Human health from fruit and vegetables: hype or hope?

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Enhancing Crop Productivity and Utilisation
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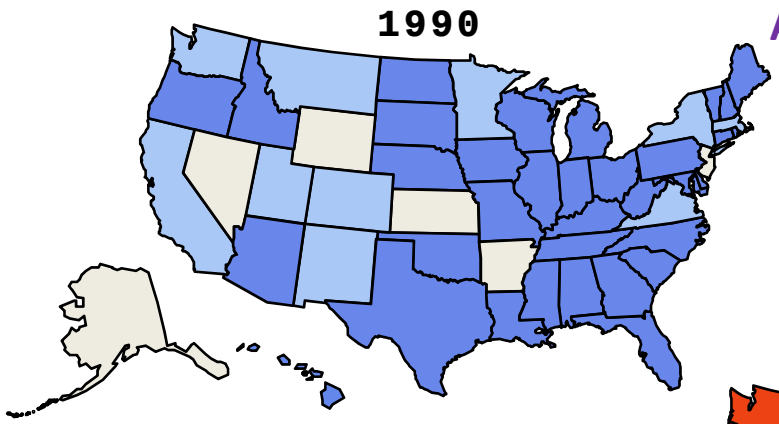
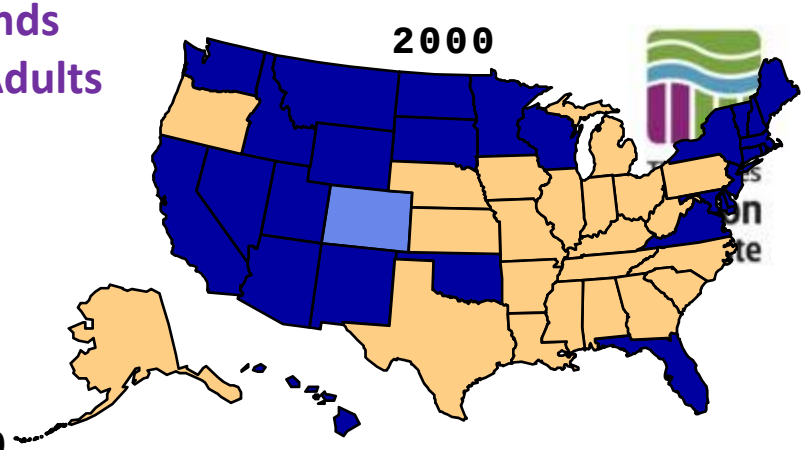
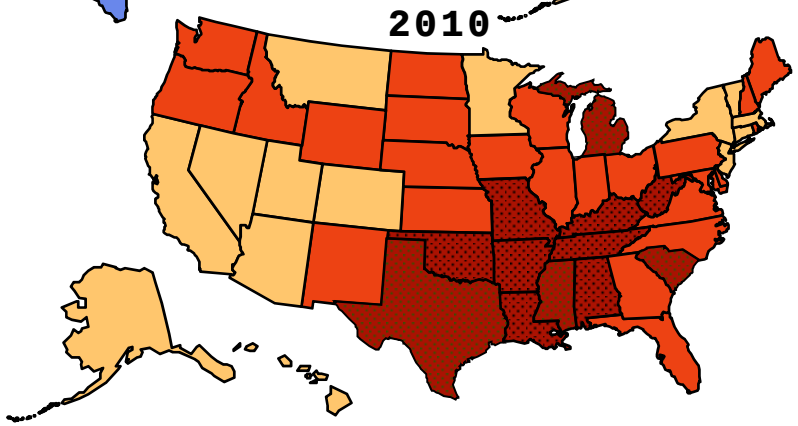
Obesity Trends Among U.S. Adults

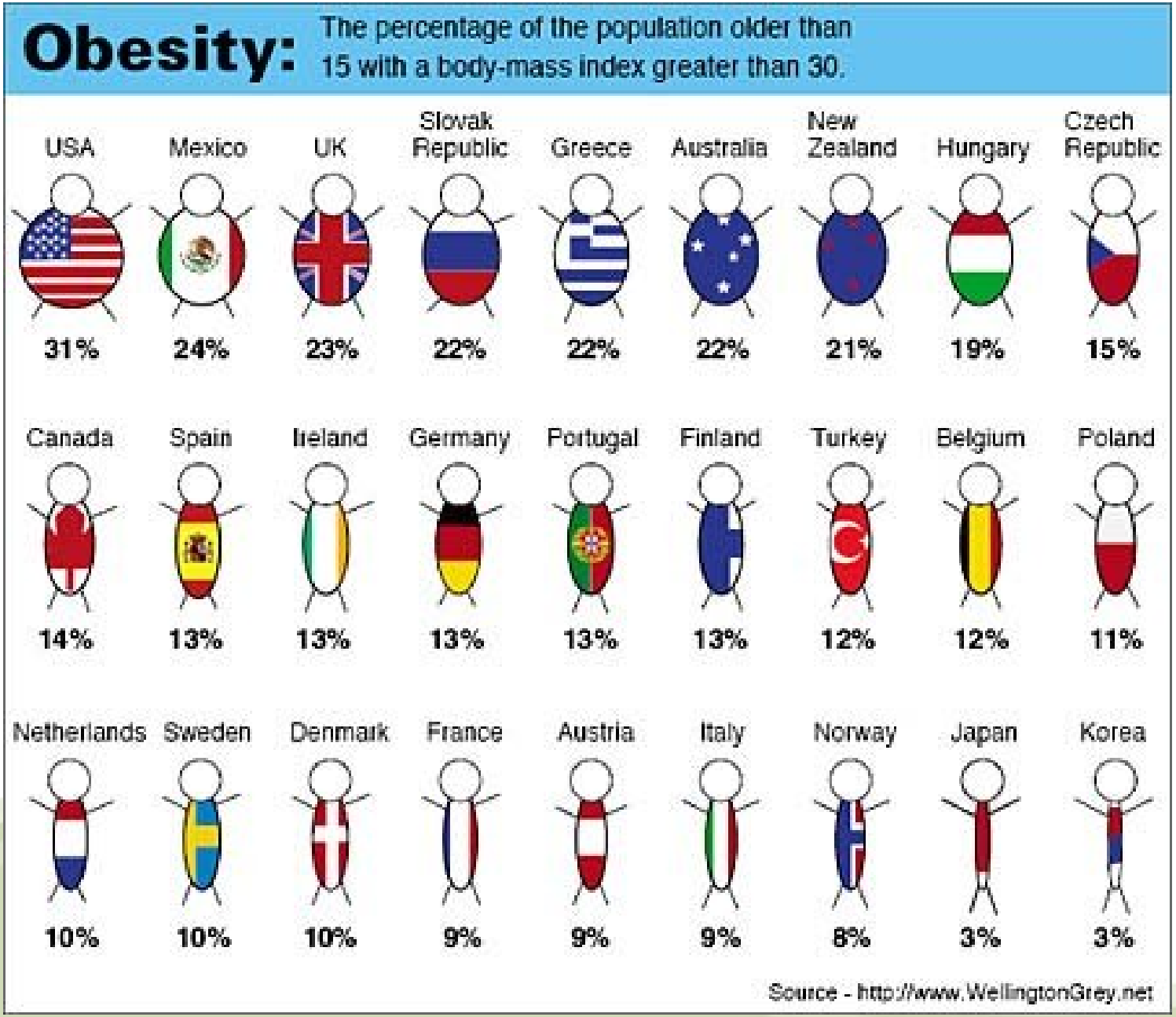






(*BMI ≥30, or about 30 lbs. overweight for 5’4” person)

No Data | <10% | 10%–14% | 15%–19% | 20%–24% | 25%–29% | ≥30%



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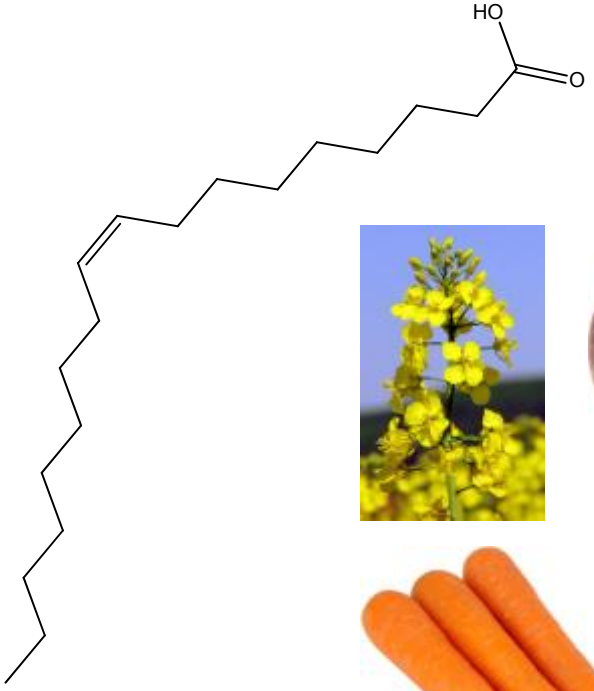
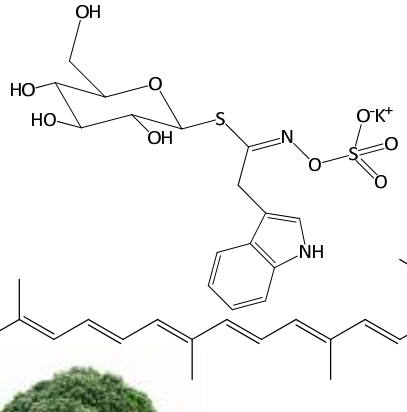
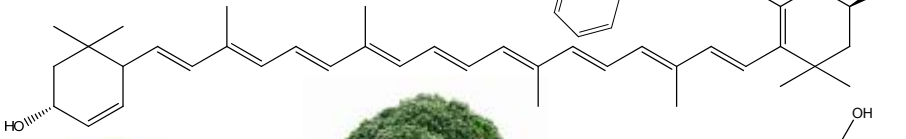
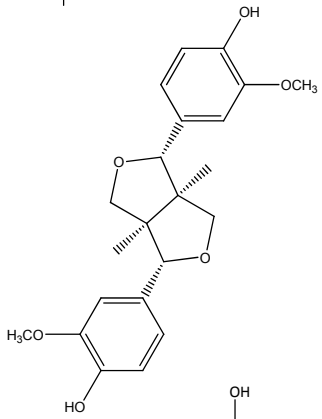
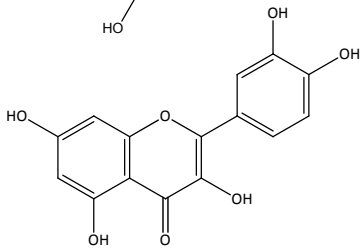
Health benefits from fruit and vegetables

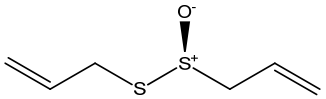


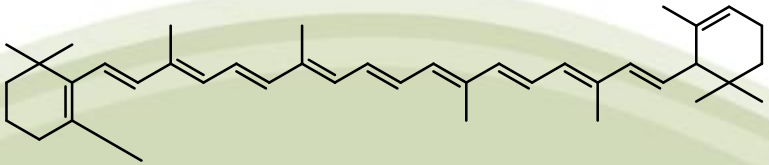


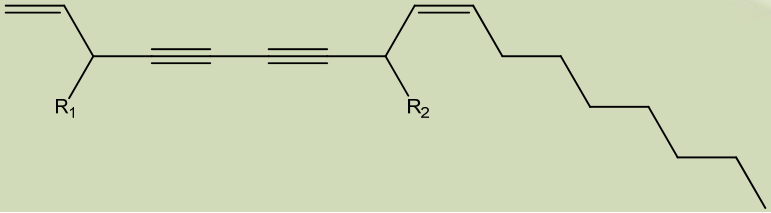










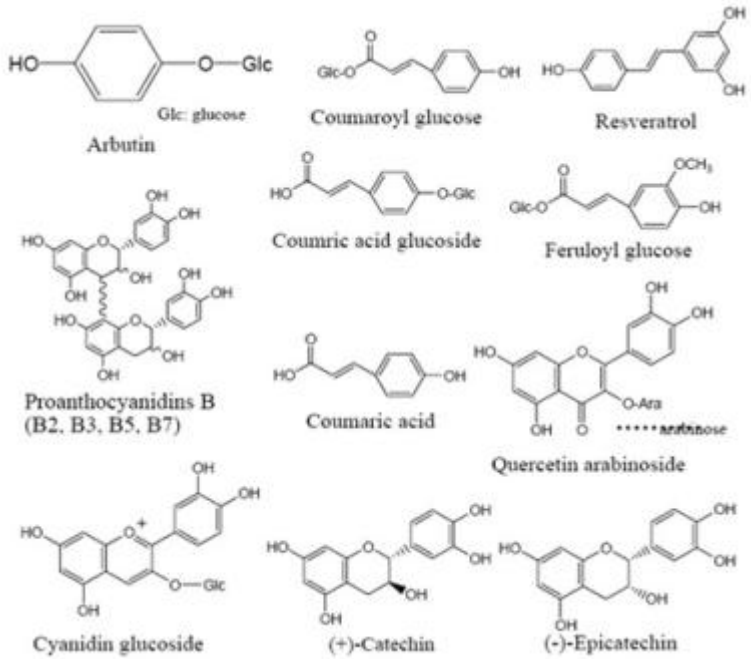


Soft Fruit

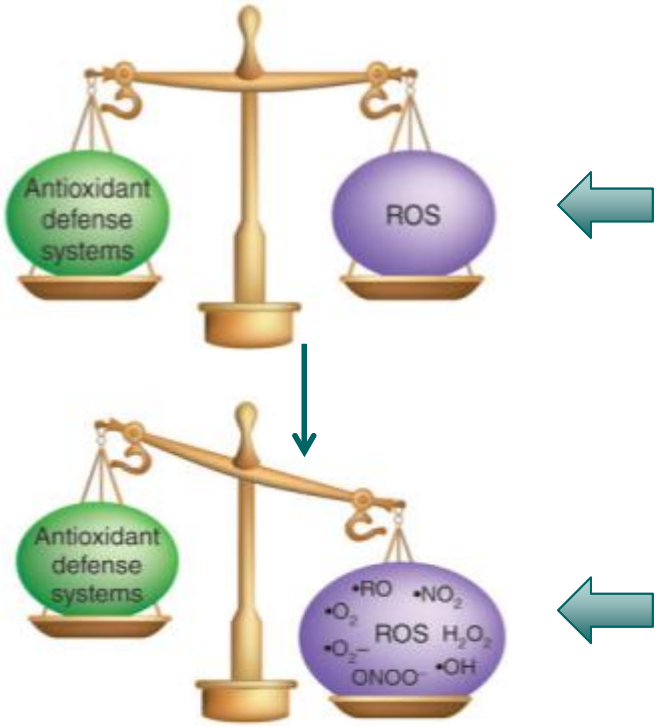
- Good nutritional value
- Excellent source of bioactive compounds called polyphenols; Health beneficial effects
- Good source of antioxidants, compounds that *in vitro* are able to scavenge free radicals cause of cell aging and death… However….!!
- Is this what happens in vivo??

