Dietary reference values of individual micronutrients and nutriomes for DNA damage prevention

Current status and a road map to the future

Michael Fenech
CSIRO Food and Nutritional Sciences
GENOME HEALTH NUTRIGENOMICS LABORATORY
michael.fenech@csiro.au
Overview

- Biomarkers used to study DNA damage in humans
- Association of DNA damage with developmental and degenerative disease
- Current knowledge of nutritional requirements for genome maintenance and stability
- Effect of nutrient-nutrient and nutrient-genotype interaction on DNA integrity
- Strategies to determine DRVs of single micronutrients and micronutrient combinations (nutriomes) for DNA damage prevention
A NUCLEOCENTRIC VIEW OF AGEING
FROM WOMB TO TOMB

David Sinclair and Philipp Oberdoerffer

BIOMARKERS OF DNA DAMAGE

Gene expression arrays

Markers up-regulated on microarray of γ-irradiated PBLs

Amundson et al., (2000)
Radiation Research, 154 (3): 342-346

γH2AX
The principal structural chromosomal aberrations which contribute acentric fragments (AF) to form micronuclei (MN) and nucleoplasmic bridges (NPB).
Genome damage

Genome damage

Micronuclei

Micronuclei

CYTOKINESIS-BLOCK MICRONUCLEUS (CBMN) ASSAY

- Oxidative stress
- Nutrient deficiency
- Excess calories

- Strand breaks in DNA
- Chromosome malsegregation
- DNA hypomethylation
- Telomere shortening

Human cells with damaged & unstable genomes
Increased lymphocyte micronucleus frequency in early pregnancy is associated prospectively with pre-eclampsia and/or intrauterine growth restriction (PEIUGR).


**RISK GROUPS**
- LOW RISK: N=45
- HIGH RISK: N=91

**PREGNANCY OUTCOME GROUPS**
- LRN: N=38
- PEIUGR: N=36
- OAO: N=38
- HRN: N=24

**LYMPHOCYTE MICRONUCLEI MEASURED AT 18 WKS GESTATION**

**PROSPECTIVE ODDS RATIO OF PEIUGR FOR ALL COHORT**
- >19.3: P=0.003
- >28.0: P=0.006
- >36.7: P=0.001
Risk of cancer increases with higher MN frequency

Hazard Ratio = 1.67
95% CI = 1.22 - 2.31
p-value = 0.002

6,983 subjects
275 cancer cases

HUMN project (www.humn.org)
Bonassi et al (Carcinogenesis 2007)
Lymphocyte Micronucleus frequency is associated prospectively with cardiovascular disease mortality in both the general population and those with known coronary artery disease.

1650 subjects
111 deaths
39 due to CVD
Mean age 65 y

178 CAD patients
Mean age 62 y

Federici C et al. Am J Cardiol 2008;102:1296–1300

E. Murgia et al. / Mutation Research 621 (2007) 113–118
GENOME DAMAGE INCREASED IN LYMPHOCYTES OF ALZHEIMER AND PARKINSON’S DISEASE PATIENTS

**DNA DAMAGE IN LYMPHOCYTES**

* P<0.001

**DISTRIBUTION OF TYPE OF MICRONUCLEI**

- MN (CHROM. LOSS)
- MN (CHROM. BREAK)

p < 0.0001

Petrozzi et al. Neurol. Sci. 2002
DNA damage increases with age ....or.... poor choices of nutrition, life-style, physical and socio-psychological environments?

What is the threshold of DNA damage we should allow?

Can we design “exposomes” that enable us to stay below this threshold?

Fenech et al. 2000
CAUSES AND EFFECTS OF GENOME DAMAGE

ENVIRONMENTAL GENOTOXINS

MALNUTRITION

LIFE-STYLE GENOTOXINS

PSYCHOLOGICAL STRESS

ENVIRONMENTAL GENOTOXINS

GENETIC AND ACQUIRED SUSCEPTIBILITY

GERMLINE DAMAGE IN:

GERMLINE

EMBRYO

FOETUS

BABY

CHILD

TEENAGER

YOUNG ADULT

OLDER ADULT

PSYCHOLOGICAL STRESS

HIGHER RISK

DEVELOPMENTAL DEGENERATIVE DISEASES

LOWER RISK
GREAT DIVERSITY IN NUTRIOMES & GENOMES

Bhutan

Chad

Egypt

Dietary patterns & 1 week’s food
Nutrition designed for diverse genetic backgrounds to optimise genome maintenance and prevent diseases caused by DNA damage.
The following genomic stability processes are modulated by vitamins or micronutrients:

<table>
<thead>
<tr>
<th>Process</th>
<th>Vitamins/Micronutrients</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA oxidation prevention</td>
<td>Vit C, Vit E, Se, polyphenols</td>
</tr>
<tr>
<td>DNA methylation, synthesis</td>
<td>folate, Vit B12, Zn, Mg</td>
</tr>
<tr>
<td>DNA repair</td>
<td>niacin, Zn, folate</td>
</tr>
<tr>
<td>Gene expression</td>
<td>folate, Vit D, Vit A</td>
</tr>
<tr>
<td>Chromosome segregation</td>
<td>folate, Vit A, Mg</td>
</tr>
<tr>
<td>Telomere length</td>
<td>niacin? via PARP, folate</td>
</tr>
<tr>
<td>Necrosis/Apoptosis</td>
<td>niacin, Zn, Vit E, Vit D, Vit C Vit A, Vit K2.</td>
</tr>
</tbody>
</table>
Folate, B12, B6 and B2 and genome maintenance

- Folic Acid
- THF
- DHF
- dUMP
- dTMP
- MTR
- MTRR
- SHMT1
- MTHFR
- DNA Methylation
- DNA Repair & Synthesis
- Homocysteine
- Methionine
- SAM
- DNA

Reactions:
- MTR
  - VIT B12 (CoII)
- MTR
  - VIT B12 (CoI)
- TS
- FOLIC ACID
- 5-MeTHF
- 5,10-MeTHF
- DNA Replication & Synthesis
- DNA Methylation
Minimally invasive High-Throughput Nutrient Array screening for Genome-Protective agents.

Development of automated CBMN Cytome assay:
MN, NPB, NBUDs, Necrosis, Apoptosis, NDI + FISH + Protein expression
<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A, B, C</td>
<td>NUCLEAR DIVISION INDEX</td>
<td>CBMN CYTOME ASSAY</td>
<td>D, E</td>
<td>CELL DEATH</td>
<td>F, G, H</td>
<td>DNA DAMAGE</td>
<td></td>
</tr>
</tbody>
</table>
[A] **MICRONUCLEATED CELLS**

- ANOVA P < 0.0001

- [A] folic acid in medium (nM)
- MNed BNs/1000 BNs

[B] **NUCLEAR BUDS**

- ANOVA P < 0.0001

- [B] folic acid in medium (nM)
- Buds/1000 BNs

[C] **NUCLEOPLASMIC BRIDGES**

- ANOVA P < 0.0001

- [C] folic acid in medium (nM)
- NPB/1000 BNs

[D] **URACIL**

- ANOVA P < 0.0001

- [D] folic acid in medium (nM)
- pg uracil / µg DNA

Crott et al. Carcinogenesis 2001
Genome damage induction by Folic acid deficiency is of a similar magnitude as that induced by unsafe doses of ionising radiation.

IAEA annual safe exposure limit i.e. equivalent to 0.1-0.5 rad X-rays
Randomised, placebo-controlled dietary intervention

<table>
<thead>
<tr>
<th>ROUND 1</th>
<th>ROUND 1</th>
<th>BASE-LINE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEREAL+</td>
<td>CEREAL+</td>
<td></td>
</tr>
<tr>
<td>ONLY</td>
<td>7ug B12+</td>
<td>700ug FOL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ROUND 2</th>
<th>ROUND 2</th>
<th>12 WEEKS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TABLET</td>
<td>TABLET</td>
<td></td>
</tr>
<tr>
<td>PLACEBO</td>
<td>20ug B12+</td>
<td>2000ug FOL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ROUND 3</th>
<th>ROUND 3</th>
<th>24 WEEKS*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* blood sample

Fenech et al. 1998 Carcinogenesis
Supplementation with 3.5 times RDI folic acid & vit B12 reduces micronucleus index by 25% in subjects with above average chromosome instability.

