

## Selenised dairy protein and colon cancer prevention

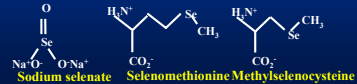


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## Selenium 34 -essential nutrient

- Essential trace element –for birds and mammals, selenocysteine-21st amino acid in functional proteins/enzymes
- Food sources : wheat/yeast - selenomethionine, or milk/eggs/meat - selenocysteine, or Organic Selenium Compounds eg allicin in garlic, isothiocyanates in cruciferae (broccoli)



## Selenoproteins (35) and functions

Selenoproteins	Functions
Glutathione peroxidase (GPx1-4)	Antioxidant function in plasma and tissues, reduce oxidative damage
Sperm mitochondrial membrane selenoprotein	Phospholipid-GPx (GPX4) protects sperm
Thioredoxin reductase	Reduces nucleotides in DNA synthesis, maintain cellular redox state, Energy metabolism- activate thyroid hormone (T3) from T4
Iodothyronine deiodinase	
Selenoprotein P	Plasma-protects endothelium, Se storage
Selenoprotein W	Redox? muscle, brain
Selenoprotein (15kd)	Redox function, prostate, thyroid

## Selenium : human health and well being

### Deficiency could result in:

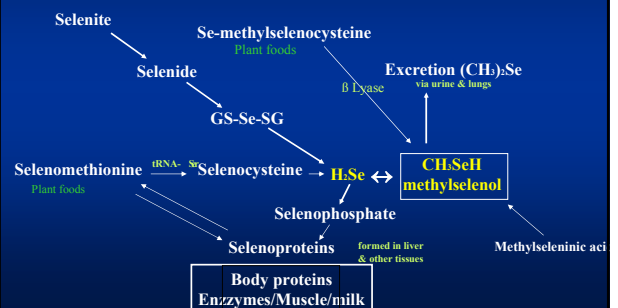
- Muscle (including heart) myopathies- ( eg Keshan disease in China), Increased ischaemic heart disease risk ?
- Diminished thyroid function
- Altered mood states-depression, anxiety, confusion, hostility
- Poor immune status-viral infections/cosackie virus-cardiomyopathy, AIDS
- Infertility- male sperm motility, female -miscarriages etc
- Increased cancer risk

## Selenium and cancer prevention

- Antioxidant enzymes responsible for peroxides and free radical removal
- Redox state -Repair of cell components (DNA) damaged by free radicals
- Inhibits activation of oncogenes, eg c-myc
- Induces apoptosis via P53 tumour suppressor gene
- Increases immunity eg N K cell activation
- Detoxification systems –phase 1 & 2 enzymes

Form in which Selenium is ingested could have significant influence

## Metabolic pathways of Se compounds



## International selenium intake data

Australian RDI (1990) – 70-85µg/d

	µg Se/day
New Zealand	38
Sweden/UK	40
France	47
Italy	49
Netherlands	67
Australia	70 ?
Finland	98
USA	113
Japan	133

nb Selenium has never been included in any Australian diet nutrient survey

## Human plasma selenium status

● Tasmania	77±13	( Daniels et al, 2000 )
● South Australia	88±20	)
● Australia	94	(Lyons et al 2003)
● New Zealand	60–88	(Thompson, 2004)
● Finland 1970 > 1985	50 > 100	(Aro, 1999)
● USA	106-120	(Clark et al 1996)

Deficiency status < 85 µg/L (plasma) (Shortt et al 1997)

● Plasma Se – 100 µg/L for optimal GsPx activity (Rayman, 2000)

## DNA stability and serum Se in high risk group for prostate cancer (NZ)

For half of male population with serum Se below 98µg/L, serum levels showed a significant inverse relationship with overall accumulated DNA damage in leucocytes, suggesting increased susceptibility to cancers and some other degenerative diseases



Karungasinghe et al (2004) Cancer Epidemiol Biomarkers Prev 13:391

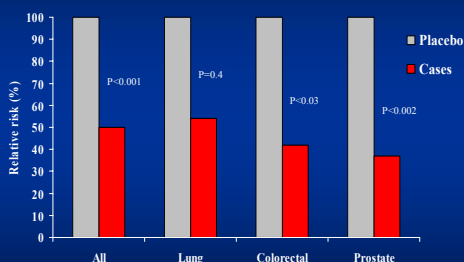
Alkaline Comet assay

## Selenium anticancer intervention trial

- Nutritional Cancer Prevention Trial- USA multicenter placebo controlled, double blind involving 1312 patients presenting with skin basal cell or squamous carcinomata
- Background selenium intake of 90µg/day
- 200µg Se per day supplement as selenised yeast over 4.5 years and 6.4 year follow up period (1983-1994)
- Primary end point- non melanoma skin cancer
- Secondary end points- total cancer incidence, prostate, colorectal and lung cancers

Clark, L.C. et al. (1996) JAMA 276: 1957-1963

## Relative risk of cancers in placebo and Se treated subjects



No beneficial effect seen in skin cancer expression, total cancer mortality reduced by 41%.

