrome $P_{450}$ isozyme, CYP4F2. CYP4F2 together with CYP4A11 are the predominant human isoforms of the enzyme that synthesise 20-hydroxyeicosatetraenoic acid (20-HETE) from arachidonic acid (AA). Considerable evidence suggests that 20-HETE plays a role in the pathogenesis of hypertension.

**Objective**
We hypothesised that sesamin could be an inhibitor of 20-HETE synthesis and investigated the effects of sesamin on 20-HETE synthesis *in vitro*, and the effect of sesame supplementation on plasma and urinary 20-HETE concentrations in humans.

**Design**
Human microsomes were used to investigate the potency and selectivity of sesamin inhibition of 20-HETE synthesis. In a randomised controlled cross over trial, overweight men and women (n=33) consumed 25 g/day of sesame (~50 mg/day sesame lignan) or an iso-caloric matched control for 5 weeks each. .

**Outcomes**
Sesamin inhibited human renal and liver microsome 20-HETE synthesis with $IC_{50}$ <20μM. It was selective towards CYP4F2 ($IC_{50}$ =1.9μM) and had reduced activity towards CYP4A11 ($IC_{50}$ >150μM), as well as CYP epoxygenation of AA ($IC_{50}$ >50μM). Relative to control, sesame supplementation resulted in a 28% decrease in plasma and a 32% decrease in urinary 20-HETE ($P$ < 0.001). Urinary sodium, potassium and blood pressure were not affected.

**Conclusion**
This study demonstrates for the first time that sesame supplementation in humans reduce plasma and urinary level of 20-HETE, likely via inhibition of CYP4F2 by sesame lignans. These results suggest that sesame lignans could be used for the investigation of potential roles of 20-HETE in humans.

**Source of funding**
National Health & Medical Research Council project grant 403957. Royal Perth Hospital Medical Research Foundation.

## Acute effects of lupin and soy on glycaemia in type 2 diabetes

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**Background**
Addition of fibre or protein to carbohydrate-rich foods could reduce their glycaemic effects in individuals with type 2 diabetes. Lupin flour is a novel food ingredient containing high levels of fibre and protein. The acute effects of lupin flour on blood glucose and insulin concentrations in Type 2 diabetes are unknown.

**Objective**
We assessed the acute effects of a lupin-based beverage on glucose and insulin responses in type 2 diabetic individuals, and compared these effects with a soy-based beverage.

**Design**
In a randomised controlled cross-over trial, 24 diabetic adults (14 men, 10 women) attended three testing sessions, each at least one week apart. At each session, participants consumed a beverage containing 50 g glucose (control), 50 g glucose plus lupin kernel flour with 12.5 g fibre and 22 g protein (lupin), or 50 g glucose plus 12.5 g fibre and 22 g protein from soy isolates (soy). Serum glucose, insulin and C-peptide were measured periodically for 4 h following beverage consumption.

**Outcomes**
Compared with control, both lupin and soy were associated with lower mean 4 h post beverage serum glucose (control: 9.7±1.6 mmol/L; lupin: 8.9±1.7 mmol/L; soy: 8.7±1.7 mmol/L) and higher insulin (control: 32±25 mU/L; lupin: 42±28 mU/L; soy: 48±37 mU/L) and C-peptide (control: 1.9±0.8 nmol/L; lupin: 2.1±0.8 nmol/L; soy: 2.2±0.9 nmol/L) concentrations (P<0.001, for all). Mean glucose (P=0.25) and C-peptide (P=0.07) concentrations did not differ significantly between lupin and soy, but lupin was associated with lower mean insulin concentrations compared with soy (P=0.013).

**Conclusion**
Adding lupin or soy to a carbohydrate-rich beverage reduces glycaemia acutely in type 2 diabetic individuals.

**Source of funding**
Western Australian Government, Department of Industry and Resources.

# Concurrent Session 6: Food Factors 1

## Iodine status in office workers

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**Background**
Iodine is essential for thyroid hormone production and deficiency results in reduced work productivity and impairs mental function in adults. Maternal deficiency results in growth and developmental effects in children. There are insufficient data on the iodine status of healthy adults in Australia.

**Objective**
To determine iodine status in healthy office workers and its relationship with iodised salt use.

**Design**
A cross-sectional study was conducted in healthy Nestlé office workers aged >18 yr who were not pregnant or breastfeeding, with a major illness or on iodine-containing medications or supplements. Anthropometric measurements (height, weight and waist circumference), and a spot urine sample were taken and questionnaires to assess iodised salt use, fish and seafood intake, work environment (position, hours per week) and physical activity were administered. Urinary iodine (UI) and creatinine (Cr) concentration were determined.

**Outcomes**
A total of 104 participants (41 males, 63 females) aged 19-64 yrs completed the study (mean±SD; age 36.2±8.6 yr, BMI 24.6±4.2 kg/m$^2$, waist circumference 76.8±11 cm). The median UI was 106±9.7 µg/L, and 49% of adults had UI<100 µg/L. Median UI/Cr ratio was 246 µg/g. Prevalence of iodine deficiency based on The World Health Organisation categories for iodine deficiency were: 51%- not deficient; 37% had mild; 13% had moderate; and severe deficiency was not present. Iodised salt users (40%) had significantly higher UI than non-users (117±19 vs. 87±10 µg/L, P<0.05). Fish and seafood avoiders (n=34) had lower, but non-significant UI than fish and seafood consumers.

**Conclusion**
These results indicate marginal iodine intake in a population of healthy adults and emphasises the importance of regular monitoring. Use of iodised salt significantly improved iodine status and therefore, universal salt iodisation may be the most cost-effective way to eliminate iodine deficiency in Australia.

**Source of funding**
Research grant from Nestlé Australia Pty Ltd.

## The sodium content of Australian processed meat products

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**Background**
The Australian Division of World Action on Salt and Health (AWASH) launched a campaign to reduce population salt intakes in May 2007 and has been calling on the food industry to reduce salt in processed foods. The government's Food and Health Dialogue has since established a reformulation working group and is currently considering the establishment of salt targets for processed meat products. The UK government has already established targets for all major food categories that contribute to dietary salt including processed meats.

**Objective**
The objective of this project was to identify sodium levels in Australian processed meat products from AWASH's 2009 branded food composition database as a baseline from which future changes can be monitored.

**Design**
Sodium data (per 100 g and per serve) were collected for 552 processed meat products. Mean levels of sodium were compared to the UK Food Standards Agency (FSA) salt targets.