Fenech et al. 1998 Carcinogenesis
MICRONUCLEUS FREQUENCY IS MINIMISED WHEN HOMOCYSTEINE < 7.5umol/l and B12 > 300pmol/l

Fenech et al. 1998 Carcinogenesis

Fenech et al. (1997) Carcinogenesis
300 times recommended intake of B12 is required to normalise holoTC, MMA and homocysteine in >70 year olds

Figure 2. Proportional effects of different doses of cyanocobalamin on mean methylmalonic acid (MMA) (A), total homocysteine (tHcy) (B), holotranscobalamin (holoTC) (C), and vitamin B₁₂ (D) concentrations after 16 weeks of supplementation. Error bars represent SD.
Future Challenges: We have started to determine the impact of common polymorphisms in folate metabolism genes on chromosome damage in Australians

*age & gender adjusted

Dhillon et al Mutation Res Fund Mech 2009
% variation in genome damage with increased intake relative to lowest tertile of intake

The combined effect of (a) calcium and folate intake and (b) riboflavin and folate intake on MN frequency. Results shown are the % variation relative to the combined lowest tertiles of intake in the pair of nutrients examined. * P < 0.05 for comparison with the referent value for the combined lowest tertile of intake for the pair of nutrients examined.

MORE RIBOFLAVIN IN A LOW FOLATE BACKGROUND MAY BE GENOTOXIC
Q. Which dietary pattern will work for your genotype?

A. It depends on the “nutriome” of the foods you prefer to eat.

Fenech, Food Chem Tox 2008
# Folate content of vegetables (DFE µg per 100g)*

*Data from USDA National Nutrient data base. DFE = dietary folate equivalent. DFE values are shown in brackets.

<table>
<thead>
<tr>
<th>High Folate (HF) Vegetables</th>
<th>Low Folate (LF) Vegetables</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pulses</strong></td>
<td><strong>Leafy or cruciferous vegetables</strong></td>
</tr>
<tr>
<td>Red Kidney beans (130)</td>
<td>Broccoli (93)</td>
</tr>
<tr>
<td>Mung beans (60)</td>
<td>Brussel sprouts (60)</td>
</tr>
<tr>
<td>Chickpeas (171)</td>
<td>Cabbage (43)</td>
</tr>
<tr>
<td>Lentils (180)</td>
<td>Endive (142)</td>
</tr>
<tr>
<td>Peas (59)</td>
<td>Spinach (146)</td>
</tr>
<tr>
<td>Lima beans (50)</td>
<td>Lettuce (73)</td>
</tr>
<tr>
<td><strong>Mean (108)</strong></td>
<td><strong>Mean (93)</strong></td>
</tr>
</tbody>
</table>

| Mean (100) | Mean (16) |

Eating the “wrong” vegetables could lead to folate deficiency.

Folate RDA 400µg requires eating:

2.5Kg LF veg/d or
0.4Kg HF veg/d
TELOMERS (TTAGGG repeats) ARE ESSENTIAL FOR CHROMOSOME STABILITY

TELOMERE SHORTENING OR DYSFUNCTION INCREASES RISK FOR CANCER AND ACCELERATED SENESCENCE
SHORTER TELOMERES ARE ASSOCIATED PROSPECTIVELY WITH INCREASED RISK FOR CANCER

Shorter telomeres in WBCs and Buccal cells of Alzheimer’s disease (AD) cases compared to controls

WBC TL <115 Kb per diploid genome
OR of being diagnosed with AD is 10.8
specificity 46% sensitivity 92.9%.

Buccal TL <40 kb per diploid genome
OR of being identified with AD is 4.6
specificity 63% sensitivity 72.7%.

young controls (N=30), old controls (N=26), younger AD (N=14), older AD (N=18).
LONGER

- FOLATE
- VITAMIN E
- VITAMIN D
- Ω3-FATTY ACIDS
- CEREAL FIBRE
- MULTIVITAMIN USE

TELOMERES

CURRENT KNOWLEDGE

SHORTER

- PUFA
- OXIDATIVE STRESS
- OBESITY
- PSYCHOLOGICAL STRESS
- PROCESSED MEAT
- HOMOCYSTEINE

ANTIOXIDANT DEFICIENCY OR OXIDATIVE STRESS

FOLATE DEFICIENCY

NIACIN OR NICOTINIC ACID DEFICIENCY

8-OHdG

URACIL

HYPO-METHYLATED SUBTELOMERE

REDUCED TRF1, TRF2 BINDING

BREAKS IN TELOMERE

LOSS OF TELOMERE LENGTH CONTROL

REDUCED TANK1 ACTIVITY

DYSFUNCTIONAL AND/OR SHORT TELOMEREs; TELOMERE END FUSIONS; AND CIN

TELOMERE DAMAGE CASCADE
High protein (TWD) or high carbohydrate (HC) weight-loss diets reverse telomere shortening in rectum in over-weight men

2 way ANOVA
P<0.0001

O’Callaghan, Clifton, Noakes, Fenech. Rejuvenation Res. 2009
IS A COMPREHENSIVE SET OF DNA DAMAGE BIOMARKERS NEEDED FOR PERSONALISED NUTRITION AND DRV DETERMINATION FOR GENOME DAMAGE PREVENTION?

