The effect of dietary micronutrients and micronutrient supplementation on telomere length

A thesis submitted for the degree of Doctor of Philosophy

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Abstract

Telomeres are nucleoprotein complexes, which cap the ends of linear chromosomes to prevent against chromosome end-to-end fusions in the cells. The underlying DNA sequence in humans is a TTAGGG hexamer that is repeated many times at each telomere. Due to the end-replication problem at DNA ends, the length of the telomere sequence shortens with each cycle of cell division. Telomere length has been associated with chronic disease, markers of oxidative stress and inflammation as well as diet patterns and individual nutrients. Evidence in the literature indicates that telomere length has both elements of heritability and may be modified by various environmental exposures including diet. However, the relationship of telomere length with dietary micronutrients in Australian populations has been largely unexplored. Additionally, the possibility that nutrients can influence telomere length in humans gives rise to the opportunity to modify telomere length through targeted dietary interventions. A double-blinded micronutrient intervention randomised controlled trial (RCT) – the *Polypill* study – provided the opportunity to assess changes in telomere length over time in a metropolitan Australian population.

Peripheral blood mononuclear cell (PBMC) telomere length in a healthy cohort of middle-aged South Australians was measured and cross-sectional associations with demographic and anthropometric variables were explored. Plasma levels of dietary micronutrients such as vitamin D and the metabolite homocysteine (associated with B vitamin deficiency) were correlated with telomere length. Vitamin D was found to be positively associated with PBMC telomere length while plasma homocysteine was inversely associated with PBMC telomere length.

The *Polypill* double-blinded RCT investigated changes in PBMC telomere length following 16 weeks of treatment with a micronutrient supplement containing folic acid, vitamin B_{12}, vitamin E, retinol, nicotinic acid and calcium (FBERNC) and compared this change to participants who received an inactive placebo. When groups were compared, there were no significant differences in the

mean changes in telomere length over time. Additionally, there was no significant difference in the proportions of individuals placed in telomere length trajectory groups defined as telomere sequence loss, maintenance or gain.

It was hypothesised that the integrity of the telomere sequence may be a more valuable biomarker than telomere length alone to assess responses to B vitamin supplementation. As uracil misincorporates in to the genome under low folate conditions, a qPCR method to detect uracil within the telomere sequence was conceived and optimised. The reproducible assay was developed with the use of synthetic uracil-containing primers and was then applied to DNA extracted from the WIL2-NS human lymphoblastoid cell line cultured *in vitro* under low folic acid and with supplemented dUTP. Next, *in vitro* modelling with various concentrations of folic acid, dUTP and additionally S-adenosyl methionine (SAM) were performed with the WIL2-NS cell line. There were no clear trends of either folic acid, dUTP or SAM on cell viability, telomere length, telomeric uracil content nor global methylation. Additionally, complex but statistically significant interactive effects for each of the experimental endpoints were noted indicating the possibility of strong homeostatic mechanisms regulating telomere integrity under conditions of folate deficiency and dUTP excess.

Candidate declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Carly Moores

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List of abbreviations

5-MeTHF	5-methyltetrahydrofolate
5,10-MeTHF	5,10-methylenetetrahydrofolate
A	adenine
ALT	alternate lengthening of telomeres
ANOVA	analysis of variance
ATCC	American Type Culture Collection
AUD	Australian dollar
B_2	vitamin B_2/riboflavin
B_6	vitamin B_6/pyridoxine
B_{12}	vitamin B_{12}/cobalamin
B	unstandardised regression coefficient
B in FBERNC	vitamin B_{12} (*Polypill*)
β	standardised regression coefficient
BER	base excision repair
BFB	breakage-fusion-bridge
BHMT	betaine homocysteine methyltransferases
BMI	body mass index
bp	base pairs
C	cytosine
C in FBERNC	calcium (*Polypill*)
CBMN	cytokinesis-block micronucleus assay
CH_3	methyl group
CO_2	carbon dioxide
CpG	cytosine phosphate guanine
CRP	c-reactive protein
CSIRO	Commonwealth Scientific and Industrial Research Organisation
C_T	cycle threshold
CV	coefficient of variation
CVD	cardiovascular disease
Δ	change
$\Delta_{0\text{-}16}$	change from week 0 to week 16
$\Delta_{16\text{-}32}$	change from week 16 to week 32
ΔC_T	change in cycle threshold
DC	dyskeratosis congenita
DDR	DNA damage response
DHF	dihydrofolate
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DNMT	DNA methyltransferase
dNTP	deoxyribonucleotide triphosphate
dTTP	deoxythymidine triphosphate
dU	deoxyuridine