**Outcomes**
Less than 10% of the 552 processed meat products met the salt targets set by the UK FSA. No salami products, 43% of bacon products, 14% of canned meat products, 5% of frozen meat products, 30% of meat burgers, 3% of sausages and 5% of sliced sandwich meats met the FSA targets.

**Conclusion**
Meat and meat products contribute a significant amount of salt in the diet, accounting for around a fifth of people's salt intakes in Australia. There is clearly huge potential for reducing the salt content of processed meat products. AWASH will continue to push for challenging targets for salt levels in processed meat products and will be carefully monitoring progress towards achieving these targets across all food categories, company by company.

**Source of funding**
Not applicable.

# Concurrent Session 6: Food Factors 1

## Bioaccessibility of sugars from fruits and vegetables as influenced by surface area

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**Background**
Glycaemic response has been associated with surface area of ingested foods and may be related to the rate and extent of sugar release under gastro-intestinal conditions.
**Objective**
To investigate the effect of surface area, fruit/vegetable type, digestion conditions and sugar type on the rate and amount of sugars available for absorption in the intestine.
**Design**
Apple, rockmelon, raw carrot, and steamed beetroot with surface areas ranging from 1080 to 2214 $mm^2$ were cubed by a specifically designed cutting device. Plant cell architectures were examined by bright field microscopy. Release of individual sugars over 2-h of in vitro digestion was quantified by HPLC-ELSD, using a complete randomised design with two replicates.
**Outcomes**
All sugar release profiles fitted well to a first-order kinetics model ($R^2 \geq 0.97$). The rate and extent of digestion were not significantly ($P>0.05$) different for glucose, fructose, sucrose, and sorbitol. The sugar release rate from raw carrot was consistently lower than that for the other food types, and was associated with the difference in cell sizes between the food types. From 71% to 87% of total sugars were accessible, and about 50% of the sugars was released during the simulated gastric digestion. The release rate of sugars was found to be a linear function of surface area. Significant correlations were found between the rate ($R=0.966$, $P<0.01$) or extent ($R=0.971$, $P<0.01$) of sugar release in the present study and a Glycaemic Index (GI) difference (calculated as the published fruit/vegetable GI - the GI of component sugars).
**Conclusion**
The rate and extent of sugar release was significantly influenced by surface area and fruit/vegetable type, but not the type of the sugar. Surface area and cell architectures of ingested fruits and vegetables may modulate the resulting glycaemic responses by affecting sugar release rates during digestion.
**Source of funding**
Australian Development Scholarships awarded to Hoerudin.

## Individual differences in oral sensitivity to oleocanthal and PROP and the relationship with nutrient intake and BMI

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**Background**
Oleocanthal is a natural NSAID in extra virgin olive oil that elicits a localised irritation in the oro-pharynx region. Large variability in sensitivity to oleocanthal has been reported, similar to variability reported for the bitter compound 6-*n*-propylthiouracil (PROP). Individual variability in sensitivity to PROP has been linked with dietary intake, but it is unknown if sensitivity to oleocanthal is related to dietary intake.
**Objective**
To determine the extent of individual variation in oral sensitivity to oleocanthal and PROP and to determine if a relationship exists between oral sensitivity to these stimuli, nutrient intake and Body Mass Index (BMI).
**Design**
Subjects (n=87, 74 female, 13 male, 20.3±0.4 years, BMI 22.0±0.3 $kg/m^2$) were screened for oral sensitivity to oleocanthal and PROP by rating perceived intensity on the gLMS. BMI was calculated from height and weight measurements and dietary intake was collected from 4-day food record questionnaire. Pearsons product moment correlations were used to analyse results. P values > 0.05 were considered statistically significant.
**Outcomes**
There was large variability in perceived intensity of oral irritation from oleocanthal (M= 32.0, SD =12.53, gLMS, range 4.26-59.70) and PROP (M=28.7 SD=19.07 glms, range 0.0 - 95.5). Oral sensitivity to oleocanthal and PROP were not correlated (r= -0.12, P= 0.92). There was no correlation between oleocanthal sensitivity and BMI (r= 0.21, P= 0.86), total energy intake (r= 0.13, P= 0.22), carbohydrate intake (r= 0.15, P= 0.18), protein intake (r= 0.12, P= 0.26) or fat intake (r= 0.12, P= 0.28). There was no correlation between PROP sensitivity and total energy intake (r= -0.41, P= 0.71), carbohydrate intake (r= -0.13, P= 0.90), protein intake (r= -0.06, P= 0.59), or fat intake (r= -0.27, P= 0.80).
**Conclusion**
Oral sensitivity to chemicals in foods is highly variable. This study found no link between oral sensitivity to oleocanthal or PROP and macronutrient intake suggesting that individual taste perception of these compounds does not play a role in dietary intake.
**Source of funding**
This research was funded by the Strategic Research Cluster, CPAN, Deakin University

# Concurrent Session 6: Food Factors 1

## *In vitro* evaluation of functional properties of probiotic combinations in fermented goat's milk

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**Background**
Goat's milk and goat's milk products are attractive to some consumers due to their therapeutic properties and nutritional value. The health promoting value of these foods could be further enhanced by the addition of probiotics. To provide beneficial health effects probiotic bacteria must survive through the gastrointestinal tract, adhere and then colonise in the gut epithelium. These functional properties can be influenced by the different combinations of probiotics used in product development due to synergistic and or antagonistic effects.

**Objective**
The objective of this study was to evaluate the effect of different probiotic combinations in fermented goat's milk on upper gastrointestinal tolerance and adhesion to intestinal epithelium *in vitro*. Production of proinflammatory cytokine, interleukin-6 (IL-6) and Tumor necrosis factor-α (TNF-α) by different probiotic combinations in human intestinal epithelial cell line Caco-2 was also evaluated.

**Design**
Newly identified potential probiotic *Propionibacterium jensenii* 702 (PJ 702) and commercially available *Bifidobacterium lactis* BB 12 (BB 12), and *Lactobacillus acidophilus* (LA) were used for this study. Fermented goat's milk was produced with three probiotics alone and with the following probiotic combinations: PJ 702 x BB 12, PJ 702 x LA, BB 12 x LA and PJ 702 x LA x BB 12. The transit tolerance of probiotics in fermented goat's milk was determined using simulated gastric juices at pH 2.0 and simulated small intestinal juices (pH 8.0) with or without 0.3% bile salts. Adhesion ability of probiotics was measured Caco-2 at 37 $^{o}$C and spread plating on selective media. ELISA assays were conducted to evaluate cytokine production.

