

WestminsterResearch

<http://www.westminster.ac.uk/westminsterresearch>

**DNA damage in paediatric obesity: a promoter and predictor of
cancer in adulthood**

Usman, M.

This is an electronic version of a PhD thesis awarded by the University of Westminster.

© Mrs Moonisah Usman, 2020.

The WestminsterResearch online digital archive at the University of Westminster aims to make the research output of the University available to a wider audience. Copyright and Moral Rights remain with the authors and/or copyright owners.

DNA damage in paediatric obesity: a promoter and predictor of cancer in adulthood

Moonisah Bajwa Usman

A thesis submitted in partial fulfilment of the requirements of the
University of Westminster for the degree of Doctor of Philosophy

College of Liberal Arts and Sciences
University of Westminster

April 2020

Abstract

Obesity in children is one of the most serious, global, public health challenges of the 21st century. The accumulation of adipose tissue is associated with a range of metabolic complications including diabetes, cardiovascular disease and dyslipidaemia. Epidemiological evidence links obesity in childhood with developing certain types of cancer later in life. It is postulated that excess adipose tissue and consequent inflammation derived oxidative stress may inflict an accumulation of deleterious DNA mutations and promote genome instability and drive carcinogenesis. Furthermore, a deficiency in micronutrients that are essential for DNA repair may exacerbate this pathological state.

This research combined the assessment of anthropometric, inflammatory, micro-nutritional and DNA damage biomarkers via non-invasive techniques. In total, 112 children were recruited from schools and NHS obesity clinics. Anthropometric markers assessed were waist to hip ratio, body fat percentage via bioelectrical impedance, and body mass index standard deviation scores (BMI-SDS). These markers were used to classify participants as obese or non-obese and used for correlational analysis. Inflammation and micronutrient status were determined via C-reactive protein and vitamin D Enzyme Immune Assay (EIA) in saliva. DNA damage assessments include a microscopic assessment of nuclear anomalies via the buccal cytome assay, salivary telomere length via quantitative Polymerase Chain Reaction (qPCR) and urinary 8-hydroxyguanosine (8-OHdG) via EIA.

The results from this study indicate obesity to be concurrent with increased inflammation and vitamin D deficiency in this cohort of participants. In addition, obesity was associated with increased oxidative DNA damage (8-OHdG) in

urine and DNA damage events in the buccal mucosa. Salivary telomere length was positively correlated with obesity and the total frequency of nuclear anomalies found in buccal epithelial cells. Furthermore, there was a negative correlation between vitamin D and the frequency of nuclear anomalies in the oral cavity. Importantly, odds ratio analysis indicates a high BMI Z-score, waist circumference, body fat percentage, salivary CRP and low salivary vitamin D to be independent risk factors for increased nuclear anomalies in the buccal mucosa.

This research is the first to accrue evidence for acquired DNA damage in multiple tissues obtained non-invasively from children with obesity. Our findings instigate that biomonitoring of 'genome health' for pre-cancerous molecular and morphological markers in obese patients may inform prioritization and severity of clinical intervention measures to prevent malignancy.

Acknowledgements

In the name of God, the most kind and the most merciful.

I would like to begin by expressing my sincere gratitude to my first supervisor Dr Emanuela Volpi. I thank Emanuela whole-heartedly for her immense academic support in every step of this journey, for inspiring me and giving me her time and trust. Undertaking this PhD has been a truly life-changing experience with thanks to her supervision. I would also like to extend my gratitude to Dr Ihab Tewfik for taking his role as my second supervisor seriously, and for motivating me. And I am thankful to Dr Sara Suliman for visiting me from the UAE to encourage and support this work.

I am very thankful to Dr John Murphy, Professor Taj Keshavarz and Professor Jane Lewis for believing in me, and for their time and support with securing a university scholarship for me.

My profound gratitude goes to our collaborators without whom this research would be incomplete. Firstly, I am incredibly thankful to Dr Murray Bain at St George's hospital, whom I thoroughly enjoyed learning from. Thank you also to Mr Ashish Desai for agreeing to meet and take this project further at King's College Hospital. I am hugely appreciative of Dr Martha Ford-Adams, Dr Simon Chapman and Bethany Glassar for giving me the opportunity to work in their clinic at King's and to learn from them too. I am also thankful to Sarah March at the Petchey Academy, her patience and cooperation has been exemplary.

I am also grateful to all the research participants and their families for engaging with this research, without whom this work would be impossible. And I am thankful to the numerous undergraduate and post-graduate students who supported the research and whom I had the opportunity to teach and learn from.

I must also thank my educators who have become my colleagues and friends over the years: Dr Romy Begum, Dr Bradley Elliott, Dr Chrystalla Ferrier and Dr Claire Robertson. I must also recognise that I have been exceptionally fortunate to have the incredible support of Dr Jennifer Fraser. Jennifer has supported the completion of this thesis by being patient and understanding with me, providing

constant encouragement and practical support with planning my writing. I am also thankful to Jennifer for sharing their office space with me as well as treats and souvenirs from their travels. Having Jennifer as a colleague made my PhD journey a lot less stressful.

My thanks also goes to everyone in the PhD office for being so supportive, sharing ideas and snacks, particularly Nasrin, Louise, Emma, Nicola, Nadeen, and Vedia. And to all the laboratory technicians, particularly Jean Pierre, Dinesh Shah, Vanita Amin, Glen Wotherspoon and Jonathan Lee.

I am also very thankful to my friends of 15 years – Fatima and Maria. They have looked after me, been wonderful listeners and great entertainers. I am very lucky to have them in my life.

And my gratitude extends to my little sister Amaan for being more like an older sister and texting me to check-in and remind me that she is proud of me – I am more proud of her. Thank you also to my wonderful older sisters - Annie and Anam for their love, care and the little angels they brought into our lives – Hannah, Soha, Ali, Amelia, Safa and Zaki. They can make me laugh regardless of how difficult the situation may be.

And thanks to my Papa for asking me every year how closer I was to getting my PhD, for getting excited about my travels and the conferences I presented at, and for wearing all the souvenir t-shirts I got him.

My most sincere gratitude goes out to my beloved husband. I am thankful to Usman for the love, care and tolerance of my moods, especially through the final phase of this work. I greatly appreciate that he helped me carry my bulky equipment and set up the community events for my research on the days he could be relaxing. I wouldn't be able to cope without his existence.

And finally but most importantly, my gratitude also extends to my Mama – she has always encouraged me to learn more and work hard with honesty and integrity. I am incredibly thankful for her loving upbringing and I dedicate this work to all her struggles.

I hope I have made you proud Mama.

Acknowledgements of Contribution

Firstly, I would like to acknowledge the work of Dr Bain, Dr Ford-Adams, Dr Chapman and Ms Glassar for the recruitment and consenting of research participants at St George's and King's College NHS trusts.

Secondly, I would like to acknowledge the work of Sarah March, Louise Thomas, Rosie Lloyd, Natacha Von Mitzinger, Fatima Ahmed, Valentina Borgognoni, Dalin Murad, Winston Ekwueme, Adhe Noviello, Jenaagan Jenakendran and Mary Momoh for the recruitment of participants from schools in London.

I would also like to acknowledge the efforts of Sophie Hawkins, Ivona Bilkevic, Jessica Britto, Saima Haider, Anamul Hoque, Emily Shorter, William Townley Stefan Lay and Tanya Awal in the early optimisation of laboratory protocols, support with recruiting participants and collecting samples. Ivona and Jessica further contributed to the microscopic analysis of buccal cell samples.

The optimisation of the qPCR analysis has been informed by Dr Shahid Chaudhary at University Hospital Zurich and Dr Louise Usher at the University of Westminster.

The creatinine analysis of urine samples was performed by Dr Chrystalla Ferrier, and I am very thankful to her and the University of Westminster, Blood Testing Service.

Statistical support was provided by Dr Claire Robertson and Mr Roger A'Hern. Claire and Roger provided support with calculation of the sample size and advised on appropriate statistical tests.

Declaration

I declare that all the material presented in this thesis, is wholly my own work, unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Moonisah Bajwa Usman

Publications

Usman, M. and Volpi, E.V., (2018). DNA damage in obesity: Initiator, promoter and predictor of cancer. *Mutation Research/Reviews in Mutation Research*. 778:23-37.

Usman, M. Tewfik, I. and Volpi, E. (2017). Genome instability in childhood pediatric obesity: a conceptual framework for an assessment, intervention and monitoring programme of inflammation and DNA damage in pediatric obesity, *IJFNPH*, **9**(1).

Table of Contents

ABSTRACT	I
ACKNOWLEDGEMENTS	III
ACKNOWLEDGEMENTS OF CONTRIBUTION	V
DECLARATION	VI
PUBLICATIONS	VII
TABLE OF CONTENTS	VIII
LIST OF TABLES	X
LIST OF FIGURES	XI
ABBREVIATIONS	XII
1. INTRODUCTION	1
1.1 OBESITY IN CHILDHOOD AND ADOLESCENCE	1
1.1.1 <i>Measurements and estimates for defining paediatric obesity</i>	1
1.1.2 <i>Epidemiology of paediatric obesity</i>	10
1.1.3 <i>Causes of paediatric obesity</i>	13
1.1.4 <i>Comorbidities in paediatric obesity</i>	18
1.1.5 <i>Cancer and later life health implications of paediatric obesity</i>	22
1.2 GENOME HEALTH.....	31
1.2.1 <i>Defining genome health and types of DNA damage</i>	31
1.2.2 <i>Threats to genome health</i>	36
1.2.3 <i>Genome instability and cancer</i>	43
1.2.6 <i>Markers of DNA damage and assessment techniques</i>	47
1.3 AIMS OF THE PROJECT	61
2. MATERIALS AND METHODS	62
2.1 OVERVIEW OF STUDY DESIGN	62
2.2 ETHICAL CONSIDERATIONS AND APPROVAL.....	62
2.3 PARTICIPANT SAMPLE SIZE CALCULATION	64
2.2 PARTICIPANT SCREENING AND RECRUITMENT	66
2.4 COLLECTION OF BIOLOGICAL SAMPLES AND ANTHROPOMETRIC DATA	69
2.6 SALIVARY C - REACTIVE PROTEIN ELISA	71
2.6.1 <i>Saliva sample collection</i>	71
2.6.2 <i>CRP ELISA sensitivity and test principle</i>	71
2.6.3 <i>Saliva sample preparation</i>	72
2.6.4 <i>Assay preparation</i>	72
2.6.5 <i>Assay procedure</i>	73
2.6.7 <i>Salivary CRP calculations</i>	74
2.7 SALIVARY VITAMIN D ELISA.....	75
2.7.1 <i>Saliva sample collection</i>	75
2.7.2 <i>Vitamin D ELISA sensitivity and test principle</i>	75
2.7.3 <i>Saliva sample preparation</i>	76
2.7.4 <i>Assay preparation</i>	76
2.7.5 <i>Assay procedure</i>	76
2.7.7 <i>Salivary vitamin D calculations</i>	77
2.8 URINARY 8-OHDG ELISA	78
2.8.1 <i>Urine sample collection</i>	78
2.8.2 <i>8-OHDG ELISA sensitivity and test principle</i>	78
2.8.3 <i>Urine sample preparation</i>	79
2.8.4 <i>Reagent and assay preparation</i>	79
2.8.5 <i>Assay procedure</i>	80
2.8.7 <i>Urinary 8-OHDG calculations</i>	81
2.8.8 <i>Creatinine quantification and correction</i>	82

2.9 BUCCAL CYTOME ASSAY	83
2.9.1 Buccal cell sample collection.....	83
2.9.2 Sample wash and preparation for fixation	84
2.9.3 Cell fixation and staining	86
2.9.4 Microscopy and Scoring.....	87
2.10 SALIVARY TELOMERE LENGTH (STL) QUANTIFICATION BY QUANTITATIVE POLYMERASE CHAIN REACTION (qPCR)	89
2.10.1 Saliva sample collection.....	89
2.10.2 DNA Extraction.....	89
2.10.3 DNA Assessments.....	90
2.10.4 qPCR Principle	91
2.10.5 qPCR Constituents.....	91
2.10.6 qPCR Run Method	92
2.10.7 Quality control and salivary telomere length calculations.....	93
2.11 STATISTICAL ANALYSIS	97
3. RESULTS	100
3.1 PARTICIPANT DEMOGRAPHICS	100
3.2 ANALYSIS OF INFLAMMATION STATUS - SALIVARY C-REACTIVE PROTEIN	107
3.3 ANALYSIS OF MICRONUTRIENT STATUS - SALIVARY VITAMIN D.....	111
3.4 URINARY 8-OHdG ANALYSIS	115
3.5 ANALYSIS OF DNA DAMAGE IN THE BUCCAL MUCOSA	120
3.6 ANALYSIS OF SALIVARY TELOMERE LENGTH.....	129
3.7 COMBINED ANALYSIS OF INFLAMMATION, MICRO-NUTRITIONAL DEFICIENCY AND DNA DAMAGE MARKERS	135
4. DISCUSSION	139
4.1 EXCESS CRP AND CHRONIC INFLAMMATION IN CHILDHOOD OBESITY	139
4.2 HYPOVITAMINOSIS D IN CHILDHOOD OBESITY.....	146
4.3 INCREASED OXIDATIVE DNA DAMAGE IN CHILDHOOD OBESITY	155
4.4 INCREASED NUCLEAR ANOMALIES IN THE BUCCAL MUCOSA OF CHILDREN WITH OBESITY	165
4.5 SALIVARY TELOMERE LENGTH IS GREATER IN CHILDREN WITH OBESITY.....	177
4.6 INTEGRATING DNA DAMAGE ASSESSMENTS IN THE CLINICAL MANAGEMENT OF CHILDHOOD OBESITY	185
5. CONCLUSION.....	193
6. FUTURE WORK	194
7. REFERENCES	196
8. APPENDIX.....	241
I. PARTICIPANT INFORMATION BOOKLET FOR PARENTS (CLINIC VERSION)	241
II. PARTICIPANT INFORMATION BOOKLET FOR 10-18 YEAR OLDS (CLINIC VERSION)	243
III. PARENTAL CONSENT FORM (CLINIC VERSION)	245
IV. ASSENT FORM (CLINIC VERSION)	246
V. PARTICIPANT INFORMATION BOOKLET FOR PARENTS (SCHOOL VERSION)	247
VI. PARTICIPANT INFORMATION BOOKLET FOR 10-18 YEAR OLDS (SCHOOL VERSION)	249
VII. PARENTAL CONSENT FORM (SCHOOLS VERSION).....	251
VIII. ASSENT FORM (SCHOOL VERSION)	253
IX. MEDICAL QUESTIONNAIRE	254
X. INVITATION LETTER (SCHOOL PARENTS/GUARDIANS)	257
XII. NORMALITY TEST RESULTS.....	258
XIII. ASSOCIATIONS BETWEEN MICRONUCLEI (MNI) FREQUENCY IN PBLs AND SITE-SPECIFIC MALIGNANCY AND PRE-MALIGNANCY.	259
XIV. ASSOCIATIONS BETWEEN ADIPOSITY AND TELOMERE LENGTH.....	261

List of Tables

Table 1.2 RCPCCH classifications of BMI for males and females aged 2-20 years.....	6
Table 1.1 Summary of techniques for assessing paediatric adiposity.	8
Table 1.3. Genetic defects associated with early-onset obesity.	17
Table 1.4. Health complications across multiple organ systems in children with obesity.	21
Table 1.5 Health complications in adulthood as a result of obesity earlier in life	25
Table 1.6 Overview of the five DNA repair pathways in humans	35
Table 1.7 Micronutrients for genome health maintenance.....	42
Table 2.1 Data for sample size calculation	65
Table 2.2 School Research Pack Components.....	66
Table 2.3 Exclusions Criteria for Participant Recruitment	67
Table 2.4 Materials for Buccal Cell Sample Collection.....	83
Table 2.5 Materials for washing and preparation of cell samples.....	85
Table 2.6 Materials for cell fixation and staining	87
Table 2.7 Scoring criteria for BMA.....	88
Table 2.8 Materials for DNA extraction from saliva	90
Table 2.9 Primer sequences and concentrations in qPCR reactions	92
Table 2.10. Standard DNA sequences.....	92
Table 2.11 Conversion of telomere standard concentrations into kbp.....	94
Table 2.12 Conversion of 36B4 standard concentrations into kbp	95
Table 2.13 Child Growth Foundation classifications of body fat % centiles	98
Table 2.14 Child Growth Foundation classifications of waist circumference centiles.....	98
Table 2.15 Risk factors and criteria for odds ratio analysis	99
Table 3.1.1 Contingency analysis of sex across cohorts arranged by RCPCCH classification of obesity	100
Table 3.1.2 Participant age and anthropometric assessments by RCPCCH classification of obesity.....	102
Table 3.1.3 Percentage of agreement between anthropometric markers and BMI Z-score	104
Table 3.1.4 R-squared values as per Spearman rank correlation between anthropometric markers	105
Table 3.1.5 Participant numbers in obese and non-obese cohorts when defined by three different classifications systems.	105
Table 3.2.1. Spearman rank correlation between salivary CRP and anthropometric assessments.....	107
Table 3.2.2. Welch's t-test analysis of average salivary CRP between Non-obese and Obese cohorts. ..	109
Table 3.3.1. Spearman rank correlation between salivary vitamin D and anthropometric assessments	111
Table 3.3.2. Welch's t-test of average salivary vitamin D between Non-obese and Obese cohorts.....	113
Table 3.4.1. Spearman rank correlation between urinary 8-OHdG and anthropometric assessments. ..	115
Table 3.4.2. Welch's t-test of average urinary 8-OHdG between Non-obese and Obese cohorts.	117
Table 3.5.1. Spearman rank correlation analysis between total DNA damage (%) in the buccal mucosa (BM) and anthropometric assessments.	121
Table 3.5.2. Welch's t-test analysis of average total DNA damage in the buccal mucosa between Non-obese and Obese cohorts.	123
Table 3.5.3. Welch's t-test analysis between frequency of individual DNA damage markers in the buccal mucosa (BM) per 1000 cells, across non-obese and obese cohorts, classified by BMI Z-Score.....	125
Table 3.5.4 Multiple Regression Analysis best-fit model for predicting total DNA damage in the buccal mucosa.....	127
Table 3.5.5 Retrospective analysis of Odds Ratio using the Fisher's exact test.	128
Table 3.6.1. Spearman rank correlation analysis between salivary telomere length (sTL) and anthropometric assessments.	129
Table 3.6.2. Welch's t-test of average salivary telomere length between Non-obese and Obese cohorts.....	131
Table 3.7.1. Summary of Spearman rank correlation coefficients of all anthropometric biomarkers with inflammation, vitamin D, DNA damage and telomere length markers.	135
Table 3.7.2. Summary of Welch's t-test analysis, reported as percentage difference in means between non-obese and obese participants classified by different anthropometric markers.....	137
Table 3.7.3. Summary of Spearman rank correlation coefficients of inflammation, vitamin D, DNA damage and telomere length biomarkers.	138

List of Figures

Figure 1.1 The distribution of body fat across multiple sites.	5
Figure 1.2 The annual frequency of childhood overweightness in the UK since 1995.	12
Figure 1.3 The eight hallmarks of cancer described by Hanahan and Weinberg (2017).	28
Figure 1.4 The Genome Health Pendulum	36
Figure 1.5 Accumulation of DNA damage over several cell cycles.....	43
Figure 1.6 Reduction and oxidation of 2-deoxyguanosine to produce 8OHdG and 8oxodG.....	49
Figure 1.7 Micronucleus formation that would be observed in a cell undergoing nuclear division and blocked prior to cytokinesis with cytochalasin-B	52
Figure 1.8 Nucleoplasmic Bridge formation as a result of dicentric chromosomes, that would be observed in a cell undergoing nuclear division and blocked prior to cytokinesis with cytochalasin-B	54
Figure 1.9 Sequential origins of the various cell types in the buccal epithelium.	56
Figure 2.1 Overview of cross-sectional study design: from planning to sample collection and testing.....	63
Figure 2.2. Flow diagram of participant recruitment from schools and NHS clinics in London.	68
Figure 2.3 Participant Work-flow diagram.....	70
Figure 2.4 Typical plate layout for CRP ELISA.	72
Figure 2.5 Typical standard curve for CRP ELISA.	74
Figure 2.6. Competitive ELISA Test Principle.....	75
Figure 2.7 Typical standard curve for vitamin D ELISA	77
Figure 2.8 Typical plate plan for 8-OHdG assay.....	80
Figure 2.9 Typical standard curve for 8-OHdG ELISA.	81
Figure 2.10 Typical qPCR standard curves.....	96
Figure 3.1.1 There is no significant difference between sex across and within cohorts.	101
Figure 3.1.2 Ethnic distribution across obese and non-obese cohorts classified by BMI Z-score.....	103
Figure 3.1.3 Correlation matrix of all anthropometric indices.....	106
Figure 3.2.1 Spearman rank correlation between anthropometric indices and salivary CRP.....	108
Figure 3.2.2 Welch's t-test of average CRP levels.....	110
Figure 3.3.1 Spearman rank correlation between salivary vitamin D and anthropometric indices.....	112
Figure 3.3.2 Welch's t-test of average vitamin D (VD) levels.....	114
Figure 3.4.1 Spearman rank correlation between urinary 8-OHdG and anthropometric indices.....	116
Figure 3.4.2 Welch's t-test of average 8-OHdG levels.....	118
Figure 3.4.3 Spearman rank analysis between urinary 8-OHdG, salivary CRP and vitamin D levels	119
Figure 3.5.1 Abnormal nuclear morphologies (DNA damage markers) scored in buccal epithelial cells.	120
Figure 3.5.2 Spearman rank correlation between anthropometric indices & DNA damage in the buccal mucosa.....	122
Figure 3.5.3 Welch's t-test of average total frequency of DNA damage in the buccal mucosa.....	124
Figure 3.5.4. The average frequency of cells with micronuclei, multiple-nuclei and nuclear buds and bridges (NBuds/Bridges) in the buccal mucosa.....	125
Figure 3.5.5 Spearman rank analysis indicates between DNA damage in the buccal mucosa, salivary CRP and vitamin D levels.....	126
Figure 3.6.1 Spearman rank correlation between anthropometric indices and salivary telomere length.....	130
Figure 3.6.2 Welch's t-test of average salivary telomere length.	132
Figure 3.6.3 Spearman rank correlation between salivary telomere length, salivary CRP and vitamin D levels.....	133
Figure 3.6.4 Spearman rank correlation between salivary telomere length, urinary 8-OHdG and DNA damage in the buccal mucosa.	134

Abbreviations

8-OHdG	8-hydroxy-2'-deoxyguanosine
25OHD	25- hydroxyvitamin D
AGEs	Advanced-glycation end products
ATM	ataxia telangiectasia mutated
BAT	Brown Adipose Tissue
BCA	Buccal cytome assay
BER	Base Excision Repair
BFB	Breakage-fusion-bridge
BI	Bioelectrical Impedance
BMA	Buccal Micronucleus Assay
BMI	Body Mass Index
BF%	Body Fat percentage
CBMN	Cytokinesis-block micronucleus assay
CGF	Child Growth Foundation
CI	Confidence interval
CIN	Chromosomal Instability
CRP	C-Reactive protein
Ct	Cycle threshold
CT	Computerised Tomography
CV	Coefficient of variation
DEXA, DXA	Dual-energy x-ray absorptiometry
DNA	Deoxyribonucleic Acid
DMSO	Dimethyl Sulfoxide
DSB	Double-strand break
EDTA	Ethylenediaminetetraacetic acid
ELISA, EIA	Enzyme-Linked Immunosorbent Assay
FISH	Fluorescence in situ hybridization
FAPY	Formamidopyrimidine
GI	Gastrointestinal
GPO	Glutathione peroxidase
GWAS	Genome-Wide Association Studies
H2AX	H2A histone family member X
H2O2	Hydrogen peroxide
HCL	Hydrochloric Acid
HPLC	High Performance Liquid Chromatography
HR	Homologous Repair
HRP	Horseradish peroxidase
hs-CRP	High sensitivity CRP
Kbp	Kilo-base pair
Kg	Kilograms
LEP	Leptin protein
LEPR	Leptin protein receptor
LC-MS	Liquid chromatography–mass spectrometry
M	Molar
mm	Millimetres
mg	milligrams
MC4-R	Melanocortin 4 Receptor
MMR	Mismatch Repair
MN	Micronucleus
MNi	Micronuclei
MIN	Microsatellite Instability
MRI	Magnetic Resonance Imaging
NBridge	Nuclear Bridge
NBUD	Nuclear Bud

NCMP	National Child Measurement Programme
NDC	Normal Differentiated Cells
NER	Nucleotide Excision Repair
ng	Nanogram
NHEJ	Non-homologous End Joining
NHS	National Health Service
NIN	Nucleotide Instability
nmol	Nanomoles
NO	Nitric Oxide
Non-Ob	Non obese
NTC	No Template Control
O ₂ -	superoxide anion radical
Ob	Obese
OD	Optical Density
•OH	Hydroxyl radical
OSA	Obstructive Sleep Apnoea
PBL	Peripheral Blood Lymphocyte
PCSK-1	Prohormone Convertase Subtilisin/Kexin1
pg	Picograms
pH	power of hydrogen
POMC	Pro-opiomelanocortin
PCR	Polymerase Chain Reaction
qPCR, RT-PCR	Quantitative Polymerase Chain Reaction
RCPCH	Royal College of Paediatrics and Child Health
RNA	Ribonucleic Acid
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RPM	Rounds per minute
RT	Room temperature
SCFE	Slipped Capital Femoral Epiphysis
SDS, Z-score	Standard deviation score
SOD	Superoxide Dismutase
SNP	Single Nucleotide Polymorphism
SSB	Single-strand break
TBE	Tris/Borate/EDTA
TMB	3,3',5,5'-Tetramethylbenzidine
UV	Ultraviolet
VD	Vitamin D
VDBG	Vitamin D binding globulin
VDBP	Vitamin D binding protein
VDR	Vitamin D receptor
WAT	White Adipose Tissue
WC	Waist circumference
WHR	Waist Hip Ratio
WHO	World Health Organization
µL	Microlitre
µM	Micromolar

1. Introduction

1.1 Obesity in childhood and adolescence

1.1.1 Measurements and estimates for defining paediatric obesity

Obesity is defined as a state of abnormal or excess adipose tissue that presents a risk to health (WHO, 2018). Adipose tissue can be divided into two subtypes - brown adipose tissue (BAT), which dissipates energy for thermogenesis, and white adipose tissue (WAT), which is the storage site for triglycerides. The latter is more prevalent in a state of obesity and can be situated in subcutaneous or visceral fat depots. There are many different techniques for the direct assessment or estimation of adipose tissue in children (Table 1.1).

The gold standard technique for assessing adiposity is Magnetic Resonance Imaging (MRI). MRI generates a magnetic field that forces protons in the body to align with that field. With the use of a radiofrequency current, the alignment can be shifted to produce detailed anatomical images (Berger, 2002). MRI is useful for examining the distribution of body fat and can differentiate between body fat depots. The interpretation of body fat as subcutaneous or visceral may be an essential phenomenon in understanding long-term disease risk. Excess visceral adiposity is associated with the metabolic syndrome and plays a role in the pathophysiology of non-alcoholic fatty liver disease in children with obesity (Mager, *et al.*, 2013). However, as a clinical tool, MRI is expensive and requires participants to be stationary for long periods of time (Shen, *et al.*, 2012). This can be an added challenge when trying to accurately assess adiposity in younger populations.