Polyphenols - bioactive compounds

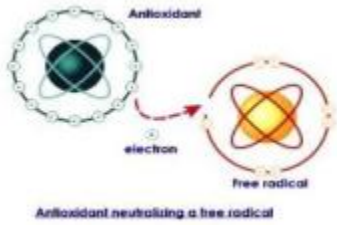
- Polyphenols represent a group of secondary metabolites commonly found in higher plants
- Polyphenols are class of organic chemicals characterized by the presence of phenol structural units
- Due to the diverse biological properties polyphenols are found to be potential candidates for use as a drugs to treat diseases such as: Diabetes types I and II, CVDs, cancer, bacterial infections, neural disorders



Oxidative stress couse of cell aging and death



Normal, balanced stage



Source of Reactive Oxygen Species

- Air pollution
- UV rays
- Bio products from food and metabolism of chemicals
- Cellular respiration

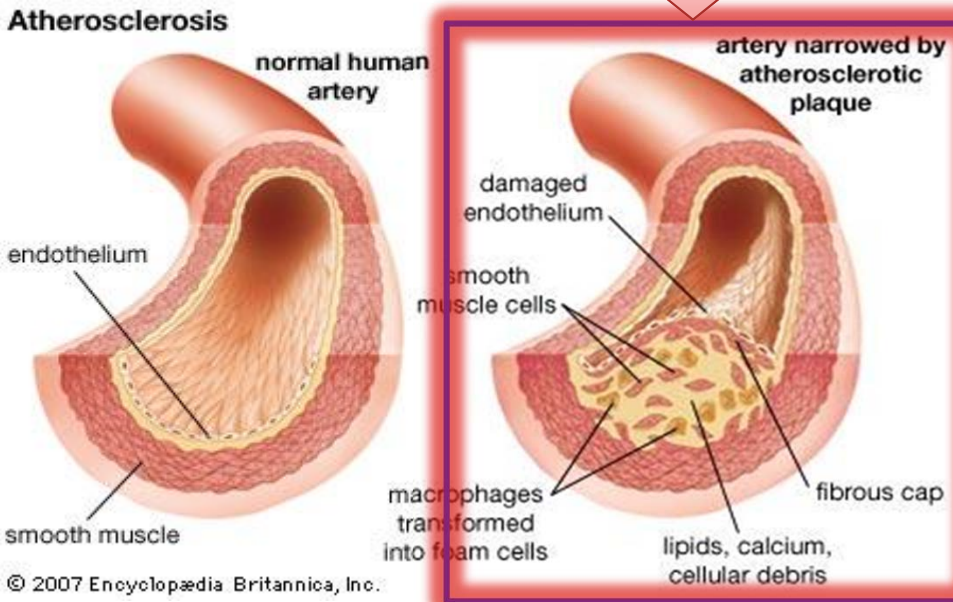
- Biomembrane damage
- DNA damage
- Protein damage
- Lipid peroxidation

Cancer CVD, Diabetes type I II, Neurodegenerative diseases

Free radicals implicated in cardiovascular disease development

Oxidative stress

Oxidative stress



- Protein damage and lipid peroxidation leads to loss of the membrane integrity, cell damage and death
- Biomembrane damage leads to the cell death
- DNA damage leads to mutations

Stroke, Atherosclerosis,

High Blood Pressure, Heart attack

Possible role of polyphenols in CVD etc…

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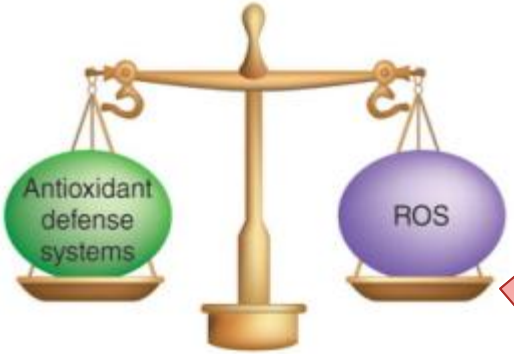
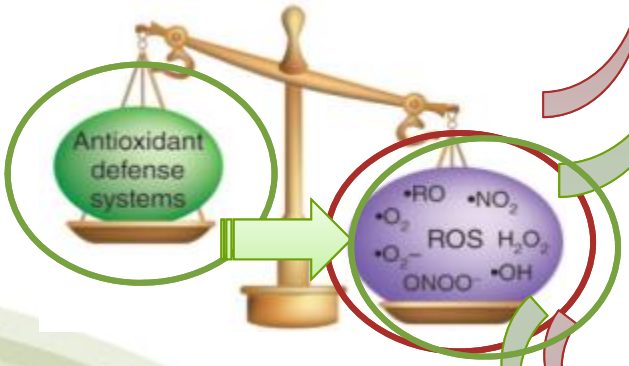
- POLYPHENOLS can act through co-action with cell membrane receptors
- induction of the cell sygnalling pahways
- modulation of the genes and proteins expression

2





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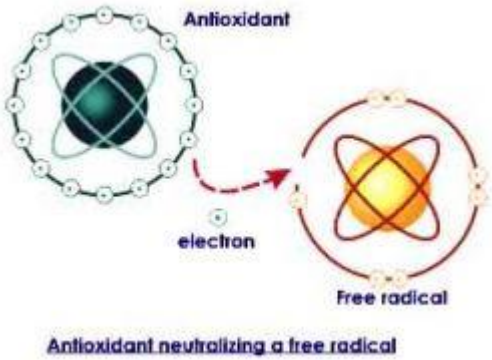
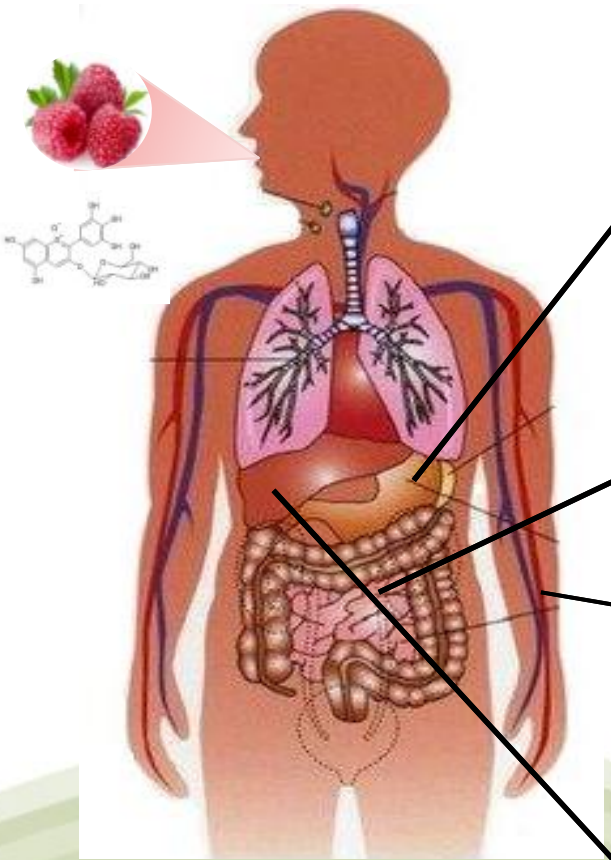


O_2, H_2O, NO_2 etc.
Not harmfull to cells anymore

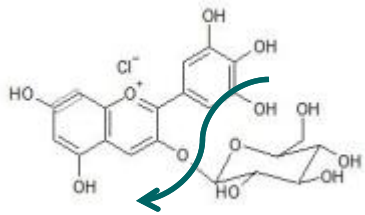
1. Direct antioxidant response

2. Indirect antioxidant response

Beware of what you measure!
Absorption and metabolism of delphinidin



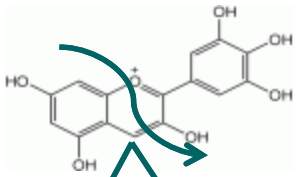
- GUT



Delphinidin-3-*O*-glucoside

- pH ~ 4

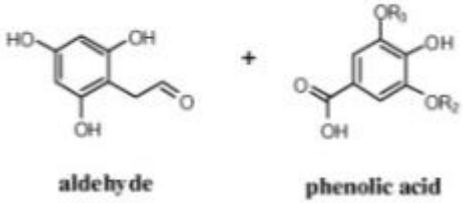
- Small intestine



Delphinidin

- pH ~ 7 - 8

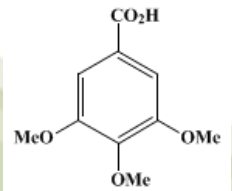
- Blood



- pH ~ 7.4

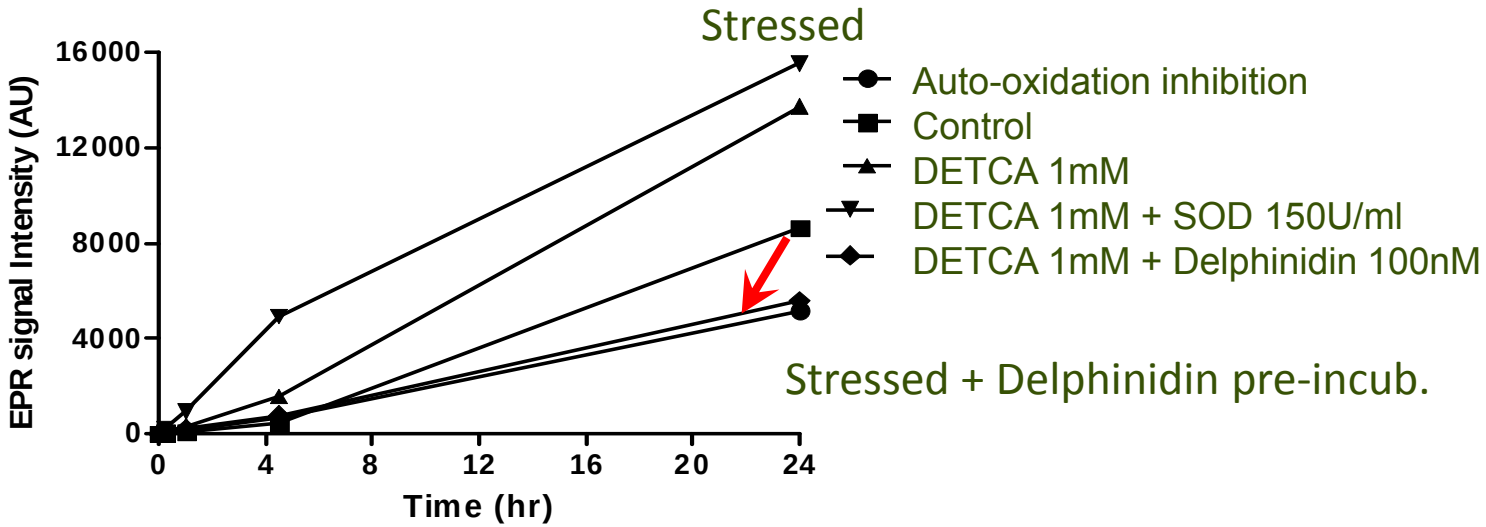
prologlucinaldehyde + gallic acid

- Liver



- Methylation
- Sulphation
- Glucuronidation

Porcine Artery model – Arterial relaxation



- DETCA induces oxidative stress in porcine artery model
- Delphinidin (acute phase) highly effective antioxidant in this model

Blackcurrant anthocyanins cause a flow-dependent increase in blood perfusion in isolated human intracerebral arteries

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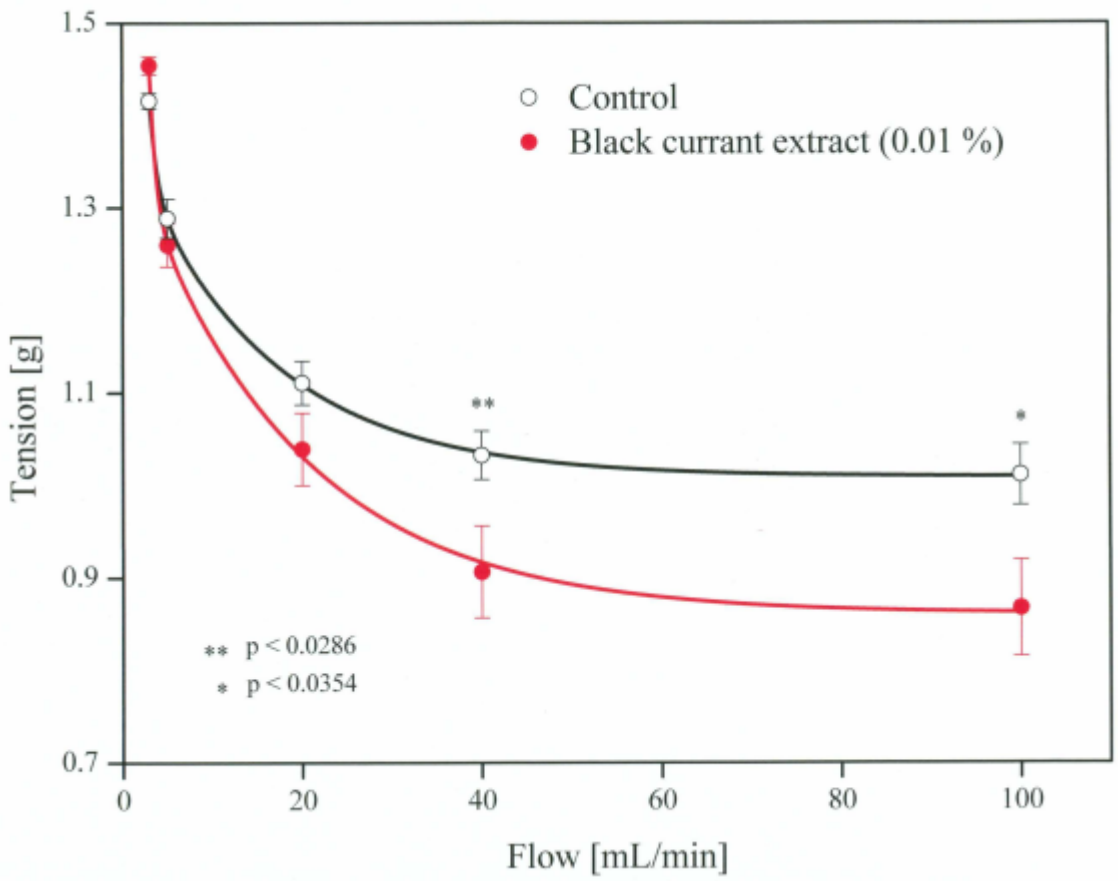
- Flow-dependent isometric tension was measured in segments of isolated human intracerebral arteries from consciousness areas: derived from brain surgery.
- The anthocyanin driven vasodilatation may have a beneficial effect on the cognitive functions in dementia of the Alzheimer type, in the prevention of TIA and stroke
- Flow-dependent relaxation is almost identical to fluvistatin.

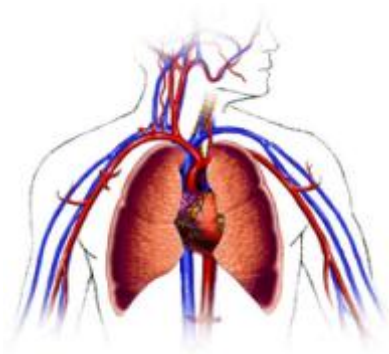


The Extract Company
BerryPharma
ARTEMIS NUTRITIONALS EUROPE

Cardiovascular function and intake of soft fruit

Intervention trial – assess effects of six week ingestion of

- **Blackcurrant berries with low vitamin C content**
- **Blackcurrant berries with high vitamin C content**
- **Blueberries (No vitamin C)**
- **Coloured flavoured water (control)**



Effects on cardiovascular function

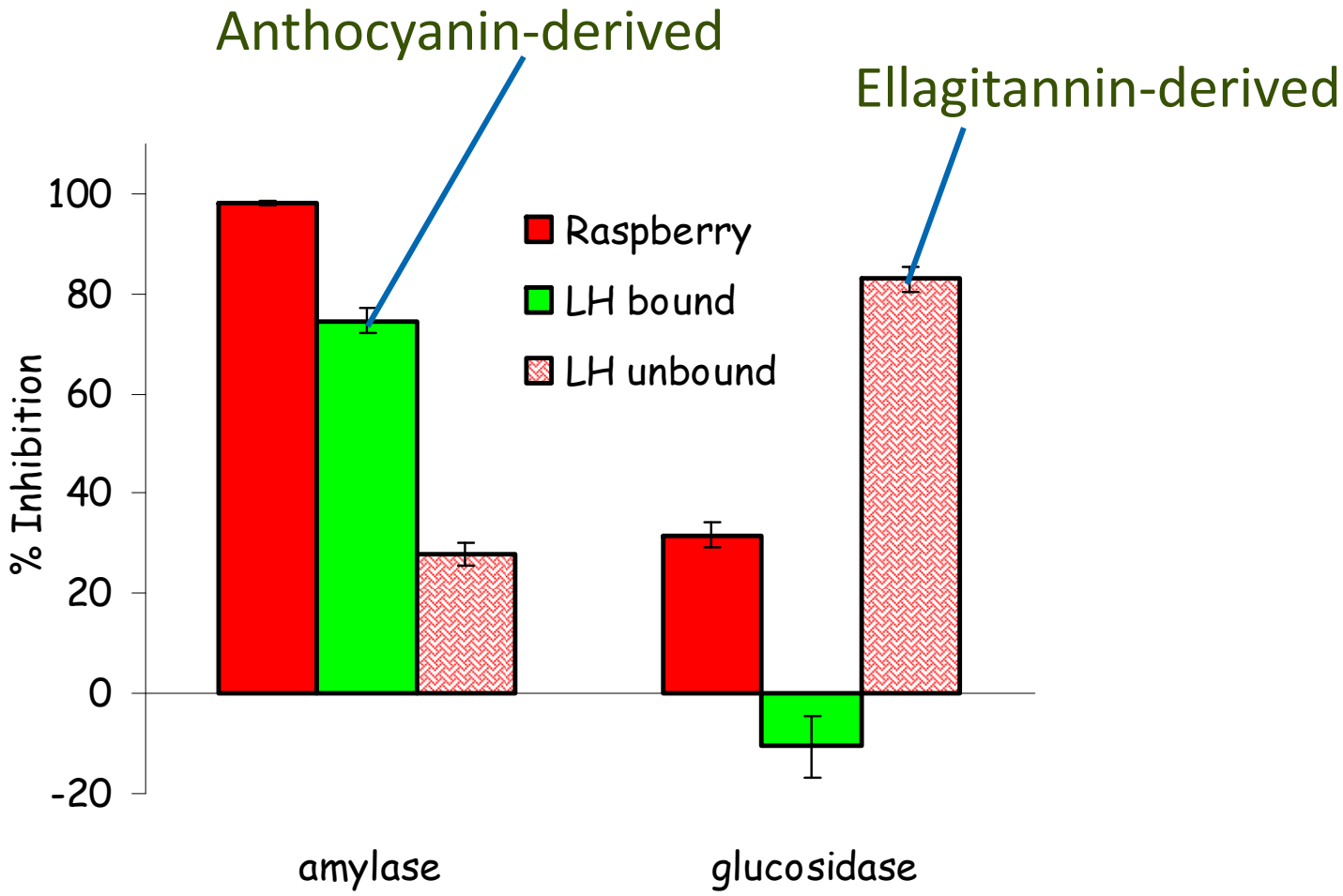
Assess Micro-circulation & macro-circulation, arterial stiffness, (SphygmoCor Pulsewave Analysis System) and carotid intima media thickness (Accuson Sequoia).