**Outcomes**
Only certain combinations (LA, BB 12 and LA x BB 12) were able to induce cytokine production by Caco-2 cells. Lower survival of probiotics was observed in the presence of 0.3% bile salts. Adhesion ability was dependent on bacterial combinations with the most significant adhesion ability observed when PJ702 was incorporated with BB12.

**Conclusion**
Probiotic combinations seemed to play an important role in gastrointestinal tolerance, adhesion ability and induction of cytokine production. Therefore, it is important to select the most suitable probiotic combinations when developing novel probiotic products to achieve maximum health benefits.

**Source of funding:** University of Newcastle.

# Concurrent Session 7: Nutrition Epidemiology and Public Health

## Coffee and coronary heart disease

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There is only space here for one of the stories in the history of research on diet and coronary heart disease (CHD)[1]. Coffee is fashionable and the coffee - CHD story is instructive.

Nearly all the large prospective studies of diet and CHD reported 1973-1996 no significant association with usual cups of coffee per day. Among these were Kaiser Permanente, Framingham, Hawaii Japanese, Evans County, Lutheran Brotherhood, Health Professional, and Nurses Health Study. But four well-run studies were exceptions, one in Norway and one a long-term follow up of graduates of Johns Hopkins Medical School (which repeated coffee consumption recording).

This situation is usually clarified by meta-analysis. One at UCLA by Greenland (1973) and another at Harvard by Kawachi (1994) neither found significant association.

There were also conflicting reports whether coffee raises serum cholesterol. Over half the 14 studies 1966-1983 found no significant correlation. Thelle (Tromso) (1983) reported a clear dose-response in 14,000 people. He asked why were others not finding this. They moved to experiment, comparing cholesterol levels when men drank the boiled coffee of northern Norway, or filtered coffee or neither. Only boiled coffee raised serum cholesterol. This was soon confirmed in Finland and Amsterdam.

Zock and Katan centrifuged boiled coffee and found a thin lipid layer on top. Large-scale equipment produced coffee lipid concentrate from boiled coffee. They used 150 kg ground coffee boiled with 1350 L water to have enough to test. This (1.3 g lipid per day for 6 weeks) in 10 volunteers raised serum cholesterol by 22%. (No triglyceride is this potent). The active substance must be in the unsaponifiable fraction.

In 1994 Katan (Wageningen) and Heckers (Giessen) independently showed this is cafestol and kahweol, two diterpenes naturally present in roasted coffee beans. It does not pass through filter paper.

[1] Truswell AS (2010) Cholesterol and beyond. The research on diet and coronary heart disease, 1900-2000. Springer, Dordrecht.

## Effects of habitual chocolate intake on vascular disease: a prospective study of risk factors and clinical outcomes in older women

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**Background**
Randomised controlled trials have shown that flavonoid-rich cocoa and chocolate can improve endothelial function and reduce blood pressure. However, it remains unclear whether regular chocolate consumption is associated with a reduced risk of vascular disease in large populations.

**Objective**
To investigate the relationship between the chocolate consumption and atherosclerotic vascular disease (ASVD) in a population based prospective study of older women.

**Design**
Chocolate consumption was assessed by questionnaire. Verified ASVD events over 9.5-years were obtained for all participants using the Western Australian Data Linkage System. Organ status and risk factors for ASVD were evaluated at various times during the study.

**Outcomes**
ASVD hospitalisation or death was less common in participants who consumed chocolate frequently, ≥ 1 serve/week (24.3 events per 1000 person years) compared to rarely, < 1 serve/week (33.2 events per 1000 person years): age-adjusted hazard ratio 0.74 (95% CI 0.59-0.94) P = 0.01. The group that consumed chocolate regularly also had reduced risk of ischemic heart disease, heart failure and a reduced prevalence of carotid atherosclerotic plaques. Individuals who consumed chocolate frequently had a 3.6% lower fat mass despite 9.3% higher energy consumption.

**Conclusion**
Frequent chocolate consumption in elderly women was associated with a reduction in the prevalence of atherosclerotic plaques and ASVD events, principally due to reduced cardiac events. Given the size of the beneficial effect in a population also receiving other accepted treatments to reduce ASVD risk these findings suggest that a randomised controlled trial of increased chocolate consumption should be supported.

**Source of funding**
The study was supported by a research grant from Healthway Health Promotion Foundation of Western Australia and by the project grants 254627, 303169 and 572604 from the National Health and Medical Research Council of Australia.

# Concurrent Session 7: Nutrition Epidemiology and Public Health

## Association of dairy product consumption with common carotid artery intima-media thickness and cardiovascular risk factors in elderly women

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**Background**
Despite the contribution of dairy foods to total dietary saturated fat intake, available data indicates that dairy consumption may lower risk of cardiovascular disease.
**Objective**
To investigate the relationship between dairy consumption, including milk, cheese and yoghurt, with common carotid artery intima-media thickness (CCA-IMT) in a cohort of elderly women.
**Design**
Dairy consumption was assessed by a validated food frequency questionnaire in 1,080 participants randomly selected from ambulant, Caucasian women aged over 70 years, living in Perth, Western Australia. CCA-IMT was assessed using B-mode carotid ultrasound. Cardiovascular disease risk factors including serum lipids and blood pressure were assessed at baseline.
**Outcomes**
Total dairy, milk and cheese consumption were not associated with CCA-IMT (P>0.05). Yoghurt consumption was negatively associated with CCA-IMT (unadjusted R= -0.081, P=0.008) and positively associated with HDLC (unadjusted R=0.075, P=0.018; multivariable-adjusted R=0.058, P=0.059). Participants with yoghurt consumption greater than 100 g/day had significantly lower CCA-IMT than participants with lower consumption (unadjusted mean difference=0.022 mm, P=0.024). This relationship remained significant after adjustment for baseline risk factors and HDLC levels (P=0.033).
**Conclusion**
Increased consumption of yoghurt, but not other dairy products, may contribute to the lowering of CCA-IMT and improvement of plasma HDLC in elderly women. The results suggest that the effect of yoghurt consumption on CCA-IMT is not accounted for by HDLC levels alone, and is independent of known risk factors.
**Source of funding**
Supported by a research grant from Healthway Health Promotion Foundation of Western Australia and by the project grants 254627, 303169 and 572604 from the National Health and Medical Research Council of Australia.