Computerised tomography (CT) scanning is another technique which can be used to assess body fat distribution. However, this technique depends on the variable resistance of different body parts to X-ray radiation. This makes CT scanning clinically impractical, particularly for long-term monitoring (Mook-Kanamori, *et al.*, 2009). Nonetheless, CT scanning is used in research to compare different techniques for measuring body fat distribution (Samara, *et al.*, 2012).

Similarly to CT scanning, Dual energy X-ray absorptiometry (DEXA, DXA) scanning incorporates the use of X-ray beams to differentiate between lean and fat mass but with a lower dose of radiation. A recent study by Dias and colleagues (2019) explored the potential of DXA scanning to monitor adiposity in 62 children with and without obesity, by comparing levels of visceral adipose tissue assessed by DXA, with results from MRI scanning. Whilst levels of total visceral fat were significantly correlated between MRI and DXA for all participants combined ($R=0.9$, $p<0.001$). Correlations between DXA and MRI were poorer for participants within the lowest and highest tertile ($R=0.38-0.51$) for visceral body fat percentage compared with those in the middle tertile ($R=0.94$). Further analysis reveals that DXA scanning overestimated visceral fat mass levels compared to MRI scanning by 163.6 grams. Furthermore, DXA is impractical for long-term monitoring due to cumulative radiation exposure. The size limit of machines can also present complications for assessing children with obesity (Horan, *et al.*, 2015).

Other techniques for measuring body composition include air displacement plethysmography (ADP) via the BOD POD. ADP depends on the principle that the volume of an object can be indirectly measured based on the volume of air it

displaces in a closed chamber. Fields and colleagues (2002) have reviewed the accuracy of the BOD POD for predicting body fat percentage in children. Their review indicates that BOP POD assessment of body fat percentage can overpredict levels of adipose tissue in children by 0.6-1.2%. Whilst this was not statistically significant, it could create a difference when categorising a child as healthy-weight or overweight based on their body fat levels.

Furthermore, bioelectrical impedance (BI) can be employed to provide an estimation of total fat-mass. BI is a measure of the resistance to an alternating electrical current that is sent through the body. Fat and bone mass increase the resistance to this current whilst body fluids and lean tissue conduct the current. Therefore, BI is a more accurate predictor of total body water than fat-mass (Lukaski, *et al.*, 1985). Based on this principle, hydration status may affect the reliability of the fat-mass prediction. Furthermore, a review of the literature suggested that although BI is a safe, cost-effective and time-efficient technique for predicting the percentage of body fat, measurement errors are possible (Talma, *et al.*, 2013). Nonetheless, Meredith-Jones and colleagues (2015) compared fat mass values from BI and DXA in 187 normal weight and obese children at baseline and after 12-months. Overall, the difference of 0.04kg in fat mass between the two techniques was not statistically significant, indicating that BI may be useful to assess changes in body composition on an individual basis in normal weight and obese children.

Total body fat percentage can also be predicted by measuring skinfold thickness with callipers. This is a straight-forward, time-efficient and cost-effective method which estimates levels of subcutaneous fat. Measurements of bicipital, tricipital, subscapular and suprailiacal skinfold thickness can be

combined and related to total body density to generate body fat percentage in sex and age specific cohorts (Weststrate, & Deurenberg, 1989). The assessment of a single skinfold may also be combined with BMI to estimate fat-mass (Pecoraro *et al.*, 2003). However, intra-subject variability, accuracy and precision are challenges particularly when assessing skinfold thickness in children with obesity (Wells, & Fewtrell, 2006). An accurate assessment may also require the removal of clothing, making this technique partly invasive. These methodological challenges are also present in the prediction of fat-mass via circumferential analysis of body parts.

The assessment of mid-upper arm (MUAC), mid-thigh, neck, wrist, waist, and hip circumference have all been proposed for evaluating the distribution of adipose tissue (Hatipoglu, *et al.*, 2010; Mazıciöđlu, *et al.*, 2010; Chaput, *et al.*, 2017). Neck circumference is convenient to assess, however, it is less predictive of overweightness when compared to the measurement of waist circumference (Hatipoglu, *et al.*, 2010). A large number of studies support the use of waist circumference to predict obesity status and body fat distribution in children (Glasser, *et al.*, 2011; Patnaik, *et al.*, 2017; Mukherjee, *et al.*, 2016; Goulding, *et al.*, 2000). Goulding and colleagues (2000) compared the sensitivity and specificity of waist circumference with DXA for the prediction of trunk fat mass across 580 children. They found that the 80th percentile for waist circumference correctly identified at least 87% of children with a high fat mass and 92% with a low trunk fat mass, indicating good sensitivity and specificity respectively. Overall, there was a strong correlation between waist circumference and trunk fat mass as determined by DXA ($R=0.92$, $p<0.0001$). Based on these results, waist circumference is a reliable predictor of high trunk fat mass in children with and without obesity.

In addition, fat distribution is relevant to consider because circumferential analyses have been linked with long-term disease risk. Figure 1.1 depicts the sites of body fat distribution that are commonly assessed via circumferential analysis and DXA. In (1956) Vague and colleagues first described the distribution of adipose tissue as being ‘android’ or ‘gynoid’, and linked these with circumferential analyses. An android distribution is described as the predominance of body fat within the upper body (abdomen, chest and neck). Whereas a gynoid distribution of body fat refers to the predominance of body fat around the lower body (hips, thighs and buttocks).

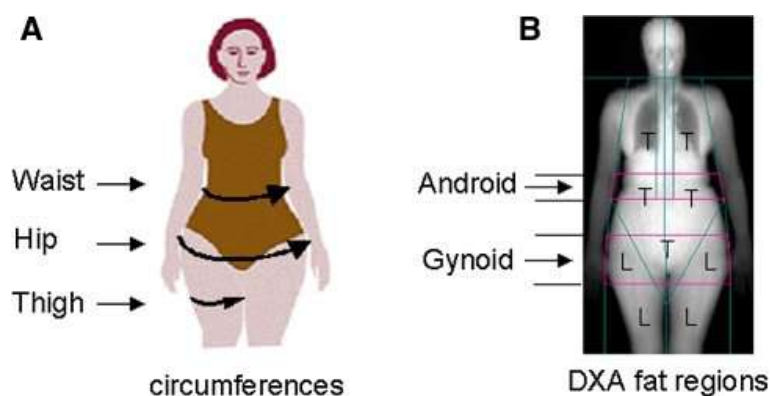


Figure 1.1 The distribution of body fat across multiple sites – showing A) circumferential assessment points for waist, hip and thigh B) body fat assessment as indicated by DXA (T indicates trunk fat (android distribution) and L indicates leg fat (gynoid distribution) (Zillikens, et al., 2010).

A larger waist circumference (>85th centile) has been associated with an increased risk of insulin resistance compared to a gynoid distribution in adolescents with obesity ($R=0.35$, $p<0.01$) (Aucouturier, *et al.*, 2009).

Furthermore, Kelishadi and colleagues (2017) report moderate correlations between neck and wrist circumference and high systolic blood pressure ($R=0.36$ and 0.37 respectively) across a cohort of 4200 7-18 year olds. Finally, there are reports that waist circumference, waist-height ratio and waist-hip ratio may also

have predictive value in the risk of metabolic disease when combined with other tools such as body mass index (BMI) (Savva, *et al.*, 2000; McCarthy, 2006; Moore, *et al.*, 2015).

At present, BMI is the most widely used surrogate measure of adiposity. It is a proportional measure of height and weight, expressed as kg/m². Due to the natural fluctuations in adipose tissue through childhood, BMI is not considered in isolation, but is specific for age and sex. Childhood BMI status is expressed with a standard deviation score (SDS or Z-score) for comparison with a population reference (Wright, *et al.*, 2002). In the UK, the UK90 growth charts are validated for a clinical diagnosis of overweightness or obesity, based on different cut-off points for BMI Z-scores (Table 1.2) (Cole, *et al.*, 2000).

However, ethnic differences in body fat distribution may undermine the current BMI Z-score classification method of body fatness (Hudda, *et al.*, 2018). There can also be children who are beneath cut-offs but may be at risk of co-morbidities (Tyson, & Frank, 2018). In such cases, combining waist circumference assessments with BMI may be useful. Savva and colleagues (2000) reported that waist circumference is a better predictor than BMI for elevated blood pressure. This result was based on findings from a multiple regression analysis where waist circumference explained 12.4% more variance in blood pressure than BMI. Similarly, de Koning and colleagues (2015), found that waist circumference explained more of the variance in blood lipid patterns compared to BMI, although this association is less striking (only 2% more). However, when waist circumference and BMI were combined, the researchers were able to explain 12% of the variance in the pro-inflammatory pattern associated with BMI above the 95th percentile and a waist circumference above the 90th centile, for age and sex.

Table 1.2 RCPCH (2013) classifications of BMI for males and females aged 2-20 years.

BMI centile/Standard Deviations (SDs)	Interpretation
<0.4 th	Underweight
≥0.4 th BMI <91 st	Healthy weight
≥91 st BMI <98 th	Overweight
≥98 th BMI <99.6 th	Obese (severely overweight)
≥99.6 BMI <3.33 SDs	Severely Obese
≥3.33 SDs	Morbidly Obese

Furthermore, even though BMI status does not differentiate between fat-mass or fat-free mass, it still remains to be the most clinically useful tool in the diagnosis of childhood obesity (Tyson, & Frank, 2018). Pietrobelli and colleagues (1998) demonstrated that BMI can reliably assess total body fat in kilograms (average $R^2=0.87$) and body fat percentage (average $R^2=0.66$). Later studies have also confirmed these findings. Steinberger and colleagues (2005) compared BMI in 130 adolescents (11-17 years old) with body fat results from DXA and reported a strong correlation ($R=0.95$). Vanderwall and colleagues (2017) compared BMI Z-scores with DXA in 663 children and found that in children above the age of 9 years, BMI-Z scores may also strongly predict total fat mass (average $R^2=0.65$). However, in the same study, this conclusion could not be applied for children under the age of 9 years (average $R^2=0.15$). There is also evidence which suggests that the combined assessment of BMI-SDS with body fat percentage via bioelectrical impedance can reliably predict total body fat as measured by MRI in 8-12 year olds ($R^2=0.89$) (Chan, *et al.*, 1998). Therefore, in addition to a clinical assessment of BMI Z-score, it would be useful to determine whether the percentage of body fat and body fat distribution via secondary methods can contribute to defining a threshold that more reliably identifies children at risk of obesity associated co-morbidities.

Table 1.1 Summary of techniques for assessing paediatric adiposity (adapted from Horan, et al., 2015).

Method	Estimates	Advantages	Disadvantages
MRI and CT	Fat mass and distribution as visceral or subcutaneous	<ul style="list-style-type: none"> •Gold standard for accuracy and precision •Valuable for research 	<ul style="list-style-type: none"> •Radiation exposure via CT •Expensive equipment •Operators require training •Children need to remain still for long periods of time •Impractical for clinical settings and monitoring on an individual basis
DEXA	Measures bone mineral density to estimate soft tissue	<ul style="list-style-type: none"> •Precise (R=0.9) when compared to gold standard techniques •Valuable for Research •DEXA scanner may be used in clinical setting 	<ul style="list-style-type: none"> •Expensive equipment •Operators require training •Body fat overestimation in heavier and underestimation in lighter individuals •Scanner size may be limited for child body size •Impractical for monitoring on an individual basis due to cumulative radiation exposure
Air displacement plethysmography (ADP) / Hydrostatic under-water weighing (HW)	Total body density	<ul style="list-style-type: none"> •HW considered as gold standard for accuracy and precision •Valuable for research 	<ul style="list-style-type: none"> •Expensive equipment •Operators require training •Hydration status can affect ADP •Body fat overestimation in heavier and underestimation in lighter individuals in ADP •Equipment not available in clinical setting •HW is Impractical for monitoring on an individual basis
Bioelectrical impedance	Fat mass	<ul style="list-style-type: none"> •Can be cost effective •Time efficient •Uncomplicated 	<ul style="list-style-type: none"> •Hydration status may affect accuracy of measurement •Underestimation of total body fat in leaner children and overestimation in obese children by 0.6-1.2%.

Skinfold thickness	Subcutaneous fat distribution	<ul style="list-style-type: none"> • Suitable for individual clinical assessments and research • Inexpensive • Time efficient • Uncomplicated 	<ul style="list-style-type: none"> • Accuracy decreases with increasing adiposity • Not useful for individual measurements • Less useful for ranking in less extreme body fatness or lean body mass • Contention over which side of the body to take measurements from • Considerable expertise and training necessary • Requires callipers which are not generally available in clinical setting
Circumference of various body parts	Subcutaneous fat distribution	<ul style="list-style-type: none"> • Inexpensive • Time efficient • Uncomplicated • Reference ranges established for waist circumference centiles • Suitable for individual clinical assessments 	<ul style="list-style-type: none"> • Lack of standardisation in measurement produces high intra-variability and inter-variability • Lack of reference ranges for other circumferential assessments • Head circumference only useful until age five • May be inaccurate during illness – eg. Waist circumference during constipation or abdominal swelling
BMI Z-Score	Body mass (weight) relative to age, height and sex.	<ul style="list-style-type: none"> • Inexpensive • Time efficient • Uncomplicated • Established reference from WHO for comparisons • Sensitivity increases with increasing adiposity • Suitable for individual clinical assessment and population based research studies 	<ul style="list-style-type: none"> • Lack of consideration for lean body mass (bone, muscle and water weight) • Lack of consideration for ethnic differences • Recommended to be used in combination with other assessments of body fatness

1.1.2 Epidemiology of paediatric obesity

Obesity is one of the greatest, global public health challenges of the 21st century. Since 1975, the global rate of obesity has nearly tripled as there were 650 million adults (>18 years of age) with obesity and 340 million adolescents (10-18 years of age) with overweightness or obesity recorded in 2016 (WHO, 2018). Latest statistics compiled by the World Obesity Federation report that the United States has the highest prevalence of childhood overweightness and obesity, as over 40% of children (aged 5-18 years) constituted this category. Secondly, nearly 30% of boys and 25% of girls in Canada are diagnosed as overweight or obese. Libya, Saudi Arabia, Iran, Australia, Brazil, Mexico, New Zealand, Southern Europe and the UK all have rates of childhood overweightness above 20%.

In the UK, the National Child Measurement Programme (NCMP) is overseen by Public Health England and is dedicated to recording height and weight of children in Reception and again in Year 6 (aged 4-5 years) of primary school. The latest report from the NCMP indicates that one in five children in reception possess a BMI >85th centile - classed as overweight, whereas one in ten children in this age group possesses a BMI >95th centile - classed as obese. In year 6 (10-11 years of age), the prevalence of obesity and overweightness is higher. One in three children in year 6 is overweight, whereas one in five is considered obese (NCMP, 2018).

Figure 1.2 illustrates the prevalence of overweightness since 1995 for ages 2-10 and 11-15 years. Firstly, It demonstrates that excess weight is more prevalent in the 11-15 year old group compared to the younger, 2-10 year old group by about 9%. Secondly, the graph suggests a steady increase in

overweightness across the UK, with the peak of overweightness in 2004 reaching over 40% in 11-15 year olds. Since then, overweightness appears to be gradually levelling out for both age groups but there is no significant decline in 2018 compared to 2006. Furthermore, the State of Child Health report (RCPCH, 2019) also presents concerning evidence that childhood obesity is positively correlated with deprivation status of the local authority in England ($R=0.66$). According to figures from the NCMP, obesity was almost four times as high in the most deprived areas (3.8%) than the least deprived areas (1.0%) in 2017/18 amongst 4-5 year olds. Overall, the Royal College of Paediatrics and Child Health (RCPCH) have predicted that the 2020 figures for overweightness amongst all children in the UK may reach 50% (RCPCH, 2020).

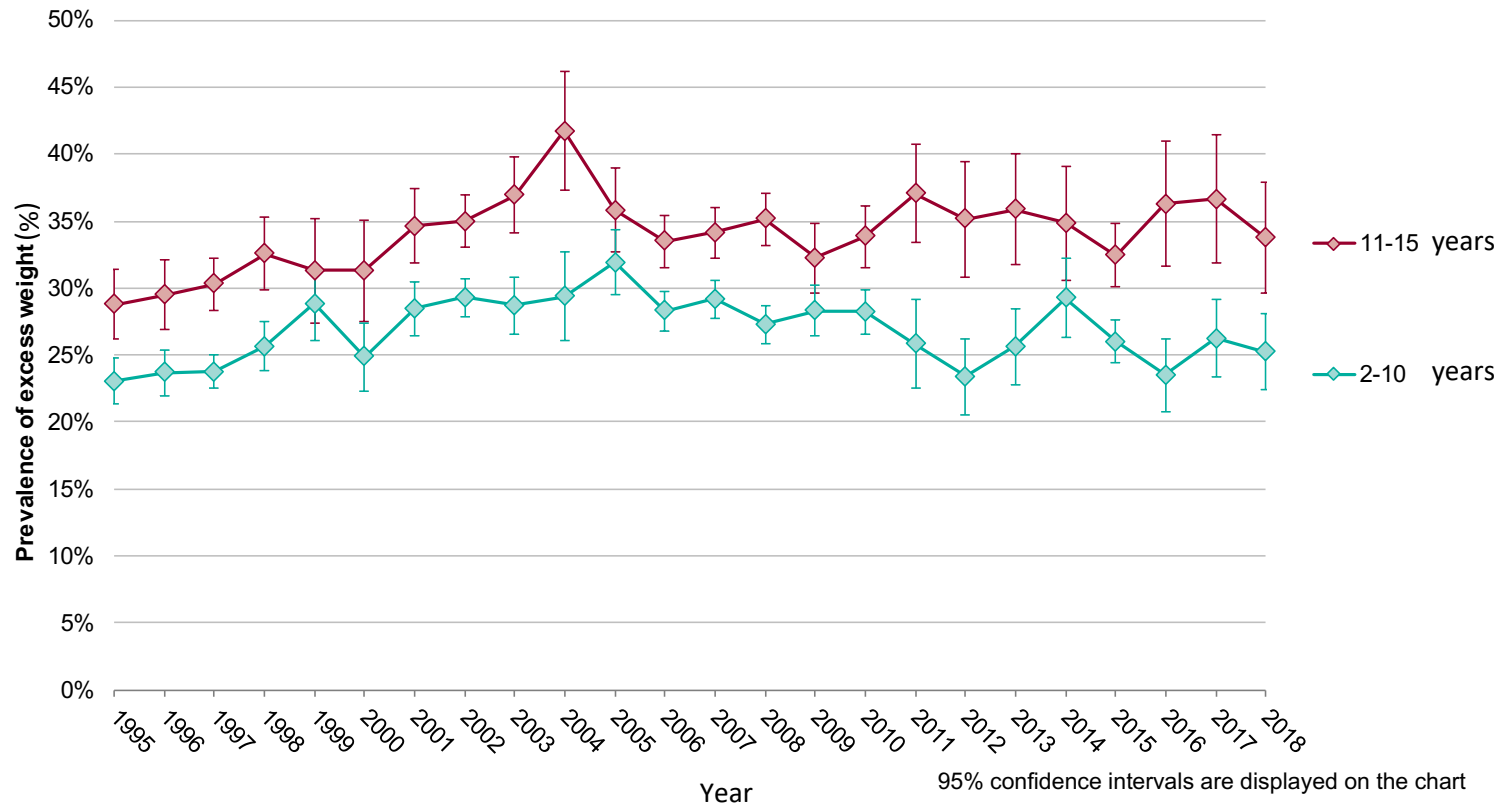


Figure 1.2 The annual frequency of childhood overweightness in the UK since 1995; presenting no significant decline in the prevalence of children within and above the 85th centile for Body Mass Index (BMI) (National Child Measurement Programme, 2019).

1.1.3 Causes of paediatric obesity

The expansion of adipose tissue is marked by an increased deposition of triglycerides as long-term stores of energy. Triglycerides are deposited under a state of excess caloric intake and reduced energy expenditure (Scientific Advisory Committee on Nutrition, 2011). The mechanisms of adipose tissue regulation are complex and influenced by a variety of exogenous factors including diet and behaviour, as well as endogenous factors such as biology and genetics.

Diet plays a critical role in the pathogenesis of childhood obesity. A report from the British Medical Association describes poor dietary patterns amongst children in the UK (Roycroft, 2015). Only 10% of children consume the recommended intake of five portions of fruit and vegetables a day. Furthermore, the report outlined an excess intake of saturated fats (>10% of total dietary energy/day), salt (>6g/day) and non-milk extrinsic sugars (>10% of total dietary energy/day). These sugars come mainly from soft drinks, fruit juice, breakfast cereals, cakes and biscuits. Research has connected the consumption of drinks with a high sugar content with the epidemiological rise in childhood obesity (Monasta, *et al.*, 2010; Fisher, & Kral, 2008). Specifically, over a 3 year follow-up of 11654 children, the consumption of one additional serving of sugar based fizzy drinks was associated with a 0.04 increase in BMI (Berkey, *et al.*, 2004). Another study conducted across 548 children, found that the odds ratio of obesity increased by 60%, for each additional serving of sugar-sweetened beverage consumed per day (Ludwig, *et al.*, 2001). Moreover, the consumption of fast food is also linked with increased adiposity as it has poor nutritional value and instead proves to be more caloric, and higher in fats and sodium (Ozuysal,

& Baccus, 2012). It is likely that these eating behaviours can be influenced by family and society.

Family culture may impact feeding-practices in multiple ways including the perception of which foods are considered healthy or unhealthy, or associating food with reward (Bruss, *et al.*, 2005). The use of food as reward is not just persuasive at home but also in classrooms and may encourage unhealthy behaviour such as the consumption of food irrespective of metabolic demands (Fedewa, & Davis, 2015). Secondly, an extensive review by Kral and Faith (2008), suggested a familial association between parental eating behaviours and child eating behaviours. They also noted that altering the types of foods available and accessible to children can have a long-term impact on food choices.

Physical activity can modulate the association between dietary intake and weight gain. Reduced physical activity has been related to increased dietary consumption and fat accumulation (Shook, *et al.*, 2015). The NHS guidelines recommend that children undertake at least 60 minutes of moderate physical activity every day, and vigorous activity at least 3 times a week (NHS, 2011). Findings from a recent longitudinal assessment of physical activity through childhood suggest that the levels of physical activity begin to decline from age 7 and continue declining through adolescence (Farooq, *et al.*, 2018). The causes for physical inactivity in children have been studied widely. Low socioeconomic conditions have been linked to increased screen-viewing and sedentary behaviour (Coombs, *et al.*, 2013). Furthermore, urbanisation may contribute to the creation of an obesogenic environment via a loss of space for physical activity (Pirgon, & Aslan, 2015).

In addition, there are a number of medical disorders that can disrupt the mechanisms of energy balance and give rise to obesity in children. Challis and colleagues (2003) have reviewed sixteen genetic, pleiotropic syndromes where obesity is one clinical feature, such as Prader-Willi and Bardet-Biedl syndromes. There are also a number of rare endocrine disorders that exhibit childhood obesity. Cushing's syndrome is marked by elevated cortisol in the bloodstream and manifests with truncal obesity (Lodish, *et al.*, 2018). Other endocrine pathologies include hypothyroidism, growth hormone deficiency and hypothalamic disorders (Aggarwal, & Jain, 2018). Thyroid hormone and growth hormone both contribute to the regulation of metabolism and food consumption (Rosenbaum, *et al.*, 2000; Vijayakumar, *et al.*, 2011). The hypothalamus plays a major role in satiety and food intake by responding to signals from the gastrointestinal tract and fat stores (Ahima, & Antwi, 2008). Tumour associated hypothalamic-pituitary lesions may disrupt these mechanisms and lead to obesity (Taylor, *et al.*, 2012). Leptin hormone is secreted from adipose tissue in proportion to stores of triglycerides and triggers the hypothalamic satiety centre to reduce food intake and increase energy expenditure (Ahima, *et al.*, 2000).

A number of genetic defects have been identified that may disrupt the signalling mechanisms between gut hormones, leptin from fat stores and the hypothalamus to cause early-onset obesity (Table 1.3). Genome wide association studies (GWAS) have linked nearly 25,000 nucleotide polymorphisms (SNPs) with obesity (Dong, *et al.*, 2018). Whilst the biological significance of most SNPs is unknown, genetic variants in the FTO gene coding for the fat-mass and obesity associated protein on chromosome 16, have been associated with regulating food choice and intake, indicating possible gene-

environment interactions (Cecil, *et al.*, 2008). Nonetheless, it has been suggested that these genetic variants explain just 2-5% of variation in BMI (Locke, *et al.*, 2015). This means that the focus of obesity interventions remains largely on dietary and lifestyle factors.

Table 1.3. Genetic defects associated with early-onset obesity.

Type of Genetic defect	Obesity causing mechanism	Reference
<i>Leptin (LEP/Ob) gene mutation</i>	Reduced stimulation of POMC neurons in hypothalamus results in hyperphagia	(Farooqi, <i>et al.</i> , 2002)
<i>Leptin receptor (LEPR) gene mutation</i>	Truncated leptin receptor leads to similar effects as seen in leptin gene mutations	(Vaisse, <i>et al.</i> , 1998)
<i>POMC gene mutations</i>	Disrupted melanocortin signaling results in hyperphagia	(Krude, <i>et al.</i> , 2003)
<i>Melanocortin-4 receptor (MC4-R) gene mutation</i>	Mutation results in MC4-R deficiency and hyperphagia due to reduced binding with α melanocyte-stimulating hormone.	(Farooqi, <i>et al.</i> , 2003)
<i>Prohormone convertase-1 (PCSK-1) gene mutation</i>	Prohormone convertase-1 deficiency results in failed conversion of gut hormones. Patients present with hyperphagia.	(Creemers, <i>et al.</i> , 2012)

1.1.4 Comorbidities in paediatric obesity

Obesity in children can manifest with a number of short-term health complications across multiple-organ systems. Studies in table 1.4 indicate that around 30% of children with obesity suffer from disorders of the endocrine, cardiovascular or pulmonary system.

A third of children and adolescents with obesity can present with insulin resistance in the UK (Viner, *et al.*, 2005; Romualdo, *et al.*, 2014). Insulin resistance in obesity is caused by excess glucose intake and chronic inflammation (Dandona, *et al.*, 2004). The progression from insulin resistance to type 2 diabetes in children for long has been considered rare (Viner, *et al.*, 2005). However, type 2 diabetes in obese adolescents is an emerging problem in the UK, particularly for high-risk ethnic groups including South Asians and Arabs (Ehtisham, *et al.*, 2008). There are currently 715 children and young adults living with type 2 diabetes in the UK (RCPCH, 2016). Type 2 diabetes is a serious health complication in children because it can lead to microvascular complications, renal disease, vision abnormalities and psychological stress (RCPCH, 2016).

In addition to insulin resistance, obese children may present with increased risk factors for cardiovascular disease. Emmerik and colleagues (2012) studied 307 twelve-year olds with severe obesity and assessed blood pressure, glucose, and cholesterol. They identified that 67% of the cohort already had at least one risk factor for cardiovascular disease. Moreover, another study conducted in 274 obese children identified that over 30% presented with dyslipidaemia (Pastucha, & Horak, 2014). Dyslipidaemia in childhood obesity may coincide with fatty liver disease (Deeb, *et al.*, 2018). A meta-analysis of 76 independent

study populations found a 30% prevalence of non-alcoholic fatty liver disease (NAFLD) in children recruited from obesity clinics (Anderson, *et al.*, 2015). They also estimated a global prevalence of nearly 8% in children that were obese but not under the care of clinicians. Although children with dyslipidaemia and fatty liver disease are usually asymptomatic to begin with, the condition may progress to cirrhosis and increase the risk of liver cancer (Marion, *et al.*, 2004).

