

**The Effects of Selenomethionine and
Wheat Biofortified with Selenium
on DNA Damage and Cell Death in
Human Lymphocytes**

Jing Wu

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the degree of Doctor of Philosophy**

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The University of Adelaide**

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Abstract

Selenium (Se) is an essential micronutrient, being a component of more than twenty seleno-proteins in humans. Previous studies suggested that increased intake of Se may reduce the risk of degenerative diseases including cancer; however, excessive intake can be toxic. Wheat is one of the major dietary sources of Se in humans, mainly in the form of L-selenomethionine (Se-met) but the impact of this source of Se on human health at the genome level was previously unexplored.

This PhD project aimed to (a) determine the safe dose-range and bio-efficacy of Se-met *in vitro*; (b) identify the optimal concentration of Se-met for reduction of genome damage *in vitro*; (c) investigate the optimal concentration of Se-met for improving resistance to gamma radiation or hydrogen peroxide induced genome damage *in vitro*; (d) determine the bioavailability and bioefficacy of Se *in vivo*, in the form of either Se-met or wheat biofortified with Se; (e) identify the nutrients and food groups that are correlated with Se intake/status and (f) identify the nutrients, food groups and plasma mineral concentrations that are correlated to baseline lymphocyte DNA damage.

The *in vitro* study was performed on the peripheral blood lymphocytes isolated from six males and cultured with media supplemented with Se-met in a series of Se concentrations from 3 to 3850 $\mu\text{g Se/l}$ while keeping the total methionine (i.e. Se-met + L-methionine) concentration constant. Baseline genome stability of lymphocytes and the extent of DNA damage induced by 1.5 Gy γ -ray or 7.5 μM hydrogen peroxide (H_2O_2) were investigated using the Cytokinesis-block Micronucleus Cytome (CBMN-Cyt) assay and the alkaline Comet assay with and without glycosylase (Fpg or Endo III) treatment after 9 days of culture. Results showed that high Se concentrations ($\geq 1880 \mu\text{g Se/l}$) caused strong inhibition of cell division, extensive DNA damage and increased cell death indicating cytotoxicity and genotoxicity. Baseline frequency of nucleoplasmic bridges (NPBs) and nuclear buds (NBud) declined significantly as Se concentration increased from 3 $\mu\text{g Se/l}$ to 430 $\mu\text{g Se/l}$ (P trend = 0.03 and 0.008, respectively); however, a significant trend of increase in Comet DNA damage was also observed (P trend < 0.05) in lymphocytes. Selenium concentration ($\leq 430 \mu\text{g Se/l}$) had no significant effect on baseline frequency of micronuclei (MN) or DNA oxidation and had no protective effect against γ -ray-induced or H_2O_2 -induced genome damage in lymphocytes.

A randomised double-blind placebo-controlled intervention trial was conducted on healthy South Australian males (n = 62, age (mean \pm SD) 56 \pm 7.0 years) with Se

dosage increased every 8 weeks for a total duration of 24 weeks. This study compared the bioavailability, by using plasma Se concentration as the biomarker, and bioefficacy of Se, by using platelet glutathione peroxidase (GPx) activity and lymphocyte DNA damage as biomarkers, from wheat process-fortified with Se-met (PROFORT) and high-Se wheat biofortified with Se (BIOFORT) compared to non-fortified normal (CONTROL) wheat. It was found that increased Se intake from BIOFORT wheat increased plasma Se concentration effectively in a dose-response manner from a baseline of 122 µg/l up to 190 µg/l (P<0.001). Increased Se intake from PROFORT wheat also increased plasma Se with a plateau at 140 µg/l, being therefore less effective than BIOFORT wheat (P<0.001). There was no significant change in Se status in the CONTROL group. Improved plasma Se concentrations had no effect on platelet GPx activity or lymphocyte DNA damage in either of the intervention groups.

Results from the food frequency questionnaire (FFQ) survey (n = 173) and plasma Se concentration survey (n = 179) suggested that the study population screened for participation in the *in vivo* trial described above had a mean plasma Se concentration (\pm SD) of 102 (\pm 12) µg/l and a mean (\pm SD) estimated Se intake of 165 (\pm 68) µg/d. This is a higher estimated Se intake than found in previous Australian studies. The major dietary sources of Se were found to be bread/cereals, fish/seafood and meat. However, increased intake of nuts/seeds, which are rich in Se, may have undesirable effects on lymphocyte DNA oxidation in this Se-replete population.

In conclusion, the *in vitro* studies suggest that (1) Se-met at higher concentrations at greater or equal to 1880 µg Se/l is cytotoxic; (2) Se-met may improve specific genome stability biomarkers such as nucleoplasmic bridge and nuclear bud at concentrations up to 430 µg Se/l, but further studies are needed to verify this effect. The *in vivo* studies in older men showed that Se from BIOFORT wheat is more effective in raising plasma Se concentration than Se from wheat process-fortified by the addition of Se-met, when both wheat products were subjected to strong heat. However, the platelet GPx activity and lymphocyte DNA damage appeared not to be modified by improved Se status.

This work contains two publications:

- 1) "The effect of selenium, as selenomethionine, on genome stability and cytotoxicity in human lymphocytes as measured by the cytokinesis-block micronucleus cytome assay". *Mutagenesis* 2009 May;24(3):225-32.
- 2) "Increased consumption of wheat biofortified with selenium does not modify biomarkers of cancer risk, oxidative stress or immune function in Australian males" *Environmental Molecular Mutagenesis*. 2009 July; 50 (6):489-501

The latter one was not able to be published in a journal of higher impact factor due to part of the data had been published elsewhere. Both articles are attached in Appendix.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Jing Wu and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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* List of publications:

"The effect of selenium, as selenomethionine, on genome stability and cytotoxicity in human lymphocytes as measured by the cytokinesis-block micronucleus cytome assay"
Mutagenesis 2009 May; 24(3):225-32.