## Salt intake is related to sugar-sweetened soft drink consumption in Australian children

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**Background**
Seventeen percent and 6% of Australian children are overweight and obese, respectively. Consumption of sugar-sweetened beverages (SSBs) is related to childhood obesity. Australian children's salt intake is known to be high and one study has found children's salt intake is associated with total fluid and SSB consumption, identifying a possible link between salt intake and obesity.
**Objective**
To examine the relationship between; dietary salt intake and total fluid and SSB consumption; and SSB consumption and Body Mass Index (BMI) in a nationally representative sample of Australian children aged 2-16 years.
**Design**
Analysis of the cross-sectional Australian 2007 Children's National Nutrition and Physical Activity Survey. Consumption of dietary salt, total fluid and SSB was determined in 4487 children via two 24-hour dietary recalls. Regression analysis was used to assess the relationship between dietary salt, total fluid, SSB consumption and BMI, with adjustment for confounders.
**Outcomes**
Thirty-nine percent of children reported consuming SSBs. Of those consuming SSBs the median intake was 203 g/d. Dietary salt intake was significantly related to total fluid (r=0.4, P<0.001) and SSB consumption (r=0.3, P<0.001). After adjustment for age, gender and BMI, one gram of salt was associated with a 50 g/d greater total fluid intake (P<0.001) and a 14 g/d increase in SSBs (P<0.001). SSB consumption was significantly related to BMI (r=0.2, P<0.001). After adjustment for age, gender and energy intake a serve of SSB (250 g) was associated with a 0.15kg/m$^2$ higher BMI (P<0.05).
**Conclusion**
The high salt diet of Australian children could be contributing to weight gain in some children, through the increased consumption of SSBs. Salt reduction strategies could assist in the prevention and management of childhood obesity.
**Source of funding**
Supported by Helen MacPherson Smith Trust and postgraduate scholarship from the Heart Foundation (Australia).

# Concurrent Session 7: Nutrition Epidemiology and Public Health

## Burning daylight: balancing vitamin D requirements while preventing skin cancer

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**Background**
The body's principal source of 25-hydroxyvitamin D (vitamin D) is exposure to ultraviolet radiation (*UVR*) from sunlight. Adherence to sun protection guidelines aimed at prevention of skin cancer has resulted in a high prevalence of vitamin D deficiency. The current recommended daily intake is 200–600 International Units (*IU*) (5-15 micrograms) vitamin D, but it has been suggested that 1000–2000 *IU* (25–50 microgram) of vitamin D daily is necessary to reduce chronic disease.

**Objective**
This article examines the feasibility of achieving these levels of vitamin D from *UVR* exposure without compromising sun protection measures.

**Design**
Hourly ultraviolet index (*UVI*) and standard erythemal dose (*SED*) for seven Australian cities were obtained to determine "efficient" *UVR* exposure periods, which we defined as the time of day when one-third of the minimal erythemal dose (*MED*) is achieved within 30 minutes, when *UVI* is below 3. One-third *MED* is adequate to produce 1000–2000 IU of vitamin D with 17–28% skin exposure without sunburn.

**Outcomes**
*UVI* and duration to achieve one-third *MED* varied according to time of day, season, and city. Theoretically, vitamin D requirements can efficiently be met for all cities and seasons, except in Townsville during spring. In summer, efficient periods occurred in the early morning or late afternoon only. In winter, at more northerly Australian latitudes, high *UVI* periods persisted during the day, whereas at more southerly latitudes, *UVI* remained below 3 throughout the day.

**Conclusion**
1000–2000 *IU* vitamin D can be efficiently synthesised from sunlight exposure at specific times in most Australian cities throughout the year. However, exposure times are limited, and as other factors reduce vitamin D synthesis, supplementation may be essential to meet recommendations.

**Source of Funding**
Not applicable

## Biomarkers of milk fat intake and metabolic syndrome risk factors in overweight adults participating in a weight loss trial

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**Background**
Dairy products are a significant source of saturated fat which has been associated with risk factors characterizing the metabolic syndrome, but given its high nutritional value, milk is usually included in advice for healthy diets. The whole blood biomarkers heptadecanoic acid (C15:0) and pentadecanoic acid (C17:0) have been validated to objectively estimate milk fat intakes. This may assist in discriminating effects from fat vs. milk as a whole food in the metabolic syndrome context.

**Objective**
To determine whether habitual intakes of milk fat as measured by whole blood biomarkers were associated with metabolic syndrome risk factors in overweight adults participating in a weight loss trial.

**Design**
A cross–sectional study of 70 overweight adults (BMI 31.6± 3.5 kg/m$^2$) participating in the SMART weight loss trial [ACTRN12608000425392] was conducted to determine whether any associations were evident between baseline disease risk factor profiles and biomarkers of milk fat intake. Whole blood concentrations of C15:0, C17:0 and C17:0+C15:0 were determined using gas-liquid chromatography. Associations between baseline weight, BMI, blood pressure, serum lipid profiles, umbilicus circumference and hip circumference were determined using Pearson's Rank Order Correlation. Results were considered significant at $P < 0.05$.

**Outcomes**
Whole blood concentrations of C17:0 were negatively associated with fasting triglycerides (r= -.245, P=.041) and BMI (r=-.263, p=.028), whilst C15:0 concentrations were negatively associated with umbilicus circumference (r= -.255, P= .033) and hip circumference (r= -.239, P= .046). Conversely, total serum cholesterol was positively associated with whole blood concentrations of C15:0 (r=.240, P=.046).

**Conclusion**
In this study sample there were indications of desirable associations between disease risk factors and biomarkers of milk intake that warrant further investigation in the context of the dietary management of metabolic syndrome.

**Source of funding**
NHMRC grant #514631 Tapsell, Batterham, Charlton

# Concurrent Session 8: Nutrients and Health

## Elevated plasma magnesium and calcium are associated with shorter telomeres

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### Background
Telomeres are structures that cap the ends of chromosomes. The integrity of the telomere structure and its DNA hexamer (TTAGGG)n repeat sequence is critical for the protection of the ends of chromosomes from degradation and in maintaining overall genomic stability. Currently, there are limited data on the influence that nutrition has on telomere length. Recent studies have suggested that folate and homocysteine may influence telomere length, with other epidemiological studies indicating that intake of multi-vitamins, vitamin E, processed meat, dietary fibre and omega-3 may influence telomere length.

### Objective
Here we examined the relationship between telomere length in lymphocytes and plasma calcium, magnesium, selenium and zinc status.