Relate to *In vivo* markers for endothelial cell function and oxidative stress.

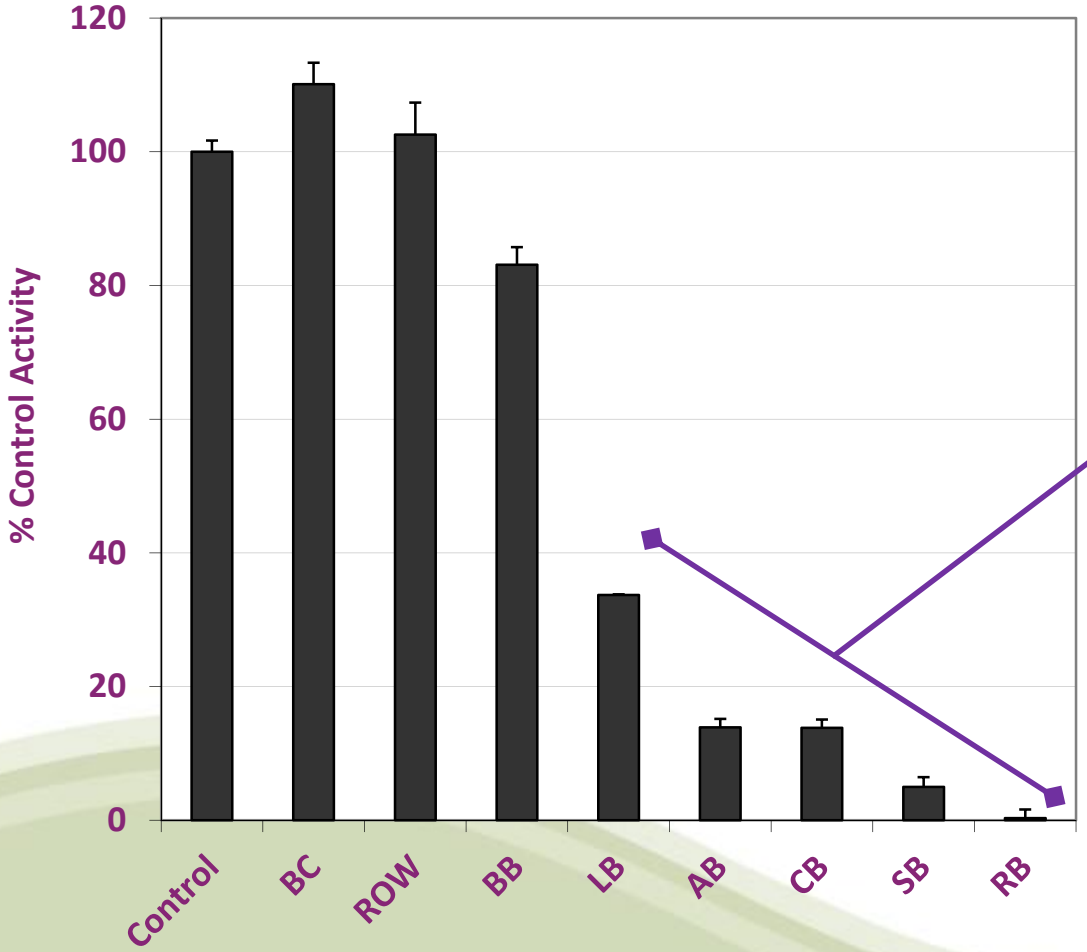
Assess bioavailability of fruit derived antioxidants.

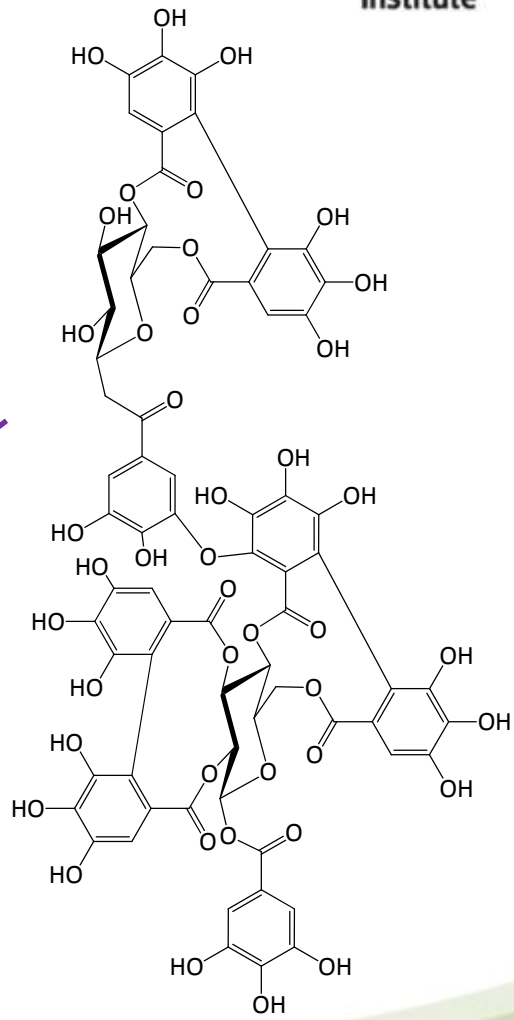
- **Blueberry group showed a modest reduction in carotid intima-media thickness**
- **Both blackcurrant groups showed reductions in isoprostanes: markers if inflammation.**

Impact of soft fruit consumption on diabetes; Inhibition of starch digestion



Inhibition of lipase by berry extracts





Enhanced faecal lipid excretion consistent with inhibition of lipase

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Human Intervention Studies

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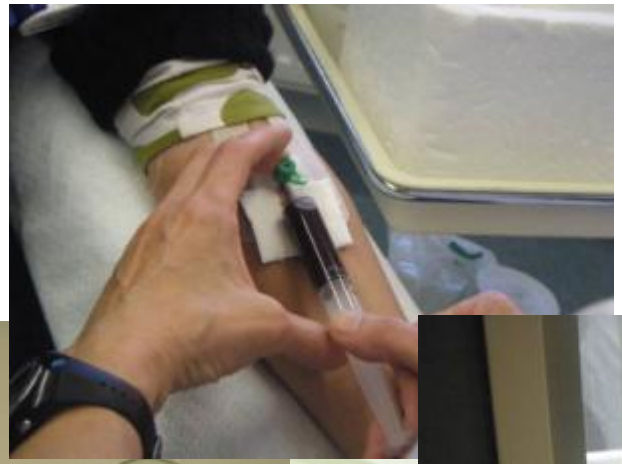
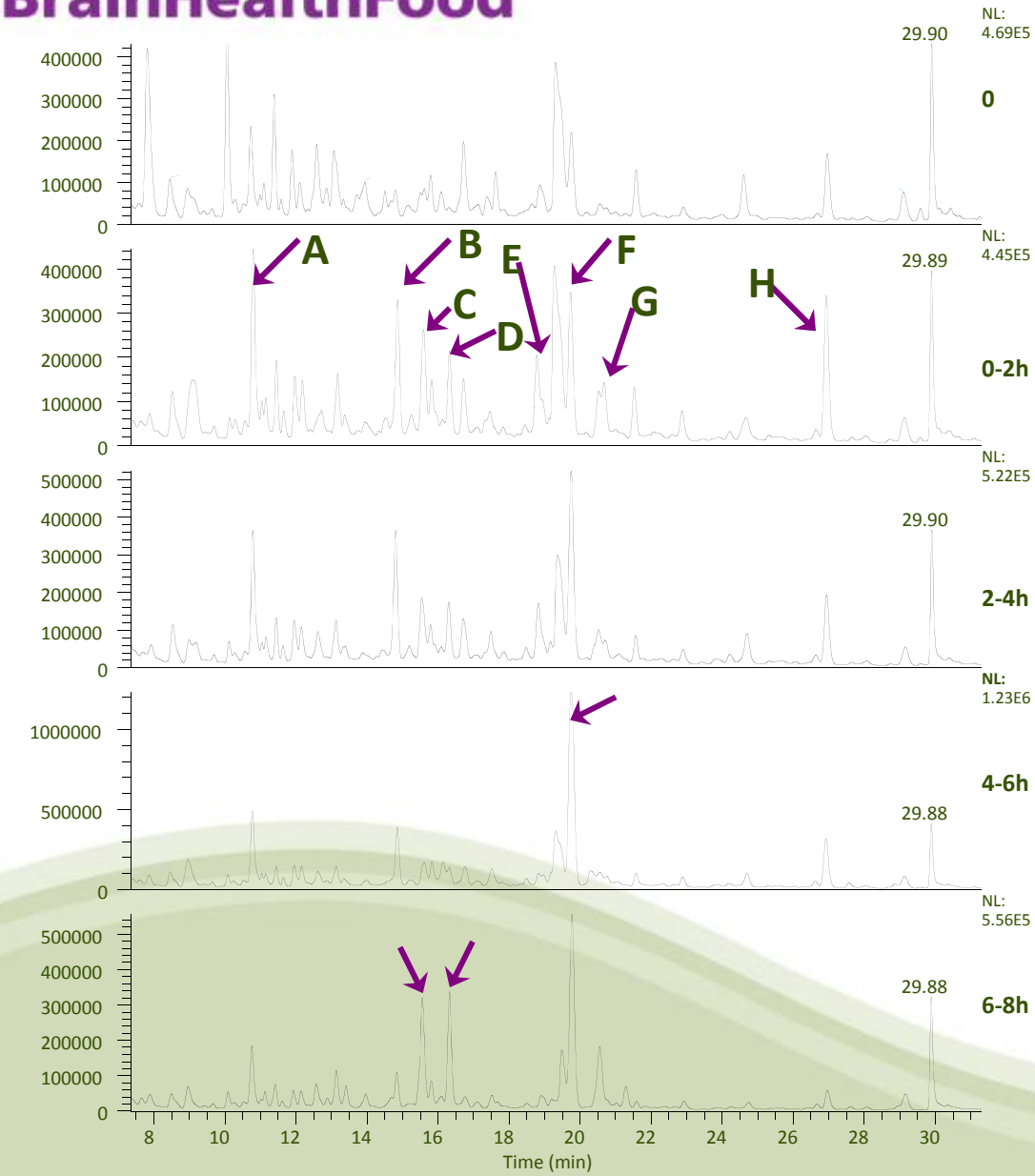
- Bioavailability of anthocyanins and other polyphenols from the two blackcurrant juices with different polyphenol contents (a control juice and a polyphenol-enriched juice)
- Randomized, controlled, double-blind cross-over study.
- Each subject in two 8-h postprandial tests, on separate days, at least 5 days apart.
- Control (pre consumption bloods taken.
- Blood taken at samples 15, 30, 45, 60, 90, 120, 150 and 180 min for plasma polyphenol, glucose and insulin measurements.
- Blood also taken at 4, 6 and 8 h for polyphenol measurements only.
- Control and trial urine taken at 0-2, 2-4, 4-6 and 6-8 hr.



Human Intervention Studies: Urine analysis



As metabolism progresses BC-related compounds start to become present in the urine. These are polyphenolic & anthocyanin derived.

At 4-6 hrs the main peak has increased by 10-fold.

The later peaks are probably a result of colonic bacterial breakdown processes followed by reabsorption and excretion

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Human Intervention Studies: Urine analysis

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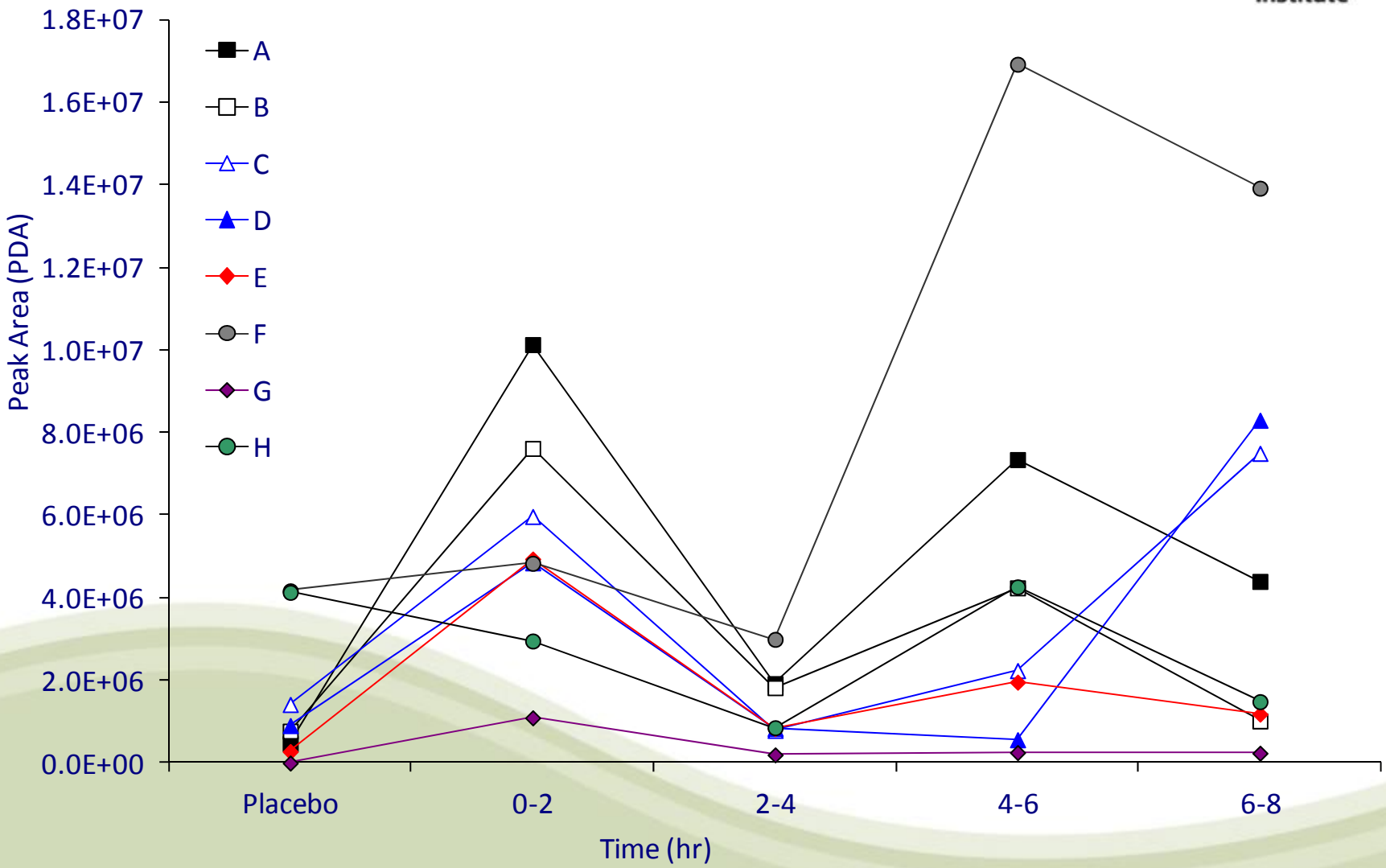
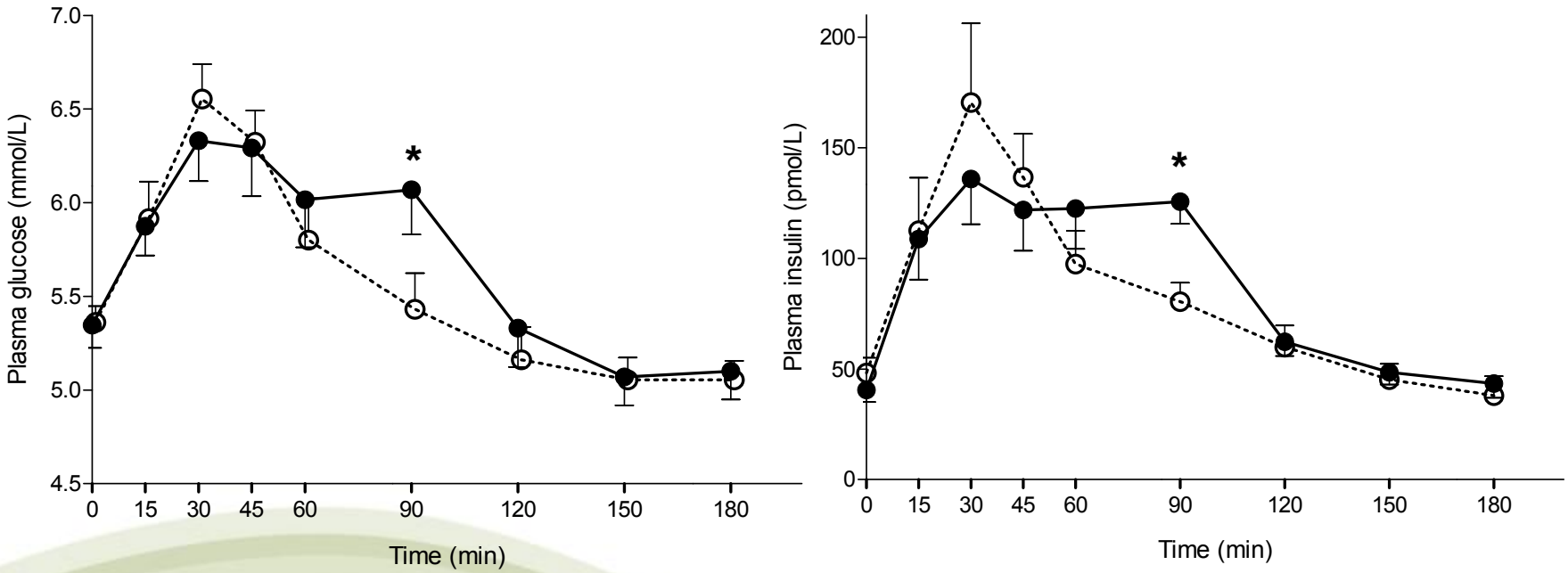
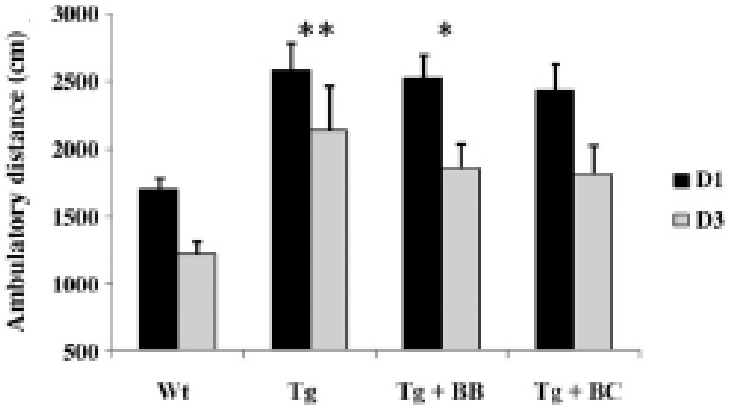


Human Intervention study

Plasma glucose and insulin concentrations (mean ± SEM) after consumption of 300 mL of sucrose-sweetened basic (○) and fortified (●) blackcurrant juices in 13 healthy subjects.