Clark et al. (1996) JAMA 276: 1957-1963

## Cancer risk relative to commencing plasma selenium concentration

NCP intervention trial -200µg Se /day as yeast for 4.5 yrs, 6.5 yrs follow up

Plasma Se (ug/L)	Cases	Controls	RR*	p
<106	28	56	0.52	0.005
106-121	39	49	0.64	0.40
>121	45	41	1.00	0.99

\* Relative Risk for lung, colon and prostate cancers

Rayman and Clark (2000) TEMA10

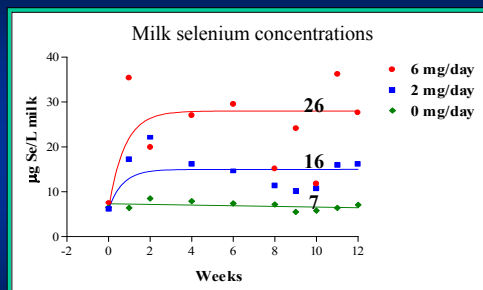
## SEL-PLEX™ organic selenium yeast



**Analysis :**  
Selenium conc. 2000ppm

**Principle organic forms :**  
Selenomethionine 50%  
Selenocysteine 20%  
Selenoproteins & organo selenium compounds 30%

## Organic selenium\* supplied in dairy feed over 12 weeks



\*Yeast selenium as Sel-Plex™-Alltech Inc

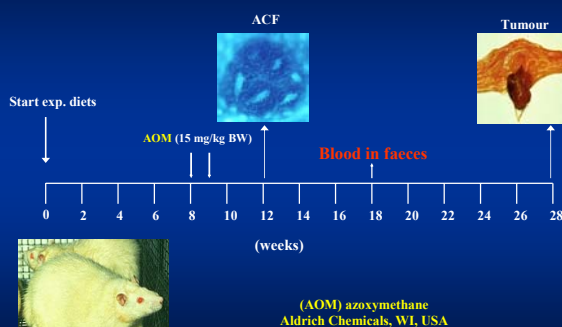
3 cows per treatment group (age/production matched)



## Selenium in dairy milk & products

	Selenium (ppb)	Protein (%)
Whole milk	17.5	4.0
Full cream milk powder	179	26.0
Casein	362	63.5
Whey	212	58.0
Casein/ colon cancer study	2310	54
Commercial casein (Aust)	143	85.0

## Animal intestinal cancer model



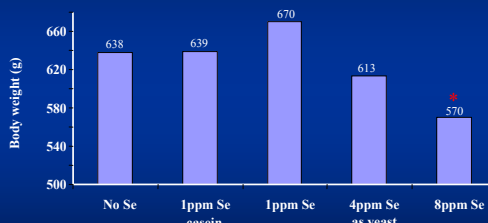
## Rat colon cancer (AOM induced) study using Se rich casein

- 25 male SD rats per treatment group
- Tumour induction - 2 x 15mg/kg AOM doses S/C one week apart
- Experimental AIN diets, post induction
  - Control AIN diet ~ 0.04 ppm
  - Se enriched casein ~ 1.0 ppm
  - AIN + Se yeast ~ 1.0 ppm
  - AIN + Se yeast ~ 4.0 ppm
  - AIN + Se yeast ~ 8.0ppm

(NOTE : no Se included in AIN mineral mix, )

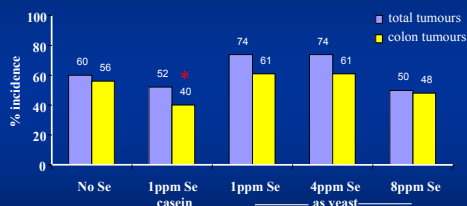
- Evaluate tumor incidence/burden/TMI\*/malignancy (Dukes classification) at necropsy TMI \* =tumor mass index

## Final body weights in colon cancer rat study



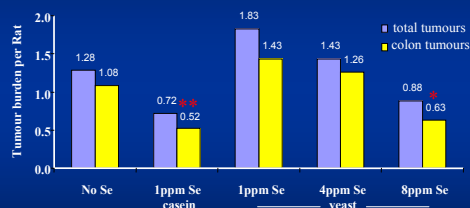
NB Body Wt of 8ppm Se rats significantly lower (9%↓) than controls \* (p = 0.01)

## Se enriched casein on tumour incidence in AOM induced rats



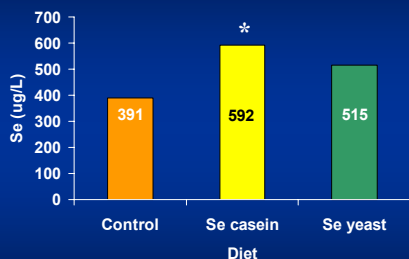
Colon tumour incidence in 1ppm Se-casein group 16% less than control ( $p=0.05$ )\*, 21% lower than 1ppm Se yeast.