### Design
Blood samples were collected from 91 volunteers. This cohort comprised 18M and 25F in the young group (aged 18-31 years), and 25M and 23F in the older group (65-75 yrs). Se, Zn, Mg and Ca were measured both in plasma by ICPMS and ICPOES. Telomere length measures were determined in lymphocytes by qPCR.

### Outcomes
We report a relationship between telomere length and mineral levels, specifically in older females where there was a strong negative association between telomere length and both plasma calcium and magnesium levels, (r=-0.47, P=0.03 and r=-0.61, P=0.001 respectively).

### Conclusion
In conclusion, our study provides new evidence indicating that magnesium and calcium impact on telomere length dynamics in lymphocytes.

### Source of funding
This study was in part funded by NCEFF.

## Steady state red blood cell folate concentration is reached within 12 months of beginning daily folate supplementation

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### Background
Daily supplementation with folate increases red blood cell folate concentrations; however, the time required for concentrations to plateau is uncertain because the duration of published supplementation trials has been too short to reveal a plateau. Predictions based on modelling the change over time in red blood cell folate concentrations suggest that a plateau should be achieved ten months after folate supplementation begins.

### Objective
To determine the time to reach a new steady state red blood cell folate concentration following the adoption of daily folate supplementation.

### Design
A two year, double-blind, placebo-controlled randomised trial in healthy participants aged 65 or over was carried out. The daily vitamin supplement contained 1000 µg of folate (5-methyltetrahydrofolate), 500 µg Vitamin $B_{12}$, and 10 mg of Vitamin $B_6$. Whole blood and plasma folate concentrations were measured by micriobiological assay at baseline and at six, 12, 18 and 24 months.

### Outcomes
Mean red blood cell folate concentration at six months was 1,784 nmol/L (95%CI, 1,615 to 1,954) higher in the vitamin group than in the placebo group. The difference in red blood cell folate concentration between the vitamin and placebo group increased significantly to 2,015 nmol/L (1,845 to 2,184) at 12 months but remained unchanged thereafter at 18 months (2,092 nmol/L [1,918 to 2,265]), and at 24 months [1,979 nmol/L (1,808 to 2,149)].

### Conclusion
The results of this two year trial show that red blood cell folate concentrations reach a plateau within 12 months of beginning daily folate supplementation.

### Source of funding
This clinical trial was supported by an Otago Research Grant.

# Concurrent Session 8: Nutrients and Health

## Diet quality and longevity in older adults

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**Background**
Diet quality indices assess compliance with dietary guidelines and represent a measure of healthy dietary patterns. While there are a number of methods for assessing diet quality, few studies compare different approaches in the same cohort.

**Objective**
The aim of this study was to compare three existing measures of diet quality and investigate the associations with all-cause mortality in a representative sample of community-living older adults.

**Design**
Analysis was based on 972 participants of the British Diet and Nutrition Survey of people aged 65 years and over in 1994/5 and who were followed-up for mortality status until 2008. Dietary intake was measured via a 4-day weighed food record. We compared the associations between all-cause mortality and three measures of diet quality which have previously been shown to be associated with all-cause mortality: the Healthy Diet Score (HDS), the Recommended Food Score (RFS) and the Mediterranean Diet Score (MDS). Higher scores on the diet quality indices reflect greater compliance with dietary guidelines. Hazard ratios for all-cause mortality by fourths of dietary index were obtained using Cox regression. Models were adjusted for age, sex, energy intake, social class, region, smoking, physical activity and BMI.

**Outcomes**
After adjustment for potential confounders, the RFS was also associated with all-cause mortality (highest vs lowest quartile; hazard ratio=0.67, 95% CI 0.52 – 0.86). Similarly, the MDS was significantly associated with mortality (highest vs lowest quartile; hazard ratio = 0.77, 95% CI 0.61 – 0.97). However, the HDS was not significant associated with mortality.

**Conclusion**
The HDS was not a predictor of mortality in this population, while the RFS and the MDS were both associated with all-cause mortality. This study indicates that simple measures of diet quality using food-based indicators can be useful predictors of longevity.

**Source of funding**
Medical Research Council (UK), National Health and Medical Research Council, National Heart Foundation of Australia.

## Development and field evaluation of a hot-weather ration pack for the Australian Defence Force

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**Background**
Appropriate drinking and eating behaviours can replace fluid and electrolyte losses for most forms and intensities of exercise in hot weather. However, it has been reported that soldiers typically discard up to 40% of combat ration pack (CRP) food items, thereby increasing their risk of dehydration and nutrient deficiencies, with consequent detrimental effects on physical and cognitive performance.

**Objective**
To develop a Hot-Weather Ration Pack (HWRP) that satisfies the nutritional requirements of soldiers in hot environments, and also leads to substantially increased energy and nutrient intake relative to existing CRP.

**Design**
A literature review was conducted to determine nutrient specifications for the HWRP. Data on food and nutrient intake and/or acceptability were obtained from a total of 295 soldiers engaged in field exercises. An in-house database (based on AUSNUT, Aus Foods and FoodWorx) was used to estimate nutrient intake. Based on the results of these studies, a prototype HWRP was designed, and then evaluated using a balanced crossover study involving 65 soldiers on a field exercise in a hot environment. Consumption rates, ration acceptability, reasons for discards and nutrient intakes were determined. Physiological variables that were measured include hydration status and energy expenditure.

**Outcomes**
The HWRP received an overall higher acceptability rating and led to significantly greater food consumption than the control ration ($P<0.01$), with snack ('eat-on-the-go') foods, new beverage powders and cold main meals being the most popular items. However, calcium availability was found to be severely limited in both ration types. There was no evidence that the HWRP led to better hydration status than the control ration—soldiers remained mildly to moderately hypohydrated throughout the study, and many were at increased risk of physiological strain.

**Conclusion**
Food intake increased when soldiers were provided with a ration pack that was based on soldier food preferences in a hot environment. However, this did not lead to improved hydration status. Discard rates were still high, and additional strategies are needed to increase consumption when ration packs are to be consumed as the sole source of nutrition in the medium to long term (ie > 10 days).

**Source of funding**
Not applicable

# Concurrent Session 8: Nutrients and Health

## Molecular interactions between bile salt micelles and soluble dietary fibres

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### Background
Soluble dietary fibres (SDF) lower plasma cholesterol, at least in part by preventing bile salts (BS) from being reabsorbed into the enterohepatic circulation. However, the nature of the interactions between bile salt micelles and SDF are poorly defined.

### Objective
Identification of molecular mechanisms of interaction of bile salt micelles with (1,3:1,4) beta glucan (βG) and arabinoxylan (AX) respectively.