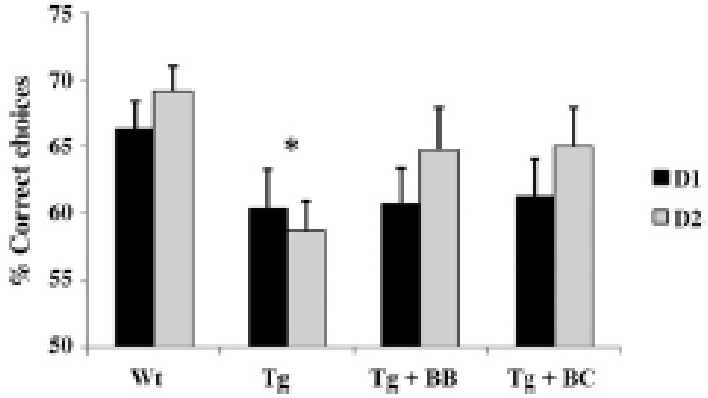


The polyphenol rich (fortified) juice attenuated both glucose and insulin levels:
An amelioration of the sugar rush and potentially a reduction in the inflammation triggers



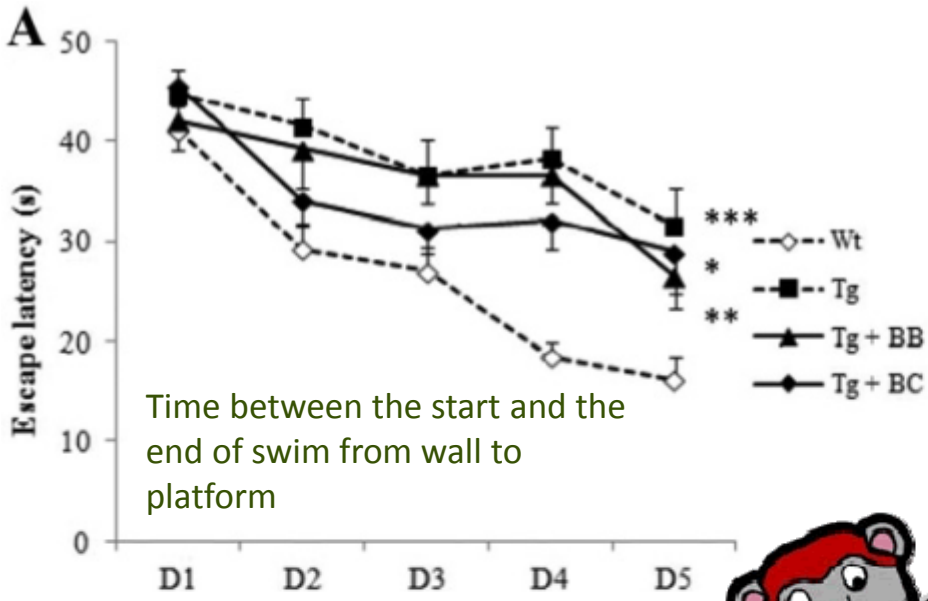
Ambulatory distance in a new test cage at two 10-min sessions on Day 1 (D1) and Day 3 (D3). Note the hyperactivity of APdE9 (tg) mice compared to their wild-type (Wt) littermates.

Anthocyanin-enriched bilberry and blackcurrant extracts alleviate hyperactivity and spatial working memory deficit in transgenic APdE9 mice.

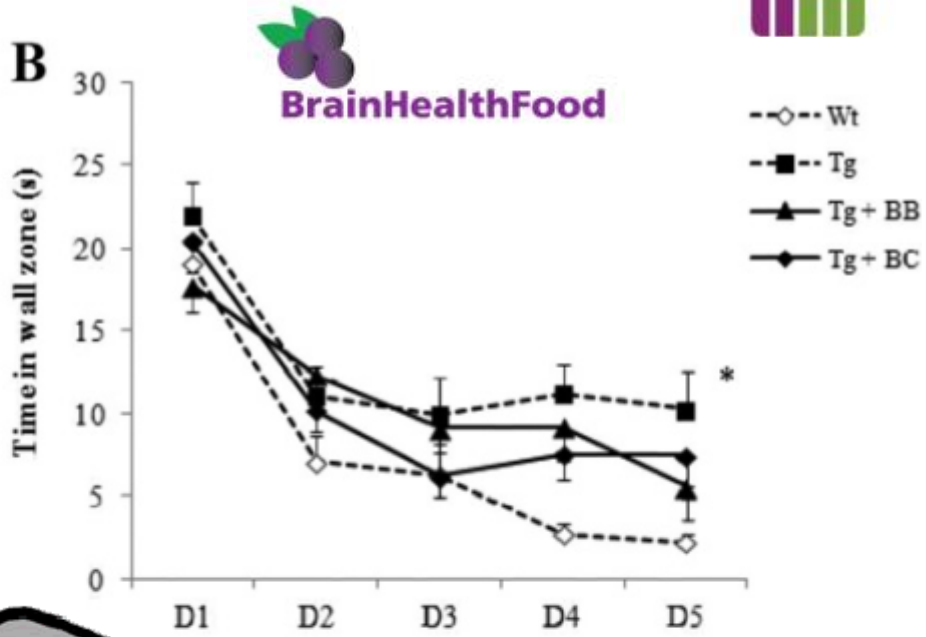


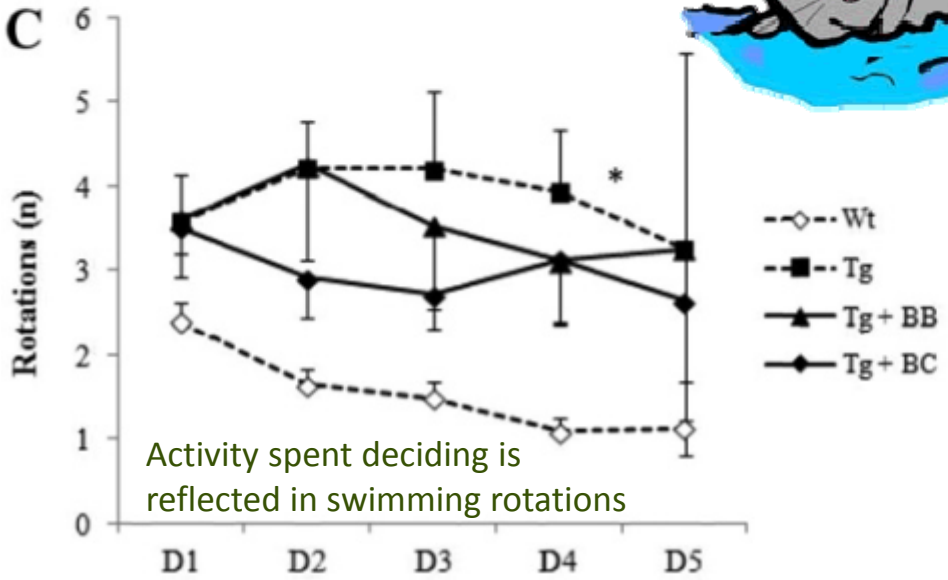
Correct choices (as %) in the delayed alternation task on D1 and D2.
Clear, but not significant, evidence of dietary polyphenol intake benefits.

APdE9 and wild type mice ability and learning tests: Fruit polyphenol effect in performance in Morris swim navigation task



Time between the start and the end of swim from wall to platform





Activity spent deciding is reflected in swimming rotations

<u>Alzheimer's model system</u>

APdE9 mice overexpress mutated human APP and presenilin-1 proteins

Water maze studies suggest that anthocyanin-rich bilberry and blackcurrant diets favourably modulate APP processing and alleviate behavioural abnormalities in a mouse model of AD

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Neural cell models highlighting polyphenol reduction in ROS: particularly important in brain metabolism

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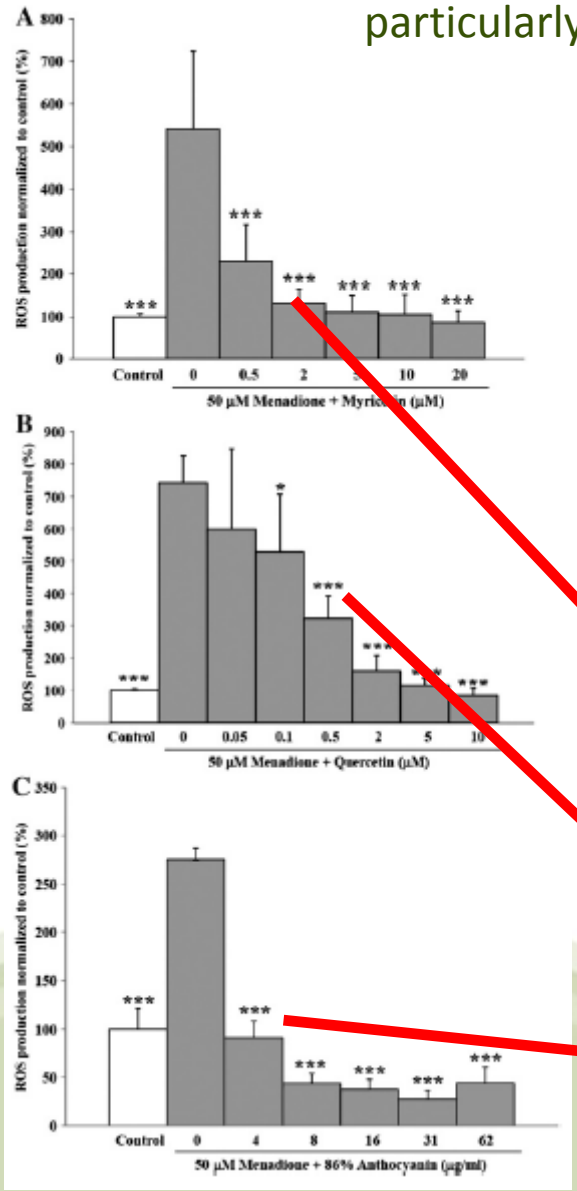


Myricetin (A), Quercetin (B) and anthocyanin-rich blackcurrant extracts alleviate menadione-induced ROS production in SH-SY5Y-APP751 cells [human neuroblastoma cells over expressing the β-amyloid protein APP751 isoform.

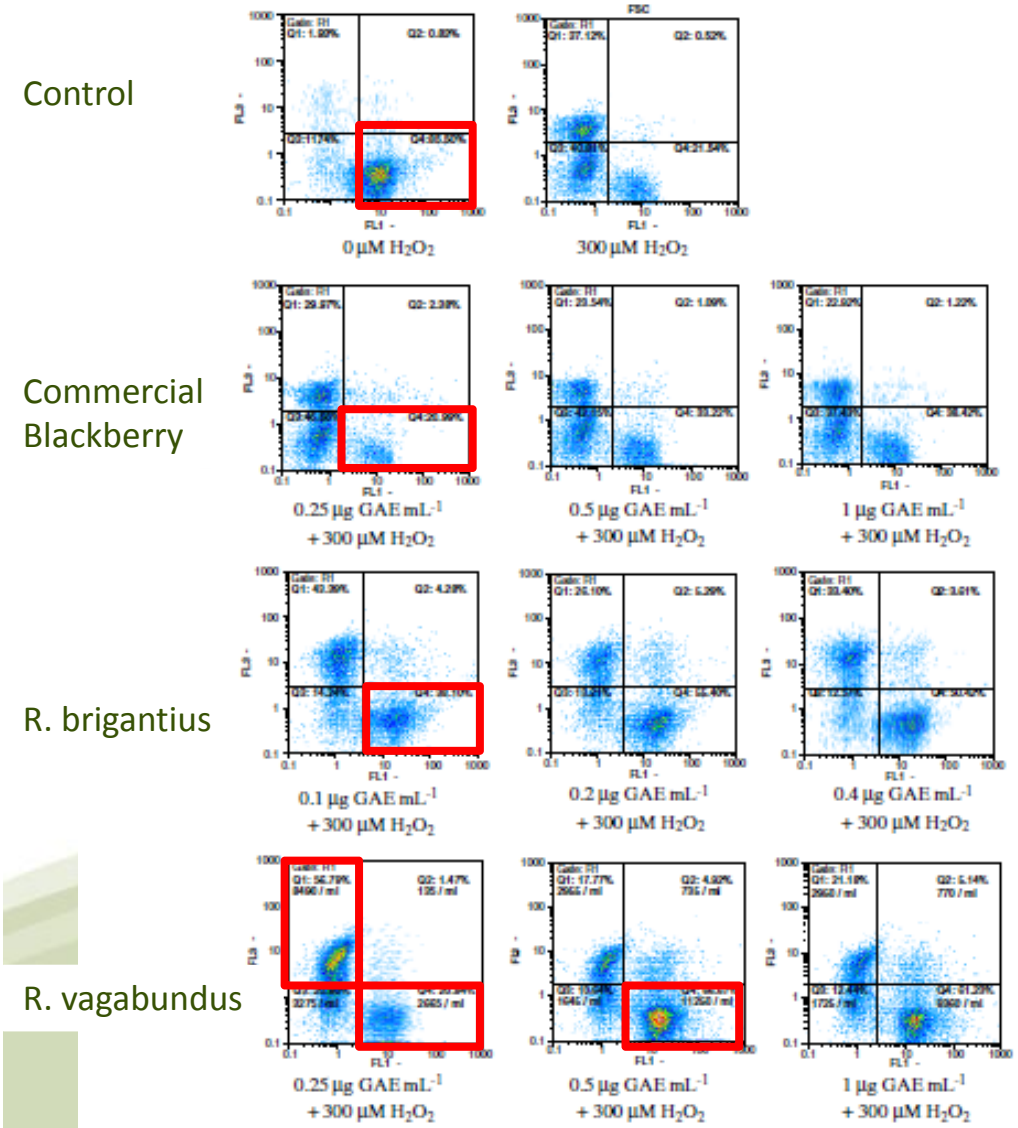
Neural cell models highlighting polyphenol reduction in ROS: particularly important in brain metabolism

Flavonols exhibit a dose response

Anthocyanins; more effective dose response and effective a physiological conditions

↓ Stress = ↓ plaque formation

Neuroprotective effects of digested polyphenols derived from wild blackberry (*R. fruticosus*) species