<table>
<thead>
<tr>
<th>DNA Damage Biomarkers</th>
<th>Association with Nutritional Status</th>
<th>Association with Developmental or Degenerative Disease or Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cross-Sectional Studies</td>
<td>Placebo-Controlled Studies</td>
</tr>
<tr>
<td>Cytokinesis-block Micronucleus Assay in Lymphocytes</td>
<td>✓ ✓ ✓ ✓</td>
<td>✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>Micronucleus Assay in Buccal Cells</td>
<td>✓ ✓</td>
<td>✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>Micronucleus Assay in Erythrocytes</td>
<td>✓ ✓ ✓ ✓</td>
<td>✓ ✓</td>
</tr>
<tr>
<td>DNA Strand Breaks in Lymphocytes by Comet Assay</td>
<td>✓ ✓</td>
<td>✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>DNA Oxidation</td>
<td>✓ ✓</td>
<td>✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>DNA Methylation</td>
<td>✓ ✓</td>
<td>✓ ✓</td>
</tr>
<tr>
<td>Telomere Length in Leukocytes or Lymphocytes</td>
<td>✓ ✓</td>
<td>✓</td>
</tr>
<tr>
<td>Mitochondrial DNA Deletion</td>
<td>✓ ✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
### PROPOSED ROAD-MAP TO DETERMINE DRVs FOR GENOME STABILITY

#### NUTRITION VARIABLES
- Single Micronutrient
- Micronutrient Combination
- Functional Food
- Food Group
- Dietary Pattern

#### STUDY DESIGN
- In Vitro Models
- In Vivo Cross-Sectional Studies
- Placebo-Controlled Trials

#### OUTCOME MEASURES
**Primary**
- DNA Damage Biomarkers:
  - Micronucleus
  - Cytome Assays
  - Comet Assay
  - DNA Oxidation
  - DNA Methylation
  - Telomere Length
  - MtDNA Deletion

**Secondary**
- Tissue Micronutrient Concentration

#### DRVs FOR GENOME STABILITY
- Databases on Vitamin & Mineral Requirements for Genome Stability in Diverse Genetic Backgrounds at the Various Life-Stages

---

LIFE-STYLE IS ALSO AN IMPORTANT DETERMINANT OF DNA DAMAGE


LIFE-STYLE HPI INDEX

<table>
<thead>
<tr>
<th>Factor</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Smoking</td>
<td>Smokers</td>
</tr>
<tr>
<td>Alcohol drinking</td>
<td>Often</td>
</tr>
<tr>
<td>Nutritional balance*</td>
<td>Poor</td>
</tr>
<tr>
<td>Exercise**</td>
<td>Good or moderate</td>
</tr>
<tr>
<td>Sleeping hours*</td>
<td>≥ 2 times per week</td>
</tr>
<tr>
<td>Working hours**</td>
<td>≥ 9 h</td>
</tr>
<tr>
<td>Mental stress</td>
<td>Excessive</td>
</tr>
<tr>
<td>Breakfast</td>
<td>No eating</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Factors</th>
<th>MN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>− 0.698</td>
</tr>
<tr>
<td>Drinking</td>
<td>− 0.174</td>
</tr>
<tr>
<td>Sleeping hours*</td>
<td>− 1.288</td>
</tr>
<tr>
<td>Mental stress</td>
<td>0.724</td>
</tr>
<tr>
<td>Exercise**</td>
<td>− 2.315</td>
</tr>
<tr>
<td>Breakfast</td>
<td>0.142</td>
</tr>
<tr>
<td>Working time**</td>
<td>− 2.194</td>
</tr>
<tr>
<td>Nutrition Balance*</td>
<td>− 2.304</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Beta, Std. error, t, p value</th>
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</thead>
<tbody>
<tr>
<td>0.63, 0.615, − 1.11, 0.27</td>
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<tr>
<td>0.174, 0.641, − 0.28, 0.78</td>
</tr>
<tr>
<td>0.618, 0.641, − 2.08, 0.04</td>
</tr>
<tr>
<td>0.724, 0.641, 1.13, 0.26</td>
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<tr>
<td>0.727, 0.748, − 3.18, 0.00</td>
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<tr>
<td>0.748, 0.682, 0.19, 0.85</td>
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<tr>
<td>0.916, 0.682, − 3.22, 0.00</td>
</tr>
<tr>
<td>0.916, 0.682, − 2.51, 0.01</td>
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</table>
ENVIROMENTAL GENOMICS