Furthermore, obesity in children can be complicated by obstructive sleep apnoea (OSA). OSA is marked by a limitation in air flow due to a reduction in the tone of the airway musculature. In obesity, this may be caused by excess adipose tissue depressing the upper airway or enlarged adenoids and tonsils (Narang, & Mathew, 2012). A retrospective study in 190 Caucasian children suggested that BMI was a significant predictor of OSA although it only explained 4.5% of the variance, indicating that other measurements of adiposity may be significant (Kohler, & van den Heuvel, 2008). Nonetheless, OSA in obese children is concerning because it may drive hypoxia, inflammation, oxidative stress and increase the risk of metabolic syndrome (Arens, & Muzumdar, 2010).

Excess adipose tissue in children may give rise to musculoskeletal complications. According to a study across 2459 participants, overweight and obese children self-reported greater musculoskeletal complications in their daily life compared to normal weight children (OR=1.86) (Krul, *et al.*, 2009). Slipped Capital Femoral Epiphysis (SCFE) is the most common cause of hip replacement surgery in young adults, caused by alterations in the shape of the hips (Chairman, *et al.*, 2012). Recently, Perry and colleagues (2018) conducted an in-depth analysis of the association between BMI and SCFE across nearly

600,000 children. They identified that severe obesity (BMI >99th percentile) was associated with a 17x higher risk of SCFE. Musculoskeletal disorders associated with obesity may complicate physical activity and quality of life.

The emotional wellbeing of children with obesity is also at risk, as they are more likely to be bullied in school and have less friendships (Eisenberg, *et al.*, 2003). This may lead to a low self-esteem and behavioural problems (Strauss, 2000). A systematic analysis representing over 143,000 children found the odds of depression to be x1.32 greater in children with obesity compared to healthy weight controls (Sutaria, *et al.*, 2019). This is concerning because depression can prevent weight-loss and cause obesity to persist in adolescence (Goodman, & Whitaker, 2002).

The treatment of obesity in children is crucial for reducing the discussed complications but also for preventing obesity in adulthood. A recent report highlighted that over 300 000 13-18 year olds are eligible for anti-obesity drugs whilst 90,000 may also be eligible for bariatric surgery in the UK (Viner, *et al.*, 2018). A systematic review of studies that followed up a total of 200,777 participants concluded that despite interventions, 70% of children with obesity may take their weight into adulthood (Simmonds, *et al.*, 2016). The researchers also identified that early interventions (prior to adolescence) are more likely to reduce the persistence of obesity.

Table 1.4. Prevalence of health complications across multiple organ systems in children with obesity.

System	Disorders	Study population	Prevalence of co-morbidity (%)	Reference
<i>Endocrine</i>	Insulin Resistance	103, 2-18 years, BMI>95 th centile	30%	(Viner, <i>et al.</i> , 2005)
<i>Cardiovascular</i>	Hypertension	103, 2-18 years, BMI>95 th centile	32%	(Viner, <i>et al.</i> , 2005)
	Dyslipidaemia	274. 10-18 year olds, BMI>97 th centile	30%	(Pastucha, & Horak, 2014)
<i>Gastrointestinal</i>	Non-alcoholic fatty liver disease (NAFLD)	Meta-analysis with 74 studies of participants aged 1-19 years, BMI>95 th centile	7.6%	(Anderson, <i>et al.</i> , 2015)
<i>Pulmonary</i>	Obstructive sleep apnoea (OSA)	190 4-12 year olds, BMI>95 th centile	33%	(Kohler, & van den Heuvel, 2008)
<i>Musculoskeletal</i>	Structural defects in upper and lower extremities, knee, back and neck.	2459, 2-17 year olds, BMI>85 th centile	21.9%	(Krul, <i>et al.</i> , 2009)
<i>Psychosocial</i>	Depression	Meta-analysis of 22 studies of participants aged <18 years, BMI>95 th centile.	10.4%	(Sutaria, <i>et al.</i> , 2019)

1.1.5 Cancer and later life health implications of paediatric obesity

Cancer is a notorious disorder characterised by abnormal cell growth and metastasis that occurs in a complex, multi-step process. In the UK, there are 360,000 new cases of cancer each year, and over a quarter of deaths are a result of cancer (CRUK, 2015). Up until 2018, obesity was described as the second largest preventable cause of cancer in adults (following smoking), as 6.3% of all cases were linked to being over-weight or obese (Brown, *et al.*, 2018). However, more recent data analysis from Cancer Research UK (CRUK) has described obesity to be the leading cause of four types of cancers; kidney, breast, ovarian and liver (CRUK, 2019). CRUK estimated that in total, obesity causes 3940 more cases of these types of cancers combined than smoking.

The epidemiological correlation between obesity and cancer has been substantiated in an extensive review by Renehan and colleagues (2008). They analysed 282,137 cases of cancer and concluded sex and site-specific differences in the association between obesity and cancer. Specifically, a 5kg/m² increase in BMI in men was associated with an increased risk of oesophageal, thyroid, colon and renal cancer (relative risk ratios (RR) between 1.24-1.52). Whereas a 5kg/m² increase in BMI in women was also associated with an increased risk of oesophageal and renal cancer but endometrial and gallbladder cancer as well (RR between 1.34-1.59). Furthermore, a large population based study of 5.24 million individuals identified that increased body mass index (BMI) heightened the risk of acquiring post-menopausal breast, endometrial, liver, ovarian, colon, gall bladder and kidney cancer (Bhaskaran *et al.*, 2014). So far, these epidemiological links have been described in adults.

However, it is likely that the pathological implications from excess adiposity and the multi-step process of tumorigenesis may begin as early as childhood.

There is growing epidemiological evidence that indicates obesity in childhood to be an independent risk factor for acquiring cancer and other non-communicable diseases in adulthood, including diabetes, cardiovascular disease, kidney disease and pulmonary disease (Table 1.5). In 2006, van Dam and colleagues published results from a cohort study which followed up 102,400 cancer-free Hispanic women in the US, aged between 24 and 44 years with their BMI status compared to that at age 18. Over a follow-up period of 12 years, 710 participants had died and the researchers identified a positive correlation between BMI status and the risk of premature death. Obesity (BMI > 30 kg/m²) was associated with a hazard ratio (HR) of 2.79 for premature death and cancer had caused more of the deaths (36%) compared to any other disease. More specifically, the HR for death by cancer in participants classified as overweight (BMI > 25 kg/m²) was 1.4. Similarly, a later longitudinal study followed up 226,678 14-19 year olds for up to 41.5 years and also found an association between BMI status in adolescents and mortality (Bjørge, *et al.*, 2008). A BMI above the 85th percentile in adolescence was associated with an increased relative risk (RR) of death from colon cancer (RR=2.0) but also from disorders of the cardiovascular (RR=2.3) and respiratory system (RR=2.5). However, this study presented with some potentially important limitations such as the lack of data for confounding variables including smoking status and dietary intake in adulthood. In 2012, Park and colleagues conducted a systematic review of 39 studies with over 1 million participants in total, to investigate the relationship between BMI status in childhood and risk of morbidity and mortality in adulthood. They identified a consistency in the

literature relating childhood overweightness with up to 40% increased risk of colorectal, kidney, cervical, and ovarian cancer. However, only a limited number of studies adjusted for BMI status in adulthood and when this was applied, the associations were not statistically significant. These findings indicate a need for more investigations about the potential effects of overweightness in childhood independently to weight status in adulthood, in light of confounding lifestyle variables.

In 2016, Llewellyn and colleagues systematically reviewed 37 studies and concluded that 20% of cases of adulthood cancer can be attributed to being overweight in childhood. In children aged 12 or over, a one-point increase in SD score of BMI was associated with an odds ratio (OR) of 1.2 for all cancers combined, including those of the GI tract, lungs, ovaries and kidneys.

Furthermore, another literature review recently emphasised the growing epidemiological link between obesity in childhood and cancer later in life (Weihrauch-Blüher, *et al.*, 2019). This review analysed the results from 6 separate studies conducted on data from 2.3 million 16-19 year olds that were followed up for 45 years in Israel. Overweightness in adolescence was associated with a significantly increased risk (HR >1) of non-Hodgkin lymphoma, acute myeloid leukaemia, pancreatic cancer, gastroesophageal adenocarcinoma, colorectal cancer and renal cell carcinoma in adulthood. Overall, these findings imply that prevention of obesity in childhood could possibly reduce the rates of cancer in adults.

Table 1.5 Health complications in adulthood as a result of obesity earlier in life (OR = odds ratio, HR = hazard ratio, RR = risk ratio)

Study type	Study population and design	Key Findings	Reference
<i>Literature Review</i>	6 longitudinal studies conducted in 2.3 million 16-19 year olds that were followed up for 45 years in Israel.	<ul style="list-style-type: none"> • An increased BMI in adolescence was independently associated with increased HR (>1) for cancers of the blood, pancreas, GI tract and kidneys in adulthood. 	(Weihrauch-Blüher, <i>et al.</i> , 2019)
<i>Meta-analysis</i>	18 longitudinal studies with a total of 317,133 2-18 year olds followed up for an average of 25 years across multiple countries.	<ul style="list-style-type: none"> • A one unit increase in childhood BMI was independently associated with OR of 1.17 for hypertension, 2.02 for impaired glucose tolerance and 3.39 for increased carotid intima media thickness in adulthood. 	(Ajala, <i>et al.</i> , 2017)
<i>Longitudinal</i>	159 7-15 year olds followed for up to 30 years.	<ul style="list-style-type: none"> • A one unit increase in childhood BMI SDS was independently associated with RR of 2.04 for left ventricular hypertrophy and 1.81 for left atrial enlargement in adulthood. 	(Yang, <i>et al.</i> , 2017)
<i>Meta-analysis</i>	26 longitudinal studies with a minimum of 1000 children in each study up to age 17.	<ul style="list-style-type: none"> • A one unit increase in BMI SDS score in childhood was independently associated with OR of 1.2 for coronary heart disease, 1.7 for diabetes and 1.2 for cancer in adulthood. 	(Llewellyn, <i>et al.</i> , 2016)
<i>Retrospective</i>	Weight status at age 18 compared with comorbidities in 1502 obese adults.	<ul style="list-style-type: none"> • Severe obesity (BMI> class II) at age 18 independently increased the risk of lower-extremity venous oedema with skin manifestations by 435%, severe walking limitation by 321%, abnormal kidney function by 302%, polycystic ovary syndrome by 74%, 	(Inge, <i>et al.</i> , 2013)

		asthma by 48%, diabetes by 42%, OSA by 25%.	
<i>Longitudinal</i>	Follow up of at least 8 years for 1877 adolescents aged between 6 and 18 years.	<ul style="list-style-type: none"> • All-cause and breast cancer death in females was up to 2.6x greater for those with an overweight BMI at age 18, independent of adult BMI status. • No significant relationship between being ever overweight and all-cause mortality in men. 	(Must, <i>et al.</i> , 2012)
<i>Retrospective</i>	1.2M adolescents aged 17 years	<ul style="list-style-type: none"> • BMI \geq 95th centile at age 17 years was associated with HR of 6.89 for end stage renal disease. 	(Vivante, <i>et al.</i> , 2012)
<i>Meta-analysis</i>	2 case control studies and 37 cohort studies (n= 1.1 million aged 0-19 years)	<ul style="list-style-type: none"> • Unadjusted results for adult BMI indicate a high childhood BMI to increase the risk of cancer by 40%. • Increased childhood BMI was independently associated with an increased risk of type 2 diabetes (OR = 1.22-2.04) and hypertension (OR=5.1). 	(Park, <i>et al.</i> , 2012)
<i>Longitudinal</i>	Up to 41.5 year follow up of 227,000 male and female adolescents aged 14-19 years.	<ul style="list-style-type: none"> • BMI >85th percentile was independently associated with RR of 2.0 for colon cancer, 2.3 for cardiovascular diseases and 2.5 for respiratory diseases and 2.2 for sudden death. 	(Bjørge, <i>et al.</i> , 2008)
<i>Longitudinal</i>	12 year follow up of 102,400 females aged 24-44 years, for a comparison with weight status assessed at age 18.	<ul style="list-style-type: none"> • BMI > 30 kg/m²) was independently associated with a HR of 2.79 for premature death. • BMI > 25 kg/m² was independently associated with a HR of 1.4. for death by cancer. 	(van Dam, <i>et al.</i> , 2006)

In addition, there are concerns that childhood obesity may increase the risk of cancer even earlier than anticipated in adulthood. A meta-analysis compared the age groups and rates of cancers that have been epidemiologically linked to obesity in adults (Berger, 2018). They found that 9 out of 13 obesity associated malignancies which were more commonly reported in the over 50 age group are being increasingly reported within the 20-44 age group in the United States. For example, an epidemiological association has been suggested between thyroid cancer and obesity, and 24% of new cases of thyroid cancer were reportedly within the 20-44 age group in 2017. In the UK, the incidence of cancer in young people aged 20-24 years has risen by 28% since the 1990s (PHE, 2019). It is likely that the growing incidence of cancer in young adults could be partly explained by the persistence of childhood obesity, making it more urgent to explore potential causative mechanisms for cancer in obesity.

To understand the obesity-cancer paradigm, it is important to first consider the molecular mechanisms that underpin tumorigenesis. The process of tumorigenesis is complex with multiple steps whereby cells acquire pathological capabilities to evade cell death and sustain proliferation. These capabilities are described as the 'Hallmarks of Cancer' (Figure 1.3), and were first introduced by Hanahan and Weinberg in 2000. There are now eight distinct hallmarks that explain the diverse and dynamic nature of cancer cells (Hanahan, & Weinberg, 2017). Firstly, cancer cells are able to sustain cell division by effectively releasing and responding to polypeptide growth factors such as platelet derived growth factor (PDGF) and insulin-like growth factor 1(IGF-1) in the micro-environment. Angiogenesis can be induced to maintain a supply of nutrients and growth factors for proliferation, and the deregulation of cellular energetics and metabolic pathways means that cancer cells are able to alter their

nutritional demands and thrive even in nutrient-poor environments. Secondly, cancer cells can evade anti-proliferative factors such as retinoblastoma protein (pRb) and tumour protein (p53) that would normally prevent progression in the cell cycle. In order to resist cell death, cancer cells must block the most prominent form of programmed cell death – apoptosis. Furthermore, cancer cells can perform re-modelling of the extracellular matrix to metastasise into other tissues and may become immortal by activating the telomerase enzyme. Finally, another hallmark of cancer is the ability to avoid destruction by the immune system by generating an immunosuppressive environment or reducing recognition by innate and adaptive defence mechanisms. It is likely that the coming years will see this blueprint of core-traits evolve, making it necessary to also develop the understanding of enabling characteristics and mechanisms that underpin these hallmarks, particularly in individuals with obesity.

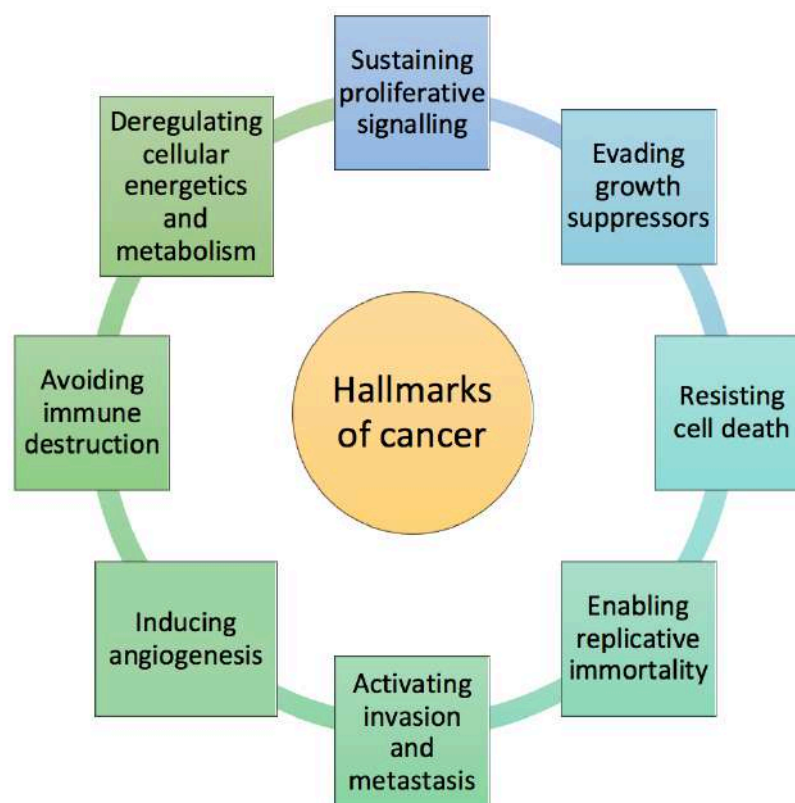


Figure 1.3 The eight hallmarks of cancer described by Hanahan and Weinberg (2017).

Several of the hallmarks of cancer have been associated with the characteristics of obesity and related co-morbidities in adults. Firstly, excess adipose tissue can coincide with excess leptin – a hormone which was described to have proliferative effects in a human epithelial colon cancer cell line (Hardwick, *et al.*, 2001). Hyperleptinaemia in obesity has been associated with a variety of malignancies including those of the breast, thyroid and liver (Dutta, *et al.*, 2012). Secondly, obesity can be marked with excess oestrogen, and sustained exposure to oestrogen has been associated with upregulation of genes that control cell proliferation and cell cycle progression, thereby increasing the risk of breast cancer (Calle, & Kaaks, 2004). Furthermore, hyperinsulinemia in obesity may promote an increased uptake of glucose by tumour cells, and consequently support the cells to meet their energy demands for proliferation (Giovannucci, 2007; Vander Heiden, *et al.*, 2009). Excess insulin may drive insulin resistance and contribute to hyperglycaemia in obesity (Friedman, *et al.*, 1992). In the context of tumorigenesis, this can be problematic because hyperglycaemia may drive metastasis by upregulating the expression of STAT3/MMP-2 to breakdown the extra-cellular matrix (Li, *et al.*, 2019). Moreover, a decline in adiponectin levels in obesity may be associated with a loss of anti-cancer effects including inhibiting inflammation (Dalamaga, *et al.*, 2012). Several studies implicate inflammation to promote cancer in obesity, suggesting that it may complicate metabolic syndrome, and drive adipose-derived stem cells and growth factors into the tumour microenvironment (Ramos-Nino, 2013; Kolb, *et al.*, 2016; Deng, *et al.*, 2016). Overall, the dysregulation of various hormones, glucose as well as inflammation may endorse the biological mechanisms associated with tumour development, sustainability and metastasis in adults with obesity.

It is also important to identify early, pre-pathological changes in childhood obesity that may enable the hallmarks of cancer to develop over time, thereby explaining the independent associations between weight status in childhood and later life risk of cancer. In 1960, Brookes and Crawley presented the first conclusive evidence which implied DNA damage to be a root cause of cancer. DNA damage may lead to faulty DNA replication and gene mutations that give rise to altered proteins. If unrepaired, such genomic alterations and the consequences may initiate the functional characteristics of cancer over time (Hanahan, & Weinberg, 2017). Thus, risk of cancer may depend on the health of the entire genome.

1.2 Genome Health

1.2.1 Defining genome health and types of DNA damage

In (2003), Fenech introduced the novel term 'Genome Health', referring to the structural integrity and stability of the entire genome. This term was derived from evidence that the genetic code plays a fundamental role in determining cellular function and health outcomes, and that there is a direct link between the absence of optimal genomic integrity and stability, and the onset of disease.

There are a number of commonly described paradigms which reflect the link between inherited or acquired genetic changes with human health and long-term wellbeing. Point mutations are associated with many inherited genetic disorders such as sickle cell anaemia and haemophilia. Other inherited genetic alterations include aneuploidies which can lead to chromosomal disorders such as trisomy 21 or chromosomal anomalies associated with infertility. Acquired genetic alterations include chromosomal rearrangements and genomic instability associated with tumorigenesis. Shortening of telomeres and accumulation of acquired DNA damage have been found in cellular ageing and may also have negative implications for 'genome health'.

Living healthily depends on our capacity to make new and accurate copies of DNA and to repair DNA correctly when it is damaged. DNA damage is defined as an alteration in DNA structure that is capable of causing cellular injury and reduces viability or reproductive fitness of the organism (Kaufmann, & Paules, 1996). There are several types of DNA damage including point mutations, base modifications, chromosomal rearrangements and breakages, loss or gain of chromosomes and modified methylation patterns. Cellular DNA repair

mechanics are fundamental for overcoming acquired DNA damage and maintaining optimal genomic integrity and stability.

There are direct and indirect DNA repair mechanisms. Direct DNA repair mechanisms actively repair DNA during replication whilst indirect repair mechanisms work post DNA replication. Five indirect DNA repair pathways that deal with various types of DNA lesions have been described (Table 1.6).

Mismatch Repair (MMR) acts on small insertions and deletions as well as base mismatches. Defective MMR due to mutations in MMR genes have been linked with acquiring hereditary non-polyposis colorectal cancer (Eshleman, & Markowitz, 1996; Wheeler, *et al.*, 2000). Furthermore, Nucleotide Excision Repair (NER) is the primary pathway for removing bulky DNA adducts such as the 6-4 photoproduct induced by UV radiation. Mutations in certain NER genes have been linked with Xeroderma Pigmentosum and an increased tendency to acquire UV-light induced skin cancer (Lehmann, 2003). Base Excision Repair (BER) removes DNA lesions caused by oxidation, deamination and alkylation. Defects in certain BER pathway genes such as MUTYH is associated with MUTYH-associated polyposis (MAP) which is characterised by multiple benign and cancerous colorectal tumours (Cheadle, & Sampson, 2007). Both Non-homologous End Joining (NHEJ) and Homologous Recombination (HR) can remove Double Strand Breaks (DSBs). However, NHEJ is the predominate pathway in mammalian cells and can repairs DSBs that occur during any phase of the cell cycle whereas HR predominantly repairs DSBs that occur during the S and G2 phase (Kanaar, *et al.*, 2008). Although HR is traditionally described as being less error-prone than NHEJ, defects in HR and NHEJ have both been associated with a number of pathologies including telomere defects, chromosomal aberrations and cancer (Sung, & Klein, 2006). Overall, DNA

damage repair pathways are of central importance in preventing adverse health outcomes caused by DNA damage. However, if DNA damage persists, then surveillance systems that monitor genomic integrity can play a fundamental role in controlling the fate of the cell through the cell cycle.

In 1989, Hartwell and Weinert introduced the concept of checkpoints in the cell cycle. Their work demonstrated that radiation induced DNA damage will stop wild-type yeast cells from dividing until the damage can be repaired. However, mutations in RAD9 allows irradiated cells to divide, leading to cell death.

Progression through the cell cycle is governed by three checkpoints. Firstly, G1 cyclin-Cdk complexes are responsible for progression from the G1 to S phase. Secondly, S cyclin-Cdk complexes are responsible for initiating and completing DNA replication. And finally, M cyclin-Cdk complexes drive cells into mitosis, preventing re-entry into the G1 phase. Progression can be inhibited by modulating the activity of these cyclin-Cdk complexes. For example, levels of cyclin can be altered by transcriptional regulation, phosphorylation of tyrosine and threonine residues close to the active site of the Cdk subunit to inactivate cyclin-Cdk complexes, and stoichiometric inhibitors (cyclin kinase inhibitors) can create inactive trimers with cyclin-Cdk complexes. DNA damage inactivates Cdk to trigger a cascade of events that will arrest the cell cycle. For example, DNA double strand breaks (DSBs) trigger phosphorylation and consequent activation of ATM and ATR that will lead to the phosphorylation of substrates that regulate DNA repair, cell cycle arrest and apoptosis. In the G1/S checkpoint, ATM and ATR phosphorylate Cdc25A, which leads to its degradation and inactivation of Cdk2 to mediate cell cycle arrest (Falck, *et al.*, 2002). A key player in these processes is p53, as it functions to maintain this arrest, activate DNA repair mechanisms or trigger apoptosis if the damage is too substantial to be repaired.

A number of pathways have been described that lead to the accumulation of p53 in the nucleus, of which one example is the inactivation of MDM2 by ATM to prevent the degradation of p53 (Shimada, & Nakanishi, 2006). Furthermore, at the intra-S checkpoint, DNA damage will inhibit replicative DNA synthesis. The progression from the S to G2 phase is dependent on the availability of licencing factors, which ensures that each chromosome replicates only once during the S phase. The intra-S checkpoint deals with stalled replication forks and prevents the transmission of un-replicated DNA. Finally, the G2/M checkpoint stops the cell entering mitosis if DNA damage is present. A key role of the mitotic checkpoint is to assess the status of kinetochore–microtubule attachment and inhibit anaphase in the presence of unattached kinetochores.

Defective cell-cycle checkpoints are associated with increased genomic instability identified in cancer cells, and are targets for anti-cancer therapies (Hartwell, 1992; Tamura, 2015). Misregulation of Cdks, particularly of Cdk1 is associated with increased chromosomal instability in cancer cells (Malumbres, & Barbacid, 2009). Mutations in the p53 gene is an extremely common occurrence in cancer (Joerger, & Fersht, 2016). Defects in the M-checkpoint are associated with aneuploidy, thereby favouring genome and chromosomal instability in cancer (Simonetti, *et al.*, 2019). Overall, the three surveillance systems are of central importance in triggering death of cells with DNA damage, and thereby preventing genomic instability.

Table 1.6 Overview of the five DNA repair pathways in humans

Repair pathway	DNA lesions repaired	Cell cycle stage when repair system is most active	Repair initiating enzymes	Reference
<i>Mismatch repair (MMR)</i>	<ul style="list-style-type: none"> • Insertion (I) • Deletion (D) • Base mismatch 	S	hMutS α recognises base-base mismatches and ID mispairs of 1-2 nucleotides. hMutS β recognizes larger ID mispairs	(Li, 2008)
<i>Nucleotide Excision Repair (NER)</i>	<ul style="list-style-type: none"> • Bulky adduct • Intrastrand crosslink • 6-4 photoproduct • Cyclobutane • Pyrimidine dimer 	Predominantly G ₁ but can occur throughout the cell cycle.	Replication protein A (RPA) and the xeroderma pigmentosum group A (XPA) recognise damage.	(Reardon, & Sancar, 2001)
<i>Base Excision Repair (BER)</i>	<ul style="list-style-type: none"> • Uracil • Abasic site • 8-oxoguanine • Single strand break • Altered base 	G ₁ and S	DNA glycosylase such as OGG1 recognises damage.	(Krokan, & Bjørås, 2013)
<i>Homologous Recombination (HR)</i>	<ul style="list-style-type: none"> • Intrastrand crosslinks • Interstrand crosslinks • Single strand breaks (SSB) • Double strand breaks (DSB) 	S and G ₂	DNA damage sensor complexes such as Mre11/Rad50/Nbs1 (MRN) detects DSBs.	(Krajewska, <i>et al.</i> , 2015)
<i>Non-homologous End Joining (NHEJ)</i>	<ul style="list-style-type: none"> • Intrastrand crosslinks • Interstrand crosslinks • Single strand break • Double strand breaks 	Throughout the cell cycle.	Ku detects DNA DSB and forms a complex with other enzymes such as DNA-PKcs.	(Yano, <i>et al.</i> , 2009)

1.2.2 Threats to genome health

There are a number of factors that may affect 'genome health' and drive genome damage over time, leading to cellular changes that are associated with frailty and disease (Figure 1.4).