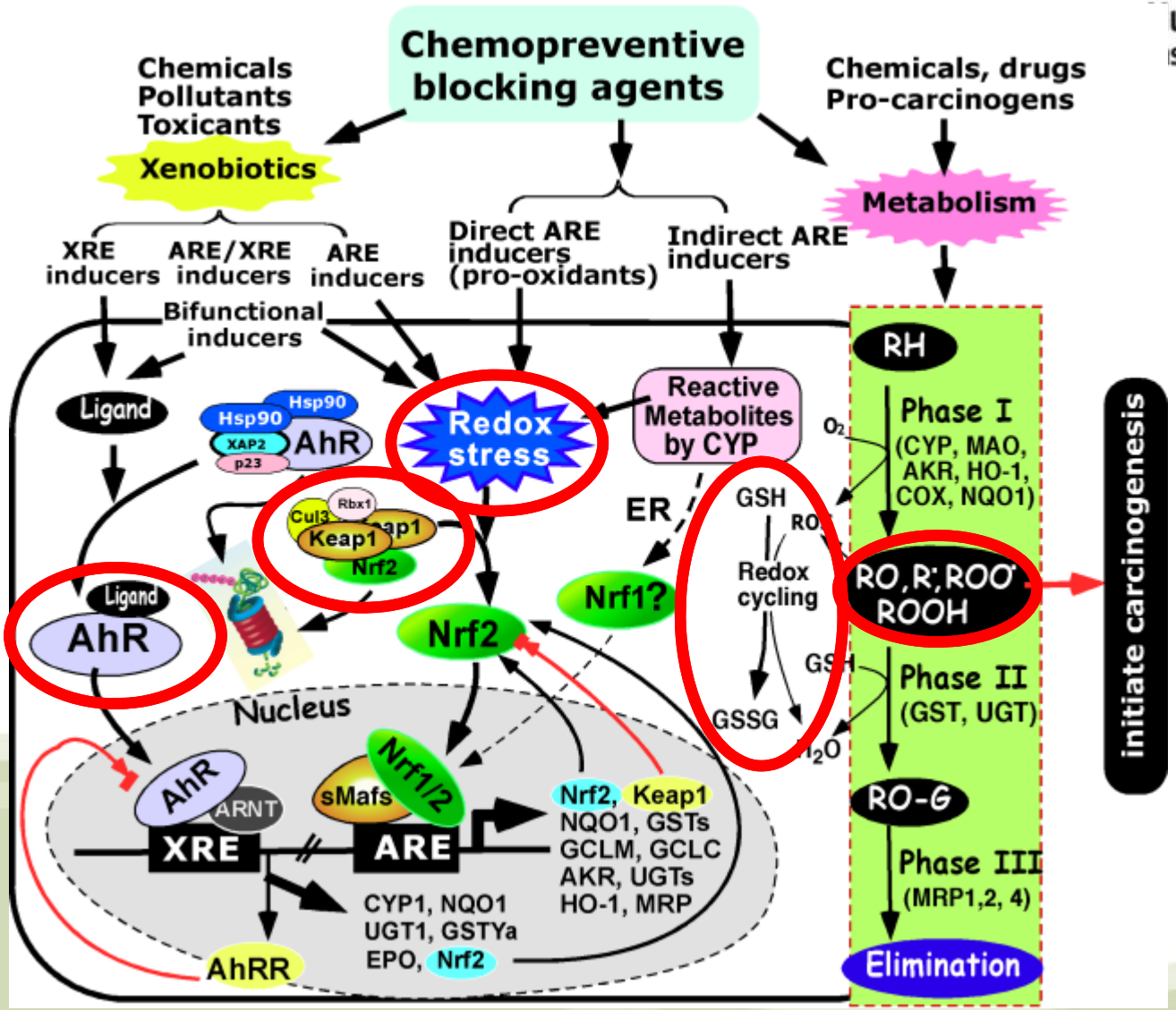


- Impact of digested polyphenols on Human neuroblastoma SK-N-MC cells responses to oxidative stress
- Flow cytometry outputs obtained with PI and DiOC6(3) allow the assessment of the percentage of viable cells [post incubation and stress] to be assed
- Q4 - cells presenting membrane integrity and high mitochondrial potential.

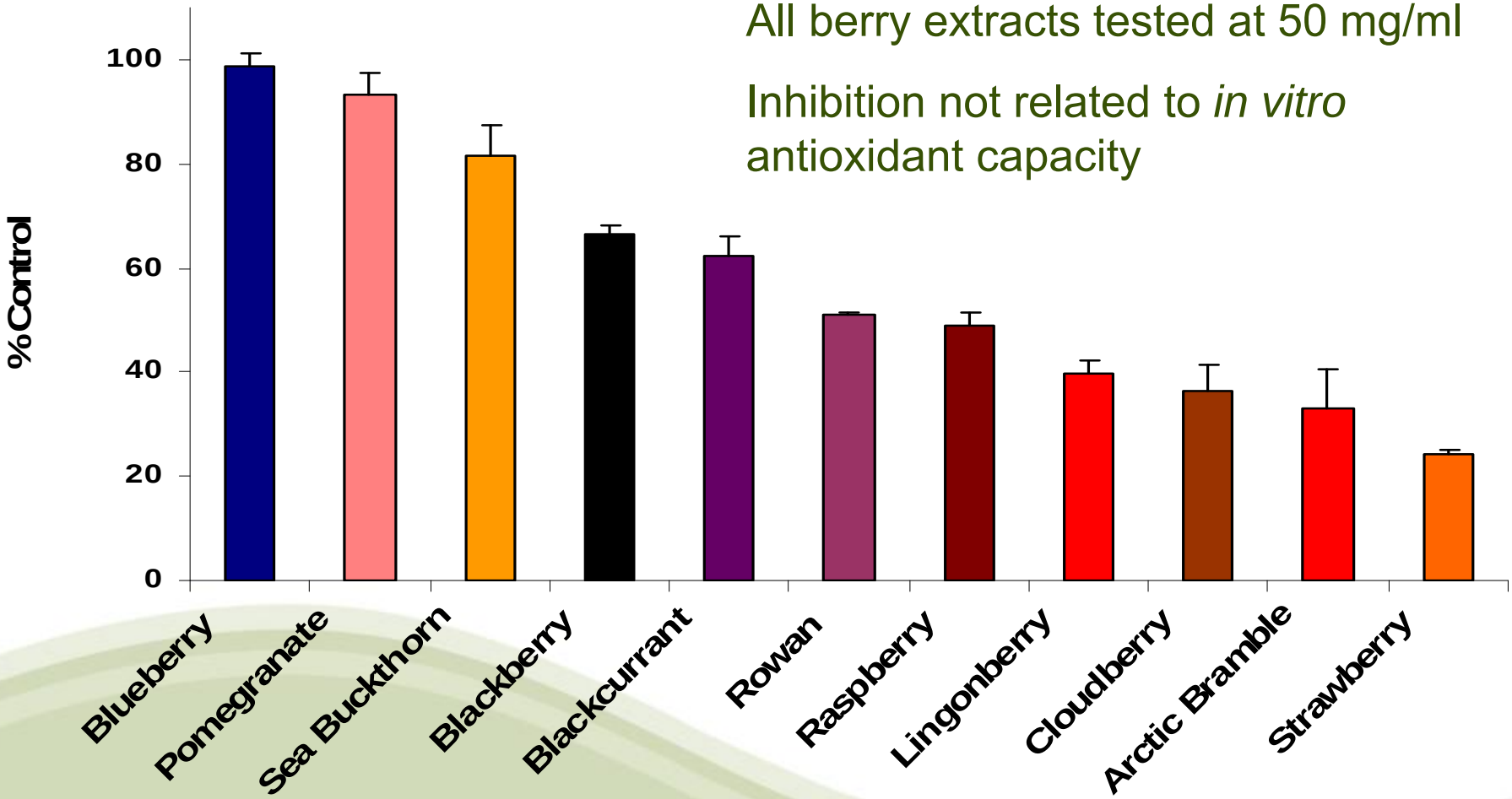
- + digested polyphenols – some deleterious effects
- + digested polyphenols then H_2O_2 – protection evident *BUT* chemistries different

Multiple signaling crosstalk between Nrf2-ARE and AhR-XRE gene regulatory networks

Dietary Polyphenols Interactions



Colon cancer cells efficacy



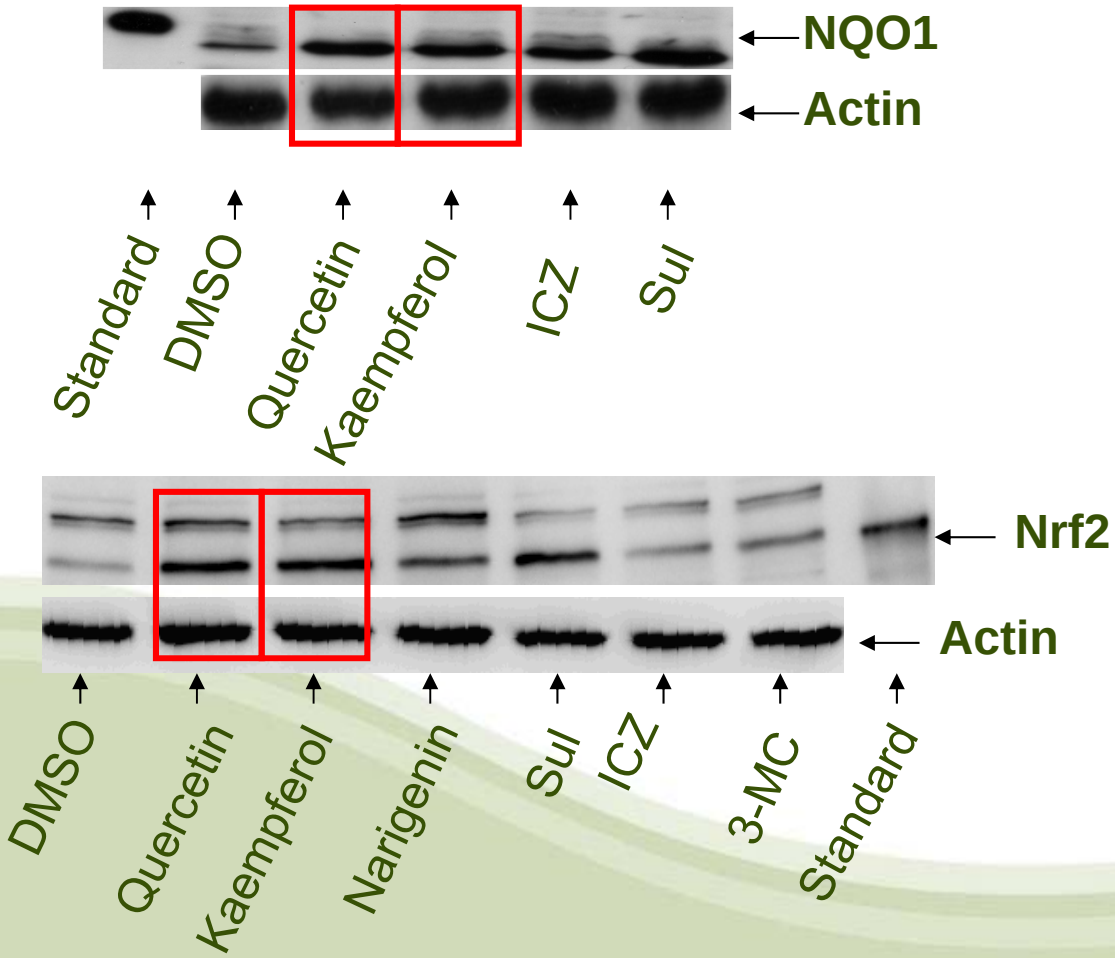
All berry extracts tested at 50 mg/ml

Inhibition not related to *in vitro* antioxidant capacity

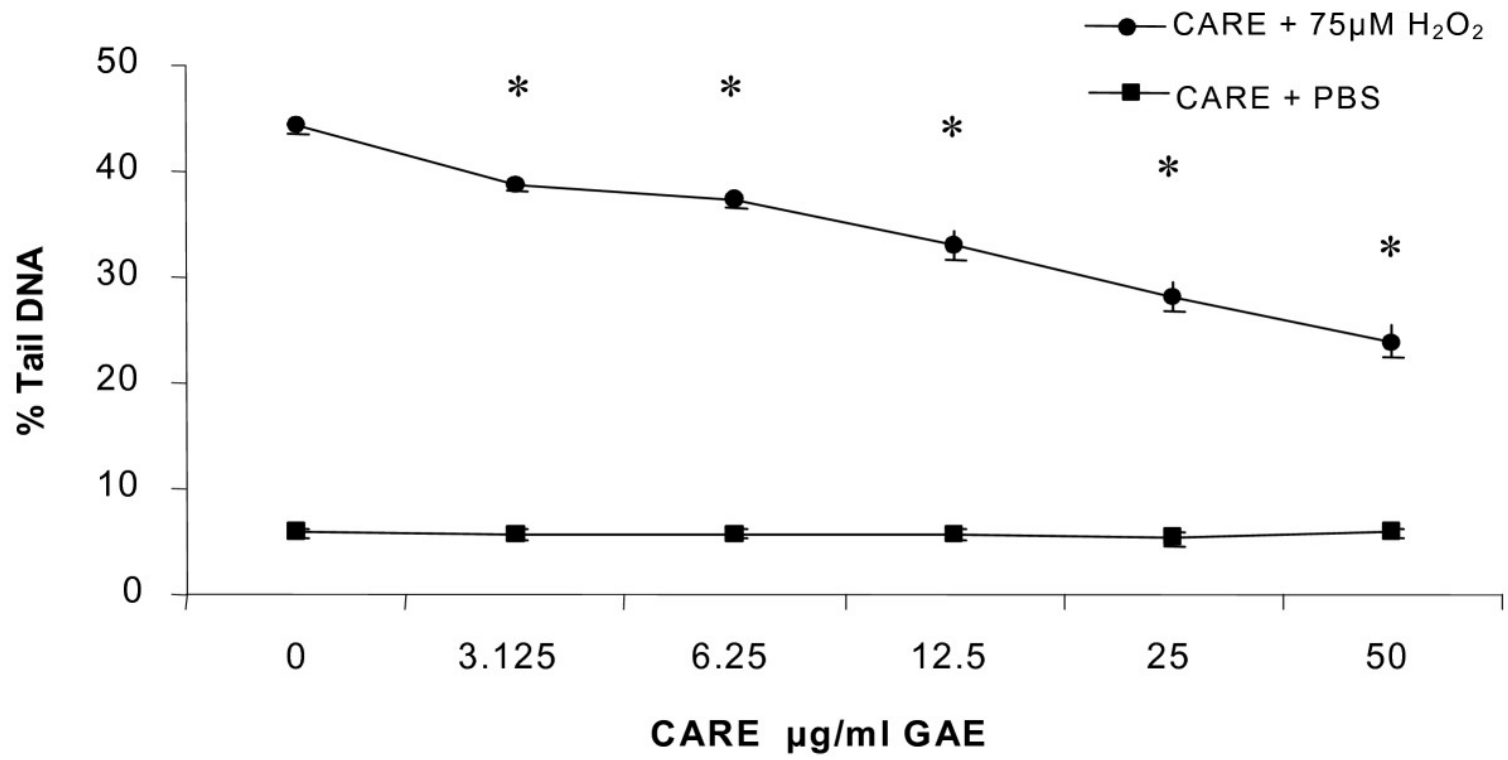
McDougall et al. (2008) JAFC 56; 3016-3023

Quercetin and Kaempferol increase the expression of NQO1 and the level of Nrf2 protein

Rat liver 34 epithelial cells

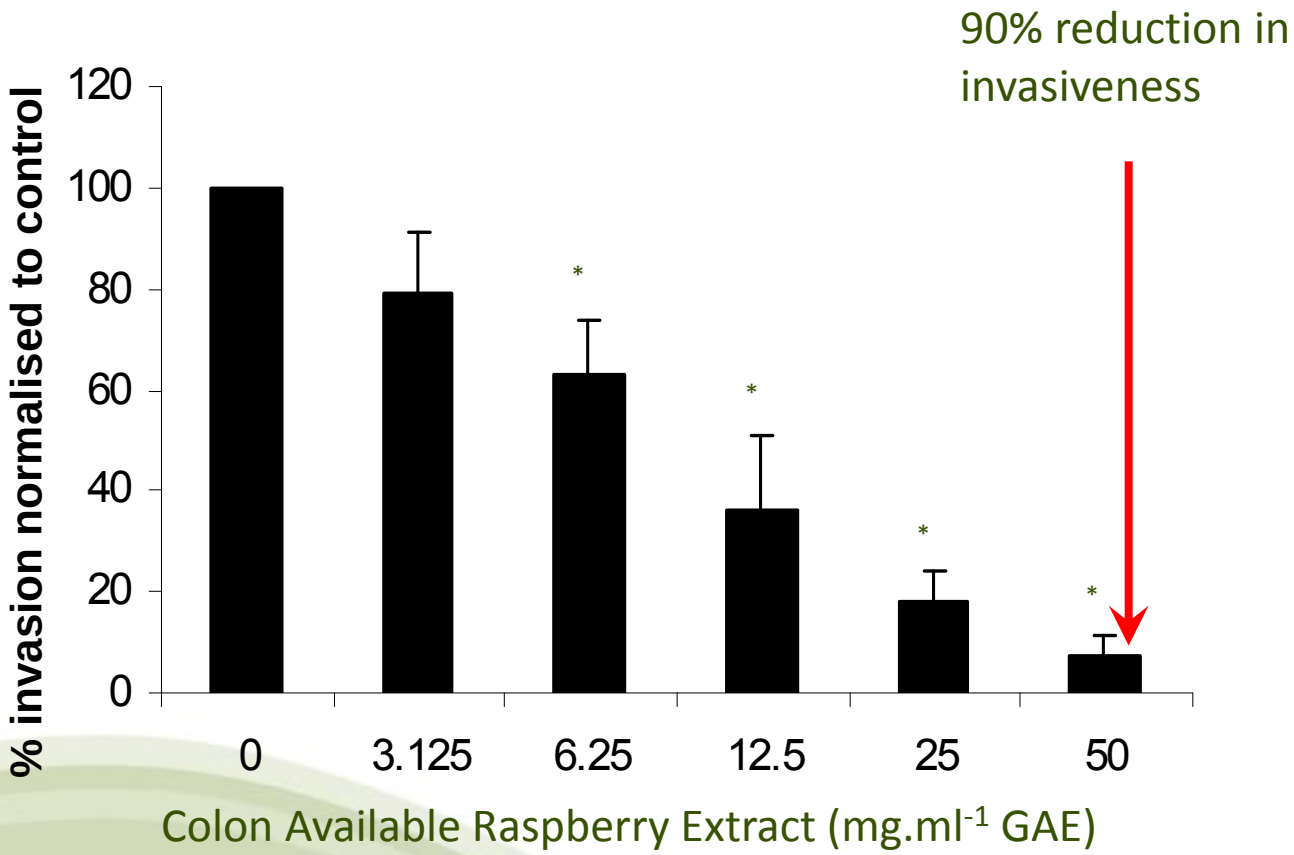


Genotoxic and anti-genotoxic effects of CARE (24 hr incubation) at different concentrations on DNA damage in HT29 cells