NUTRITION GENOMICS

TOXICOGENOMICS

GENOME HEALTH STATUS OF THE POPULATION

PSYCHOGENOMICS

LIFESTYLE GENOMICS

PUBLIC HEALTH GENOMICS
AUTOMATED DIAGNOSTICS
GENOME HEALTH - NUTRIENT STATUS - GENOTYPE

NUTRIGENOMICS
EXPERT ADVICE SYSTEM
DATA BASE

INDIVIDUALISED NUTRITION
DIETARY PATTERNS - FUNCTIONAL FOODS - SUPPLEMENTS

GENOME HEALTH OPTIMISED
DEVELOPMENTAL & DEGENERATIVE DISEASE PREVENTED

Funding: Reach 100, NCEFF
ABC Catalyst, DNA Doctor Story
“We are at the threshold of a new era in which harm to the genome, which is the most fundamental pathology, can be efficiently diagnosed and prevented.

A person’s DNA damage profile is likely to become the ultimate routine biomarker of health status.

Prevention of DNA damage will soon achieve its rightful place as one of the most important objectives of global health strategies.”

Michael Fenech October 2010
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THE GENOME HEALTH NUTRIGENOMICS TEAM

Felicia Bulman
Julie Turner
Carolyn Salisbury
Philip Thomas
Jimmy Crott
Will Greenrod
Josy Rinaldi
Clare Aitken
Sally Record
Maryam Hor
Theodora Hua Haen
Jing Wu
Caroline Wu
Nathan O’Callaghan
Wayne Leifert
Glen Patten
Erin Symonds
Bianca Benassi
Sasja Beetstra

NO CONFLICT OF INTEREST
REDUCTION IN FAT CORRELATES WITH INCREASE IN TELOMERE LENGTH

- Change in Telomere Length (kb/diploid genome) vs Change in weight (kg)
  - $r = -0.65$, $P = 0.01$
- Change in Telomere Length (kb/diploid genome) vs Change in percentage body fat
  - $r = -0.56$, $P = 0.05$
- Change in Telomere Length (kb/diploid genome) vs Change in abdominal fat (g)
  - $r = -0.65$, $P = 0.02$

ANOVA $P = 0.02$

- Baseline
- Week 12
- Week 52
Telomere length in older men is significantly associated with plasma folate and homocysteine.

Telomere length is negatively correlated with plasma homocysteine in older men.

Males: \( r = -0.57, p = 0.004 \) (n = 24).
Females: \( r = 0.092, p = 0.68 \) (n = 23)

Telomere length is positively correlated with plasma folate in males but not in females.

Males: \( r = 0.42, p = 0.04 \) (n = 24).
Females: \( r = -0.11, p = 0.61 \) (n = 23)

Bull, O’Callaghan, Mayrhofer & Fenech Rejuvenation Res (2009)
Nutriomes and nutrient arrays - the key to personalised nutrition for DNA damage prevention and cancer growth control

Prof. Michael Fenech
michael.fenech@csiro.au

Nutritional Genomics and DNA Damage Diagnostics Laboratory
CSIRO Food & Nutritional Sciences

Minimally invasive High-Throughput Nutrient Array screening for Genome-Protective agents.

Development of automated CBMN Cytome assay: MN, NPB, NBUDs, Necrosis, Apoptosis, NDI + FISH + Protein expression
Is it possible to identify the nutriome that prevents the growth of each cancer?

- One of the greatest challenges in ageing populations is the need to prevent the proliferation of cancers which accumulate with age.

- Currently there is no rational advice on the appropriate diet to adopt once a person is diagnosed with cancer because our knowledge on nutrient-gene interaction with respect to cancers is rudimentary.

- Furthermore there is concern that supplementation with certain nutrients that are required for genome maintenance and cell growth (e.g. folate, methionine) may stimulate the cancer growth.
FOLATE: A DOUBLE-EDGED SWORD IN CANCER DEPENDING ON DOSE AND TIMING

FOLATE DEFICIENCY
<300 mcg/day*

promotes
NORMAL  →  ADENOMA  →  CANCER

inhibits??