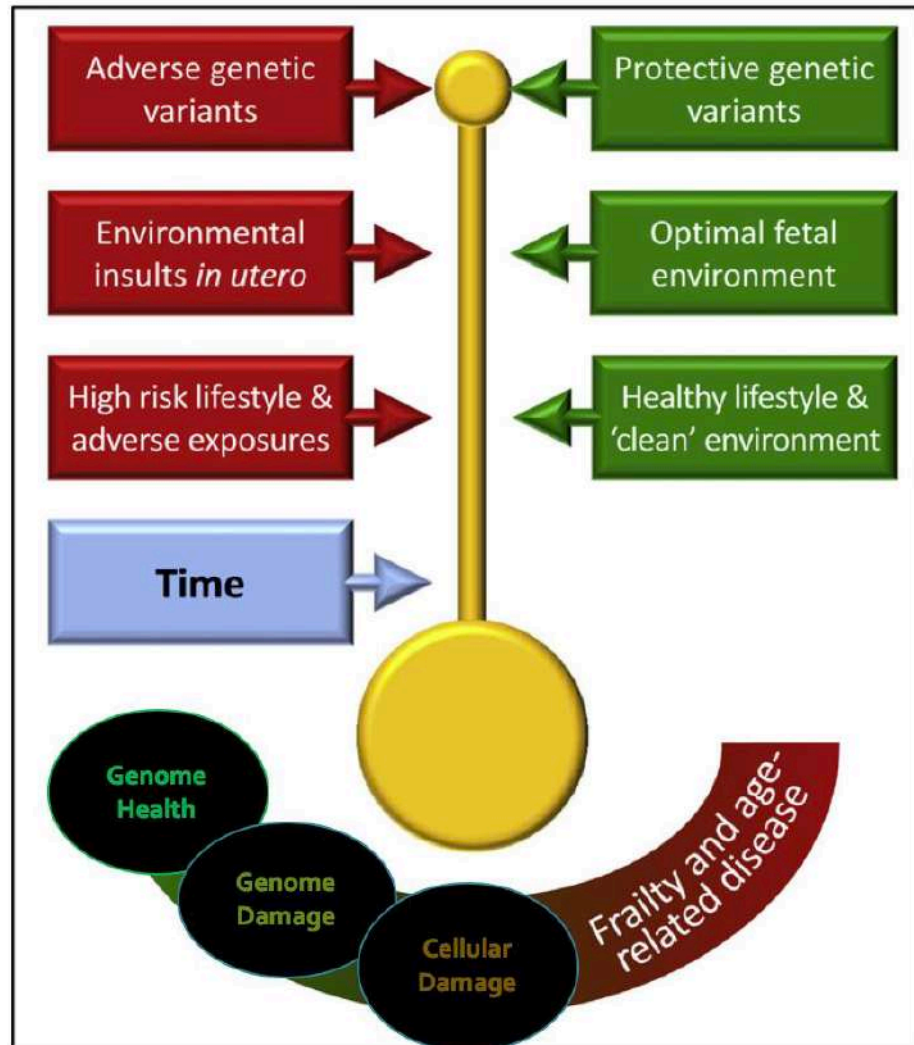


Figure 1.4 The Genome Health Pendulum (adapted from Langie, *et al.*, 2012)

Firstly, DNA can become compromised in response to environmental insults in utero. Perera and colleagues (2002) identified that transplacental exposure to carcinogenic air pollutants is associated with DNA damage and somatic mutations in new-borns. Similarly, in utero exposure to arsenic has been linked with increased DNA damage in new-borns, as well as being associated with a

higher risk of acquiring cardiovascular disease, respiratory disease and cancer later in life (Navasumrit, et al., 2019; Farzan, et al., 2013). Furthermore, there is evidence that somatic mutations can be generated over time, and increase with age (Yizhak, et al., 2019). There are multiple normal tissues whereby a large number of somatic mutations and microscopic mutational clones have been identified in cancer associated genes (Tomasetti, 2019). It is important to understand the dynamics of mutational mosaicism in normal tissues and their relevance in generating a pre-cancerous genome. This is because they could provide useful information for the prediction of age-dependent cancer risk on an individual basis.

Finally, the lifestyle of an individual may also play a causative role in acquiring somatic mutations and DNA damage. A recent review has highlighted the relevance of multiple physical, chemical and biological factors that are associated with acquired genomic instability and increased risk of cancer (Lewandowska, et al., 2019). These include exposure to electromagnetic fields, ultraviolet radiation, ionizing radiation, alcohol consumption, a poorly balanced diet, lack of physical activity and smoking. It is undisputed that smoking tobacco is associated with increased chromosomal instability and plays a role in the initiation and progression of lung cancer (Sanchez-Cespedes, et al., 2001). Overall, a priority for optimal 'genome health' and prevention of disease requires identification of all the exogenous and endogenous threats that may exacerbate DNA damage on an individual level, including the levels of oxidative stress and micronutrients.

Oxidative stress

Over the course of life, DNA can be exposed to exogenous mutagens such as ionizing radiation, UV light and certain chemicals. However, there is also a constant endogenous threat from mutagens such as reactive oxygen species (ROS), a product of cellular metabolism. Although cells are equipped with complicated anti-oxidant defence mechanisms to scavenge ROS, certain pathological states such as chronic inflammation can ultimately lead to an overdrive of ROS production, thereby increasing the risk of DNA damage.

Inflammation is the classic response to tissue injury which also underlies multiple cellular pathologies, including cancer. Inflammatory pathways can be activated by endogenous factors released directly from tissues (Medzhitov, 2008). Macrophages can release inflammatory cytokines including TNF-alpha, interleukin-1 and interleukin-6 which activate the recruitment of leukocytes and release of acute-phase proteins such as C-reactive protein (CRP) from the liver. With a half-life of about 19 hours, plasma CRP levels greatly represent the rate of synthesis by hepatocytes in response to inflammatory cytokines (Pepys, & Hirschfield, 2003). CRP promotes inflammation by activating the classical pathway of complement to generate anaphylatoxins (Gruys, *et al.*, 2005). As a result, mast cells and other leukocytes are recruited and may undergo a 'respiratory burst', releasing ROS into the environment (Reuter, *et al.*, 2010).

Early studies pointed out some of the most potent ROS to include •OH, O₂⁻, and H₂O₂ as they can cause base modifications, react with the deoxyribose sugar, cause DNA single and double strand breakages (SSB, DSBs) and create DNA-protein cross links (Bandyopadhyay, *et al.*, 1999; Vilenchik, & Knudson, 2003). ROS can induce oxidation of purine and pyrimidine bases resulting in

DNA abasic sites (Takeshita, & Eisenberg, 1994). Although this transformation is not always lethal, it can be very mutagenic and halt DNA polymerase activity. Furthermore, Cadet and Wagner (2013) have extensively reviewed a plethora of single and tandem based lesions in DNA, formed via oxidation. The oxidation of guanine is one of the most commonly described mutagenic lesions (Korkmaz, *et al.*, 2018).

Nitric oxide (NO) is another genotoxic agent that is released during inflammation by activated macrophages (Fang, & Vazquez-Torres, 2002). NO can cause deamination, oxidation and strand breakages in DNA and has been linked with causing the G-T transversion mutation commonly identified in the p53 gene of human cancers (Tamir, *et al.*, 1996; Ambs, *et al.*, 1999). In addition, there is evidence that NO can reduce the efficacy of DNA repair by inhibiting the FAPY glycosylase DNA repair enzyme (Jaiswal, *et al.*, 2001).

On the other hand, there have been reports that indicate ROS might play a tumour suppressive role. In human fibroblasts and cancer cells, elevated ROS was shown to activate cell cycle arrest and apoptotic pathways via ASK1/JNK and ASK1/p38 (Ichijo, *et al.*, 1997; Moon, *et al.*, 2010). However, more recent studies conducted in human liver cancer cells show that hydrogen peroxide can downregulate many tumour suppressor genes such as *USP28*, *DRAM*, *TIGAR*, and *CYLD* (Kim, *et al.*, 2013). Overall, whilst there are some discrepancies, there is more evidence that chronic inflammation and consequent overproduction of ROS and RNS can be a causative factor for DNA damage and may contribute to carcinogenesis.

Micronutrient deficiencies

Micronutrients are essential dietary components that also play a role in the maintenance of genomic integrity during DNA replication. A comprehensive review by Fenech (2010) has highlighted the significance of dietary nutrients for genome health. They can function as part of anti-oxidant defence mechanisms or act as co-factors for DNA repair enzymes. Therefore, a deficiency in the intake of a number of micronutrients has been linked with DNA damage in animal and human models (Table 1.7).

A deficiency of co-factors for DNA repair enzymes including magnesium, zinc and iron are associated with increased oxidative lesions in DNA and can also cause chromosome malsegregation errors. Secondly, vitamin C, E, selenium and manganese are important components of anti-oxidant defence mechanisms and a deficiency of these is also linked with oxidative DNA damage but also chromosomal aberrations.

Micronutrients can also play a key role in the regulation of mitosis. An interesting review by Shalliker and colleagues (2012), has brought to light the various roles vitamin D plays in regulating DNA replication and cell division. There are two main forms of vitamin D: cholecalciferol (vitamin D₃) and ergocalciferol (vitamin D₂). The biologically active hormone is called calcitriol (1-25OHD), and is produced after vitamin D₃ and D₂ first undergo hydroxylation in the liver and then in the kidneys. Vitamin D₂ is cleared much faster from the circulation as it has a lower affinity than vitamin D₃ for vitamin D binding protein (Lips, 2006). Clinically, vitamin D status is determined by assessing 25OHD levels in serum rather than the biologically active vitamin D, because 1-25OHD has a shorter half-life (Holick, 2007). 1-25OHD acts on the vitamin D receptor

(VDR) to regulate gene expression and calcium homeostasis. The VDR is widely expressed and not just limited to the bone, gut and kidneys (Bikle, 2014). This presence of the VDR may be explained by the role of vitamin D in protecting cells from DNA damage, inducing cell-cycle arrest and promoting apoptosis (Krishnan, *et al.*, 2012). There is evidence that Vitamin D treatment can reduce oxidative DNA damage as well as the formation of cyclobutane pyrimidine dimers – a DNA damage signature following UV-light exposure (Wong, *et al.*, 2004; Fedirko, *et al.*, 2010). Therefore, there is a potential role for vitamin D in the maintenance of genomic stability and integrity.

Furthermore, specific micronutrient deficiencies have also been connected with DNA damage and disease. Folate deficiency is associated with increased genome damage and neural tube defects in the developing foetus (Fenech, 2001; Green, 2002). Choline deficiency has been related to increased DNA damage and epidemiological evidence has linked an increased consumption of choline with a lowered risk of cancer (da Costa, *et al.*, 2006; Sun, *et al.*, 2016). Other micronutrients have also been suggested to play a role in the risk of acquiring cancer. A recent review has concluded that vitamin D deficiency may be associated with an increased risk of cancer (Grant, 2018). Omega-3 fatty acid deficiency has been associated with metastatic melanoma (Denkins, *et al.*, 2005). Finally, there is also epidemiological evidence linking magnesium deficiency with colorectal cancer (Trapani, *et al.*, 2015). In conclusion, certain micronutrients play a significant role in genome maintenance and may also be of importance in cancer risk and prevention.

Table 1.7 Micronutrients for genome health maintenance (adapted from Fenech, 2010).

Micronutrients	Role in Genome stability	Possible Consequence of Deficiency	Model Systems	Reference
<i>Vitamin D</i>	Regulates telomerase activity Component of anti-oxidant defence	Increased Oxidative DNA Lesions including 8-OHdG Chromosomal aberrations	Mice, rats and humans	(Nair-Shalliker, <i>et al.</i> , 2012)
<i>Vitamin C and E</i>	Component of anti-oxidant defence	Increased oxidative DNA lesions and chromosomal aberrations	Adult humans	(Fenech, <i>et al.</i> , 2005)
<i>Niacin</i>	Telomere length maintenance	Chromosomal aberrations and increased sensitivity to mutagens	Mice, rats and humans	(Hageman, & Stierum, 2001)
<i>Zinc</i>	Co-factor for DNA repair enzymes	Increased oxidative DNA lesions and chromosomal aberrations	Rats	(Ho, & Ames, 2002)
<i>Iron</i>	Co-factor for mitochondrial enzymes	Increased oxidative DNA lesions in mitochondrial DNA	Rats	(Walter, <i>et al.</i> , 2002)
<i>Magnesium</i>	Co-factor for DNA repair enzymes	Reduced DNA repair Chromosomal segregation errors	Human fibroblasts	(Hartwig, 2001)
<i>Manganese</i>	Component of anti-oxidant defence	Increased oxidative damage to mitochondrial DNA and reduced resistance to radiation-induced damage to nuclear DNA	Mice	(Bakthavatchalu, <i>et al.</i> , 2012)
<i>Calcium</i>	Co-factor for regulating mitosis	Chromosomal aberrations	Human cervical cancer cells	(Phengchat, <i>et al.</i> , 2016)
<i>Selenium</i>	Component of anti-oxidant defence	Increased oxidative DNA lesions and chromosomal aberrations	Chickens	(Zoidis, <i>et al.</i> , 2018)

1.2.3 Genome instability and cancer

Genomic instability is marked by an increased propensity of the genome to undergo alterations during the cell cycle (Shen, 2011), and has been described as an enabling characteristic for the complex, multi-step process of tumorigenesis (Hanahan, & Weinberg, 2016). The accumulation of DNA damage may lead to modifications in gene expression that transform a healthy progenitor cell into becoming pre-cancerous. Through a series of subsequent cell cycle divisions, the acquisition of multiple genomic alterations advances the cells into being clinically classified as invasive cancer (Figure 1.5). The identification and mapping of genomic instability to the stages of tumorigenesis may inform the optimal windows for prevention, diagnosis and therapy of cancer.

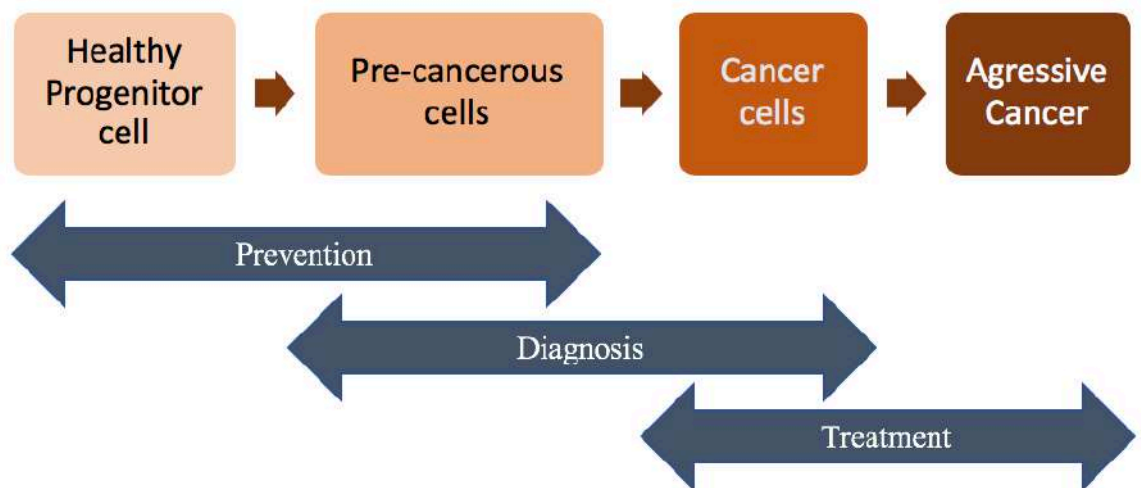


Figure 1.5 Accumulation of DNA damage over several cell cycles may drive progenitor cells into becoming cancerous (smaller block arrows represent events of DNA damage and genomic alterations), mapping the genomic alterations may inform the optimal windows for prevention, diagnosis and treatment (adapted from Shen, 2011).

Genomic instability can be divided into three forms; nucleotide instability (NIN), microsatellite instability (MIN) and chromosomal instability (CIN). NIN is associated with early stages of cancer and is where one or a few nucleotide bases are changed due to errors in replication or DNA repair genes such as *MYH* and *XPC* (Bester, *et al.*, 2011). NIN is less common compared to the other forms of genomic instability in sporadic cancers (Negrini, *et al.*, 2010).

MIN is caused by defective Mismatch Repair (MMR) within microsatellite regions which are long repetitive sequences of DNA (10 to 60 base pairs) (Liu, *et al.*, 1995). In 1993, MIN was first identified to be a phenomenon in both sporadic and hereditary forms of colorectal cancer (Aaltonen, *et al.*, 1993). MIN has now also been associated with at least 15 different types of sporadic cancers (Lawes, *et al.*, 2003). Most MIN occurs in non-coding regions, but when mutations occur in short coding microsatellite sequences, then it may lead to frameshift mutations and increased cancer risk. For example, 90% of cases of colorectal cancer that are positive for MIN in *TGF β -R2*, result in the inactivation of the TGF β -R2 protein and a consequent loss of inhibition of proliferation (Bacher, *et al.*, 2016). Furthermore, MIN may provide important prognostic information. Interestingly, high microsatellite instability (where at least 2 out of 5 microsatellite markers are positive) is associated with a better prognosis when identified during stage II of colorectal cancer (Kawakami, *et al.*, 2015). However, in advanced stages this trend is controversial and may not be applicable for all types of cancers, including those of the endometrium (Wang, *et al.*, 2019; An, *et al.*, 2007).

Chromosomal instability (CIN) is an increased loss or gain of one or more whole chromosomes or chromosomal fragments during cell division. It is the most

predominant type of genomic instability and can be marked by several karyotypic abnormalities including aneuploidy, deletions, translocations, inversions and amplification (Gagos, & Irminger-Finger, 2005). The early detection of CIN may also play an important role in cancer prevention as it can be identified in pre-malignant cells of the GI tract (Garnis, *et al.*, 2009; Yang, *et al.*, 2006). Furthermore, complex CIN such as aneuploidies have been associated with a poorer prognosis in several different types of cancer including lymphomas, breast cancer, pancreatic cancer, lung cancer, thyroid cancer and glioblastomas (Bakhoun, *et al.*, 2011; Bakhoun, & Cantley, 2018). CIN may support cancer cells with evading recognition from the immune system and promote the survival of cancer cells under stressful conditions (Bakhoun, & Cantley, 2018). Thereby, causing resistance to cytotoxic treatment and increasing their capability for metastasis (Tanaka, & Hirota, 2016).

One of the most important indications of genomic instability, is the heterogeneity of the genome across cell sub-populations. For example, non-recurrent abnormalities (NCCAs) are aberrations which are detected at a frequency of less than 4% among 50–100 mitotic figures and therefore characterise an unstable or 'chaotic genome' (Heng, *et al.*, 2016). It has been suggested that this type of karyotype heterogeneity is vital for the evolution of cancer cells (Heng, *et al.*, 2013). Research conducted in patients with chronic myeloid leukaemia found that centrosome aberrations may contribute to the gain of karyotype heterogeneity and correlate positively with aggressiveness of the disease (Giehl, *et al.*, 2005). Similarly, the most lethal form of epithelial ovarian cancer is marked by chromosomal aberrations and DNA repair defects that give rise to genetic diversity and clonal evolution (Salomon-Perzyński, *et al.*, 2017).

Overall, genome instability enables cancer cells to acquire the hallmark capabilities that are required to sustain proliferation. Several forms of genomic alterations can be identified across the multiple step process of tumorigenesis. Therefore, the assessment of early, pre-pathological genomic alterations may play a key role in the prevention of cancer.

1.2.6 Markers of DNA damage and assessment techniques

Genotoxicity assessments depend on the identification of DNA damage biomarkers. Such biomarkers can include DNA damage lesions such as 8-oxo-deoxy-guanosine (8-OHdG), nuclear anomalies, telomere length as well markers of chromosomal aberration including comet tail frequency and induction of gamma foci.

1.2.6.1 8-OH-2-deoxy Guanosine

8-OH-2-deoxy Guanosine (8-OHdG) or 8-oxodG is one the most commonly described lesions of oxidative DNA damage. It can be derived due to an excess endogenous build-up of reactive oxygen species (ROS) coupled with a reduced efficiency of anti-oxidant enzymes. Generation of the hydroxyl radical (HO•) can cause a reaction with guanine in DNA to produce deoxyguanosine and radical adducts (Valavanidis, *et al.*, 2009). The removal of an electron from the C8-OH adduct then produces 8-OHdG or its tautomer 8-oxodG (Figure 1.6).

8-OHdG was first described as a mutagen in 1984 by Kasai and Nishimura using HPLC. Today, 8-OHdG can be detected in serum, saliva and urine, making it an ideal DNA damage biomarker for large population based studies. The quantification of 8-OHdG is particularly useful in urine because it is the primary route of excretion (Wu, *et al.*, 2004). However, multiple studies have suggested the measurement of 8-OHdG in plasma to be more sensitive than in urine to risk factors such as BMI and smoking status, and interventions including exercise (Wang, *et al.*, 2016; Karpouzi, *et al.*, 2016). Nonetheless, Wang and colleagues (2016) researchers also identified a moderate, significant correlation between plasma and urinary 8-OHdG levels ($R = 0.31$, $p < 0.01$),

which means the measurement of 8-OHdG in urine could be a reliable alternative when plasma is inaccessible.

The absolute quantification of 8-OHdG can be achieved via HPLC and LC-MS with high sensitivity, but these techniques first require the isolation of genomic DNA (Korkmaz, *et al.*, 2018). Furthermore, immunohistochemistry and immunocytochemistry can also be employed to semi-quantify 8-OHdG. One of the major issues with this technique is the interference from cytoplasmic staining of RNA (Korkmaz, *et al.*, 2018). There are a number of commercially available ELISA assays that although have lower sensitivity and specificity when compared to LC-MS and HPLC, are more time efficient and cost-effective (Korkmaz, *et al.*, 2018). Besides the assessment of 8OHdG, toxicology and genotoxicity assays can also assess the oxidised form of the nucleoside guanine (8-OHG) when it is attached to a ribose sugar and when it is oxidised on its own (8-OHGua).

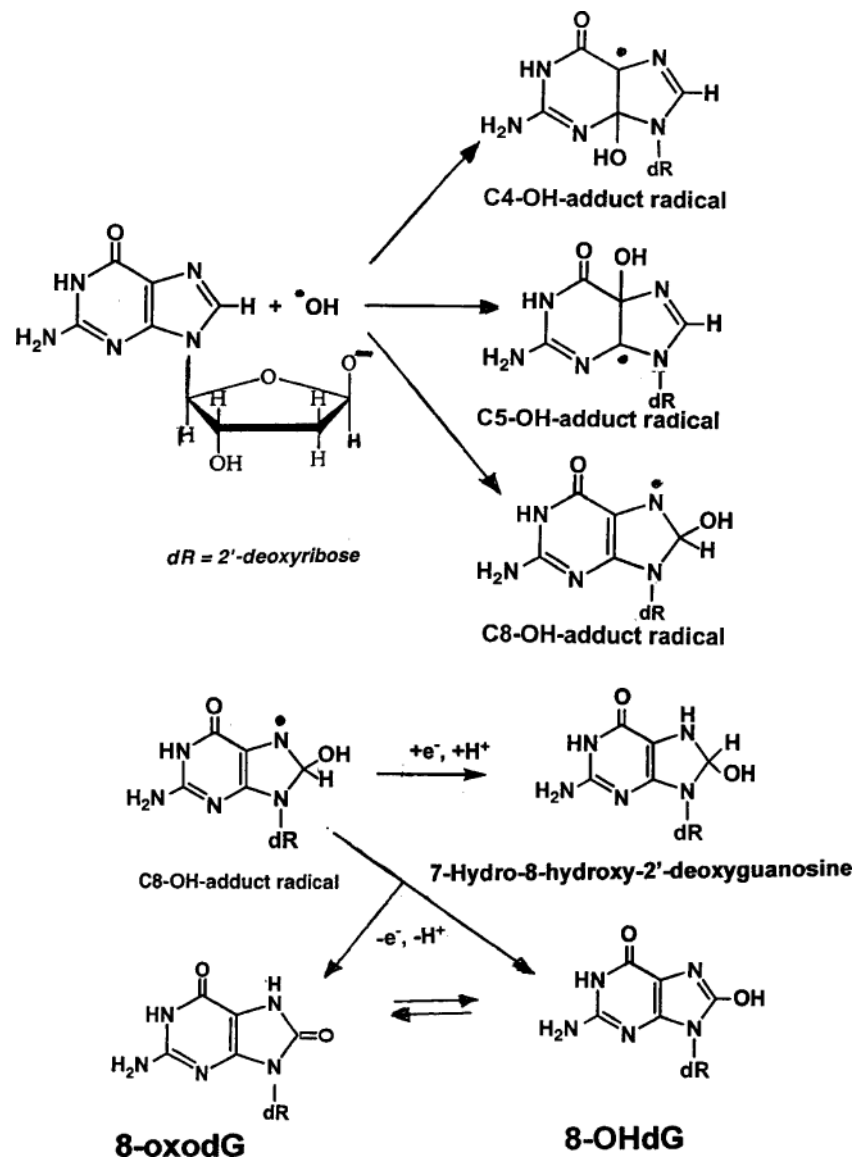


Figure 1.6 Reduction and oxidation of 2-deoxyguanosine to produce 8OHdG and 8oxodG (Valavanidis, *et al.*, 2009)

1.2.6.2 Morphological nuclear anomalies

Nuclear anomalies can be biomarkers of genotoxic events related to chromosomal aberrations or defective nuclear architecture. A number of morphological nuclear anomalies can be quantified in cells to assess genomic instability. These include micronuclei, nuclear buds and bridges, as well as the retention of multiple nuclei in cells that would normally have a single nucleus.

Micronuclei

Micronuclei are well-recognised markers of genotoxic stress and genomic instability. By definition, a micronucleus is an extra-nuclear body within the cell cytoplasm containing acentric chromosomal or chromatid fragments, or whole chromosomes (Thomas, *et al.*, 2011). Micronucleus formation is caused by *clastogenic* or *aneugenic* events that lead to a failure of the damaged genetic material attaching appropriately to spindle fibres during anaphase of mitosis and therefore not being incorporated in the main nucleus (Figure 1.7).

Clastogenic events result in chromosomal breakages and may lead to the deletion, addition or rearrangement of genetic material (Beedanagari, *et al.*, 2014). Whereas *aneugenic* events disrupt spindle fibre mechanisms, affecting chromosomal segregation and causing aneuploidy (Parry, *et al.*, 1996).

