Dietary Flavonoids: Modulators of Brain Ageing?

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The Sensitivity of the Brain to Oxidative Stress

- High oxygen consumption
- Rich in polyunsaturated fatty acids.
- High levels of transition metals
- Lower antioxidant defences
- Presence of oxidisable neurotransmitters
Why is there interest in diet?

- Increasing life expectancy
- Increasing number of patients with neurodegenerative diseases
- To develop therapeutic or dietary interventions to delay the onset or counteract the progression of neurodegenerative diseases

5 portions a day!
Flavonoids: source

Fruit and vegetables: (All classes)

Red wine: (Flavanol, Flavonols)

Tea: (Flavanols)

Cocoa: (Flavanols and procyanidins)

Citrus: (Flavanone)

Berries: (Anthocyanins)
Flavonoids: structure

- Flavanol
- Isoflavone
- Anthocyanin
- Flavonol
- Flavanone
Ageing and Incidence of Neurodegenerative Diseases

Life Expectancy is increasing

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Prevalence of AD (%)</th>
<th>Prevalence of PD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35-59</td>
<td>0.2</td>
<td>0.07</td>
</tr>
<tr>
<td>60-69</td>
<td>0.3</td>
<td>0.18</td>
</tr>
<tr>
<td>70-79</td>
<td>3.2</td>
<td>1.72</td>
</tr>
<tr>
<td>80-89</td>
<td>10.8</td>
<td>6.2</td>
</tr>
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</table>

Increased health care costs and general demand on the NHS.

- Reduced quality of life for the elderly population.
Evidence for Flavonoid Action?

- Flavonoids as beneficial compounds against degenerative diseases.
- Molecular Mechanisms?
- Supplementation studies in humans and animals
- Epidemiological Studies

Flavonoids may reduce the risk of death from coronary heart disease and cancer


Flavonoid extracts from fruit and vegetables have been reported to attenuate cognitive decline and neuronal dysfunction in animal models and humans.

How Might They Work at the Cellular Level?
Flavonoids as Antioxidants

Classical Antioxidant Property:

- B-ring catechol group
- Un-saturation in the C-ring
- Carbonyl group
- Presence of transition metal ion binding functions

Powerful scavengers of ROS and RNS:

- Fenton systems: $\text{Fe}^{2+} \text{Cu}^+$ and peroxide
- $\text{ONOO}^-$ and $\text{HOCl}$
- Metal chelators
Biotransformation of flavonoids in the body

1. Oral Ingestion of flavonoid → Oligomeric Flavonoids → Monomeric units
   - Stomach
   - Small Intestine (jejunum, ileum)
   - Colon

2. Gut microflora → Phenolic acids
   - Flavonoid metabolites

3. Further metabolism
   - A-ring glucuronides
   - O-methylated glucuronides
   - O-methylated sulphates
   - Aglycone

4. Blood-brain barrier
   - Neurons glia
   - Blood vessels

5. Renal excretion of glucuronides
   - Liver
   - Kidney
   - Urine
Metabolism Reduces Antioxidant Potential

Increasing level in the circulation

Reduction in Antioxidant potential

3'-O-Methyl-epicatechin

Epicatechin 7-O-β-D-glucuronide

Epicatechin
Do Flavonoids Access the Brain?
Pelargonidin

**2h**

- **Brain**: Conjugated (0.05 nmol/g tissue), Free (0.02 nmol/g tissue)
- **Lungs**: Conjugated (0.03 nmol/g tissue), Free (0.02 nmol/g tissue)
- **Heart**: Conjugated (0.04 nmol/g tissue), Free (0.02 nmol/g tissue)
- **Liver**: Conjugated (0.06 nmol/g tissue), Free (0.03 nmol/g tissue)
- **Spleen**: Conjugated (0.08 nmol/g tissue), Free (0.04 nmol/g tissue)
- **Kidney**: Conjugated (0.12 nmol/g tissue), Free (0.06 nmol/g tissue)
- **Plasma**: Conjugated (1.1 nmol/g tissue), Free (0.5 nmol/g tissue)

**18h**

- **Brain**: Conjugated (0.01 nmol/g tissue), Free (0.005 nmol/g tissue)
- **Lungs**: Conjugated (0.02 nmol/g tissue), Free (0.01 nmol/g tissue)
- **Heart**: Conjugated (0.03 nmol/g tissue), Free (0.015 nmol/g tissue)
- **Liver**: Conjugated (0.05 nmol/g tissue), Free (0.025 nmol/g tissue)
- **Spleen**: Conjugated (0.07 nmol/g tissue), Free (0.035 nmol/g tissue)
- **Kidney**: Conjugated (0.09 nmol/g tissue), Free (0.045 nmol/g tissue)
- **Plasma**: p-HBA (0.2 nmol/ml), Conjugated (0.1 nmol/ml), Free (0.05 nmol/ml)
Levels of antioxidants in brain

- Total GSH
- Vitamin E
- Vitamin C
- Pelargonidin

Values in µmol/g brain tissue and nmol/g brain tissue.
Possible Mechanisms of Bioactivity

Oxidative Stress

Mitochondrial dysfunction

MAPK Signaling

Apoptosis
Necrosis

Mitochondrial Swelling
Cytochrome c release
Respiratory complexes

MAP kinase
JNK, c-jun, AP-1

Neurodegeneration

Age-Related Decline
Alzheimer’s Disease
Parkinson’s Disease
Ischaemia/Reperfusion

Biomarkers of damage
i.e. 8-OH-Guan. Lipid ox.
Plasma membrane

Signal

Small G-protein

MAPKKKs/ MAPKK

MAPK

p38 JNK ERK

Signal

PI3-kinase Ca(II)