dUTP	deoxyuridine triphosphate
dUMP	deoxyuridine monophosphate
E	vitamin E/alpha-tocopherol (*Polypill*)
EAR	estimated average requirement
EDTA	ethylenediaminetetraacetic acid
ex.	excludes
F	folate (*Polypill*)
FA	folic acid
FBS	foetal bovine serum
FFQ	food frequency questionnaire
FISH	fluorescence in-situ hybridisation
FPG	formamidopyridine DNA glycosylase
G	guanine
H_2O	water
HBSS	hanks balanced salt solution
hOGG1	human 8-oxoguanine DNA N-glycosylase 1
HPLC	high performance liquid chromatography
HREC	human research ethics committee
hTERT	human telomerase reverse transcriptase
ICF	immunodeficiency, centromere instability and facial anomalies syndrome
IEO	index of education and occupation
IER	index of economic resources
IRSD	index of relative socio-economic disadvantage
IRSED	index of relative socio-economic advantage and disadvantage
IU	international unit
IQR	interquartile range
kb	kilobase
LDL	low density lipoprotein
m^5C	5-methyl cytosine
hm^5C	5-hydroxymethyl cytosine
MN	micronuclei
MTHFR	methylene tetrahydrofolate reductase
MTR	methionine reductase
MTRR	methionine synthase reductase
n	number
N	nicotinic acid (*Polypill*)
n-3	omega 3
n-6	omega 6
NAD	nicotinamide adenine dinucleotide

NADP	nicotinamide adenine dinucleotide phosphate
NaK	sodium-potassium
NER	nucleotide excision repair
NHANES	National Health and Nutrition Examination Survey
NHMRC	National Health and Medical Research Council
NO	nitric oxide
NTC	no template control
P	probability
PARP	poly ADP ribose polymerase
PASW	predictive analytical software
PBL	peripheral blood lymphocytes
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PUFA	polyunsaturated fatty acid
qPCR	quantitative polymerase chain reaction
qFISH	quantitative fluorescence in-situ hybridisation
R	Pearson correlation coefficient
R in FBERNC	retinol (*Polypill*)
R^2	coefficient of determination
RCT	randomised controlled trial
RDA	recommended daily allowance
RDI	recommended daily intake
RNA	ribonucleic acid
RP-HPLC	reverse phase high performance liquid chromatography
SAM	s-adenosyl methionine
SD	standard deviation
SDS	sequence detection software
SE	standard error
SEIFA	socio-economic index for areas
SEM	standard error of the mean
SHMT	serine hydroxymethyltransferase
SNP	single nucleotide polymorphism
T	thymine
TERRA	telomeric repeat-containing RNA
TERT	telomerase reverse transcriptase
TGA	Therapeutic Goods Administration
THF	tetrahydrofolate
TL	telomere length
TPE	telomere position effect
TRAP	telomere repeat amplification protocol
U	uracil
UDG	uracil DNA glycosylase
UL	safe upper level of intake

USER	uracil specific enzyme reagent
UTR	untranslated region
UV	ultra violet
VNTR	variable number tandem repeat

Standard international units of measure

°C	degrees Celsius
Gy	Gray
h	hour
L	litre
m	metre
mol	mole
M	molar
min	minute
s	second

Orders of magnitude

M	mega	($\times 10^{6}$)
k	kilo	($\times 10^{3}$)
m	milli	($\times 10^{-3}$)
µ	micro	($\times 10^{-6}$)
n	nano	($\times 10^{-9}$)
p	pico	($\times 10^{-12}$)
f	femto	($\times 10^{-15}$)
a	atto	($\times 10^{-18}$)