CARE- Colon Available Raspberry Extract (digested)

Impact of fruit polyphenolics of invasiveness of HT115 colon cancer cells *in vitro:* Cancer spread



Anticancer Activity in Berry Extracts
Cell models of polyphenol efficacy post digestion and colonic fermentation

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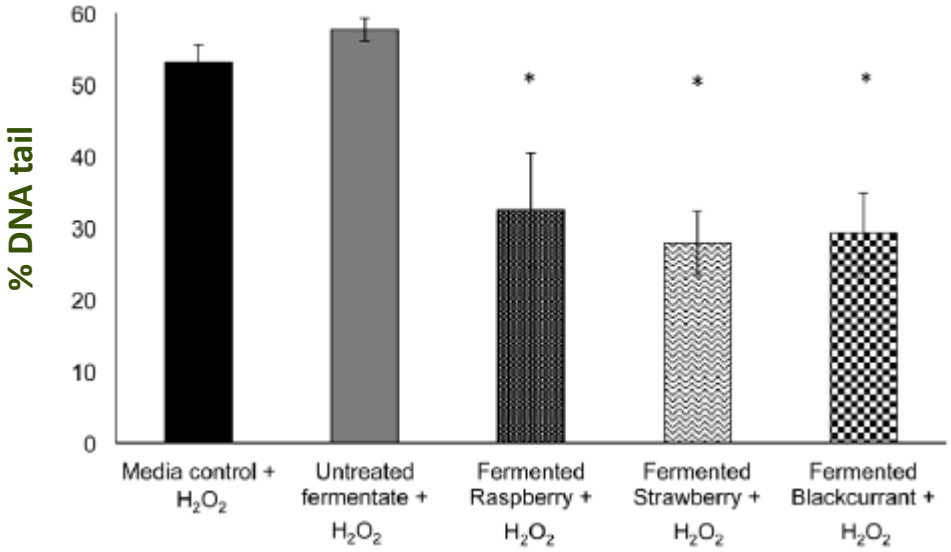
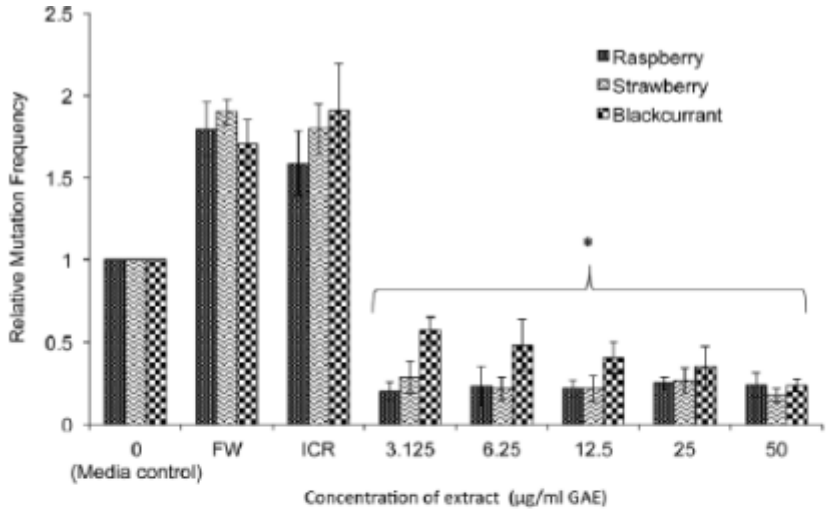
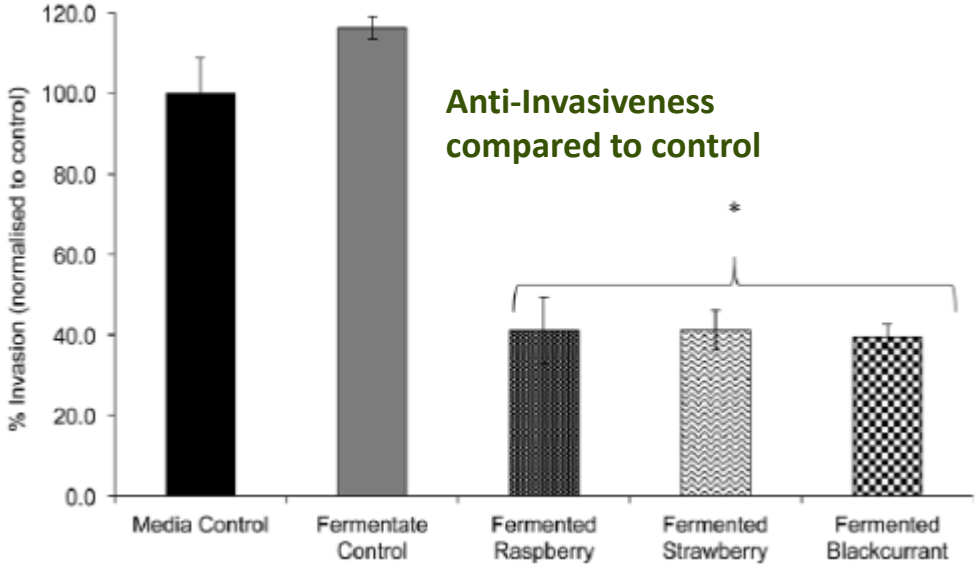
DNA damage

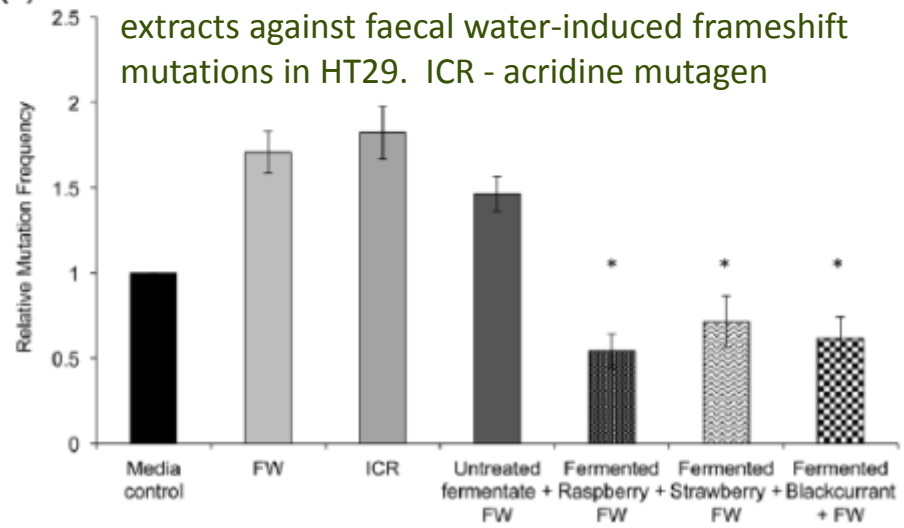




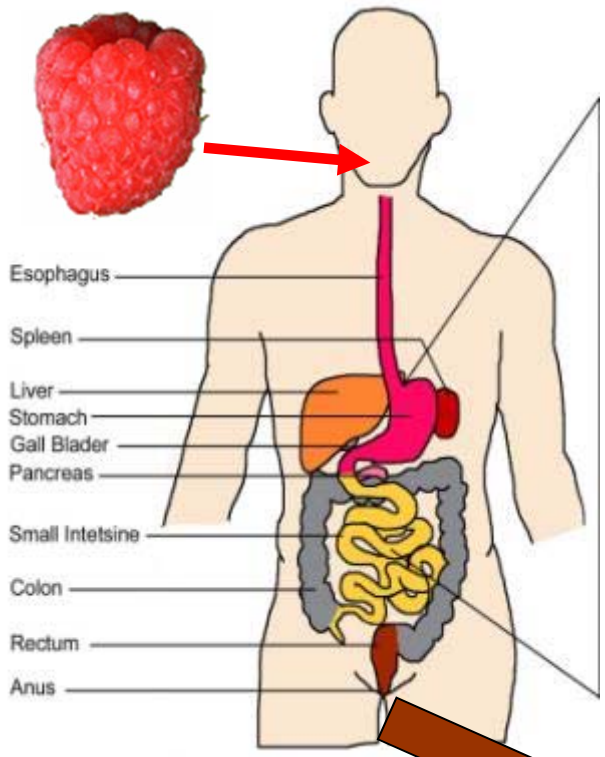


(b)

Anti-mutagenic effects of various types of berry extracts against faecal water-induced frameshift mutations in HT29. ICR - acridine mutagen



Faecal metabolism of berry polyphenols



- Metabolomic Profiling of faecal water metabolites in 10 free-living students after intake of raspberry puree (200 g/d for 14 d) by gas-chromatography mass spectrometry (GC-MS [and LC-MS]): Metabolomics
- Substantial ingestion of anthocyanins, ellagitannins etc.
- Focus on major phenolic metabolites.
- Some common metabolic patterns noted.

Faecal metabolism of berry polyphenols

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But not the same subjects!

Phenylacetic acid increased in 7/10 subjects

4-Hydroxy phenylacetic acid increased in 6/10 subjects

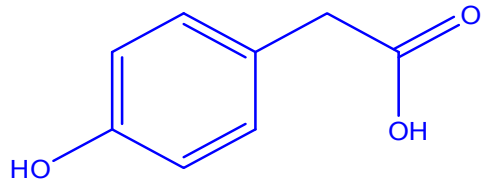
3-Hydroxy phenylacetic acid increased in 5/10 subjects

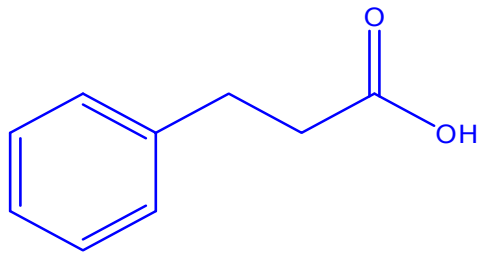
3-Phenylpropionic acid increased in 6/10 subjects

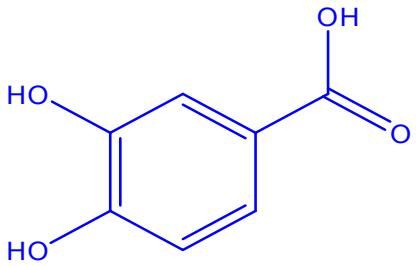
3-(4-Hydroxy)-phenylpropionic acid increased in 5/10 subjects

3,4-Dihydroxy benzoic acid increased in 7/10 subjects

4-Hydroxy benzoic acid increased in 2/10 subjects

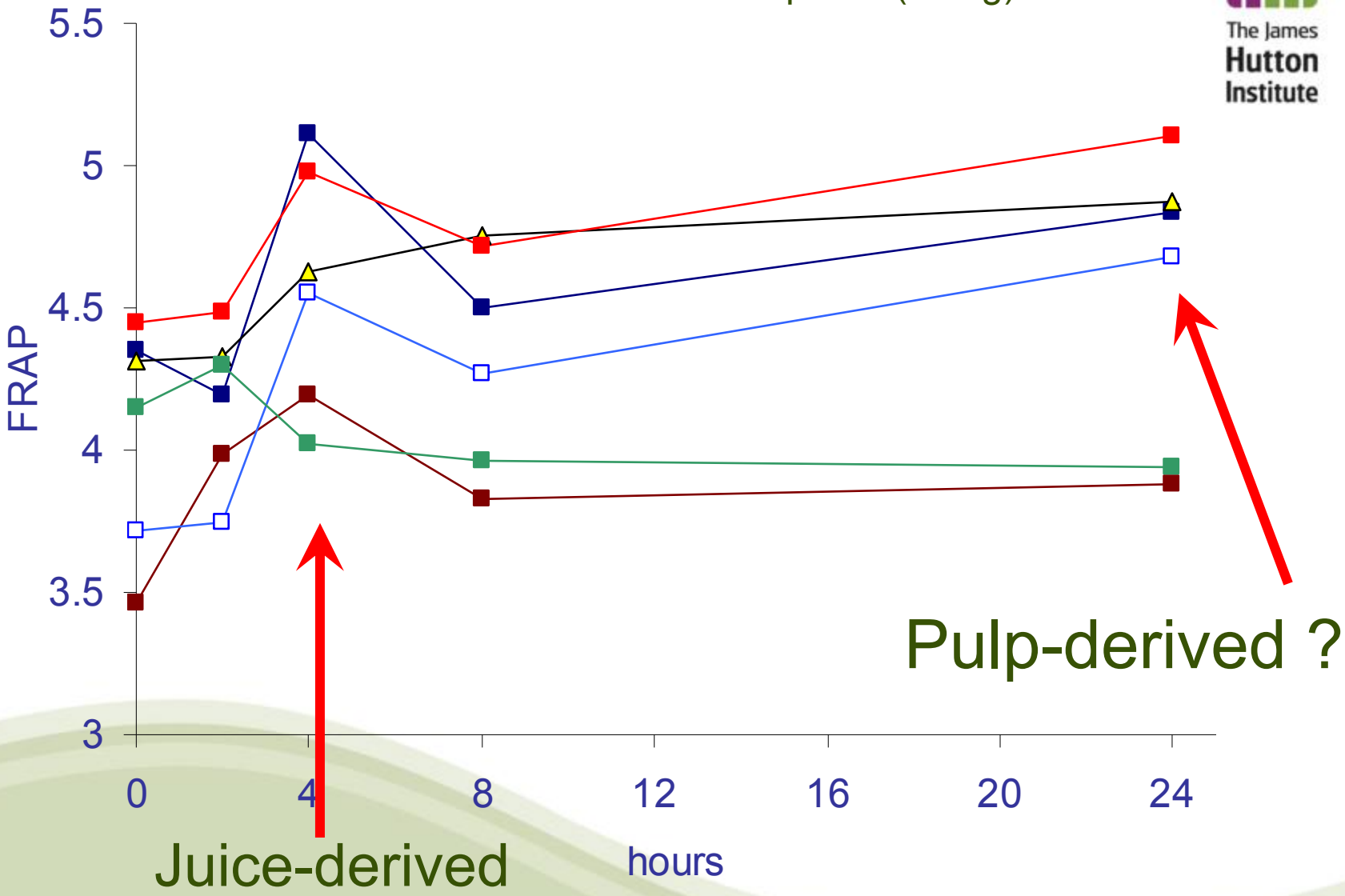




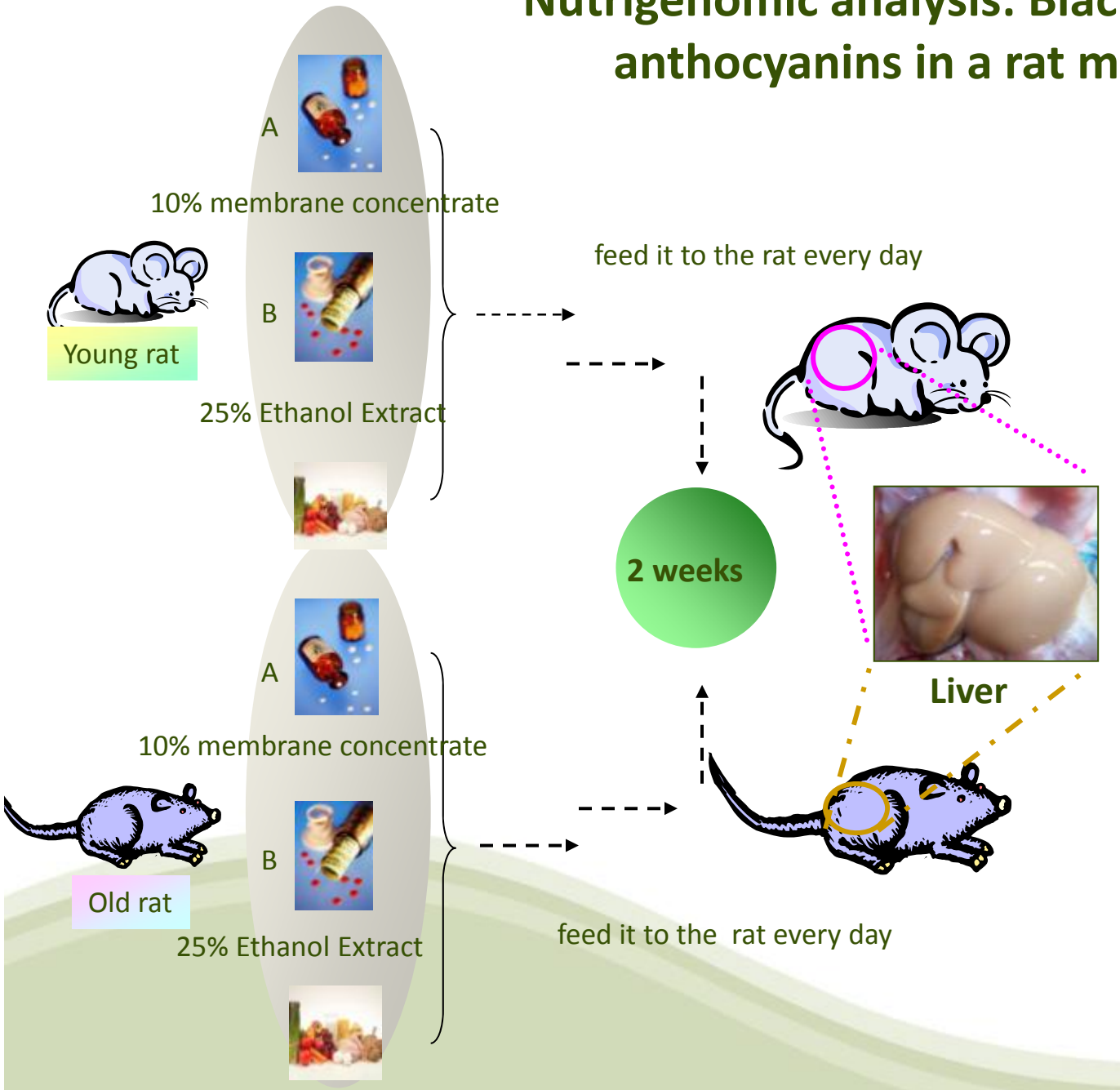


- Predominantly anthocyanin derived
- Fits evidence from model studies with faecal inocula but shows large inter-individual variation.
- Due to differences in diet or microflora?
- A proper confined study is required: defined diet, labelled fruit/anthos?