ADEQUATE FOLATE
300-500mcg/day

inhibits

EXCESSIVE FOLATE
> 1000mcg/day

promotes

* folate deficiency increases risk of cancer initiation, dementia, stroke, osteoporosis
A nutrient array system could interrogate which nutrient restrictions or supplementations could control any cancer

- Cancers are genetically and epigenetically very different from normal tissue in the same person and from each other across persons.

- The multiple mutations in a cancer may make it difficult to rely on genotyping or gene expression patterns to work out an appropriate dietary control strategy.

- Some cancers amplify the high affinity folate receptor and may benefit from folate restriction.

- Other cancers may have defects in methionine metabolism making them susceptible to methionine restriction.

- A nutrient array system could identify the appropriate nutriome to control a cancer without knowing its genotype/epigenotype.
We now live in an era when stem cells are taken out of the body and expanded in vitro before being returned to the body.

Stem cell or iPS cell cytogenetic abnormalities constitute a roadblock to regenerative therapies because they increase the rate of senescence and the risk of oncogenic transformation.

Determining the optimal nutriome in culture medium that prevent chromosomal instability for each stem cell or iPS culture is therefore important not only to predict in vivo requirements but also in vitro nutrient requirements for DNA damage prevention.
The oral epithelium is a stratified squamous epithelium. It consists of four layers:
- The keratinised layer at the surface
- The prickle cell layer
- The basal layer
- Lamina propria
Buccal Micronucleus Cytome is a powerful diagnostic of accelerated ageing.

Fenech & Thomas 2007 Mutagenesis
Buccal Micronucleus Cytome Assay & Alzheimer Disease Risk

PPV = 98%; NPV = 77%;
Sensitivity = 82%, Specificity = 97%
LR = 25, OR = 140 for Biomarker 1+2 < 41

Fenech & Thomas 2007 Mutagenesis
Protective effects of grape seed polyphenol and curcumin consumption on Aβ plaque burden in brain section spanning hippocampus and DNA damage in blood and buccal cells of APPSwe/PS1dE9 transgenic mice
Chromosomal abnormalities in protein-calorie malnutrition


Rajeev Gupta,² M.B., B.S., Mukesh Gupta,³ M.D., and I. N. Ramdeo,⁴ M.D.


<table>
<thead>
<tr>
<th>Types of chromosomal aberrations in malnourished children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>16</td>
</tr>
</tbody>
</table>

TABLE 4
Observations

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Karyotype</th>
<th>No. of metaphase plates analyzed</th>
<th>No. of abnormalities</th>
<th>Percent abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>46 XY</td>
<td>30</td>
<td></td>
<td>3.22</td>
</tr>
<tr>
<td>2</td>
<td>46 XX</td>
<td>31</td>
<td>1</td>
<td>4.00</td>
</tr>
<tr>
<td>3</td>
<td>46 XX</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>46 XY</td>
<td>25</td>
<td>1</td>
<td>4.00</td>
</tr>
<tr>
<td>5</td>
<td>46 XX</td>
<td>36</td>
<td>2</td>
<td>1.31%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>152</td>
<td>2</td>
<td>1.31%</td>
</tr>
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</table>

Malnutrition group

<table>
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<tr>
<th>Case no.</th>
<th>Karyotype</th>
<th>No. of metaphase plates analyzed</th>
<th>No. of abnormalities</th>
<th>Percent abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46 XY</td>
<td>34</td>
<td>2</td>
<td>5.88</td>
</tr>
<tr>
<td>2</td>
<td>46 XY</td>
<td>40</td>
<td>4</td>
<td>10.00</td>
</tr>
<tr>
<td>3</td>
<td>46 XX</td>
<td>32</td>
<td>2</td>
<td>6.25</td>
</tr>
<tr>
<td>4</td>
<td>46 XY</td>
<td>34</td>
<td>2</td>
<td>5.88</td>
</tr>
<tr>
<td>5</td>
<td>46 XX</td>
<td>46</td>
<td>6</td>
<td>13.04</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>186</td>
<td>16</td>
<td>8.60%</td>
</tr>
</tbody>
</table>
Accumulated lymphocytic mtDNA deletions depend upon
(a) dietary folate deprivation,
(b) depletion of cellular folate storage

Lymphocyte mtDNA deletions are positively correlated
with brain mtDNA deletions ($r = 0.917$, $P < 0.0001$).