"Increased consumption of wheat biofortified with selenium does not modify biomarkers of cancer risk, oxidative stress or immune function in Australian males"
Epub ahead of print in *Environmental Molecular Mutagenesis*. DOI: 10.1002/em

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Signature:..... Date:

Jing Wu

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List of abbreviations

| | |
|-------------------|--|
| ACCV | Anti-Cancer Council of Victoria |
| AIDS | Acquired immunodeficiency syndrome |
| ALS | Alkali labile site |
| ANOVA | Analysis of variance |
| <i>ATM</i> | Ataxia telangiectasia mutated gene |
| ATP | Adenosine tri-phosphate |
| <i>ATR</i> | Ataxia telangiectasia mutated and Rad3-related gene |
| AU | Arbitrary unit |
| | |
| BIOFORT | Wheat biofortified with selenium |
| BNed | Binucleated |
| <i>BRCA</i> | Breast cancer gene |
| | |
| Ca | Calcium |
| CBMN Cyt assay | Cytokinesis-block micronucleus cytome assay |
| <i>CHK2</i> | Background checkpoint kinase 2 gene |
| CSIRO | Commonwealth Scientific and Industrial Research Organisation |
| Cu | Copper |
| CuSO ₄ | Copper sulphate |
| CV | Coefficient of variation |
| Cyto-B | Cytochalasin B |
| | |
| DAN | Diaminonaphthalene |
| DI | Deiodinase |
| DMABP | 3,2'-dimethyl-4-aminobiphenyl |
| DMSO | Dimethyl sulfoxide |
| DSB | Double strand break |
| | |
| EDTA | Ethylenediaminetetraacetic acid |
| Endo III | Endoneclease III |
| | |
| FBS | Foetal bovine serum |
| Fe | Iron |
| FFQ | Food frequency questionnaire |
| Fpg | Formanidopyrimidine-DNA glycosylase |

| | |
|---------------|--|
| <i>Gadd45</i> | Growth arrest and DNA damage gene |
| GPx | Glutathione peroxidase |
| GSH | Reducing glutathione |
| GSSG | Oxidized glutathione |
| H_2O_2 | Hydrogen peroxide |
| HBSS | Hanks balanced salt solution |
| $HClO_4$ | Perchloric acid |
| HDL | High-density lipoprotein |
| HIV | Human immunodeficiency virus |
| HNO_3 | Nitric acid |
| hTERT | Human telomerase reverse transcriptase |
| ICP-MS | Inductive coupled plasma mass spectrometry |
| ICP-OES | Inductively coupled plasma optical emission spectrometry |
| IDI | Iodothyronine deiodinase |
| IFN- γ | Interferon gamma |
| IL-2 | Interleukin-2 |
| IMVS | Institute of Medical and Veterinary Science |
| K | Potassium |
| KCl | Potassium chloride |
| KH_2PO_4 | Potassium dihydrogen phosphate |
| LDL | Low-density lipoprotein |
| Mg | Magnesium |
| MN | Micronuclei |
| MNed | Micronucleated |
| MT | Metallothionein |
| Mtase | Cytosine-5-methyltransferase |
| MTHFR | Methylenetetrahydrofolate reductase |
| MUFA | Monounsaturated fatty acid |

| | |
|----------------------------------|---|
| Na | Sodium |
| NaCl | Sodium chloride |
| Na ₂ CO ₃ | Sodium carbonate |
| Na ₂ HPO ₄ | Sodium phosphate |
| NADPH | Reduced nicotinamide adenine dinucleotide phosphate |
| NaOH | Sodium hydroxide |
| NBud | Nuclear bud |
| NCEFF | National Centre of Excellence in Functional Foods |
| NDI | Nuclear division index |
| NHMRC | National Health and Medical Research Council |
| NOAEL | No observable adverse effect level |
| NPB | Nucleoplasmic bridge |
| 8-OHdG | 8-hydroxy-2-deoxyguanosine |
| P | Phosphorus |
| PBS | Phosphate buffered saline |
| PHA | Phytohaemagglutinin |
| PHGPx | Phospholipid hydroperoxide glutathione peroxidase |
| PROFORT | Wheat process-fortified with selenomethionine |
| PUFA | Polyunsaturated fatty acid |
| p-XSC | 1,4-phenylenebis(methylene)selenocyanate |
| RDA | Recommended daily allowance |
| RDI | Recommended daily intake |
| ROS | Reactive oxygen species |
| S | Sulphur |
| SAM | S-adenosylmethionine |
| SARDI | South Australia Research and Development Institute |
| SCGE | Single-cell gel electrophoresis |
| SDG | Selenodiglutathione |
| SE | Standard error |
| Se | Selenium |
| SeAM | Se-adenosylmethionine |
| SECIS | Selenocysteine insertion sequence |

| | |
|--------|---------------------------------------|
| SE-EMP | Selenium exchangeable metabolic pool |
| Sel | Selenoprotein |
| Se-met | Selenomethionine |
| SD | Standard deviation |
| SNP | Single nucleotide polymorphism |
| SPS2 | Selenophosphate synthetase-2 |
| SSB | Single strand break |
| Top II | Topoisomerase II |
| TrxR | Thioredoxin reductase |
| UTR | Untranslated region |
| WAS | Waite Analytical Services |
| XPA | Xeroderma pigmentosum group A protein |
| Zn | Zinc |