Measure serum antioxidant capacity in free-living students after intake of soft fruit puree (200 g)



Nutrigenomic analysis: Blackcurrants anthocyanins in a rat model.



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Nutrigenomic analysis: Blackcurrants anthocyanins in a rat.

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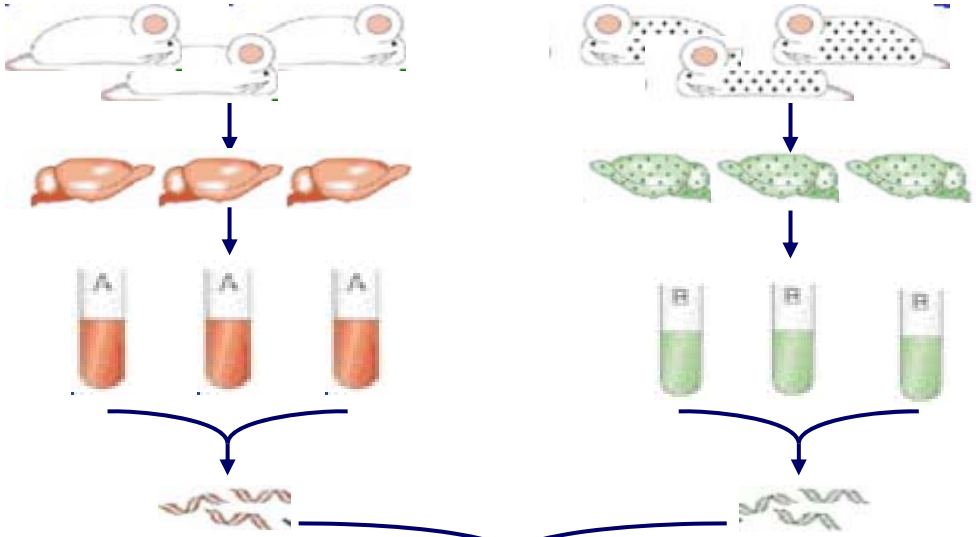
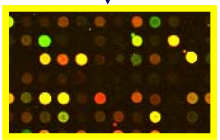
Control (No feed) **Experiment (feeded)**



Specimen collection & storage

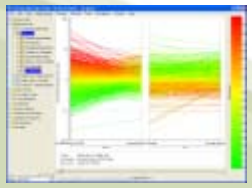
RNA isolation

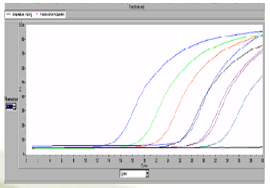


Rat Nutrigenomics array

Microarray experiment (Differential Gene Expression Profiling)

Bioinfomatics analysis





Targeted genes Validation by Q-PCR

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Blackcurrant anthocyanin intervention
Rat transcriptomic analysis

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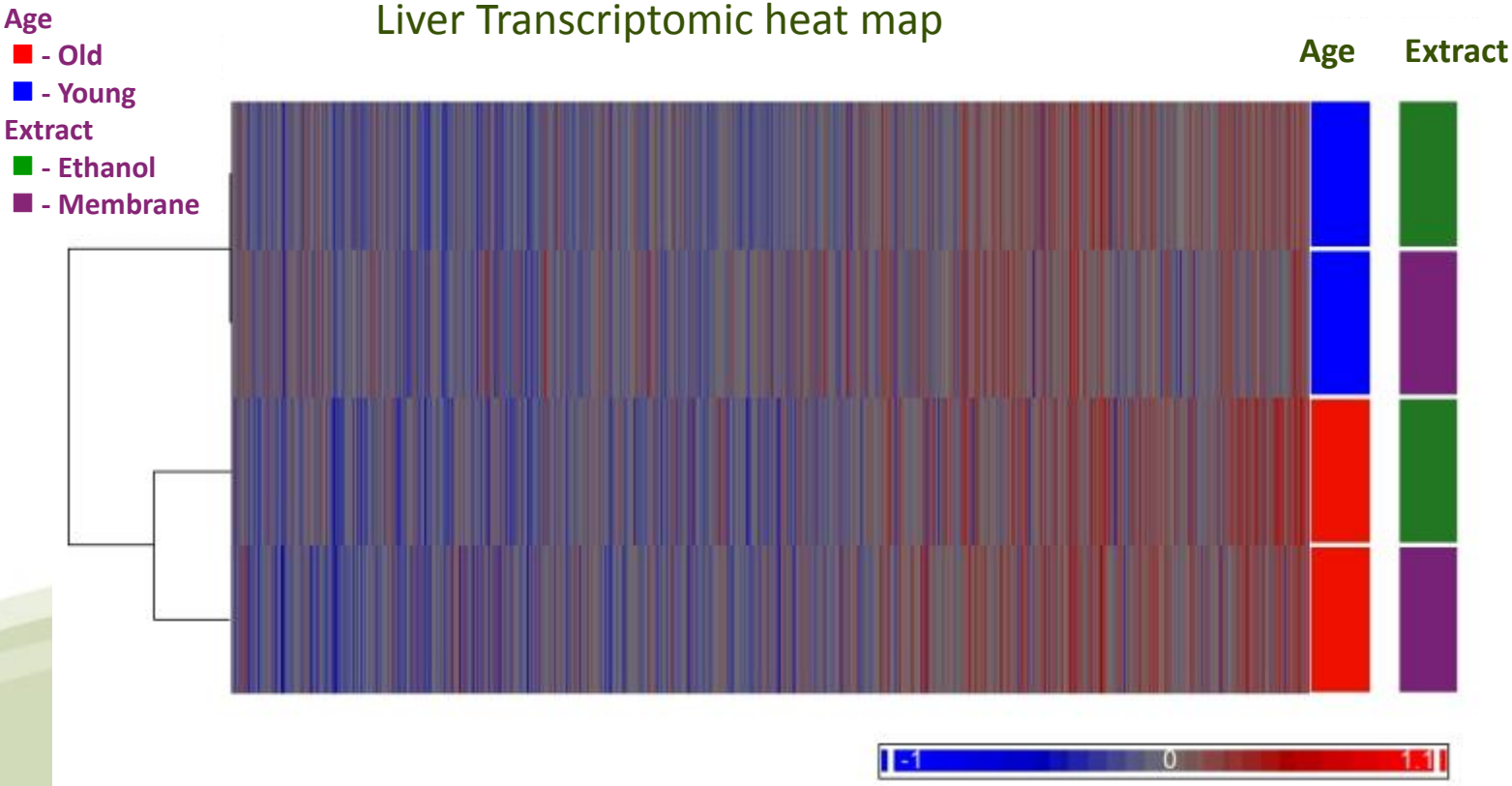
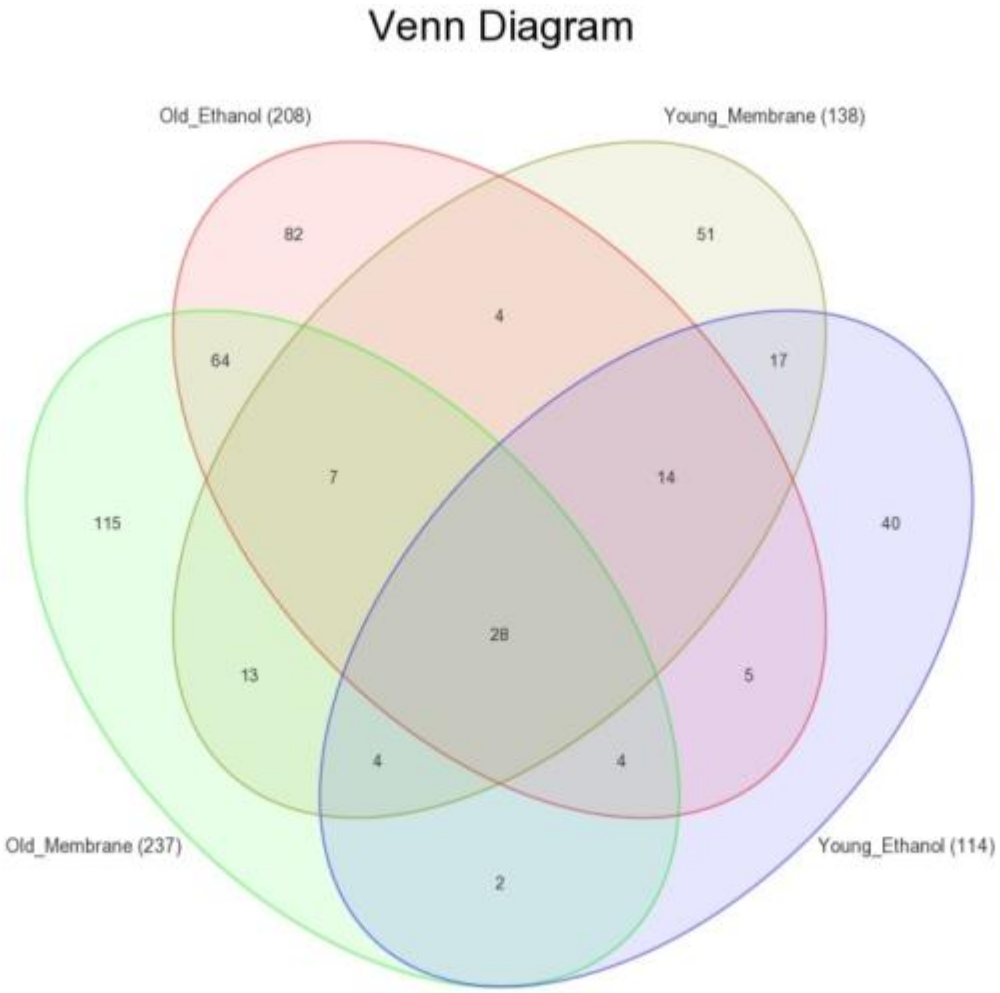
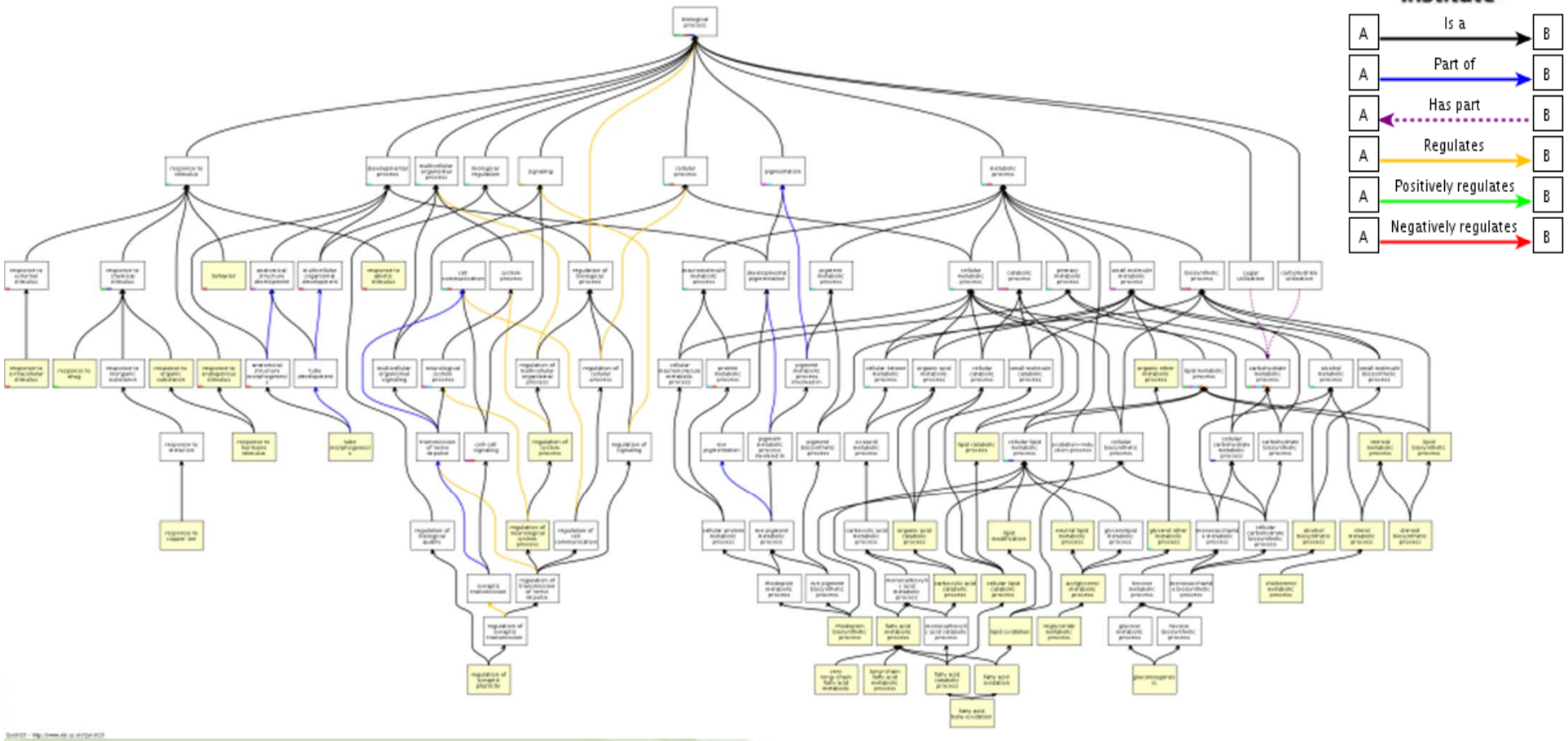
Liver Transcriptomic heat map



Blackcurrant anthocyanin intervention
Rat transcriptomic analysis



Pathway enrichment/regulation and ontology as a consequence of blackcurrant anthocyanin intervention



Database for **A**nnotation, **V**isualization and **I**ntegrated **D**iscovery (**DAVID)** - http://david.abcc.ncifcrf.gov/
QuickGO (http://www.ebi.ac.uk/QuickGO/)

Health benefits: what next?