When DNA damage repair capacity is exceeded by DNA damage, double strand breaks (DSBs), a *clastogenic* event, may be unrepaired or mis-repaired (Thomas, *et al.*, 2011). Acentric chromosomal fragments lack a centrosome and can be a consequence of unrepaired DSBs where there are errors in the non-homologous end joining (NHEJ) repair mechanism (Hartlerode, & Scully, 2009). DSB formation can also occur as a result of inappropriate bases or damaged lesions such as 8-hydroxyguanosine being incorporated in DNA (Thomas, *et al.*,

2011). Aneugenic events and malsegregation of chromosomes may be a consequence of hypomethylation of centromeric repeat sequences (Schueler, & Sullivan, 2006). It has been demonstrated that hypomethylation of cytosines in centromeric DNA of chromosomes 1, 9 and 16 can lead to elongation and possible kinetochore defects resulting in chromosomal malsegregation and increased micronuclei formation (Suzuki, *et al.*, 2002). Furthermore, micronucleus formation due to chromosomal malsegregation may also be caused by defects in proteins that regulate the dynamics of kinetochore assembly and microtubule interaction (Bakhoun, *et al.*, 2009). Other mechanisms for micronucleus formation as a result of malsegregation include abnormal DNA amplification in centrosomes, defective mitotic check points and telomere-end fusions that can result in detachment from mitotic spindle (Thomas, *et al.*, 2011). Ultimately, a micronucleated cell can undergo apoptosis, have the DNA repaired and reincorporated into the main nucleus, have the micronucleus ejected from the cell or retain the micronucleus and persist in the tissue (Kirsch-Volders, *et al.*, 2011).

Overall, it is clear that micronucleus formation is a consequence of genotoxic events. Therefore, the quantification of micronuclei has been described as the most widely used *in vivo* tool to assess mutagenicity for hazard identification and risk assessment (Hayashi, 2016). However, the significance of micronuclei goes beyond being a marker of chromosomal instability, as new insights indicate micronuclei to have a pathological role in cancer progression as they could complicate chromosomal instability and trigger inflammation (Guo, *et al.*, 2019).

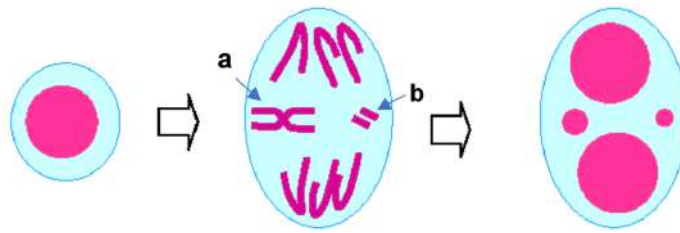


Figure 1.7 Micronucleus formation that would be observed in a cell undergoing nuclear division and blocked prior to cytokinesis with cytochalasin-B. Micronuclei can contain (a) whole chromosomes or (b) acentric fragments (adapted from Thomas, *et al.*, 2011).

Nuclear Buds and Bridges

One other morphological marker of genomic instability is the identification of nuclear bud and nucleoplasmic bridge formation in cells. A nucleoplasmic bridge is characterised as a fine thread-like structure that connects two nuclei (Figure 1.8). It has been established that this structure may be an anaphase bridge, resulting from the pulling of dicentric chromosomes to opposite poles of the cell during mitosis via their centrosomes (Thomas, *et al.*, 2011). There are two main genome damage events that can lead to the formation of dicentric chromosomes and nuclear bridges (NBridges). Firstly, as with micronuclei formation, the occurrence of dicentric chromosomes can be a consequence of mis-repaired chromosomal breaks (Thomas, *et al.*, 2011). Secondly, dicentric chromosomes can form due to dysfunctional telomeres and consequent Breakage-Fusion-Bridge (BFB) cycles (Murnane, 2006). First explained by McClintock in the 1930s, a BFB cycle begins with breakage of telomeric regions, exposing chromosomal ends (Kass, & Chomet, 2009; Zakov, *et al.*, 2013). Next, the telomere-free ends of sister chromatids undergo fusion and as they are pulled apart to opposite ends of the cell during anaphase, a chromosomal bridge is formed. However, further cycles lead to a breakage of

the chromosomal bridge, leading to daughter chromatids deficient in telomeric regions and a continuum in the BFB cycle.

In some instances, a nucleoplasmic bridge can be attached to a smaller structure with the same morphology as a micronucleus – termed a nuclear bud (NBUD). If a nuclear bridge breaks, it can also shrink and migrate towards the nucleus which is where it will be detected as a nuclear bud (Thomas, *et al.*, 2011). *In vitro* experiments in human and mouse cells have demonstrated that NBUD formation is a mechanism to expel extrachromosomal material from the main nucleus (Shimizu, *et al.*, 2005). Although NBUDs are morphologically similar to micronuclei, it has been demonstrated in cultured human lymphocytes that NBUDs more frequently contain amplified DNA without centromeric or telomeric regions (Lindberg, *et al.*, 2007).

Overall, NBUDs and NBridges are well recognised markers of genotoxicity as exposure to undisputed mutagenic agents have all been associated with their increased prevalence. The frequency of NBridges was x50 higher in response to hydrogen peroxide treatment in cultured human neutrophils (Umegaki, & Fenech, 2000). Cheong and colleagues (2013) identified a significant dose-dependent increase in micronuclei and NBUDs/Bridges in human lymphoblastoid cell lines in response to treatment with neutron and γ -radiation. A study conducted in 83 adult males, demonstrated that smoking non-filtered cigarettes was associated with an increase in NBUDs and micronuclei within the oral mucosa (Nersesyan, *et al.*, 2010). Interestingly, this study indicated that NBUDs may be a more sensitive marker for genotoxicity than micronuclei, as smoking moderately filtered cigarettes significantly increased the levels of NBUDs but not micronuclei.

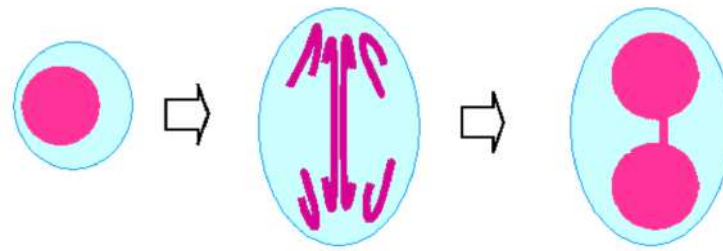


Figure 1.8 Nucleoplasmic Bridge formation as a result of dicentric chromosomes, that would be observed in a cell undergoing nuclear division and blocked prior to cytokinesis with cytochalasin-B (adapted from Thomas, *et al.*, 2011).

Multinucleated cells

There are some human cells whereby multiple nuclei play an important physiological role for organ functioning. These include osteoclasts, hepatocytes and cardiomyocytes (Bar-Shavit, 2007; Kreutz, *et al.*, 2017; Paradis, *et al.*, 2014). However, multinuclear or polynuclear cells in some cell types may be an indicator of cytokinesis failure (Bolognesi, *et al.*, 2013). Cytokinesis is the final step in the cell division process which begins during chromosomal segregation and ends in abscission – complete separation of cytoplasmic contents. It has been demonstrated that malsegregation of damaged chromosomes (non-disjunction) may trigger cytokinesis failure (Shi, & King, 2005). Furthermore, DNA damage response proteins such as BRCA2 and BCCIP, DNA damage checkpoint kinase Rad53 and Ku70, a DNA-binding protein required for DNA damage repair have all been suggested to interfere with the cytokinesis process (Normand, & King, 2010). More recently, telomere dysfunction in human epithelial cells was also associated with increased failure of cytokinesis (Pampalona, *et al.*, 2012). Finally, patients with Down's syndrome, a disorder marked by aneuploidy, presented with a two-fold increase in binucleated buccal epithelial cell frequency compared to matched healthy controls (Thomas, *et al.*, 2008). Overall, it can be viewed that the persistence of more than one nuclei in

a cell may be a marker of cytokinesis failure and possibly chromosomal aberrations as well.

Assessment of nuclear anomalies

The assessment of nuclear anomalies can be conducted in multiple cell types. First pioneered by Michael Fenech in the early 1980s, the cytokinesis-block micronucleus cytome assay can be employed in peripheral blood lymphocytes to assess micronuclei and NBUD/Bridge status whilst cells are arrested in the cell cycle (Thomas, & Fenech, 2011). This assay has been extensively employed to establish the index of nuclear anomalies observed in lymphocytes upon exposure to *in-vitro* and *in-vivo* radiation, nutritional deficiency and various other genotoxic agents (Jacociunas, *et al.*, 2013; Bull, *et al.*, 2012; Vral, *et al.*, 2011). The advantages of the lymphocyte CBMN assay include the incorporation of probes to detect centromeric and telomeric DNA (Thomas, & Fenech, 2011). Most importantly, a prospective study revealed that this assay can be employed to predict the risk of cancer in humans (Bonassi, *et al.*, 2007). However, the need for blood sampling complicates the implementation of this tool for biomonitoring. Instead, a minimally invasive assessment of nuclear anomalies can be conducted in exfoliated cells. The buccal cytome assay has also been in use since the 1980s to evaluate genotoxic effects of environmental and lifestyle factors as well as dietary deficiencies and different diseases (Holland, *et al.*, 2008).

The structural organisation of the buccal epithelium is such that it takes 7-21 days for basal cells to mature and migrate from the stratum germinativum (basal cell layer) to the stratum corneum (dying cell layer). Differentiated cells can be exfoliated from the oral cavity and scored for a number of different cytogenetic

events in addition to micronuclei, multiple nuclei and nuclear buds or bridges (Figure 1.9). Karyorrhectic, pyknotic and condensed chromatin cells can indicate stages of apoptotic cell death whilst karyolytic cells may indicate necrotic cell death. In order to avoid false positive identification of genetic material in the cytoplasm, the buccal epithelial cells must be stained with DNA-specific staining agents. For this reason, Feulgen and Fast Green stains are used more frequently (Bolognesi, *et al.*, 2013). A further advantage of this staining technique is that an adequate microscopic analysis can be performed via bright-field but also fluorescence. Moreover, comparisons can be drawn from the typical cell frequencies that have been detailed for healthy young and old populations (Thomas, *et al.*, 2009). Overall, the buccal cytome assay is a relatively simple tool that can be employed in vulnerable populations to assess the efficacy of cell regeneration and genome damage events in epithelial tissue.

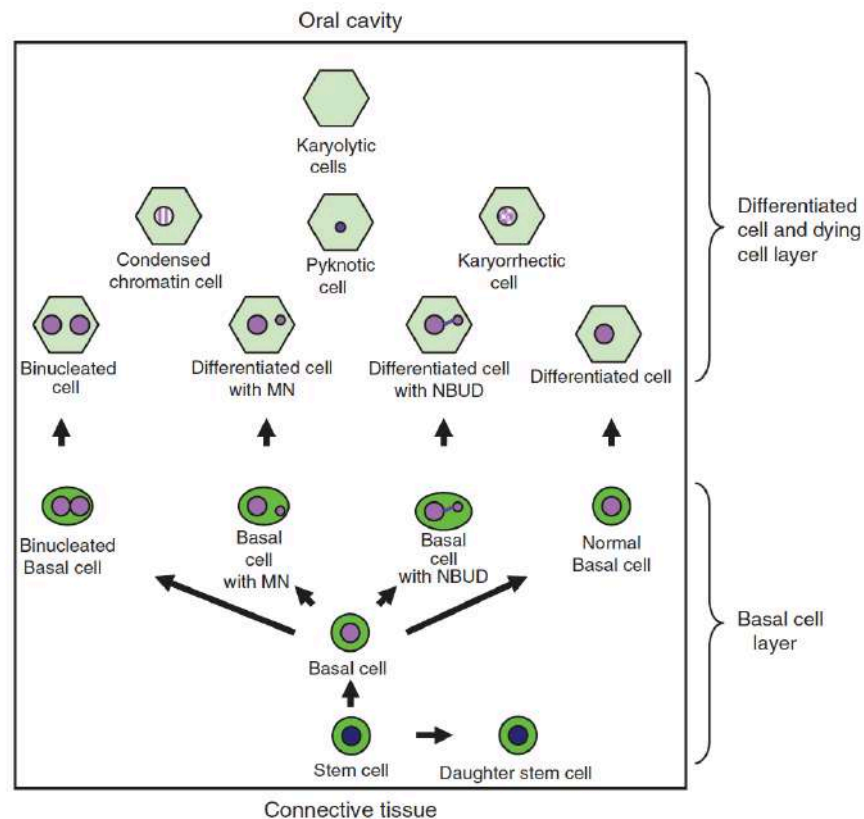


Figure 1.9 Sequential origins of the various cell types in the buccal epithelium (Thomas and Fenech, 2009).

1.2.6.3 Telomere Length

Telomeres are vital segments of non-coding DNA repeat sequences (TTAGGG) that are located at the ends of linear chromosomes and play a fundamental role in maintaining chromosomal integrity. They were first identified by Elizabeth Blackburn and Joseph Gall (1978) in *Tetrahymena thermophila*. Telomeres have a 3' overhang and T loop structure which is bound by proteins of the shelterin complex and protects telomeric repeats from being detected as DSBs (Verdun, & Karlseder, 2007). With each mitotic division, telomeres shorten in length and upon reaching a critical length, telomeres activate p53 to initiate cellular senescence or apoptosis (Deng, & Chang, 2007).

Optimal telomere functioning is crucial for preventing chromosomal DNA from degradation and depends on two factors; adequate telomere length and the ability of telomeric DNA to attract proteins of the sheltering complex (O'sullivan, & Karlseder, 2010). Therefore, the protective function of telomeres can be lost once they reach a critically short length and are no longer protected from DNA damage machinery. On the other hand, even in the presence of long telomeric DNA repeats, uncapped telomeres can be detected as DNA breaks which may result in BFB cycles and genome instability. Therefore, dysfunctional telomeres can be critically short or long.

In germ line cells, expression of the enzyme telomerase can elongate telomeres to maintain cell proliferation and lineage. There is also evidence for activity of the telomerase enzyme in human epithelial tissue, albeit the activity of the enzyme is weaker in these cells (Yasumoto, *et al.*, 1996). Nonetheless, telomere length can be modulated by expression of the telomerase enzyme – a

phenomenon also associated with increased telomere length in some types of cancer.

Telomere length can be assessed in multiple tissue types via a variety of techniques that include quantitative Polymerase Chain Reaction (qPCR), the use of fluorescence probes and the gold standard - Terminal Restriction Fragmentation (TRF) (Montpetit, *et al.*, 2014). TRF provides an average quantification of telomere length via digestion of chromosomes and removal of non-telomeric DNA from the sample. The remaining sample can be visualised via gel electrophoresis to determine the size of the telomeric DNA. However, this technique requires large amounts of DNA and is rather labour intensive – decreasing its application in large scale studies. Secondly, non-invasively obtained tissues such as saliva do not contain sufficient DNA to withstand this process. Similarly, other techniques such as Quantitative fluorescence in situ hybridization (Q-FISH), Primed in situ subtype of Q-FISH (PRINS), Flow-FISH, and HT-Q-FISH require mitotically active cells for analysis, another challenge when sampling exfoliated, largely non-viable cells.

The most suitable technique for assessing telomere length in large scale population studies is qPCR. qPCR provides an average length of telomeres in a DNA sample by normalising values against a single copy gene. qPCR is less labour intensive and can be conducted on small (ng) amounts of DNA. DNA can be extracted from any tissue sample to perform qPCR. However, accurate results depend also on the quality of DNA. Therefore, adequate purification steps and caution must be taken during DNA extraction and storage.

1.2.6.4 γ -H2AX foci

Induction of nuclear γ -H2AX foci or gamma foci signal the start of a crucial repair process that follows a DSBs. Phosphorylation of the H2AX histone by the ATM protein on serine residues is the first step before further DNA repair proteins such as NBS1 and BRCA1 are recruited to the site as part of the DNA damage response (DDR) pathway (Kobayashi, 2004). Each single gamma foci is indicative of one DSB repair, thereby it is a highly sensitive indicator of an earlier DNA damage event (Rothkamm, & Löbrich, 2003). However, the suitability of γ -H2AX foci as a DNA damage marker has been questioned as its presence has been observed in the absence of recognisable DNA damage (Tu, *et al.*, 2013).

Typically, the assessment of gamma foci is conducted by employing immunostaining or immunoblotting techniques followed by microscopy or flow cytometry. This process can be eased by the use of commercially available antibodies. Gamma foci have been assessed via these techniques in multiple cell types including PBLs, splenocytes, bone marrow cells, and keratinocytes. (Redon, *et al.*, 2011). However, an analysis of gamma foci via fluorescence microscopy is not recommended for when higher levels of foci are expected (Reddig, *et al.*, 2018). Furthermore, the assessment of gamma foci in buccal epithelial cells can be complicated by high levels of background signals (Palla, *et al.*, 2017). It has also been suggested that current ELISA based methods are less sensitive and can be affected by total cell concentration (Reddig, *et al.*, 2018). More research is required to confirm the suitability of this assay for monitoring DNA damage via non-invasive techniques for vulnerable populations.

1.2.6.5 Comet tails

Comet tails are sensitive biomarkers of deteriorating chromosomal integrity as they enable inference of chromosomal breakages. The comet assay, used to detect these tails is a well-established laboratory assay that was first pioneered by Ostling & Johanson in 1984. After treating cells to remove nuclear membranes and histone material, single cells are embedded in agarose for gel electrophoresis. The application of a current allows broken, uncoiled DNA loops to migrate faster towards the anode, creating the shape of a comet, with intact DNA remaining in the comet head whilst damaged, 'broken' DNA migrates in the comet tail. Subsequently, the DNA is treated with an intercalating dye and visualised with fluorescence microscopy. The length of the tail and percentage of DNA inside the tail is taken as a directly proportional measure of DNA damage (Olive, & Banath, 2006). The comet assay has been extensively applied to measure genotoxicity, responsiveness to chemotherapy, and is now evolving into a diagnostic marker of cancer (Apostolou, *et al.*, 2014).

The comet assay can be employed even with a small number of cells per individual, and enables a robust statistical analysis based on the level of DNA damage in each cell (Collins, *et al.*, 2008). Whilst the comet assay is relatively cheap and fast to perform, there are some technical problems associated with conducting the assay in buccal epithelial cells (Sanchez-Alarcon, *et al.*, 2016). Due to possessing specialised cell membranes, the lysis process required for the comet assay can be complicated. High pH treatment can disintegrate cells whereas a low pH may not adequately lyse the cells. Secondly, a lack of viability of exfoliated buccal cells and increased atypical comet formation can also complicate their analysis (Pinhal, *et al.*, 2006). Therefore, non-invasive sampling to perform the comet assay remains a challenge.

1.3 Aims of the project

The increasing evidence that childhood obesity is a risk factor for cancer and other morbidities in adulthood, calls for an investigation of the mechanisms that may underpin this association.

The aim of this research is to explore the potential role of DNA damage as a link between obesity in childhood and increased risk of cancer later in life. This research will evaluate genomic integrity and stability in relation to inflammation and vitamin D status in children with obesity. Secondly, this research will establish the applicability of a non-invasive tool-kit for the assessment of adiposity, inflammation, micro-nutritional deficiency and 'genome health', for long-term monitoring of pre-cancerous pathological states in children with obesity.

Hypothesis:

Excess inflammation and low vitamin D are associated with elevated DNA damage in children with obesity.

Research objectives:

1. To develop a non-invasive laboratory 'tool-kit' for a personalised and combined assessment of adiposity, inflammation, micro-nutritional deficiencies and 'genome health' in obese and non-obese individuals.
2. To conduct a correlational analysis of adiposity, inflammation, micronutrient status and DNA damage in children.

2. Materials and Methods

2.1 Overview of Study Design

A cross-sectional study was designed to compare markers of adiposity, inflammation, vitamin D and DNA damage in children aged 10-18 years (Figure 2.1). Firstly, laboratory protocols and sample collection techniques were optimised in a cohort of adults recruited from the University of Westminster. The study documentation and sample collection and testing protocols were then adapted for use in children. Participant recruitment, data collection and analysis were led by the author of the thesis. Overall, medical history, anthropometric data, and four biological samples; urine, unstimulated saliva, stimulated saliva and a cheek swab were obtained from each study participant.

2.2 Ethical Considerations and Approval

A research ethics application was prepared and approved by the Human Research Authority and NHS Research Ethics committee (Integrated Research Application System ID: 212869). A subsequent application was submitted to and approved by the University of Westminster (ETH1617-1943).

Data and samples were treated with great care when being transported to the university and stored securely in a laboratory with restricted access. Data was protected in accordance with the Data Protection Act 1998, and later updated according to the General Data Protection Regulation and Data Protection Act 2018. Written consent was obtained from the parents of all participants that were screened and included in the study. The data and tissue samples were only used for the purposes consented for and outlined in the methodology of the study. Biological samples were stored in line with the Human Tissue Act 2004.

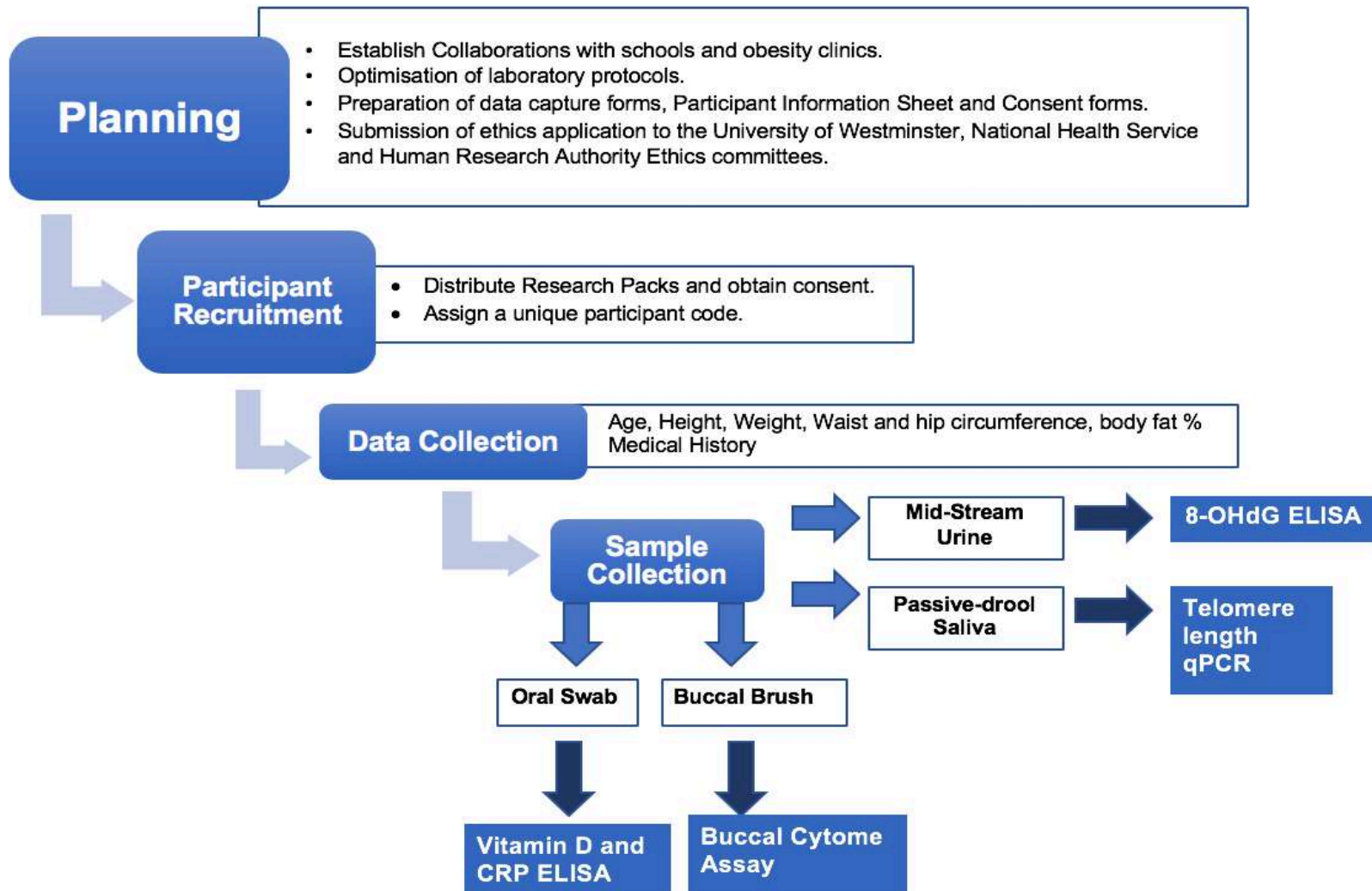


Figure 2.1 Overview of cross-sectional study design: from planning to sample collection and testing

2.3 Participant Sample Size Calculation

Firstly, the endpoints for this study were evaluated to assign a single primary endpoint for sample size calculation. The factors taken into consideration were; the extent to which the endpoint addresses the main research question, the accuracy at which it represents the outcome of interest (DNA damage) and the precision of the test. Out of the three DNA damage biomarkers that were intended to be measured (urinary 8-OHdG, salivary telomere length and buccal micronuclei frequency), associations for buccal micronuclei frequency were most consistent between obese and non-obese participants to date (Usman, & Volpi, 2018). For these reasons, buccal micronuclei frequency was selected as the primary endpoint for this study.

To calculate the sample size, buccal micronuclei data was extracted on mean values and variance in adults with a healthy weight (n=21) and adults with obesity (n=84) (Donmez-Altuntas, *et al.*, 2014) (Table 2.1). This data was entered into G*Power (v3.1) software for *A priori* calculation of sample size based on a two-tailed, independent means test at an error rate of 1%. The total sample size was calculated to be 80, with 40 participants as controls and 40 participants with obesity. Whilst it would have been most appropriate to source data from a study conducted in children, there was a lack of literature reporting mean and variance values for buccal micronuclei in a cohort of children with and without obesity; also highlighting the novelty of the research approach of this thesis. In order to account for this potential source of bias, and in consideration of feasibility regarding laboratory costs, time frame for work completion, and to cover issues such as missing data, this sample size was

increased by 20% with an aim to include a minimum of 48 healthy weight children (5th-85th BMI percentile) and 48 obese children (>98th BMI percentile).

Table 2.1 Data for sample size calculation (sourced from Donmez-Altuntas, *et al.*, 2014).

	Control cohort	Obese cohort
Age (years)	34.81 ± 11.56	37.95 ± 10.52
BMI (kg/m ²)	22.38 ± 1.72	37.98 ± 7.46
Mean (%) +/- S.D	0.71 ± 0.51	1.24 ± 0.45
Sample size in study	21	83
Calculated sample size	40	40

2.2 Participant Screening and Recruitment

Over 200 research packs were distributed across secondary schools in London to pursue non-selective recruitment of children aged 10-18 years. Table 2.2 outlines the components of the research packs sent to schools. St George's London NHS trust and King's College Hospital London supported the recruitment of obese patients by distributing information sheets to parents (Appendix I) and patients (Appendix II) prior to their clinic appointment. Written parental consent (Appendix III) was obtained from all parents and participants gave written assent (Appendix IV) prior to data or sample collection.

Table 2.2 School Research Pack Components

Document	Appendix
Participant Information Booklet for Parents	V
Participant Information Booklet for 10-18 year olds	VI
Parental Consent Form	VII
Assent Form	<u>VIII</u>
Medical Questionnaire	<u>IX</u>
Invitation Letter	<u>X</u>

In total, four schools and two NHS obesity clinics agreed to collaborate for participant recruitment and 171 participants were screened for inclusion (Figure 2.2). The screening process required all participants to complete a medical questionnaire (Appendix VIII). After applying the exclusion criteria (Table 2.3), 132 participants were included in the study.