### Design
$^{13}$C NMR titration experiments were used to study molecular interactions between purified bile salts with different concentrations of both barley (1,3:1,4) beta glucan, and wheat arabinoxylan. Small angle X-ray scattering (SAXS) was used to study the nature of polymer - micelle interactions.

### Outcomes
**1.** $^{13}$C NMR results show that the polymers behave differently in the presence of BS micelles:
- βG: (i) Systematic chemical shift changes in BS resonances observed as a function of βG concentration suggesting close molecular interactions between BS micelles and βG, (ii) No measurable change in line widths for either BS or SDF resonances suggest that interactions are dynamic.
- AX: Increased line widths without systematic chemical shift changes for BS micelle resonances suggest local aggregation of arabinoxylan causes entrapment and reduced mobility of micelles.

**2.** SAXS results show mixed aggregates of βG and BS, consistent with adsorption of micelles on to the polymer chain. No such change in AX scattering occurred in the presence of BS micelles, and neither polymer caused a detectable change in micelle dimensions.

### Conclusion
$^{13}$C NMR and SAXS show that the nature of the molecular interaction between SDF and BS micelles depends on the type and source of the SDF.

### Source of funding
This study was supported by a grant from the CSIRO Flagship Collaboration Fund to the High Fibre Grains Cluster via the Food Futures Flagship.

## Fructose intake in West Australian adolescents

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### Background
Fructose consumption has been linked to the development of ill-health, including non-alcoholic fatty liver disease and the metabolic syndrome. Although the health effects of fructose are being researched, there is little evidence of how much fructose is being consumed in Australia from fructose-containing foods, such as soft drinks, fruit juices, yoghurts, and sweetened grain products. Dietary intake of fructose is of particular interest in the adolescent age group as it is a time of rapid growth and development and the stage when individuals are establishing independent eating patterns.

### Objective
This research aimed to quantify fructose consumption in adolescents participating in the 14 yr follow-up of the Western Australian Pregnancy Cohort (Raine) Study.

### Design
Subjects were 822 adolescents aged 13 – 15 years participating in the Raine Study. Dietary intake was assessed by 3-day food records and entered into the FoodWorks dietary analysis program. Fructose values for individual foods were linked from the NUTTAB 2006 Online Version, or directly from product manufacturers.

### Outcomes
Mean ± SD dietary fructose was 47.0 ± 2.6 g, with males higher than females (51.3 ± 2.9 g vs 42.8 ± 2.1 g, $P<0.001$). Fructose intake was positively associated with BMI ($P<0.001$), total energy intake (kJ) ($P<0.001$), and how frequently the participant skipped breakfast within the dietary record period ($P=0.001$).

### Conclusion
Fructose intake has not previously been described in Australian adolescents and it is unknown whether intake has increased over time. However comparison of national nutrition surveys shows a significant increase in carbohydrate intake, with the greatest increase in the adolescent age group. This study quantifies the amount of fructose being consumed in West Australian adolescents and highlights associations with subject characteristics.

### Source of funding
The Western Australian Pregnancy Cohort (Raine) Study is funded by the Raine Medical Research Foundation at The University of Western Australia, the National Health and Medical Research Council of Australia, the Telstra Research Foundation, the Western Australian Health Promotion Foundation, the Australian Rotary Health Research Fund and the National Heart Foundation of Australia and Beyond Blue. Curtin University supplied funding for obtaining fructose values.

# Concurrent Session 9: Omega-3 and Other Fats

## The Omega-3 Index in adolescents: dietary associations and relationships with cardiovascular and metabolic risk factors

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**Background**
The Omega-3 Index, a measure of long-chain omega-3 fats in red blood cell membranes, predicts heart disease mortality in adults, but its association with cardiovascular risk factors in younger populations is unknown.

**Objective**
To determine the Omega-3 Index in adolescents participating in the Western Australian Pregnancy (Raine) Cohort, assess associations with diet, lifestyle and socioeconomic factors, and investigate independent associations with cardiovascular and metabolic risk factors.

**Design**
Red blood cell fatty acid analysis was determined for 1301 adolescents aged 13-15yrs. Dietary intake was derived from 3-day food diaries. Risk factors examined were blood pressure, fasting blood insulin and glucose concentrations, and fasting blood lipids including ratios.

**Outcomes**
Mean Omega-3 Index was 4.90±1.04% (range 1.41-8.42%). When compared with Omega-3 Index categories identified in adults, 15.6% of adolescents were in the high risk category (Index <4%). Age (P<0.01), maternal education (P<0.01) and BMI (P=0.05) were positively associated with the Omega-3 Index. The Index was positively associated with dietary intakes of eicosapentaenoic and docosahexaenoic acid (P<0.01), protein (P<0.01), omega-3 fats (P<0.04), and food groups of fish and wholegrains (both P<0.01), and negatively associated with intakes of soft drinks and crisps (both P<0.01). In boys, Omega-3 Index was independently associated with total (b=0.06, P=0.01) and HDL-cholesterol (b=0.03, P=0.01), and diastolic blood pressure (b=-0.68, P=0.04).

**Conclusion**
Significant associations between Omega-3 Index and some cardiovascular risk factors in boys were observed in our adolescent cohort, however the predictability of the Index for risk of cardiovascular disease later in life may be limited, particularly in girls.

**Source of funding**
NH&MRC, UWA, Raine Medical Research Foundation, Telstra Research Foundation, WA Health Promotion Foundation, Australian Rotary Health Research Fund, NHF and Beyond Blue.

## Development and validation of a database for approximating the percentage fish in canned fish products

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**Background**
Habitual fish consumption is associated with numerous health benefits, such as decreased incidence of heart failure and stroke. Consequently, a number of clinical trials have been developed to investigate the health outcomes of fish consumption. Food label data from canned fish products, which are of increasing popularity in Australia, indicates that other ingredients such as flavourings and sauces make up a substantial proportion of the weight of these products. Failing to account for these ingredients in clinical trials investigating fish consumption may lead to overestimation of the amount of fish consumed.

**Objective**
To develop and validate a database of the percentage fish and seafood contained in common canned fish and seafood products, for the use in clinical trials.

**Design**
Six major supermarkets in the Illawarra region, NSW were audited for canned seafood products, and a database of reported percentage fish and seafood in each product was developed. Mean ± SD of each type of product was then determined. To validate the database, a representative sample of canned tuna products were weighed according to Codex Alimentarius methods. The weighed percentage fish was then compared to reported percentage via independent sample t-test and Mann-Whitney test for parametric and non-parametric data, respectively.