Chou and Huang (2009) EJN 48:429-436
Department of Nutritional Science, Fu-Jen University,
Hsinchuang 242, Taipei County, Taiwan.
POSSIBLE MECHANISMS BY WHICH MICRONUTRIENT DEFICIENCY COULD CAUSE DAMAGE TO THE GENOME

- Folate/B12 choline deficiency
- Deficiency of antioxidant Vitamins and Cofactors of Antioxidant enzymes
- Deficiency of Cofactors of DNA Repair enzymes

- Cytosine hypomethylation
- Uracil
- Oxidised DNA bases
- Unrepaired DNA adducts
- DNA break misrepair
- Unrepaired DNA breaks

- Telomere dysfunction
- Telomere shortening
- mtDNA deletion
- Base sequence mutation or deletion
- Dicentric chromosomes
- Unrepaired DNA adducts
- Acentric Chromosome fragments

- Chromosome loss or Malsegregation, Micronucleus formation
- Telomere end fusion

ACCELERATED SENESCENCE → BFB CYCLES → CHROMOSOME INSTABILITY PHENOTYPE & ABERRANT KARYOTYPE

- Telomere dysfunction
- Telomere shortening
- Unrepaired DNA breaks
Ageing causes hypomethylation of satellite DNA which leads to loss of ch 1, 9, 16 and micronucleus formation.
THE RABIT: A RAPID AUTOMATED BIODOSIMETRY TOOL FOR RADIOLOGICAL TRIAGE

Guy Garty,* Youhua Chen,† Alessio Salerno,‡ Helen Turner,* Jian Zhang,† Oleksandra Lyulko,* Antonella Bertucci,* Yanping Xu,* Hongliang Wang,† Nabil Smaan,‡ Gerhard Randers-Pehrson,* Y. Lawrence Yao,‡ Sally A. Amundson,* and David J. Brenner*

Health Phys. 98(2):209–217; 2010
THE HORMESIS HYPOTHESIS OF CALORIC/NUTRIENT RESTRICTION

CR or other biological stress

- Increased cell defenses
- Attenuation of stress-induced cell death
- Altered metabolism, cell cycle
  - Cell-cell communication, IGF-1, insulin, other humoral factors

- Reduced ROS, cellular damage, mutations
- Increased secretion of factors, fat mobilization, longevity of critical cells

- Insulin sensitization

Protein/Methionine restriction

Glucose Restriction (CR)

Caloric Restriction Mimetics

Nitrogen restriction

NAM depletion

VISFATIN

Heat shock

SIRT1

Longevity

KNOWN CALORIC RESTRICTION MIMETICS:

- 2-deoxyglucose
- Metformin
- Resveratrol
- Fisetin

XENOHORMETIC AGENTS??
DISCOVERY OF CALORIC RESTRICTION MIMETICS

DECELERATED AGEING

HEALTHY GENOME
- DNA repair
- Oncogenes silenced
- Compact chromatin

DAMAGED GENOME
- Oxidants
- Excess Calories
- DNA misrepair

SIRTUIN DEACETYLASE ACTIVATION
- NAD
- NICOTINIC ACID TRYPTOPHAN

RESVERATROL

OPTIMAL FAT LEVEL
- PPAR-γ
- EXCESS FAT

ACCELERATED AGEING

Howitz et al Nature 2004
ENVIRONMENTAL STRESSORS
(work, home, neighbourhood)

MAJOR LIFE EVENTS

TRAUMA, ABUSE

INDIVIDUAL DIFFERENCES
(genetics, development, experiences)

PERCEIVED STRESS

PHYSIOLOGICAL RESPONSE

BEHAVIOURAL RESPONSES
(fight or flight, smoking, drink, diet, exercise)

ALLOSTASIS

ADAPTATION

ALLOSTATIC LOAD

McEwen BS Physiol Rev (2006)
Accelerated telomere shortening in response to life stress

Elissa S. Epel1,2*, Elizabeth H. Blackburn1, Yue Lin1, Firdaus S. Dhabhar3, Nancy E. Adler4, Jason D. Morrow5, and Richard M. Cawthon6

17312–17315 | PNAS | December 7, 2004 | vol. 101 | no. 49

Correlations

<table>
<thead>
<tr>
<th></th>
<th>Perceived stress, n</th>
<th>Years of caregiving, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telomere length</td>
<td>−0.31* (−0.27*), 54</td>
<td>−0.40* (−0.43*), 36</td>
</tr>
<tr>
<td>Telomerase activity</td>
<td>−0.24* (−0.24*), 59</td>
<td>−0.35* (−0.32*), 37</td>
</tr>
<tr>
<td>Oxidative stress index</td>
<td>0.27* (0.22), 44</td>
<td>0.33* (0.38*), 30</td>
</tr>
</tbody>
</table>