Table 2.3 Exclusions Criteria for Participant Recruitment

Exclusions Criteria	Purpose
Dental treatment within last six weeks or local inflammation including pain, swelling and other evidence of tooth decay.	Local inflammation and increase in inflammatory cytokines (CRP) is possible.
General illness (flu, cold, fever) on the day of sample collection.	As CRP is one of the first inflammatory cytokines to be released during infection, there is some evidence that CRP levels may be raised during the first (2-3) days of illness (Melbye, <i>et al.</i> , 2004).
Intense physical activity one hour prior to sample collection.	Increase in inflammatory cytokines (CRP) is possible.
Consumption of food and drink thirty minutes prior to sample collection.	To avoid contamination of saliva samples with food and drink.
Medical history of inflammatory conditions (asthma, eczema etc) and cancer.	May cause elevation in biomarkers of inflammation and DNA damage.
Consumption of medications including multivitamins.	May cause elevation or suppression of all biomarkers.
X-rays of the head and neck within last six months.	X-rays can induce micronuclei formation.
Smoking	Smoking can induce micronuclei formation.

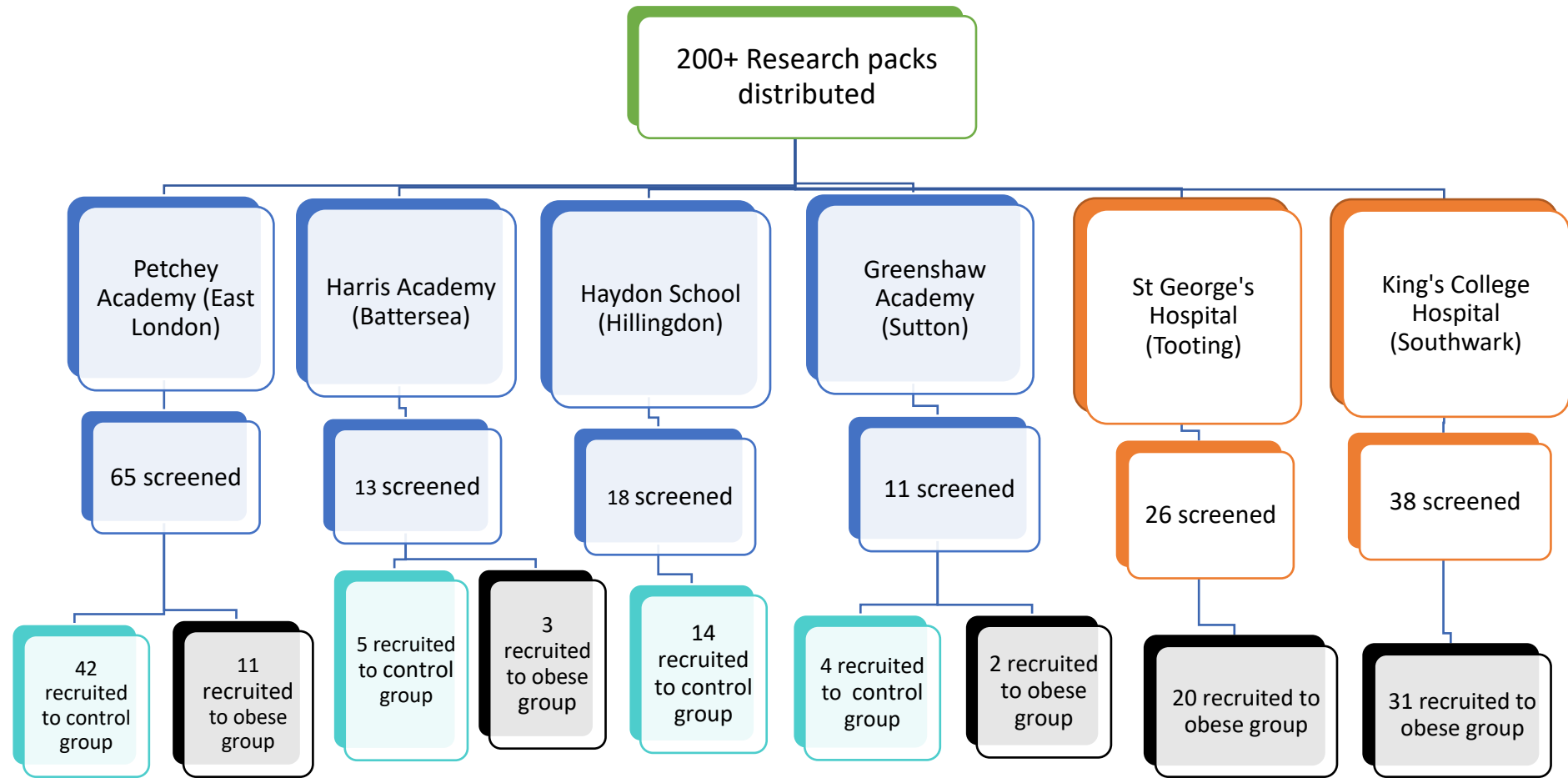


Figure 2.2. Flow diagram of participant recruitment from 4 schools (blue) and two NHS clinics in London (orange), and allocation into 'control' and 'obese' groups following screening against exclusion criteria and analysis of BMI Z-score.

2.4 Collection of biological samples and anthropometric data

A workflow was implemented with each participant to enable the collection of anthropometric data and biological samples (Figure 2.3). Following consent, participants were assigned a unique participation code to pseudo-anonymise the data. For participants recruited through schools, the code began with a letter F (females) or M (males) and was followed by the number in sequence of their enrolment to the study. For participants recruited through NHS hospitals, the code began with the letters KCH (King's College Hospital) or SGH (St George's Hospital) and also followed the same numbering system.

Sample and anthropometric data collection was conducted by a group of students and staff at the University of Westminster. In order to standardise the techniques for collecting anthropometric measures and biological samples, the author of the thesis provided a briefing sheet that described the reference points for anthropometric measures and also provided training to the data collection team. Participant's height was recorded using a standard, portable stadiometer (Marsden Weighing Machine Group) to the nearest mm. Height, age (years) and sex was entered into the TANITA BC54N body composition scales to determine weight and body fat percentage via bioelectrical impedance. Waist and hip measurements were recorded to the nearest mm using a standard measuring tape. Waist was measured at the level midway between the lower rib margin and iliac crest, with the tape placed horizontally and firmly all the way around. The hip measurement was determined as the maximal circumference of the buttocks.

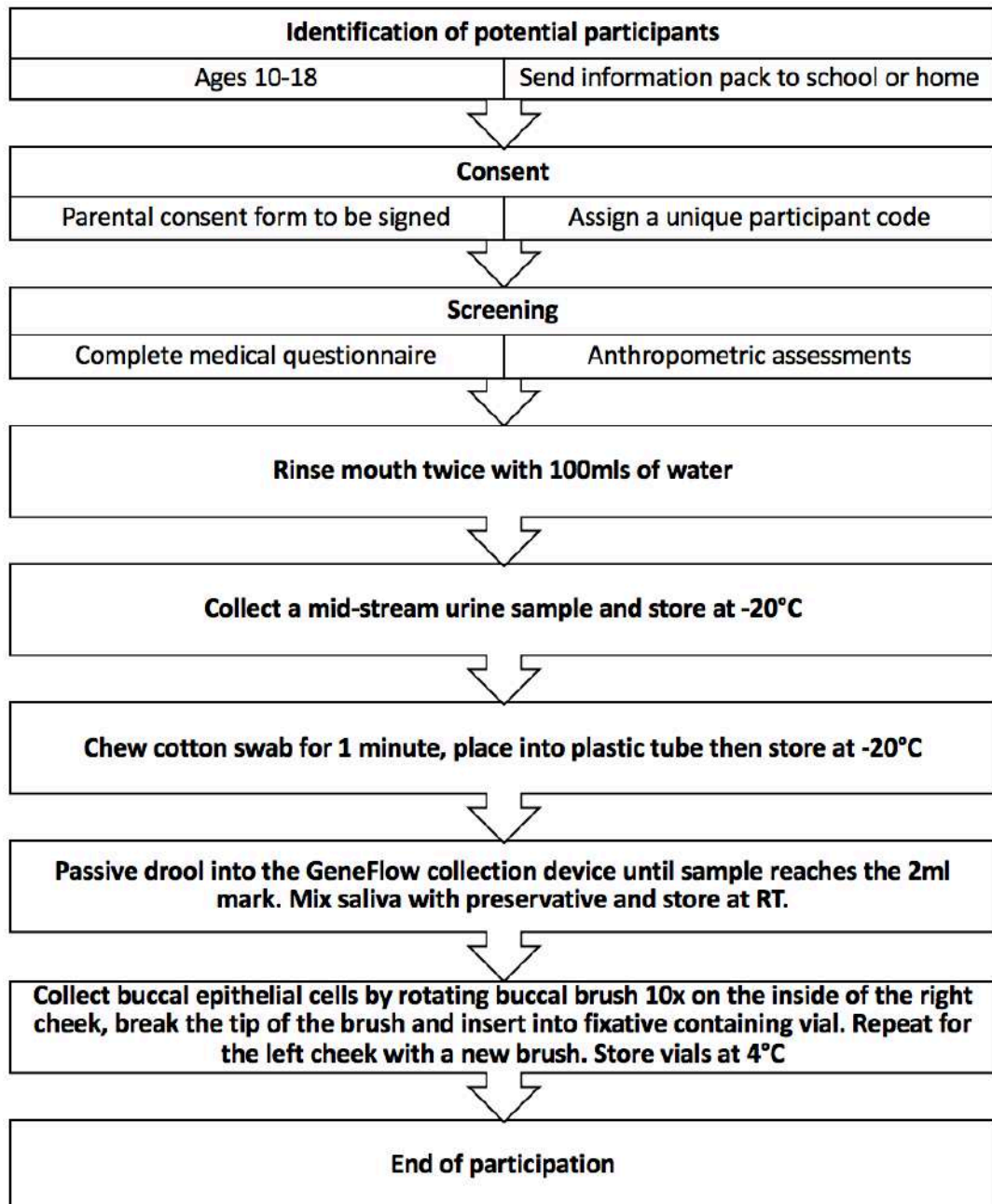


Figure 2.3 Participant Work-flow diagram

2.6 Salivary C - reactive protein ELISA

2.6.1 Saliva sample collection

Saliva was collected from participants using the Salimetrics Oral Swab (SOS) (Stratech, 5001.02-SAL-50) which was placed on the floor of the oral cavity for one minute before being transferred into a Swab Storage Tube (Stratech, 5001.05-SAL-50). Saliva samples were transported on ice to the laboratory and immediately centrifuged at x1500g before the swab was discarded. Saliva was stored at -20 °C until analysis.

2.6.2 CRP ELISA sensitivity and test principle

C-Reactive Protein was quantified in saliva samples using the Salimetrics CRP ELISA kit (Stratech, 1-3302). The analytical sensitivity of the kit, or lower limit of detection was 0.042pg/ml. The manufacturers report no cross-reactivity between the antibody used in the kit and known protein markers found in saliva. The test is based upon the principles of an indirect sandwich ELISA. Anti-CRP antibodies are immobilised on a 96-well plate and bind CRP from saliva or standard samples. A 'sandwich' is formed when CRP bound to anti-CRP is detected and bound by anti-CRP detection antibody labelled with horseradish peroxidase (HRP) enzyme conjugate. TMB substrate is added which reacts with the enzyme to produce a blue colour. The reaction is stopped with 0.16M sulphuric acid and the final colour of the wells turns to yellow. The colour intensity of the wells is directly proportional to the concentration of CRP in the sample.

2.6.3 Saliva sample preparation

Saliva samples were allowed to thaw at room temperature before they were vortexed and centrifuged at x1500g for fifteen minutes, in order to remove mucins and particulate matter that can interfere with the assay. 15µL of saliva was diluted in 135µL of CRP sample diluent to perform a ten-fold dilution and allow for assessment in duplicates.

2.6.4 Assay preparation

All reagents and the microtitre plate were brought to room temperature for a minimum of 1.5 hours. A 1X wash buffer was prepared using 100mL of Wash Buffer Concentrate (10X) and 900mL of deionized water. High and low CRP control vials were reconstituted with 500µL of deionized water and mixed thoroughly by inversion then left to sit for 20 minutes at room temperature. A two-fold serial dilution of the CRP standard was performed with CRP sample diluent to produce six concentrations from 3000pg/mL to 93.75pg/mL. CRP diluent was used alone as a 'zero' standard. 36 samples were run in each microtitre plate, and a plate plan was prepared to determine the layout of standards and unknown samples (Figure 2.4)

	1	2	3	4	5	6	7	8	9	10	11	12
A	3000 Std	3000 Std	Ctrl-L	Ctrl-L								
B	1500 Std	1500 Std	SMP-1	SMP-1								
C	750 Std	750 Std	SMP-2	SMP-2								
D	375 Std	375 Std	SMP-3	SMP-3								
E	187.5 Std	187.5 Std	SMP-4	SMP-4								
F	93.75 Std	93.75 Std	SMP-5	SMP-5								
G	0 Std	0 Std	SMP-6	SMP-6								
H	Ctrl-H	Ctrl-H	SMP-7	SMP-7								

Figure 2.4 Typical plate layout for CRP ELISA.

2.6.5 Assay procedure

50µL of standards, controls and saliva samples were loaded into the appropriate wells. A multi-channel pipette was used to transfer the CRP antibody enzyme conjugate into all wells. Next, the plate was covered with adhesive and placed on a plate rotator for 2 hours at 500RPM and room temperature. After incubation, a squirt bottle was used to load each well with wash buffer before discarding the solution. This wash stage was repeated four times before a clean paper towels were used to pat the plate dry. Then, a multi-channel pipette was used to add 200µL of TMB substrate solution to each well and the plate was incubated in the dark (covered with foil) at room temperature for 30 minutes mixing constantly on a plate rotator at 500 rpm. Finally, 50µL of stop solution was added to each well and the plate rotated again until the colour of the well changed from green to yellow (after approximately three minutes). The bottom of the plate was wiped with a water-moistened, lint-free towel and the plate was read immediately with the SPECTROstar^{Nano} plate reader at 450 nm.

2.6.7 Salivary CRP calculations

An average of the optical density (OD) of each standard and sample was calculated and the average OD of the zero wells subtracted from each average OD to calculate the net OD. The net OD of the standards were imported into GraphPad Prism (v7.0) to generate a linear curve (Figure 2.5). The same software was used to interpolate the standard curve and retrieve the concentration of CRP in participant's samples. The concentration of CRP was corrected for the dilution by multiplying by ten. Participant's samples that were outside the linear range of the curve were further diluted and re-assayed. In total, four assays were run to analyse 132 saliva samples. The average r-squared value of standard curves was 0.99. The inter-assay coefficient of variation was 14%.

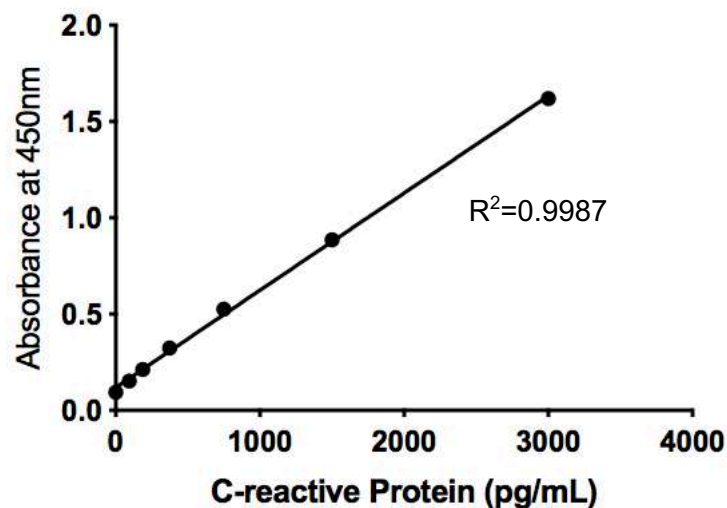


Figure 2.5 Typical standard curve for CRP ELISA.

2.7 Salivary Vitamin D ELISA

2.7.1 Saliva sample collection

Surplus saliva collected via the Salimetrics Oral Swab method was used for the vitamin D ELISA assay.

2.7.2 Vitamin D ELISA sensitivity and test principle

Vitamin D was quantified in saliva samples using the 25-OH Vitamin D (total) ELISA kit (DX-EIA- 5396, Oxford Biosystems). The analytical sensitivity of the kit, or lower limit of detection was 2.89ng/ml. The test was based upon the principle of competitive binding (Figure 2.6). Vitamin D binding globulin (VDBG) is immobilised to the 96-well plate. Endogenous 25-OH Vitamin D from the participant's sample competes with a 25-OH Vitamin-D3-biotin conjugate for binding to the VDBG. 25-OH Vitamin D-biotin that binds to VDBG is detected by peroxidase-labelled streptavidin after a washing stage is performed to remove unbound components. A colour reaction is started by addition of TMB enzyme substrate and stopped with a solution of 0.5M sulphuric acid. The colour intensity is inversely proportional to the concentration of 25-OH Vitamin D in the sample.

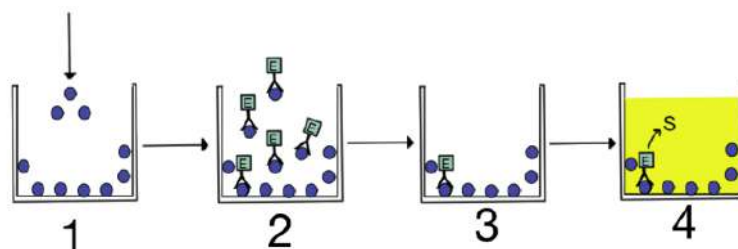


Figure 2.6. Competitive ELISA Test Principle: (1) addition of saliva samples or standards, (2) add Vitamin-D3-biotin conjugate to compete with salivary and standard vitamin D for VDBG (3) wash away unbound proteins (4) addition of substrate for colour reaction.

2.7.3 Saliva sample preparation

Saliva samples were allowed to thaw at room temperature before they were vortexed and centrifuged at x1500g for fifteen minutes, in order to remove mucins and particulate matter that can interfere with the assay. Saliva was not diluted for this assay.

2.7.4 Assay preparation

All reagents and the microtitre plate were brought to room temperature for a minimum of 30 minutes before use. Reagents were mixed thoroughly by inversion to avoid foaming, prior to use. 10mL of Working Conjugate Solution was prepared by mixing Enzyme Conjugate with Enzyme Complex in a 1:1 ratio. Next, a 1X wash buffer was prepared using 30mL of Wash Buffer Concentrate (40X) and 1170mL of deionized water. Six standard samples ranging from 0ng/mL to 130ng/mL were provided with the kit. 39 samples were run in each microtitre plate, with all samples run in duplicates.

2.7.5 Assay procedure

Firstly, total 25-OH Vitamin D had to be extracted from VDBP in saliva, standard and control samples. This step involved the incubation 25µL of these samples with 50µL of denaturation buffer into separate 1.5mL Eppendorf tubes. These tubes were incubated at 37°C for 30 minutes. Next, 200 µL of Neutralization Buffer and 100µL of Working Conjugate Solution was added to each vial and the tubes were mixed thoroughly by inversion for 10 seconds. 150 µL of this mixed solution of each mixed solution was transfer to the appropriate wells in the microtitre plate using new disposable tips. The plate was sealed using an

adhesive and incubated at 37°C for 60 minutes. After incubation, a squirt bottle was used to load each well with wash buffer before discarding the solution. This wash stage was repeated four times before a clean paper towels were used to pat the plate dry. Using a multi-channel pipette, 150µL of TMB substrate solution was added to each well and the plate incubated at room temperature. After 15 minutes, 100µL of sulphuric acid stop solution was added to the wells and the turned into a yellow colour. The plate was read within 10 minutes using the SPECTROstar^{Nano} plate reader at 450 nm.

2.7.7 Salivary vitamin D calculations

An average of the OD of each standard and sample was calculated. The average OD of the standards were imported into GraphPad Prism (v7.0) to generate a 4-parameter logistic curve (Figure 2.7). The same software was used to interpolate the standard curve and retrieve the concentration of vitamin D in participant's samples. In total, four assays were run to analyse 132 saliva samples. The average r-squared value of standard curves was 0.99. The inter-assay coefficient of variation was 3.6%.

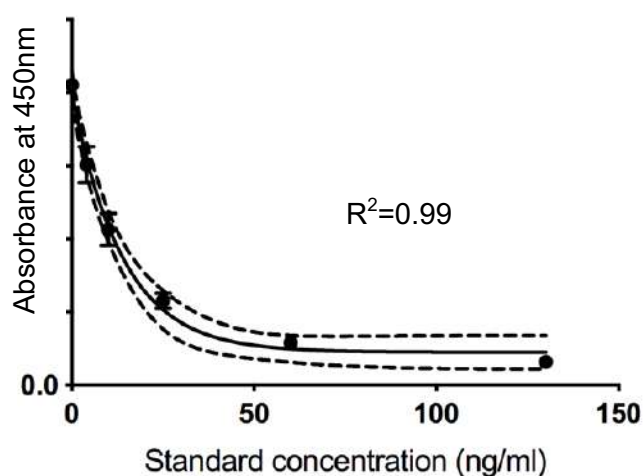


Figure 2.7 Typical standard curve for vitamin D ELISA

2.8 Urinary 8-OHdG ELISA

2.8.1 Urine sample collection

Participants collected one mid-stream urine sample into a polypropylene universal container. Between 10-30mls of sample was obtained per participant and transported to the laboratory on ice, where it was aliquoted into three 1ml solutions in separate Eppendorf tubes. 3 μ L of Gentamycin (Sigma) was added to each tube to prevent microbial growth and stored at -80°C. Surplus urine was stored at -20°C. A 24-hour collection of urine for 8-OHdG analysis could have provided a more precise result, as 8-OHdG levels can vary between different spot samples collected in one day (Barregard, *et al.*, 2013). However, this would have been impractical for this investigation and could possibly have affected our participation rates. To control for intra-individual variation in urinary 8-OHdG levels, a creatinine correction was applied. Barregard and colleagues (2013) report that in creatinine-adjusted levels, the variation is low (CV of 12%).

2.8.2 8-OHdG ELISA sensitivity and test principle

The DNA Damage EIA Kit (AD-EKS-350, Enzo Life Sciences) was used to perform the quantification of 8-OHdG in urine samples via a competitive ELISA reaction. The analytical sensitivity of the kit, or lower limit of detection was 0.59 ng/ml. The assay incorporates the use of a 96-well plate with pre-bound 8-OHdG. 8-OHdG in urine samples, or known concentration standards, competes with 8-OHdG monoclonal antibodies to bind to 8-OHdG coating the wells. Anti-8-OHdG that successfully bind become immobilized in the wells whilst unbound 8-OHdG and antibodies are washed away. HRP conjugate is the secondary antibody which binds to the immobile anti-8OHdG antibody. The substrate: tetramethylbenzidine is added to develop the assay and produce a yellow

colour. The intensity of the yellow colour is inversely proportional to the concentration of 8-OHdG. The absorbance of the known concentrations of 8-OHdG are used to generate a 4-parameter logistic curve and calculate the unknown concentrations of 8-OHdG in samples.

2.8.3 Urine sample preparation

Prior to each assay run, 39 urine samples were allowed to thaw and centrifuged at x2,000g for ten minutes at room temperature. 150uL of urine was prepared in a 1:20 dilution with sample diluent (Part#: 80-150 of ADI-EKS-350 Kit). Samples were vortexed for ten seconds before being loaded into the assay plate.

2.8.4 Reagent and assay preparation

All reagents were brought to room temperature and mixed gently prior to the assay procedure. A 2-fold serial dilution of the 8-OHdG standard (Part#: 80-1513 of ADI-EKS-350 Kit) was undertaken to produce seven concentrations ranging from 60ng/ml to 0.94ng/ml using sample diluent (part 80-150). A 2X wash buffer (Part#: 80-1287) was diluted to a 1x concentration with distilled water. Anti-8-OHdG (Part#: 80-1514) and Anti-Mouse IgG: HRP Conjugate (Part#: 80-1515) were also diluted as per the manufacturer's guidelines. A plate plan was produced (Figure 2.8) to determine the number of wells required and appropriate locations for loading samples and standards in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	7.5 ng/mL	0 ng/mL									
B	Blank	7.5 ng/mL	0 ng/mL									
C	60 ng/mL	3.75 ng/mL	US1									
D	60 ng/mL	3.75 ng/mL	US1									
E	30 ng/mL	1.875 ng/mL	US2									
F	30 ng/mL	1.875 ng/mL	US2									
G	15 ng/mL	0.94 ng/mL	US3									
H	15 ng/mL	0.94 ng/mL	US3									

Figure 2.8 Typical plate plan for 8-OHdG assay, C3→H12 are loaded with unknown samples (US) in duplicates and labelled with participant code.

2.8.5 Assay procedure

50µL of *sample diluent* was loaded into the ‘zero’ wells to serve as a blank.

50µL of prepared standards and unknown samples were loaded into appropriate wells followed by the Anti-8-OHdG antibody, excluding the ‘zero’ wells. The plate was covered and incubated at room temperature (RT) for one hour. The contents of the plate were then emptied and the plate was loaded with wash buffer to thoroughly remove 8-OHdG and unbound antibodies. A squirt bottle was used to load the plate with wash buffer and rinse off five times. On the sixth occasion, the plate was patted dry on clean paper towels. 100µL of Anti-Mouse IgG: HRP Conjugate was loaded into all wells except the blank and the plate incubated for one hour at RT. The washing stage was repeated again, followed by addition of 100µL of TMB Substrate into all wells and incubation for fifteen minutes in the dark at RT. Immediately after, 100µL of stop solution was added to all wells to stop further colour development. The absorbance of all wells was measured at 450nm in the SPECTROstar^{Nano} plate reader.

2.8.7 Urinary 8-OHdG calculations

An average of the optical density (OD) of each standard and sample was calculated and the average OD of the blank wells subtracted from each average OD to calculate the net OD. The net OD of the standards were imported into GraphPad Prism (v7.0) to generate a 4-parameter logistic curve (Figure 2.9). The same software was used to interpolate the standard curve and retrieve the concentration of 8-OHdG in participant's samples. The concentration of 8-OHdG was corrected for the dilution by multiplying by twenty. In total, four assays were run to analyse 132 urine samples. The average r-squared value of standard curves was 0.99. The inter-assay coefficient of variation was 12.7%.

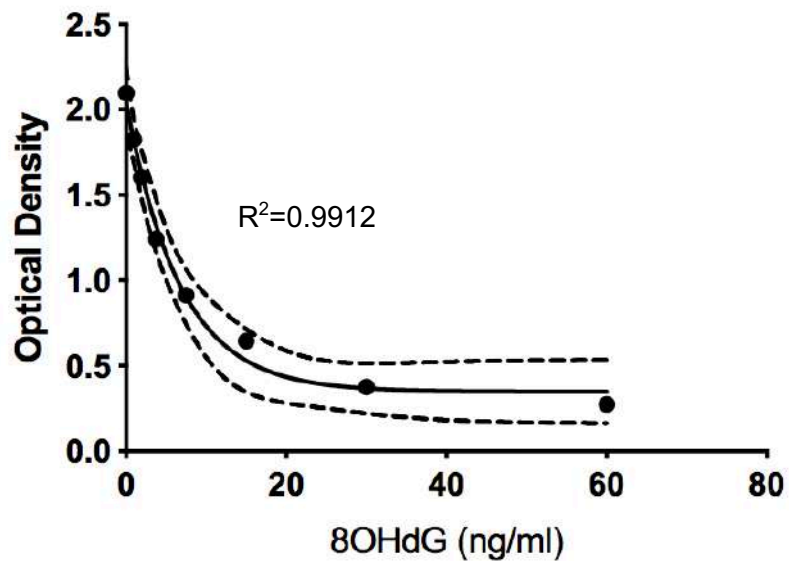


Figure 2.9 Typical standard curve for 8-OHdG ELISA.