## Se enriched casein on AOM induced colon tumour burden



Colon tumor burden in 1ppmSe-casein group 50% of control ( $p=0.01$ )\*, and 36% of 1ppm Se yeast group 8ppm group 58% of control\* ( $p=0.05$ ).

## Plasma selenium assay



The plasma selenium concentration of the three diets are significantly different

## Cell dynamics in rat colonic crypts-measured as cells/crypt wall



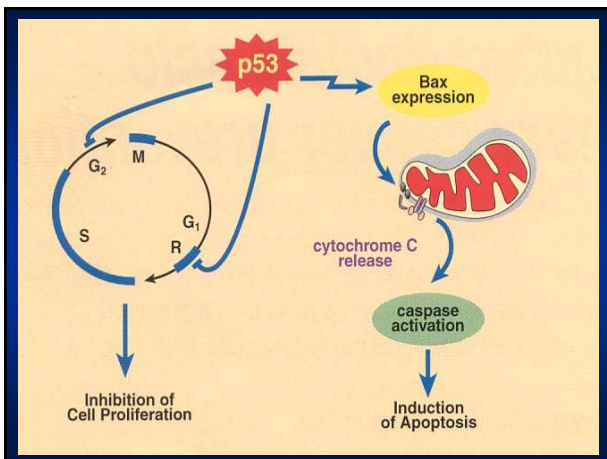
There was a significant difference ( $P=0.001$ ) between the height of the crypt of Se casein rats relative to control and Se yeast rats

Height reflects proliferation / apoptotic indices – a significantly lower height indicates a protective effect

## Possible mechanisms of selenium in cancer chemoprevention

- Modulating tumour suppressor gene P53-initiates apoptosis
- Inducing cell oxidative changes in mutated cells which triggers apoptosis
- Inducing Fas ligand and Jun NH2-terminal kinase-mediators of apoptosis
- Inhibiting protein kinase C, reacting with S groups of catalytic domain- critical intracellular signal molecule in cell proliferation
- Binding nuclear regulatory transcription factors- eg Nuclear Factor  $\kappa$ B
- Modulating phase 1 and 2 enzyme activities-carcinogen inactivation
- Inhibiting matrix metalloproteinases and tumour vascular endothelial growth factor production – blocking angiogenesis

Nutrition and Cancer (2001)- V40:1



## Conclusions

- Selenium has significant cancer inhibiting effect – rat cancer studies, human intervention studies and epidemiological studies show Se status is related inversely to cancer risk
- Colon cancer (AOM induced) in rats was significantly reduced when fed 1ppm Se as casein, not seen in rats fed Se yeast at 1 or 4 ppm. Reduced colon crypt height in Se casein rats - was inversely related to plasma selenium status
- Australians and New Zealanders may be marginal for Se status in a significant proportion of mature population; evaluated by some relevant biological markers of health and selenium status
- To optimise anticancer benefits intakes exceeding several fold current RDI (50 µg/d) may be required. Identify specifically Se rich foods or food fortification with Se.
- Dairy foods appear to offer a potentially beneficial option

## Acknowledgements

- Peter Royle and Ben Scherer
- Alltech Biotechnology Pty Ltd-Selplex™
- Dairy Australia-supporting research
- Food Science Australia- casein production
- Dr Peter Doyle and Glen Walker  
Department of Primary Industries- Vic



## Seleen- greek goddess

“bright side of the moon”

	6	7	8	9	10
1	C	N	O	F	Ne
2	12.01	14.01	16.00	19.00	20.18
3	14	15	16	17	18
4	Si	P	S	Cl	Ar
5	28.09	30.97	32.07	35.45	39.95
6	32	33	34	35	36
7	Ge	As	Se	Br	Kr
8	72.61	74.92	78.96	79.90	83.80
9	50	51	52	53	54
10	Sn	Sb	Te	I	Xe
11	118.7	121.8	127.6	126.9	131.3
12	82	83	84	85	86

Discovered by Jons Brezelius in 1818, naturally occurring element ranks 17<sup>th</sup>

## Wheat selenium and colon cancer prevention – ACF data

- Aberrant crypt foci (ACF) – preneoplastic lesions / colon cancer risk biomarker
- Chemically induced in rats by azoxymethane (AOM)
- Methylene blue stained & visible under low power microscopy

n=23 per group F 344 rats Signif p<0.05	0.1 ppm Se wheat	0.5 ppm Se broccoli + Se wheat	2.0 ppm Se wheat	2.0 ppm Se wheat (1.5ppm) +Se broccoli (0.5ppm)
ACF	76 <sup>b</sup>	82 <sup>b</sup>	50 <sup>a</sup>	57 <sup>ab</sup>
Total AC	180 <sup>b</sup>	198 <sup>b</sup>	125 <sup>a</sup>	152 <sup>ab</sup>
AC/ACF	2.4	2.4	2.5	2.7

Finley & Davis (2001) BioFactors 14:191-196