Childhood Adversities Are Associated with Shorter Telomere Length at Adult Age both in Individuals with an Anxiety Disorder and Controls

Laura Kananne1,2, Ida Surakkana2, Sami Pirkolan3,5, Jaana Naivisaara5, Jouko Lönqvist5,6, Leena Peltomäen3,5,7,8, Samuli Rippli3,5, Iliris Hovatta1,2,8

Regressions model | β | se | P-value |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiety disorder</td>
<td>−0.080</td>
<td>0.069</td>
<td>0.224</td>
</tr>
<tr>
<td>GHQ-12 score</td>
<td>−0.002</td>
<td>0.009</td>
<td>0.838</td>
</tr>
<tr>
<td>Number of childhood adversities</td>
<td>−0.090</td>
<td>0.032</td>
<td>0.005</td>
</tr>
</tbody>
</table>
LIFE-STYLE IS ALSO AN IMPORTANT DETERMINANT OF DNA DAMAGE

LIFE-STYLE HPI INDEX

<table>
<thead>
<tr>
<th>Smoking</th>
<th>Smokers</th>
<th>Non-smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol drinking</td>
<td>Often</td>
<td>No or moderate</td>
</tr>
<tr>
<td>Nutritional balance*</td>
<td>Poor</td>
<td>Good or moderate</td>
</tr>
<tr>
<td>Exercise**</td>
<td>No or seldom</td>
<td>≥2 times per week</td>
</tr>
<tr>
<td>Sleeping hours*</td>
<td>&lt; 7 h</td>
<td>≥7 h</td>
</tr>
<tr>
<td>Working hours**</td>
<td>&gt; 9 h</td>
<td>&lt; 9 h</td>
</tr>
<tr>
<td>Mental stress</td>
<td>Excessive</td>
<td>Slight or mild</td>
</tr>
<tr>
<td>Breakfast</td>
<td>No eating</td>
<td>Eating</td>
</tr>
</tbody>
</table>

Factors | MN
|---------|-----------------
|        | Beta | Std. error | t   | p value |
| Smoking | −0.698 | 0.63 | −1.11 | 0.27 |
| Drinking | −0.174 | 0.615 | −0.28 | 0.78 |
| Sleeping hours* | −1.288 | 0.618 | −2.08 | 0.04 |
| Mental stress | 0.724 | 0.641 | 1.13 | 0.26 |
| Exercise** | −2.315 | 0.727 | −3.18 | 0.00 |
| Breakfast | 0.142 | 0.748 | 0.19 | 0.85 |
| Working time** | −2.194 | 0.682 | −3.22 | 0.00 |
| Nutrition Balance* | −2.304 | 0.916 | −2.51 | 0.01 |
THE GENOME HEALTH CLINIC CONCEPT

• DNA DAMAGE IS THE MOST FUNDAMENTAL DISEASE

• DNA DAMAGE CAN BE EFFICIENTLY DIAGNOSED

• DNA DAMAGE CAN BE PREVENTED

• DNA DAMAGE DIAGNOSTICS SHOULD BECOME ROUTINE IN INTEGRATIVE & PREVENTIVE MEDICINE PRACTICES
GENOME HEALTH CLINIC

AUTOMATED DIAGNOSTICS
GENOME HEALTH - NUTRIENT STATUS - GENOTYPE

NUTRIGENOMICS
EXPERT ADVICE SYSTEM
DATA BASE

INDIVIDUALISED NUTRITION
DIETARY PATTERNS - FUNCTIONAL FOODS - SUPPLEMENTS

GENOME HEALTH OPTIMISED
DEVELOPMENTAL & DEGENERATIVE DISEASE PREVENTED

VERIFICATION OF EFFICACY
FEEDBACK TO DATABASE

RADICAL INNOVATION IN NUTRITION

Funding: Reach 100, NCEFF


ABC Catalyst, DNA Doctor Story
WHAT IS THE GENE EXPRESSION PATTERN ASSOCIATED WITH MICRONUCLEUS FORMATION?

NETWORK OF GENES ASSOCIATED WITH MN FORMATION BASED ON DATA IN LITERATURE

Dedicated network showing customised MN-related gene–gene interactions with p53 as central hub and validated against actual occurrence of MN in exposed individuals has been constructed.

Van Leeuwen DM et al Mutagenesis (in press)