2.8.8 Creatinine quantification and correction

Creatinine was assessed in a fresh aliquot of urine by the *University of Westminster Blood Testing Service* - a UKAS accredited service. Samples were analysed in the ILab Aries based on the colorimetric methodology between the reaction of creatinine with picric acid under alkaline conditions. Urinary creatinine (mg/ml) was calculated by multiplying the concentration of creatine (mmol/L) in urine samples by the molecular weight of creatine (113.12g/mol) then divided by one hundred to correct for units. Final 8-OHdG (ng/ml creatinine) was calculated by dividing urinary 8-OHdG (ng/ml) by urinary creatinine (mg/ml).

2.9 Buccal Cytome Assay

The buccal cytome assay (BCA) was performed within one week of buccal cell sample collection according to the protocol published by Thomas and colleagues (2009), with modifications detailed below.

2.9.1 Buccal cell sample collection

Participants rinsed their mouth with one cup of water immediately before sample collection. A soft, small-headed brush was rotated ten times in a spiral-outward motion to collect a sufficient number of cells from the right cheek. The tip of the brush was broken off and placed inside a container with Saccomanno's fixative. This process was then repeated for the left cheek. Samples were immediately placed on ice and transported to the laboratory where they were stored at 4°C in Saccomanno's fixative until processing and fixation onto a microscopic slide. Sample processing was carried out within seven days of sample collection. The product numbers and suppliers for buccal cell sample collection equipment are listed in Table 2.4.

Table 2.4 Materials for Buccal Cell Sample Collection

Product	Purpose	Source	Catalogue/reference Number
Cervibrush LBC Sampler	Buccal cell collection	CellPath Ltd.	NCA-0780-02A
Sacomanno's Fixative (0.2% Carbowax in 50% Alcohol)	Cell preservative during sample storage and transfer	Clin-Tech	641999
25ml Flat Base Tubes, 54x27mm 25ml Polystyrene Screw Cap Sterile	Storage of cell sample until processing stage	Sarstedt	60.9922.115

2.9.2 Sample wash and preparation for fixation

The tip of the brush was removed from the Saccomanno's fixative and scraped against the edge of the container to dislodge any attached cells. The cell sample solution from the left and right cheeks was then poured into separate 10mL tubes to proceed with the washing stage. The washing stage consisted of centrifugation at *581g* for ten minutes at room temperature followed by aspiration of the supernatant to leave behind a pellet of cells in 1mL of fixative. 5mL of buccal cell buffer was added prior to vortexing of the sample for 10 seconds. The wash stage was repeated four times per tube of sample.

At the end of the fourth wash, 4ml of buccal cell buffer was added and both left cheek and right cheek samples were pooled into one 30mL container. A 25G needle and syringe were used to draw up the sample and flush it back into the container for homogenization. The sample was drawn into the syringe again and filtered through a nylon membrane into a new 10mL tube to further remove debris and break up large aggregates of cells. Next, the tube was centrifuged at *581g* and all the supernatant was removed to leave behind a pellet of cells. Cells were then resuspended in 1mL of fresh buccal cell buffer. 50 μ L of DMSO was added to promote cell separation, prior to a ten second vortex of the sample. The product numbers and suppliers for buccal cell sample washing equipment are listed in Table 2.5.

Table 2.5 Materials for washing and preparation of cell samples

Product	Purpose	Source	Catalogue/reference Number
10ml Polystyrene screw cap sterile tubes	Storage of cells during processing	Sarstedt	60.9921.829
Trizma Hydrochloride	Component of Buccal Cell Buffer for sample washing	Sigma	T3253-250G
Ethylenediaminetetraacetic acid powder	Component of Buccal Cell Buffer for sample washing	Sigma	ED-500G
Sodium Chloride	Component of Buccal Cell Buffer for sample washing	Sigma	S5886-500G
12ml NORM-JECT Syringe	Homogenizing and filtration	Henke Sass Wolf	4100-000V0
Sterican 0,50 x 16mm, 25G sterile needles	Homogenizing and filtration	Fisher	465-7853
Swinnex Filter Holders	Filtration of cell sample	Merck Millipore	SX0002500
Nylon Net Filters 100um	Filtration of cell sample	Merck Millipore	NY1H02500
Dimethyl sulfoxide (DMSO)	Cell separation	Sigma	D2650-6X5ML

2.9.3 Cell fixation and staining

Glass microscope slides were cleaned with 70% ethanol and left to air dry before being labelled with the date and participant code. Slides were assembled into apparatus for cytocentrifugation – a filter card and cytocentrifuge cup held within a meal clip. 150µL of cell sample was added to each cytocentrifuge cup. Slides were then centrifuged at 600RPM for five minutes at room temperature, and allowed to air dry for ten minutes.

Slides were fixed for ten minutes in a 3:1 ethanol/acetic acid solution, and left to air dry vertically in a rack for a further ten minutes. Subsequent steps were taken to dehydrate the sample in two concentrations of ethanol (50% and 20%) for one minute each and re-hydrated for two minutes in distilled water. Slides were immediately transferred into a coplin jar containing a 5M solution of HCL for thirty minutes, and next placed under a running tap for three minutes. Slides were then placed inside a coplin jar of Schiff's reagent for one hour in the dark. Next, slides were placed under a running tap for six minutes and then rinsed with distilled water before and after being taken to stain with light green for thirty seconds. Slides were assessed under a light microscope to assess cell concentration and staining efficacy. Unsatisfactory slides (low cell counts, debris obscuring cells or poor staining) were repeated and two slides were produced per participant. Each slide was placed in a rack, covered with foil and left to dry overnight at room temperature. Ultimately, DPX was used to apply a cover glass and slides were stored in a box at room temperature. The product numbers and suppliers for buccal cell sample fixation and staining equipment are listed in Table 2.6.

Table 2.6 Materials for cell fixation and staining

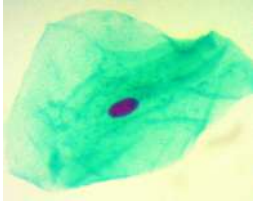
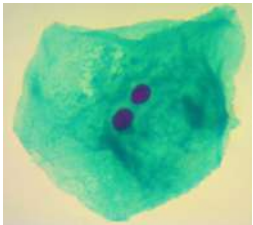
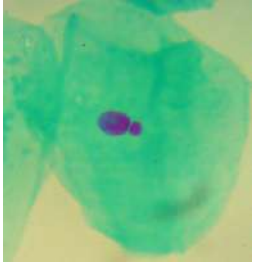
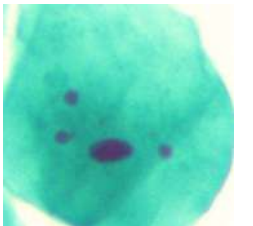
Product	Purpose	Source	Catalogue/reference Number
Single Cytofunnel Disposable Sample Chambers with White Filter Cards	Cell transfer onto microscope slide	Thermo Scientific	5991040
Superfrost Microscope slides 76x26mm	Microscopy	Thermo Scientific	SFG90 Blue
Ethanol absolute	Fixation	VWR	20821.330
Acetic Acid	Fixation	Sigma	45726-1L-F
Hydrochloric Acid	Fixation	Amresco	E484-500ML
Feulgen Stain (Schiff's Reagent)	Staining Nuclei	Cell Path	HS265-500
Light Green (Masson) Stain	Staining Cytoplasm	Cell Path	HS405-500
DPX	Mounting Medium	Cell Path	SEA-1300-00A
Cover glasses 22x22mm	Mounted to microscope slide	Thermo Scientific	12372108

2.9.4 Microscopy and Scoring

A Carl Zeiss Primo Star Light Microscope (37081) was used for analysis. The microscope was connected to a JENOPTIK ProgRes CT5 USB C Camera (D-07739 Jena) and images were captured using ProgRes Software. One thousand cells per participant were imaged from the top right edge of the slide to the bottom left, at a magnification of x1000 with immersion oil. Slides were scored for their frequency of normal differentiated cells (NDCs), cells with micronuclei (MNI), multi-nucleated cells (MNCs) and cells with nuclear buds/nucleoplasmic bridges (NBUDs/NBridges). The criteria for each cell type are described in Table 2.7. The frequency of each cell type was reported as per 1000 cells. In an attempt to increase the accuracy of the scoring, the author of

thesis attended the 'Human Micronucleus Network Workshop' held in London for training, led by Professor Michael Fenech in 2018.

Table 2.7 Scoring criteria for BMA (images captured by the author of the thesis).

<p>Normal Differentiated cell (NDCs)</p>		<p>NDCs have a uniformly stained nucleus, which is oval or round in shape. These cells are larger in size than basal cells and have a smaller nuclear: cytoplasmic ratio.</p>
<p>Multi- nucleated Cells (MNCs)</p>		<p>MN cells contain two or more main nuclei. The nuclei can be in very close proximity and may also touch each other. The morphology of the nuclei is that observed in NDCs.</p>
<p>Nuclear buds/nucleop lasmic bridges (NBUDs/NBri dges)</p>		<p>Nuclei appear to have a fine 'thread' like structure connecting a constriction of the main nucleus with a smaller segment of itself: suggestive of elimination of nuclear material by a budding process.</p>
<p>Micronuclei (MNi)</p>		<p>Cells with MNi are marked by the presence of a main nucleus and one or more smaller nuclear structures. MNi are round or oval in shape and their diameter range between 1/3 and 1/16 of the main nucleus, have the same staining intensity and texture as the main nucleus and are located within the cytoplasm. Only cells with a 'normal' main nucleus are scored for MNi. Multiple MNi can be identified in once cell.</p>

2.10 Salivary Telomere Length (STL) Quantification by Quantitative Polymerase Chain Reaction (qPCR)

2.10.1 Saliva sample collection

2mLs of saliva was collected from each participant via an un-stimulated passive drool method, whereby the participant tilted their head forward and drooled into the Saliva DNA Collection and Preservation Device (RU35710, Norgen Biotek Corp) through a collection funnel. This device incorporated a 'preservation' ampoule which is emptied into the saliva sample after discarding the funnel, and mixed by inversion ten times. A 4mL mixed saliva sample was suitably stored at room temperature as per the manufacture's guidance.

2.10.2 DNA Extraction

DNA was extracted from saliva using the DNA isolation kit (RU35700, Norgen Biotek Corp). Firstly, saliva samples were gently mixed by inversion before 500 μ L of saliva was transferred to a 2mL micro-centrifuge tube. Next Proteinase K was thoroughly mixed and 20 μ L was added to the tube. The sample was vortexed for ten seconds and incubated at 55°C for 15 minutes. 200 μ L of Binding Buffer B was added to the sample prior to another round of vortex for ten seconds and incubation at 55°C for 5 minutes. An equal volume (720 μ L) of isopropanol was added and the sample was gently inverted ten times before centrifugation for 3 minutes at x20,000g. The supernatant was carefully discarded and the micro-centrifuge tube was inverted on a paper towel to remove residual isopropanol. 500 μ L of a 70% ethanol solution was added and the tube was left to stand at room temperature. After one minute, the sample was centrifuged again at x20,000g for one minute and the supernatant was discarded. The tube was inverted on paper towels for 5 minutes to remove

excess ethanol and to air dry the DNA pellet. Next, the DNA pellet was resuspended in 50µL of TE buffer, vortexed for thirty seconds and incubated at 55°C for 5 minutes to ensure complete rehydration. A final centrifugation stage followed at x20,000g for 1 minute to pellet any insoluble material. The clear liquid was then carefully transferred to a 1.5mL sterile micro-centrifuge tube and labelled with the participant code and date. Following assessment of DNA for purity and yield, the sample was stored at -20°C until qPCR analysis. The product numbers and suppliers for DNA extraction equipment are listed in Table 2.8.

Table 2.8 Materials for DNA extraction from saliva

Product	Purpose	Source	Catalogue/reference Number
1.5ml BRAND Sterile microcentrifuge tubes	DNA Extraction and qPCR Reaction Preparation	Fisher	780400
DNA Extraction Kit with Proteinase K and Binding Buffer B	DNA Extraction	Norgen Biotek Corp	RU35700
Ethanol absolute	Fixation	VWR	20821.330
TE Buffer	DNA storage and Gel Electrophoresis	Sigma	93283

2.10.3 DNA Assessments

The purity and concentration of all DNA samples was assessed using a NanoDrop 1000 Spectrophotometer. 1µL of purified DNA was analysed using the default setting for DNA. The 260/280 ratio and concentration per microliter were recorded. During the optimisation stage, DNA was visualised on a 1% agarose gel. The gel was prepared by heating 0.5g of agarose (Appleton Woods, Birmingham, UK) in 50mL of 1X Tris/Borate/EDTA (TBE) (Thermo Fisher Scientific) buffer using a microwave to dissolve all solid particles. The

heated solution was allowed to cool for 10 minutes and 2µl of SYBR Gold DNA staining dye (Thermo Fisher Scientific) was added to the gel. The agarose solution was then poured into a gel tank and allowed to solidify. DNA was concentrated to 20ng/µl using nuclease free water (Invitrogen) and loading buffer (1.6µl per 5µl of DNA) and between 8-10µl was loaded per well. 1µl of pre-dyed, 1kb DNA ladder (Thermo Fisher Scientific) was loaded and the gels were run at 100 volts for 30 minutes prior to UV analysis.

2.10.4 qPCR Principle

A qPCR protocol established by O'Callaghan and Fenech (2011), was adapted to assess telomere length in DNA extracted and purified from saliva. Two qPCR reactions were carried out on each participant's DNA sample. The first reaction was performed to quantify copies of a single copy gene (36B4) and the second was performed to quantify the number of telomere repeats (TTAGGG). Salivary telomere length (sTL) was calculated by dividing the number of telomere repeats by the number of copies of the 36B4 gene.

2.10.5 qPCR Constituents

10µL of qPCR reaction mixture was prepared and composed of 1X PowerUp SYBR Green Master Mix (Life Technologies), RT-PCR grade water (Life Technologies), 10ng of purified DNA extracted from participants and varied concentrations of primers (Integrated DNA Technologies) (Table 2.9) to compliment the telomere sequence or 36B4 sequence. A 96-well plate was used to run qPCR reactions in the ABI 7500 Fast RT-PCR System (Life Technologies). Each plate was run with a 7-point standard curve with concentrations ranging from 5ng to 5×10^{-5} of standard DNA (Integrated DNA Technologies) (Table 2.10). Plasmid DNA (Pbr322 Vector, New England

Biolabs) was added to wells with standard DNA to ensure the total concentration of DNA was 10ng per well. Participant samples were loaded into the plate in triplicates. The wells were loaded as 'No template controls' (NTC) these negative controls contained all qPCR constituents except DNA.

Table 2.9 Primer sequences and concentrations in qPCR reactions

Primer	Sequence	Final Concentration
Forward Telomere (Tel-1)	CGGTTTGGTTGGGTTTG GGTTTGGGTTTGGG TTTGGGTT	0.3µM
Reverse Telomere (Tel-2)	GGCTTGCCTTACCCTTA CCCTTACCC TTACCCTTACCCT	0.3µM
Forward 36B4 (36B4F)	CAGCAAGTGGGAAGGT GTAATCC	0.3µM
Reverse 36B4 (36B4R)	CCCATTCTATCATCAAC GGGTACAA	0.5µM

Table 2.10. Standard DNA sequences

DNA	Sequence
36B4	CAGCAAGTGGGAAGGTGTAATCCGTCTCCACAGACAAGGCC AGGACTCGTTTGTACCCGTTGATGATAGAATGGG
Telomere	(TTAGGG) ¹⁴

2.10.6 qPCR Run Method

All qPCR run cycles were started at 95°C for 10 minutes for the initial denaturing stage, and then proceeded into 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds (annealing stage), and 72°C for 30 seconds (extending stage). Each reaction was followed with the default setting for melt curve analysis on the ABI 7500 RT-PCR machine.

2.10.7 Quality control and salivary telomere length calculations

The concentration of the telomere standards were converted to their corresponding Log_{10} Telomere length in kbp using Avagadro's constant by following the method of O'Callaghan and Fenech (2011). Table 2.11 shows the calculation of the telomere standard across it's serial dilution. This calculation was based on the principle that the oligomer standard is 84 bp in length with a molecular weight of 26667.2, and the weight of one molecule is equal to the molecular weight/Avogadro's number. Similarly, this process was repeated for the single copy gene standard (Table 2.12), based on the principle that the synthesised 36B4 oligomer standard is 75 bp in length with a MW of 23268.1. Next, the average cycle threshold (C_t) values of each standard and sample was calculated in MS Excel. The average C_t of the standards were imported into MS Excel to generate two linear curves (Figure 2.10). Standard curves were checked for qPCR efficiency using the Thermofisher qPCR efficiency calculator to ensure an efficiency between 90-110% was achieved. Reactions with an efficiency outside this range were repeated. Reactions with positive amplification signals in the NTC were also repeated. In total, qPCR analysis was adequately conducted over 18 runs. The average r-squared value for linear standard curves was 0.99 and the average qPCR efficiency was 98.88%. The equations of linear standard curves were used to calculate Log_{10} Telomere length and the number of 36B4 amplicons in a haploid genome. Absolute telomere length was calculated by dividing Log_{10} Telomere length by the respective value of Log_{10} 36B4 amplicons for each participant.

Table 2.11 Conversion of telomere standard concentrations into kbp (Courtesy of Dr Shahid Chaudhary, University Hospital Zurich).

Concentration	concentration in ng	molecules in gm	Mol weight/Avogadros number	Molecules of oligomer in gm std	Amount of telomere*84	Amount of telomere (Kbp)	log₁₀ (TL/Kb)
50	1.00E-09	5.00E-08	4.40E-20	1.14E+12	9.55E+13	9.55E+10	10.97979661
5	1.00E-09	5.00E-09	4.40E-20	1.14E+11	9.55E+12	9.55E+09	9.979796614
0.5	1.00E-09	5.00E-10	4.40E-20	1.14E+10	9.55E+11	9.55E+08	8.979796614
0.05	1.00E-09	5.00E-11	4.40E-20	1.14E+09	9.55E+10	9.55E+07	7.979796614
0.005	1.00E-09	5.00E-12	4.40E-20	1.14E+08	9.55E+09	9.55E+06	6.979796614
0.0005	1.00E-09	5.00E-13	4.40E-20	1.14E+07	9.55E+08	9.55E+05	5.979796614
0.00005	1.00E-09	5.00E-14	4.40E-20	1.14E+06	9.55E+07	9.55E+04	4.979796614
0.000005	1.00E-09	5.00E-15	4.40E-20	1.14E+05	9.55E+06	9.55E+03	3.979796614
0.0000005	1.00E-09	5.00E-16	4.40E-20	1.14E+04	9.55E+05	9.55E+02	2.979796614

Table 2.12 Conversion of 36B4 standard concentrations into kbp (Courtesy of Dr Shahid Chaudhary, University Hospital Zurich).

Concentration	concentration in ng	molecules in gm	Mol weight/Avogadros number	Molecules of oligomer in gm std	Amount of 36B4 amplicons in diploid genome	Log₁₀ 36B4 amplicons
50	1.00E-09	5.00E-08	3.80E-20	1.32E+12	6.58E+11	10.818156412
5	1.00E-09	5.00E-09	3.80E-20	1.32E+11	6.58E+10	9.818156412
0.5	1.00E-09	5.00E-10	3.80E-20	1.32E+10	6.58E+09	8.818156412
0.05	1.00E-09	5.00E-11	3.80E-20	1.32E+09	6.58E+08	7.818156412
0.005	1.00E-09	5.00E-12	3.80E-20	1.32E+08	6.58E+07	6.818156412
0.0005	1.00E-09	5.00E-13	3.80E-20	1.32E+07	6.58E+06	5.818156412
0.00005	1.00E-09	5.00E-14	3.80E-20	1.32E+06	6.58E+05	4.818156412
0.000005	1.00E-09	5.00E-15	3.80E-20	1.32E+05	6.58E+04	3.818156412
0.0000005	1.00E-09	5.00E-16	3.80E-20	1.32E+04	6.58E+03	2.818156412

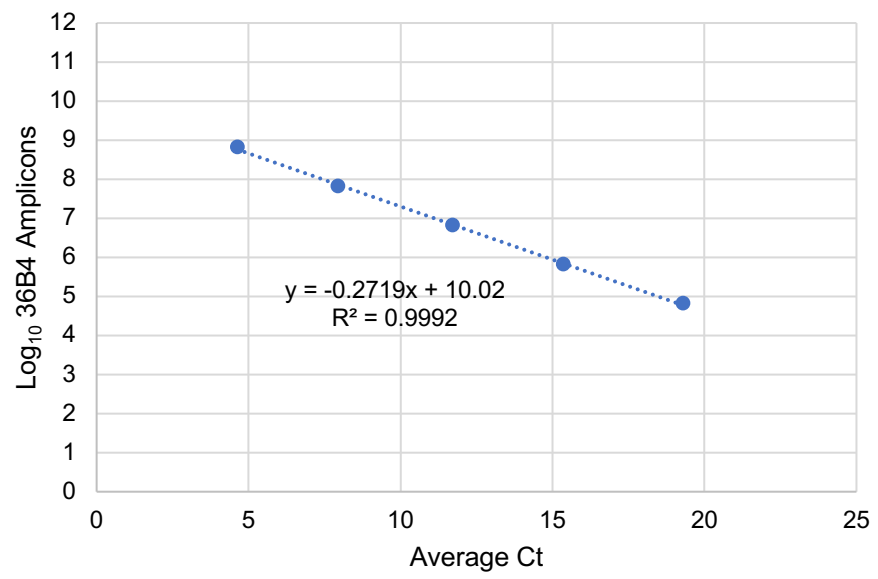
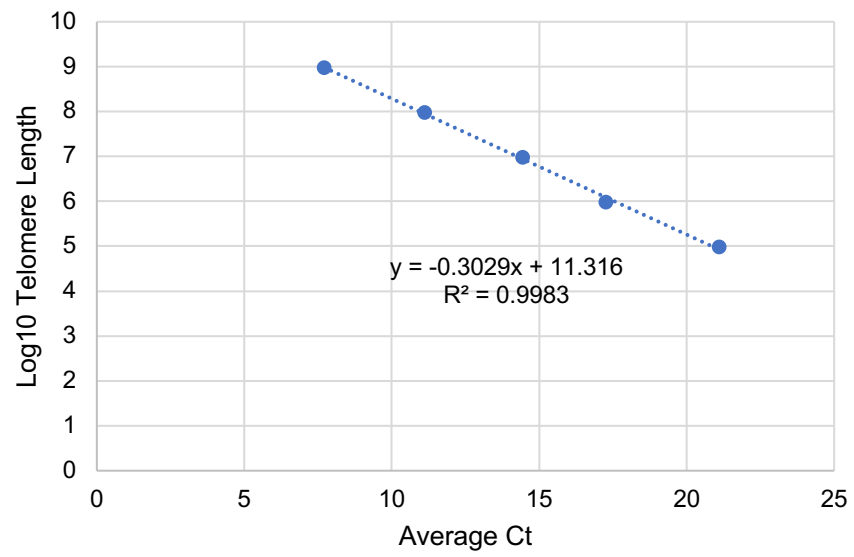


Figure 2.10 Typical qPCR standard curves

2.11 Statistical analysis

A total of 132 participants were recruited for statistical analysis. Participants were excluded due to specific exclusions criteria (Table 2.3) and due to missing data. Secondly, the ROUT test was applied to remove outliers from the dataset. The Q (false discovery rate) value was set at 0.2% in order to exclude data with most confidence and reduce false positive associations (Motulsky, & Brown, 2006). The test was applied to all dependent variables (CRP, vitamin D, 8-OHdG, DNA damage in the buccal mucosa and telomere length) leading to a further exclusion of 20 participants. The final sample size for statistical analysis was 112.

As part of the primary analysis, participants were arranged into two groups based on the RCPCH classification of BMI percentiles as non-obese ($>0.4^{\text{th}}$ BMI percentile $<91^{\text{st}}$) or obese ($3.33 > \text{BMI Z-score}$). As this investigation compared two extreme groups (omitting underweight and overweight participants), a normal gaussian distribution was not expected (Appendix XI) and the two-sample t-test with Welch's correction was considered for the analysis of means at a confidence level of 95%.

Furthermore, participants were classified as obese and non-obese via body fat centiles (Table 2.13) and waist circumference centiles (Table 2.14) and the same statistical analysis of means was repeated. In order to assess correlations between biomarkers and anthropometric ranks, the Spearman rank correlation test was selected and applied also at 95% confidence.

Next, based upon the results from the Spearman rank analysis, the most significant data was assessed for multiple linear regression to formulate a

prediction model. Due to co-linearity between anthropometric markers, only one anthropometric was selected to create this model (body fat %).

Finally, participants with a total DNA damage in the buccal mucosa above the average frequency identified in healthy children (Thomas, *et al.*, 2009) were separated from those with a frequency of DNA damage below the average frequency for a retrospective analysis of odds ratio. Five risk factors were established based on recommended cut-offs or the upper 95%CI of the average value for each biomarker assessed (Table 2.15). Odds ratio was calculated using the Baptisa-Pike method and the Fisher's exact test was used to assess significance at a confidence level of 95%.

All statistical analysis described above were completed in GraphPad Prism v7.0.

Table 2.13 Child Growth Foundation classifications of body fat % for males and females aged 5-18 years (McCarthy, *et al.*, 2006).

Body Fat % centile	Interpretation
$\leq 2^{\text{nd}}$	Under Fat
$2^{\text{nd}} > \text{BF}\% < 85^{\text{th}}$	Healthy Fat
$\geq 85^{\text{th}} \text{BF}\% < 95^{\text{th}}$	Over Fat
$\geq 95^{\text{th}}$	Obese

Table 2.14 Child Growth Foundation classifications of waist circumference centiles for males and females aged 5-20 years (McCarthy, *et al.*, 2001).

Waist circumference centile	Interpretation
$< 0.4^{\text{th}}$	Underweight
$\geq 0.4^{\text{th}} \text{WC} < 91^{\text{st}}$	Healthy weight
$\geq 91^{\text{st}} \text{WC} < 98^{\text{th}}$	Overweight
$\geq 98^{\text{th}}$	Obese

Table 2.15 Risk factors and criteria for odds ratio analysis

Risk Factor	Cut-off	Classified by
Obesity	BMI >98 th centile	RCPCH
Obesity	Body fat % >95 th centile	Child Growth Foundation
Obesity	Waist circumference >95 th centile	Child Growth Foundation
Inflammation	CRP >2248 pg/mL	Upper 95% CI
Vitamin D deficiency	Vitamin D <6.229 ng/mL	Upper 95% CI

3. Results

3.1 Participant Demographics

Data analysis was conducted on a total of 112 participants aged 10-18 years. Primarily, participants were classified into control (healthy weight) and case (obese) via the RCPCH classification system. Under this classification system, the total number of control participants was 58 and the total number of obesity cases was 54 (Table 3.1.1).