**Outcomes**
Percentage fish data was collected for n=214 canned fish products. The mean percentage contained in canned tuna products was 60.4±11.3% (n=144), 72.0±14.7% in canned salmon products (n=31) and 70.9±9.3% in canned sardine products (n=23). Weighed validation of the database was carried out in n=59 tuna products. There was no significant difference between the reported and weighed percentage fish in any of the products; however, flavoured tuna was the only variety to have a lower reported percentage fish than was weighed.

**Conclusion**
A validated database of the percentage fish in canned fish and seafood products highlighted the substantial proportion of additional ingredients found in these products. Given the popularity of such products, future studies investigating the health outcomes of fish consumption should use a similar database in order to accurately measure fish intake.

**Source of funding**
School of Health Sciences, University of Wollongong Higher Degree Research student support.

# Concurrent Session 9: Omega-3 and Other Fats

## Comparison of the Cancer Council Food Frequency Questionnaire (FFQ) to a Validated PUFA FFQ to determine PUFA intakes

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**Background**
Polyunsaturated fatty acids (PUFA) including the long chain omega-3 PUFA (LC n-3 PUFA) are important for health.

**Objective**
To assess if the Cancer Council Food Frequency Questionnaire (FFQ) accurately determines PUFA intakes by comparison to the recently validated PUFA FFQ.

**Design**
Forty-one study volunteers were recruited from the local Illawarra region of New South Wales. The method of triads was used to 1) determine validity co-efficients by comparing the electronic PUFA FFQ intakes against a 3 day weighed food record and appropriate blood biomarkers (erythrocyte and plasma fatty acids) and 2) determine validity co-efficients by comparing the Cancer Council FFQ intakes against a 3 day weighed food record and appropriate blood biomarkers (erythrocyte and plasma fatty acids). The validity co-efficients from each of the FFQ were subsequently compared.

**Outcomes**
Using erythrocytes as the biomarker, the electronic PUFA FFQ had much higher validity co-efficients compared to the Cancer Council FFQ, for EPA (0.92 vs 0.19), DHA (0.69 vs 0.26) and total LC n-3 PUFA (0.78 vs 0.23), respectively, whereas omega-6 PUFA were comparable. Using plasma as the biomarker, the electronic PUFA FFQ had much higher validity co-efficients compared to the Cancer Council FFQ, for ALA (0.96 vs 0.49), EPA (0.87 vs 0.19), DHA (0.64 vs 0.24) and total LC n-3 PUFA (0.73 vs 0.21), respectively, whereas omega-6 PUFA were comparable.

**Conclusion**
The electronic validated PUFA FFQ is better suited to determine PUFA intakes especially the omega-3 PUFA intakes.

**Source of funding**
Metabolic Research Centre at the University of Wollongong funded this study.

## Eicosapentanoic acid as a novel treatment for cancer induced cardiac atrophy and cachexia

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**Background**
Cachexia is a common complication of cancer and is responsible for marked weight loss and negative outcomes. Studies show that levels of reactive oxygen species potentiated through disruption of the enzymes: xanthine oxidase (XO) and superoxide dismutase (SOD) are elevated in patients and animal models of cachexia. The disruption of these pro and antioxidant systems have been linked to protein breakdown pathways. One consequence of elevated protein breakdown is the loss of heart tissue which can adversely affect patient outcomes. Currently there is no treatment available for cachexia.

**Objective**
This study investigated the potential of eicosapentaenoic acid (EPA) a SOD agonist and the xanthine oxidase antagonist oxypurinol to ameliorate weight loss and cardiac atrophy associated with cancer cachexia.

**Design**
8 week old nude mice were placed on a standard chow and injected with murine adenocarcinoma cell line 16 (MAC16) then randomised to one of four groups EPA, Oxypurinol, EPA/Oxypurinol in combination or no treatment, for a period of 29 days. Body weight and tumour size was measured daily, whilst food and water intake was assessed on a weekly basis. At the conclusion of the study hearts were collected and weighed.

**Outcomes**
Food and water intake did not vary between groups. All animals injected with MAC16 cells eventually lost a significant amount of body weight. However, the EPA group was able to delay the onset of weight loss (cachexia) by 9 days (P=<0.05). Similarly EPA and Oxypurinol delayed onset of cachexia by 4 days. Whereas, Oxypurinol accelerated cachexia by 3 days in comparison to control. Heart weight in the EPA group was maintained whereas the no treatment, oxypurinol and the combination of EPA/Oxypurinol all lost significant amounts of cardiac tissue. All treatments delayed the initial appearance of tumours in animals (P=<0.05). However, the final tumour size was not different between groups.

**Conclusion**
EPA has shown a protective effect against cancer induced cachexia and subsequent cardiac atrophy. The mechanisms responsible may be related to the delay of tumour formation in mice. Oxypurinol also had anti tumour activity however accelerated the development of cachexia. The combination of EPA and Oxypurinol had mixed synergistic effects.

**Source of funding:** Deakin University and EPX pty ltd.

# Concurrent Session 9: Omega-3 and Other Fats

## Longitudinal relationship between omega-3 polyunsaturated fatty acids and well being in an older community dwelling population

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**Background**
Increasing evidence suggests that omega-3 polyunsaturated fatty acids (n-3 PUFAs) play an important role in mental health and well being. To date research results have been mixed, possibly due to large differences in methodologies employed. These range from the use of different populations including clinical and healthy groups, different methods used to assess n-3 PUFA status, and study design.

**Objective**
The relationship between n-3 PUFAs and measures of well being will be examined at baseline and longitudinally using data obtained as part of the EPOCH (Older people, omega-3 and cognitive health) trial; a randomised, double-blind, placebo-controlled 18-month clinical trial.

**Design**
Participants (N=393; MMSE>23; 53.7% female; Age: *M*=72.32, *SD*=5.55, range = 65-90) completed self-report measures of well being (including affect, life satisfaction, mental health and depression) and two food frequency questionnaires to measure general nutritional status and dietary intake of n-3 PUFAs. Measures of n-3 PUFA status were further assessed in erythrocyte membranes.

**Outcomes**
Regression analyses suggested that at baseline, EPA was associated with both global (β=0.189, P<0.05) and domain (β=0.209, P<0.05) levels of Life Satisfaction. Structural equation modelling will be used to further examine the causal direction of the relationship between EPA and life satisfaction longitudinally whilst controlling for measurement error and other potential confounders such as demographics, physical activity, physical health and nutritional status. Preliminary independent samples t-tests revealed no significant differences (P>0.05) between the n-3 and placebo group for any of the measures of well being.