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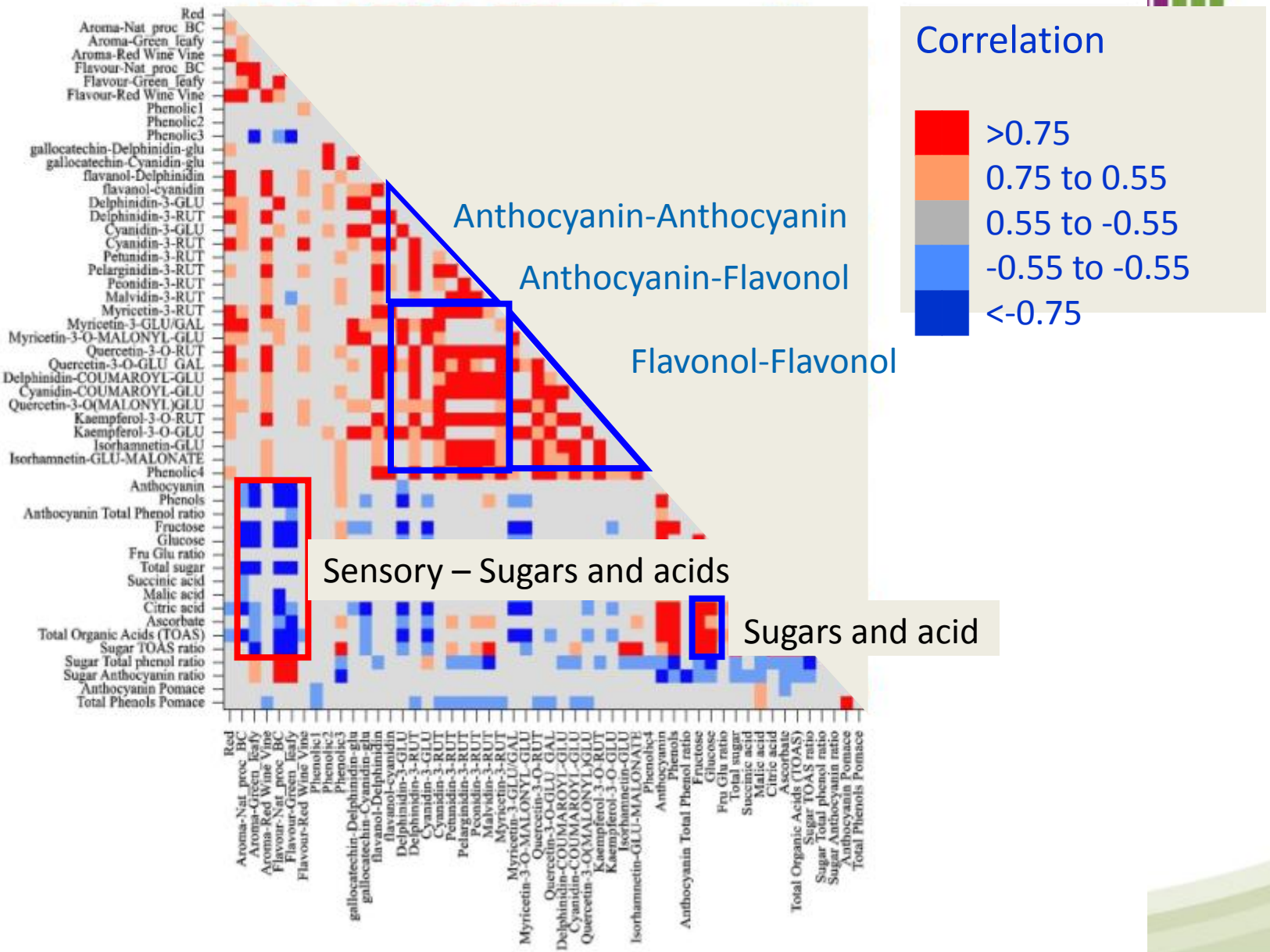
- Fruit and vegetables impact beneficial for human heath beyond nutritive value.
- Should be included as part of a health diet.
- Promoted as a route to remediate a poor (western?) healthy diet?
- Will the benefits sell more product.

Probably IF everything else stays the same

Soft fruit: Phytochemical Correlation Matrix



Correlation of chemical composition (matrix & volatiles) with sensory traits

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▲◆ matrix components

● volatiles

- ● Sulfur compounds
- ● Hydrocarbons
- ● Furan and furfural
- ● Aromatic aldehyde
- ● Short chain br-aldehydes
- ● Saturated aldehydes & ketones
- ● Unsaturated aldehydes & ketones
- ● Alcohols
- ● Furan derivatives (sub at position 2)
- ● Sesquiterpenes
- ● Methyl esters

- ◆ Glycoalkaloids
- ▲ Amino acids
- ▲ Sugars
- ◆ Nucleotides

allnames	Aroma:2 (Ar)	Intensity:2 (Int)	Sweetness: (Sw)	Savoury:2 (Sa)	reaminess (Cr)	Off:2 (Of)
Aroma						
Intensity	-0.47					
Sweetness	0.49	-0.70				
Savoury	-0.34	0.92	-0.64			
Creaminess	-0.53	0.89	-0.41	0.74		
Off	-0.28	-0.45	0.19	-0.56	-0.39	
Furfural	0.82	-0.74	0.50	-0.69	-0.74	-0.08
Furan	0.75	-0.87	0.58	-0.77	-0.84	0.01
%2_Propanone	0.73	-0.75	0.43	-0.67	-0.75	-0.12
Benzaldehyde	0.65	-0.91	0.50	-0.77	-0.95	0.19
Dimethyltrisulfide	0.63	-0.73	0.43	-0.67	-0.69	-0.15
Uunknown	0.62	-0.89	0.44	-0.78	-0.98	0.32
Dimethyldisulfide	0.61	-0.74	0.54	-0.60	-0.65	-0.23
Solanine_mg_100g_FW	0.57	-0.95	0.62	-0.92	-0.91	0.48
%2_Methylpropanal	0.56	-0.88	0.50	-0.76	-0.85	0.12
%3_Methylbutanal	0.56	-0.85	0.47	-0.74	-0.82	0.05
%2_Methylbutanal	0.54	-0.87	0.43	-0.74	-0.86	0.10
Methional	0.52	-0.78	0.48	-0.69	-0.69	-0.07
n_Nonanal	0.51	-0.64	0.15	-0.59	-0.86	0.48
Chaconine_mg_100g_FW	0.47	-0.92	0.61	-0.90	-0.89	0.64
sesquiterpene_b_Cubebene	0.46	-0.26	-0.06	-0.19	-0.57	0.19
%1_Penten_3_one	0.46	-0.92	0.82	-0.83	-0.69	0.19
Benzeneacetaldehyde	0.42	-0.81	0.33	-0.67	-0.84	0.15
%2_2or1_pentenylfuran	0.41	-0.83	0.63	-0.68	-0.66	0.10
%2_Ethylfuran	0.39	-0.61	0.27	-0.39	-0.82	0.47
%4_or_2_hepten_1_al	0.34	-0.85	0.61	-0.73	-0.68	0.14
%2_Heptanone	0.34	-0.60	0.27	-0.42	-0.56	-0.15
Ethylacetate	0.33	-0.77	0.21	-0.75	-0.75	0.21
%2_2or1_pentenyl_furan_or_other	0.32	-0.86	0.65	-0.74	-0.67	0.21
Aspartate_g_100gFW	0.31	-0.22	0.61	-0.23	0.02	-0.43
%2_Pentylfuran	0.30	-0.81	0.62	-0.70	-0.59	0.13
%1_penten_3_ol	0.29	-0.74	0.12	-0.53	-0.88	0.26
a_isopropylidene_2_furancarboxal	0.29	0.04	-0.56	0.03	-0.31	-0.23
Unknown	0.28	-0.89	0.78	-0.79	-0.64	0.31
%2_Nonenal	0.28	-0.91	0.50	-0.83	-0.79	0.33
%2_4_Heptadienal	0.26	0.03	-0.47	-0.04	-0.31	0.23
n_Decanal	0.23	-0.11	-0.16	-0.13	-0.41	0.37
n_Octanal	0.23	-0.41	0.03	-0.43	-0.64	0.61
%2_2_pentenylfuran	0.21	-0.90	0.78	-0.80	-0.65	0.44
n_Pentanal	0.20	0.35	-0.74	0.40	-0.05	-0.41
%1_pentanol_or_i_amyl_alcohol	0.18	-0.01	-0.61	0.09	-0.36	-0.21
n_Heptanal	0.17	-0.75	0.57	-0.65	-0.52	0.16
%2_Methylfuran	0.14	-0.38	-0.06	-0.22	-0.64	0.57
%5_methylhexanal_or_2_hepten_1_o	0.11	-0.23	0.27	-0.26	-0.30	0.56
Propanal	0.09	-0.74	0.49	-0.65	-0.74	0.78
Glutamate_g_100gFW	0.09	-0.02	0.53	-0.05	0.19	-0.22
Pentane	0.08	-0.46	-0.15	-0.31	-0.58	0.25
%2_Butylfuran	0.07	-0.68	0.63	-0.56	-0.39	0.17
Fructose_mg_gFW	0.03	-0.03	0.54	-0.03	0.30	-0.39
%3_Ethyl_2_Methyl_1_3_hexadiene_	0.01	-0.38	-0.38	-0.35	-0.65	0.38
%2_Hexenal	-0.01	-0.81	0.65	-0.69	-0.63	0.58
Dimethylsulfide	-0.01	-0.25	-0.25	-0.31	-0.48	0.74
Sucrose_mg_gFW	-0.03	0.34	0.27	0.42	0.52	-0.53
a_Copaene	-0.08	0.01	0.08	0.01	-0.09	0.51
%2_Propylfuran	-0.17	-0.12	-0.11	-0.05	-0.31	0.67
%2_Octenal	-0.20	-0.62	0.57	-0.62	-0.29	0.42
%2_Heptenal	-0.21	0.27	-0.48	0.16	-0.01	0.40
Methylsalicylate	-0.24	0.93	-0.77	0.84	0.69	-0.41
Glucose_mg_gFW	-0.36	-0.36	0.40	-0.31	-0.08	0.44
EUC	-0.51	0.65	-0.14	0.60	0.72	-0.14
%3_or_2_methylbutanoic_acid_me_	-0.54	0.76	-0.50	0.75	0.64	0.10
%2_or_4_pentenal	-0.58	0.07	0.03	-0.04	0.14	0.70
AMP_g_100gFW	-0.63	0.76	-0.61	0.71	0.63	0.11
GMP_g_100gFW	-0.69	0.74	-0.57	0.70	0.65	0.12

Ar: Aroma

Int: Intensity

Sw: Sweet

Sa: Savoury

Cr: Creaminess

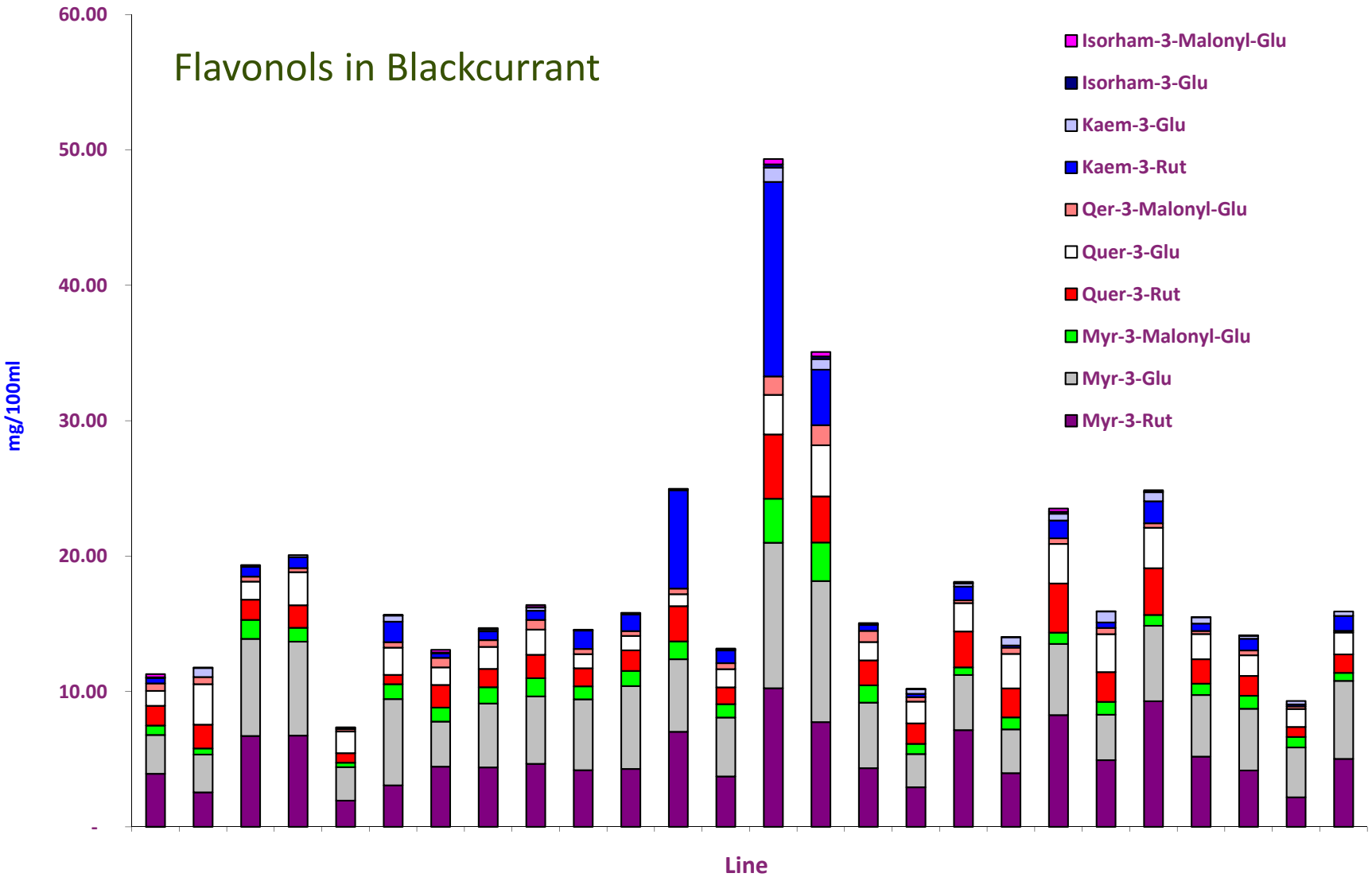
Of: Off

+Ve correlation > 0.5

-Ve correlation < -0.5

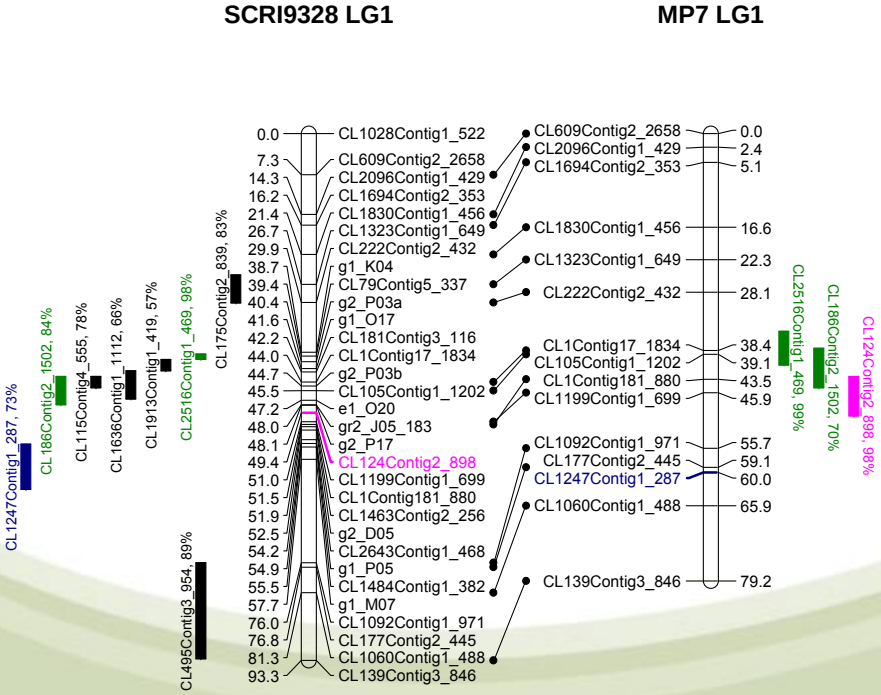
Morris et al., (2010) *Phytochemistry 71*, 1765-1773.

Polyphenolic Diversity



Next Generation Sequencing in *Ribes and Rubus*

- Large scale 454 transcriptomic sequencing of two *Ribes* genotypes (9328 reference mapping parents)
- > 700k reads (117.9 Mbp of blackcurrant transcriptome)
- Reads assembled into 46411 contigs
- 7245 SNPs and 3179 SSRs discovered
- Set of 384 SNPs selected using `Tablet' programme, range of germplasm assessed on Illumina BeadXpress platform
- New 384-SNP under development, also Genotyping By Sequencing is being investigated.
- Trait associations in development through field phenotyping and detailed metabolomics



Conclusions

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- The time line for health claims on non-nutrients looks mid-long term: antioxidant Vs pharmacological Vs signalling.
- Further *in vitro/vivo* studies need to use the appropriate metabolites of polyphenols; anthocyanins Vs metabolites
- Appropriate concentrations ***must*** be used in order to fully ascertain their mode of action *in vivo.*
- Polyphenols have significant cytotoxic effects at concentrations at or above 10 µM so enhancement may have an upper limit? However this may enhance colonic activity and benefits.
- More expert exploitation of germplasm is needed to effectively identify the active components: acylation, differential glycosyation, +/- anthocyanins etc.
- The explosion in genome sequencing will mean that bespoke fruit should soon be within our grasp especially when linked to metabolomics.
- This will also facilitate identification of environment driven changes.
- Care needs to be taken not to alter the MAIN selling points: appearance, smell and taste

Hope ~~or Hype~~

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