Akt/PKB

Pro-survival pathway

ERK: Pro-survival
JNK and p38: Pro-death

Cytosolic Targets

Nuclear Targets

Tau PLA₂

CREB c-jun

Neuronal Death/Survival
Effects of Flavonoids on Neurons
Morphological Changes

Control

Ox. Stress

EC + Ox. Stress

MeEC + Ox. Stress
Epicatechin/ 3’MEC attenuate oxLDL-induced JNK activation

(EC/ 3’MEC 10 µM, oxLDL 12.5 µg/ml)

Flavonoid modulation of Death Signalling
Flavonoid Activation of Pro-survival Signalling

(15 min; 310 K; n=4)

Relative Band Intensity

pERK2
pERK1

vehicle 0.1 0.3 1.0 µM

44 42

pERK1/2
Total ERK

Vehicle
EC 0.1
EC 0.3
EC 1
EC 3
EC 10
µM
Flavonoids mediate CREB Activation

Epicatechin [µM]

<table>
<thead>
<tr>
<th>Epicatechin [µM]</th>
<th>basal</th>
<th>0.1</th>
<th>0.3</th>
<th>1</th>
<th>3</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCREB (Ser-133)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total CREB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(picatechin 300 nM; 15 min; UO126 10 µM, LY294002 35 µM)
Possible route to EC-induced CREB phosphorylation

Neuronal Survival

CREB

ERK

EC

PI3K

UO126

LY294002
Similarity between flavonoids and kinase inhibitors

MEK Inhibitor
PD98059

Quercetin

PI3 Kinase Inhibitor
LY294002

Epicatechin
Effects of a blueberry-rich diet on age-associated decline in cognitive function in rats
Study design

Animal groups

<table>
<thead>
<tr>
<th>Young</th>
<th>Old</th>
</tr>
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<tbody>
<tr>
<td>(5-6 month)</td>
<td>(22-24 month)</td>
</tr>
<tr>
<td>Control diet</td>
<td>Control diet + 2% freeze-dried blueberries</td>
</tr>
</tbody>
</table>

Old (22-24 month) Control diet
Results

Daily Food Intake

Intake (g)

YOUNG
OLD
SUPP
Measurement of spatial learning and memory

T-maze alternation task
Baseline and 3, 6, 9, 12 weeks

Animals have been trained for 5 weeks to find food placed in opposite arms.
Training phase (5 weeks)

Supplement Diet

Test phase I
animal allowed to eat one treat

Alternate phase II
Spatial learning

Test phase II
Number of correct choices + Time to chose measured

Test phase II

mins
Performance on T-maze alteration task

Correct Choices (All trials)

<table>
<thead>
<tr>
<th>Time</th>
<th>Young</th>
<th>Old</th>
<th>Blueberry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>8</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>3 weeks</td>
<td>8</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>6 weeks</td>
<td>8</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>9 weeks</td>
<td>8</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>12 weeks</td>
<td>8</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

* indicates significant difference.
Ongoing work: Investigate the mechanism underlying the beneficial effect of blueberries on cognitive decline.

At the end of feeding period
Sacrifice of animals

Hippocampal tissue

Coronal slices

Total/active CREB
AKT, ERK, PKA
BDNF

DNA Microarray
Gene expression

Immunohistochemistry
p-CREB
in hippocampal CA1

Lipofuscin
CREB in the Brain

- Transcription factor

- CREB is expressed in numerous tissues but plays a large regulatory role in the nervous system.

- CREB is believed to play a key role in promoting neuronal survival, proliferation, neurite outgrowth and neuronal differentiation in certain neuronal populations.

- Additionally, CREB signaling is involved in learning and memory in several organisms.
CREB, BDNF and Memory

LTP
Memory
Neuronal survival
Neuronal Outgrowth
Differentiation

activated receptor
Extracellular

Cytoplasm

Nuclear envelope

Transcription initiation

Nucleus

BDNF

nNOS

Akt

ERK

CREB

CREB P
Hippocampal changes in CREB

![Image showing changes in CREB levels in different conditions: Control, Old, and Blueberry.](image)

**Graph:***
- **X-axis:** CREB 1, CREB 2
- **Y-axis:** Count
- **Legend:**
  - Control
  - Old
  - Blueberry

*Significant differences indicated by asterisks (*).
Hippocampal changes in pro-BDNF

• All neurotrophins are synthesized as preproneurotrophin precursors

• Proneurotrophin precursors also mediate biological functions

• Polymorphism that replaces valine for methionine at position 66 of the pro domain, is associated with memory defects and abnormal hippocampal function in humans
Hippocampal changes in ERK
Hippocampal changes in Akt

![Graph showing changes in Akt activation across different conditions.](image-url)
Normal Learning

PI3K → Akt

Akt → CREB

CREB + + BDNF

BDNF → Memory/LTP

CREB → Neuronal Survival

ERK + +

ERK → PKA

PKA → Ca(II)/Calmodulin

Ca(II)/Calmodulin + + Anthocyanins
Increase in synaptic density of proteins such as HOMER 2

mTOR signalling pathway
Summary

• Ageing and neurodegeneration are linked with oxidative processes in the brain.
  
  • The effects of dietary flavonoids/metabolites are seemingly independent of their antioxidant potential.
  
  • Flavonoids appear to induce cellular effects via specific interactions within cell signalling cascades such as the MAP kinase pathway.
  
  • The beneficial effects of flavonoid-rich foods on the reversal of the age-associated cognitive decline might be mediated through modifications of CREB and CREB-dependant gene expression cascades
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