**Conclusion**
Results thus far suggest that previously reported associations between n-3 PUFAs and mental health may be due to the specific relationship between EPA and life satisfaction. It is recommended that future research assess both n-3 PUFA status and well being at the individual component level.

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## The effect of a brief oral fat exposure on oral fatty acid taste sensitivity

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**Background**
Excessive dietary fat consumption contributes to obesity. Animal and human studies show that fatty acid taste sensitivity may be affected by preference for dietary fat and fat consumption, which in turn influences body weight regulation. For example, those who consume more dietary fat over a prolonged period of time have been found to be less orally sensitive to fatty acids, whereas those who consume less dietary fat are more orally sensitive to fatty acids. Whether or not a brief oral fat exposure has an effect on fatty acid taste sensitivity remains unknown.

**Objective**
To establish whether or not a brief oral fat exposure, as would occur during a normal eating event, has an effect on oral fatty acid sensitivity, and determine if there is an association with liking for high and low fat foods.

**Design**
Twenty-nine subjects (10 males, age 22 ± 0.3 yrs, BMI 23.8 ± 1.0; 19 females, age 21 ± 0.6 yrs, BMI 21.2 ± 0.5) attended laboratory sessions where oleic acid (C18:1) detection thresholds using 3-Alternate Forced Choice Methodology (3-AFC) and food liking tests were performed. Prior to completing the C18:1 3-AFC test, subjects rinsed their mouth with either cream or water for 30 seconds. Subjects also completed a liking task where they rated their liking for three types of cupcakes and custard, each with a different fat content on a 9-point hedonic scale. Subjects also completed the NWLRC Fat Intake Scale and BMI was calculated from height and weight measurements.

**Outcomes**
The oral fat exposure had no influence on oral C18:1 detection thresholds (6.29 ± 0.7 mM C18:1 compared to 6.35 ± 0.8 mM C18:1). In addition, there was no difference in liking of high or low fat cupcakes after a brief oral fat exposure (oral fat exposure; high-fat; 6.07 ± 0.7, low-fat; 7.68 ± 0.7; no-fat; 5.36 ± 0.6, water rinse; high-fat; 7.02 ± 0.8, low-fat; 5.80 ± 0.9, no fat; 6.07 ± 0.6) or custard (oral fat exposure; high fat; 5.92 ± 0.7, low-fat; 6.25 ± 0.7, no-fat; 8.67 ± 0.5, water rinse; high-fat; 6.29 ± 0.8, low-fat; 6.98 ± 0.8, no-fat; 4.97 ± 0.6). No relationship between BMI and C18:1 detection thresholds was found (r= 0.13, P= 0.51).

**Conclusion**
The data shows that short-term oral exposure to a high fat food has no influence on C18:1 thresholds and also no short-term effect on an individual's liking of high and low fat foods.

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Dairy Australia Lecture

# Evolution of lactation: Protection then nutrition

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## Overview

The evolutionary origin of the mammary gland has been difficult to establish because little knowledge can be gained on the origin of soft tissue organs from the fossil records. Although other animals such as pigeons, sharks, salamanders and skinks can nourish their young from various bodily secretions, mammals are the only animals that secrete a complex nutritive and protective fluid from complex skin glands to provide the sole source of nourishment for the growth, development and protection for either their hatchlings or neonates. The development of a fully functional lactating mammary gland predates the origin of mammals; the primitive beginning for the mammary gland probably date back more than 310 Mya to the Pennsylvanian epoch when the Synapsida branch of the Amniota evolved to have soft glandular skins rather than the scales of related taxa. Three recent innovative approaches have been taken to explain the ancestral development of the mammary gland and lactation. Oftedal looked for evidence in support of a developing role for lactation during the course of synapsid evolution that could have led to the development of mammals. This approach included identification of skin glands that could have been the ancestral mammary glands, analysis of the comparative anatomy of existing 'primitive' mammals, including the platypus and kangaroo, and the conditions required for successful reproduction in animals that laid porous parchment (soft) shelled eggs. He proposed that the evolutionary precursor to milk was secreted from hypertrophied skin glands that were associated with hair follicles and the secretion was favoured by natural selection because it prevented desiccation and microbial attack of the parchment-shelled eggs of the synapsids. Furthermore, this secretion gradually became nutrient rich and this paralleled a progressive decline in egg size with a possible dual role of providing some transfer of nutrients during egg incubation and subsequently an enteral supply of nutrients for the hatchlings.

On the other hand, Vorbach et al. took a metabolic and molecular approach. They examined the metabolic and molecular synergy between the highly conserved innate immune system and both milk composition and the regulation of the synthesis and secretion of milk. They proposed that lactation partly evolved from the highly conserved inflammatory response to tissue damage and infection. Therefore they concluded that the sequence of mammary gland development was first to provide protection and subsequently the nutritional components evolved to nourish either hatchlings or newborn mammals. Xanthine oxidoreductase (XOR) and lysozyme were highlighted as two important antimicrobial enzymes of the innate immune system that became key regulators in the development of the nutritional components of milk. Due to gene sharing, the housekeeping enzyme XOR is required for the secretion of the milk fat globules. $\alpha$-Lactalbumin, a key whey protein and a subunit of lactose synthase that catalyses the synthesis of lactose from glucose and UDP galactose, evolved from gene duplication of lysozyme (40% amino acid homology). Lactose provides both dietary carbohydrate and the osmotic gradient for the formation of the aqueous phase of milk in most mammals. Vorbach et al. also dated the ancestral origin of the mammary gland back to the soft-skinned Synapsids of the Pennsylvanian epoch.

Lefevre et al. recently reviewed comparative genomic and transcriptomic evidence and concluded that lactation evolved gradually along the Synapsid lineage and that a complex lactation system was present 200Mya in the ancestors of the mammals. The earliest split in the mammalian phylogeny occurred about 166 to 220 Mya with the split to the monotremes and the Theria. Subsequently, about approximately 140 Mya the Theria split into the marsupials and placental mammalian lineages.

It is of interest to explore these distinctly different approaches and to evaluate their contribution of the understanding of the role of lactation (the most energy demanding component of mammalian reproduction) in mammals. The approaches have resulted in reasonably consistent conclusions on the development of lactation and it is of interest to explore them in more detail to determine how they relate to Darwin's theory of natural selection.

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