In order to detect a possible sex bias, the Mann-Whitney test was applied at a significance level of 95%. The test confirmed that there were no significant differences in the number of male and female participants across and within both cohorts ($p>0.99$) (Figure 3.1.1).

Table 3.1.1 Contingency analysis of sex across cohorts arranged by RCPCH classification of obesity status (Chi-squared test, $n=112$).

	Controls (Non-Ob)	Cases (Ob)	Difference between Means	p-value
Sex (n)				
<i>Males</i>	27	23	NS	>0.9999
<i>Females</i>	31	31		
<i>Total n</i>	58	54		

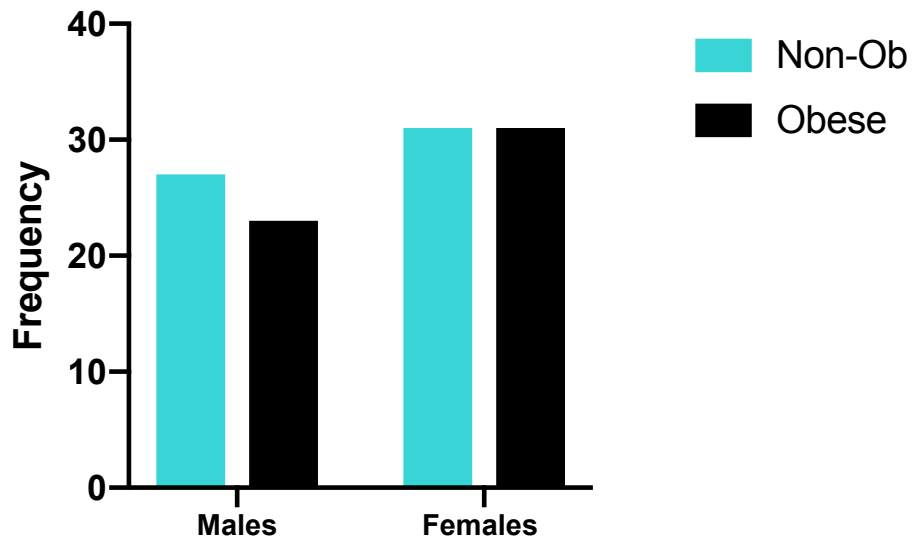


Figure 3.1.1 There is no significant difference between sex across and within cohorts via the Mann-Whitney test, $p > 0.999$, $n = 112$.

Furthermore, the demographic means of control and case participants were also analysed to deduce differences in age and anthropometric indices. The average age of the participants in the healthy weight cohort was 13.6 years and the average age of the obese group was 14.7 years. Analysis of this data via the t-test with Welch's correction reveals that participants in the obese cohort were on average 1.15 years older than the healthy weight group ($p < 0.01$) (Table 3.1.2). In addition, all anthropometric indices of adiposity were significantly greater in the obese group when compared to the non-obese cohort ($p < 0.0001$).

Table 3.1.2 Participant age and anthropometric assessments by RCPCH classification of obesity status, (t-test with Welch's correction, n=112).

	Range		Mean \pm SD		Difference between Means	p-value
	Controls (Non-Ob)	Cases (Ob)	Controls (Non-Ob)	Cases (Ob)		
Age (years)	10-18	10-18	13.55 \pm 2.32	14.71 \pm 2.16	**1.154	0.0075
BMI	15.1 – 25.0	25.2 - 63.6	19.72 \pm 2.677	40.49 \pm 10.08	****20.78	<0.0001
BMI (z-score)	-2.3 – 1.53	1.99 - 4.86	0.29 \pm 0.93	3.48 \pm 0.81	****3.189	<0.0001
BF (%)	5 – 34.8	25.3 - 58.9	22.87 \pm 6.74	43.18 \pm 7.58	****20.31	<0.0001
WC (mm)	520 – 870	430 - 1590	697.1 \pm 68.25	1090 \pm 313.9	****392.6	<0.0001
WHR	0.68 – 0.97	0.57 - 1.38	0.812 \pm 0.066	0.907 \pm 0.106	****0.0955	<0.0001

In this research, participants were recruited from multiple ethnic groups. These groups have been reported as African, Arab, Caucasian, South Asian and mixed ethnicity. To deduce differences in the frequency of ethnicities within the case and control cohorts, the Chi-squared test was employed at a significance level of 95%. As per Figure 3.1.2, there were no significant differences in the number of participants from each ethnic group between the obese and control cohort. Overall, the total number of Caucasian participants was significantly greater than the number of participants from the Arab and Mixed ethnic groups ($p < 0.05$).

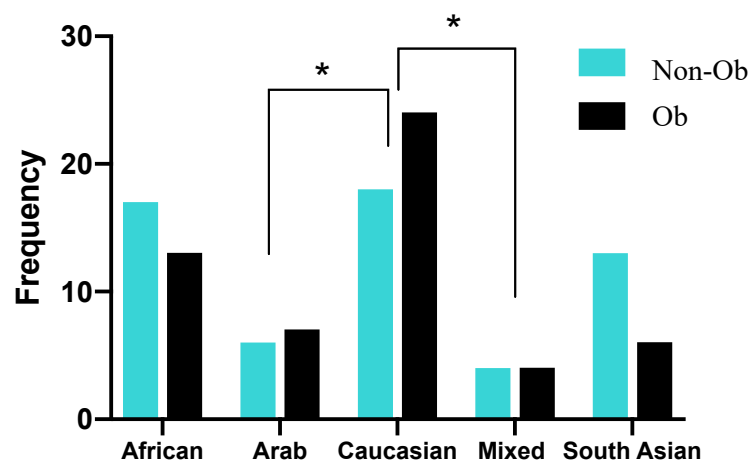


Figure 3.1.2 Ethnic distribution across obese and non-obese cohorts classified by BMI Z-score. Chi-square test analysis reveals no significant difference in ethnicities between case and control groups ($p=0.4185$). There is a higher frequency of total Caucasian participants compared to Arab ($*p=0.03$) and Mixed-ethnicity ($*p=0.0162$), $n=112$.

To assess agreement between the different methods of measuring obesity, each anthropometric parameter was divided into quartiles and compared against the primary parameter of BMI Z-score. Participants that were within the same or adjacent quartile were considered to be appropriately classified. Participants that were more than one quartile away from their classification based on their BMI Z-score, were considered to be 'grossly misclassified'. The assessment of body fat percentage showed most agreement with BMI Z-score as all participants were within the same or adjacent quartile (Table 3.1.3). Waist circumference was also an appropriate measure of adiposity as it classified 97.3% of participants in agreement with BMI Z-score and only 2.7% were 'grossly misclassified'. The greatest 'gross misclassification' occurred when participants were defined via waist to hip ratio (WHR, 13.4%).

Table 3.1.3 Percentage of agreement between anthropometric markers and BMI Z-score across participants.

	Same quartile classification	Adjacent quartile classification	Gross misclassification
<i>Body fat (%)</i>	61	39	0
<i>WC (mm)</i>	65	32.3	2.7
<i>WHR</i>	36.6	50	13.4

To further assess agreement between anthropometric indices, the Spearman rank correlation test was applied (Figure 3.1.3). As per Table 3.1.4, the analysis indicated a significant correlation between all anthropometric measures ($p < 0.001$). However, the strongest correlation existed between BMI Z-score and BF%, and the weakest correlations were between WHR and all other anthropometric assessments.

Table 3.1.4 R-squared values as per Spearman rank correlation analysis between anthropometric markers, $p < 0.0001$, $n = 12$.

	BMI Z vs BF %	BMI Z vs WC	BMI Z vs WHR	BF% vs WC	BF% vs WHR	WC vs WHR
<i>R squared</i>	0.8900	0.8668	0.5245	0.7955	0.4367	0.5830

Finally, in order to assess the clinical applicability of different obesity classification systems, participants were also classified as obese or non-obese via the Child Growth Foundation (CGF) cut-offs for bioelectrical impedance and waist circumference (Table 2.11.2 and 2.11.3). The obese cohort defined by body fat percentage consisted of 49 participants whereas the obese cohort defined by CGF criteria for waist circumference consisted of 55 participants (Table 3.1.5).

Table 3.1.5 Participant numbers in obese and non-obese cohorts when defined by three different classifications systems.

	Non-Obese (n)	Obese (n)
<i>BMI (Z-score)</i>	58	54
<i>BF (%)</i>	63	49
<i>WC (mm)</i>	57	55

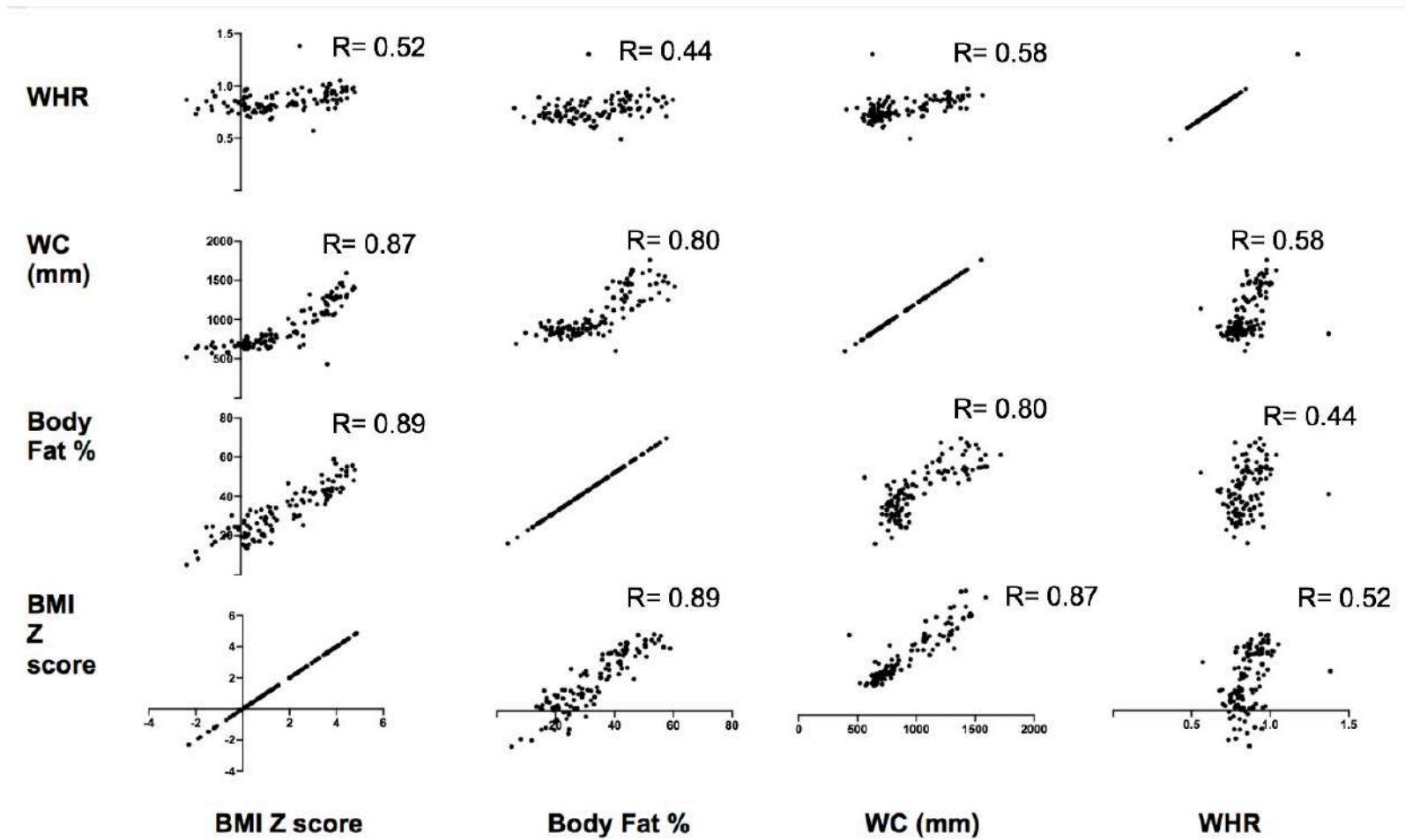


Figure 3.1.3 Correlation matrix of all anthropometric indices. Spearman correlation analysis indicates a strong correlation between all indices (**** $p < 0.0001$, $n = 112$).

3.2 Analysis of inflammation status - salivary C-reactive protein

For the purpose of assessing inflammation, C-reactive protein (CRP) level was measured in saliva. Across all 112 participants, salivary CRP was detected in the range of 341.79pg/ml to 7789.2 pg/ml.

Spearman rank correlation analysis was applied to detect associations between multiple anthropometric indices and inflammation status (Figure 3.2.1). The analysis indicated that levels of CRP in saliva were positively and significantly correlated with BMI Z-score and body fat percentage ($p < 0.01$) but had no significant correlation with waist circumference and waist to hip ratio. Salivary CRP levels were more strongly correlated with BMI-Z score than body fat percentage, indicated by a greater R value (Table 3.2.1).

Table 3.2.1. Spearman rank correlation analysis between salivary CRP and anthropometric assessments (n=112).

CRP (pg/ml)	BMI Z score	Body fat (%)	WC (mm)	WHR
<i>R value</i>	0.2920	0.2619	0.1780	0.1089
<i>95% CI</i>	0.107 – 0.458	0.075 - 0.431	-0.015- 0.357	-0.085 - 0.292
<i>p value</i>	0.0018	0.0053	0.0624	0.2601
<i>Significance</i>	**	**	ns	ns

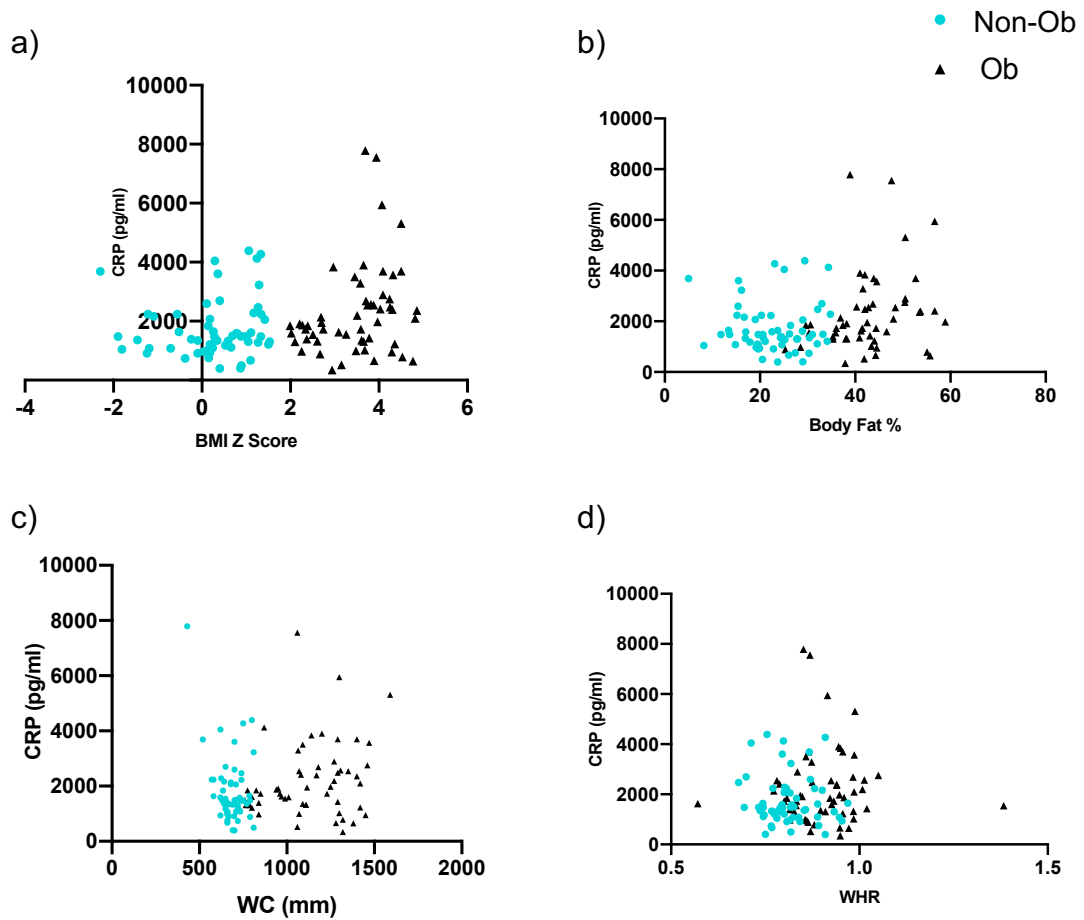


Figure 3.2.1 Spearman rank correlation analysis between anthropometric indices and salivary CRP (pg/ml) across all 112 participants coded as obese or non-obese via BMI percentile. a) there is a significant positive correlation between BMI Z-score and CRP ($R=0.292, p<0.01$), b) there is a significant positive correlation between body fat (%) and CRP ($R=0.262, p<0.01$), c) there is no significant correlation between WC (mm) and CRP ($p>0.05$), d) there is no significant correlation between WHR and CRP ($p>0.05$).

Furthermore, the Welch's t-test was applied to the average value of salivary CRP when participants were divided based on their BMI, BF and WC percentile (Figure 3.2.2). The Welch's t-test analysis indicated that average salivary CRP were significantly higher in the cohort of children with obesity when classified by BMI ($p < 0.05$) and body fat percentage ($p < 0.01$), whereas the difference in salivary CRP when participants are classified via waist circumference was not significant. The most significant difference in salivary CRP was seen in children with a body fat percentage above 95th centile, who had a 1.4x greater concentration than children with a body fat percentage below the 95th centile ($p < 0.01$) (Table 3.2.2).

Table 3.2.2. Welch's t-test analysis of average salivary CRP between Non-obese and Obese cohorts classified by BMI, BF % and WC percentile.

CRP	Non-Obese BMI < 91st	Obese BMI > 98th	Non-Obese BF% < 95th	Obese BF% > 95th	Non-Obese WC < 98th	Obese WC > 98th
<i>n</i>	58	54	63	49	57	55
<i>Average (pg/ml) ± SD</i>	1724 ± 972.5	2300 ± 1556	1650 ± 972.0	2276 ± 1477	1778 ± 1232	2211 ± 1373
<i>Difference in means (pg/ml)</i>	576.3		626		411.2	
<i>p value</i>	0.0222		0.0082		0.0985	
<i>Significance</i>	*		**		ns	

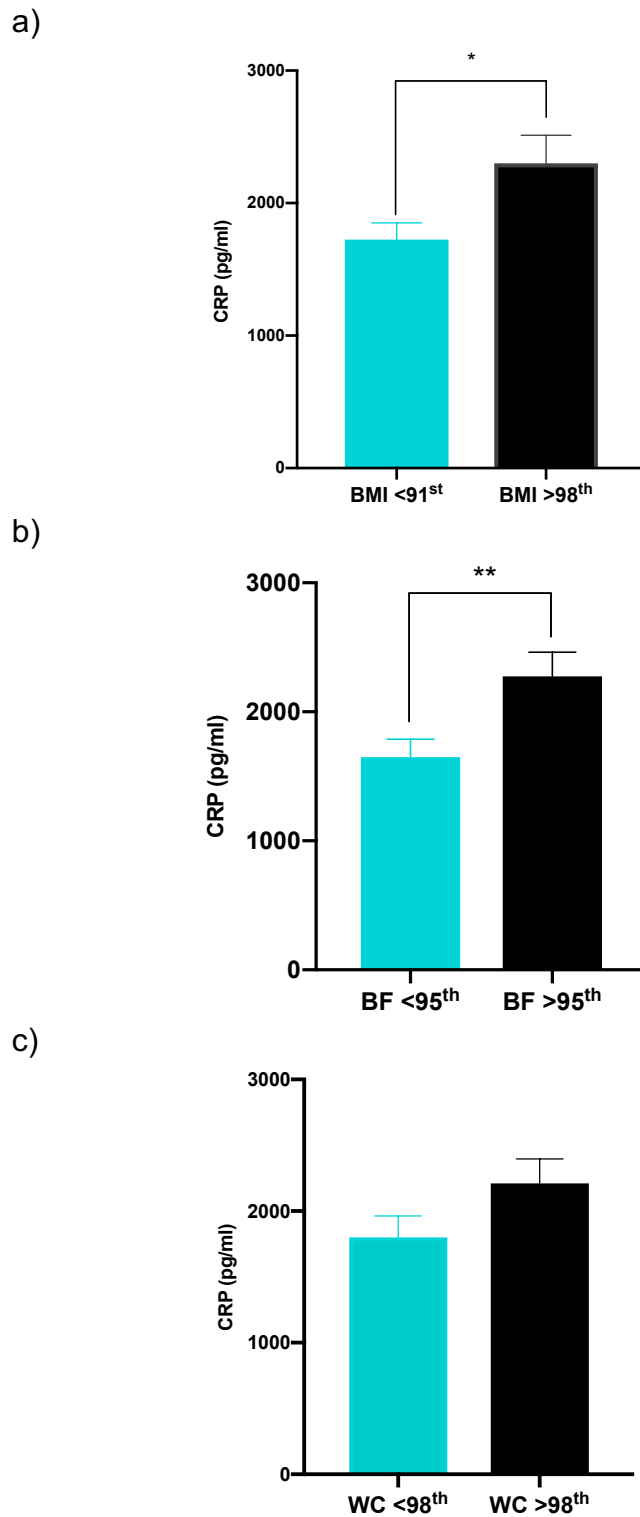


Figure 3.2.2 Welch's t-test of average CRP levels. a) average CRP is higher in the obese (n=54) cohort when compared to the non-obese cohort (n=58) when participants are classified by **BMI percentiles** (*p<0.05), b) average CRP is higher in the obese cohort (n=49) when compared to the non-obese cohort (n=63) classified by **body fat percentiles** (**p<0.01), c) no significant difference in average CRP in the obese cohort (n=55) when compared to the non-obese cohort (n=57) classified by **waist circumference percentiles** (p>0.05).

3.3 Analysis of micronutrient status - salivary vitamin D

To determine micronutrient status in obesity, Vitamin D levels were detected in saliva. There were six participants that had salivary vitamin D levels that could not be interpolated from the standard curve. Five of these participants were classified as obese via BMI Z-score. All six participants were removed from the data set for statistical analysis in this section. Across the 106 participants, the range of Salivary vitamin D was 0.07ng/ml to 17.44ng/ml.

Firstly, spearman rank correlation analysis was applied to detect correlations between multiple anthropometric indices and salivary vitamin D status (Figure 3.3.1). The analysis indicates all anthropometric markers were significantly and negatively correlated with salivary vitamin D ($p < 0.01$). Based on an analysis of R-values, body fat percentage had the most significant inverse correlation with salivary vitamin D ($R = -0.32$, $p < 0.001$), followed by WHR ($R = -0.262$, $p < 0.01$) (Table 3.3.1).

Table 3.3.1. Spearman rank correlation analysis between salivary vitamin D and anthropometric assessments (n=106).

<i>Vitamin D (ng/mL)</i>	BMI Z score	Body fat (%)	WC (mm)	WHR
<i>R value</i>	-0.2512	-0.3206	-0.2523	-0.2618
<i>95% CI</i>	-0.426 - -0.058	-0.486 - -0.133	-0.427 - -0.059	-0.436 - -0.071
<i>p value</i>	0.0094	0.0008	0.0091	0.005
<i>Significance</i>	**	***	**	**

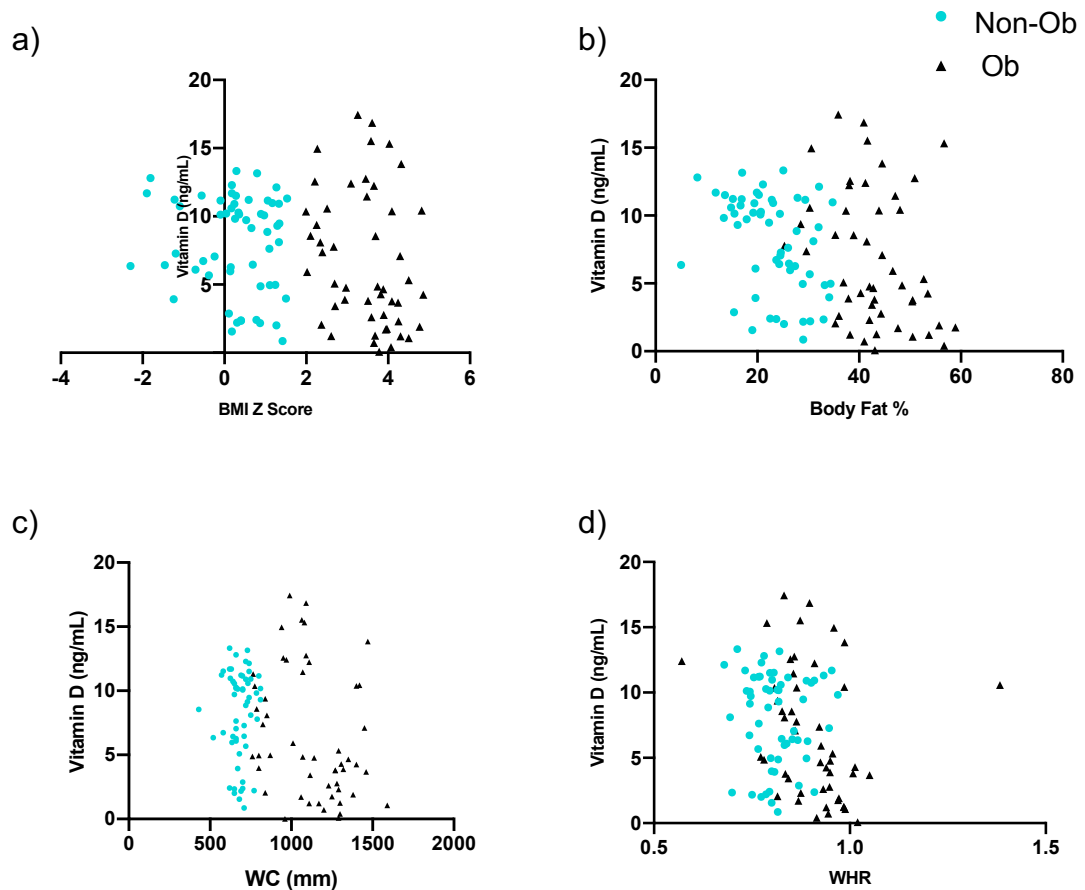


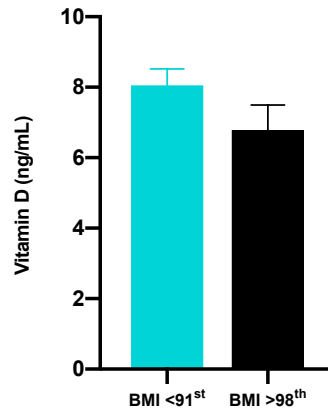
Figure 3.3.1 Spearman rank correlation analysis between salivary vitamin D and anthropometric indices across all 112 participants coded as obese or non-obese via BMI percentile. a) there is a significant inverse correlation between BMI Z-score and vitamin D ($R=-0.25$, $p<0.01$), b) there is a significant inverse correlation between body fat (%) and vitamin D ($R=-0.32$, $p<0.001$), c) there is a significant inverse correlation between WC (mm) and vitamin D ($R=-0.25$, $p<0.01$), d) there is a significant inverse correlation between WHR and vitamin D ($R=-0.26$, $p<0.01$).

Furthermore, the Welch's t-test was applied to the average value of salivary vitamin D when participants were divided based on their BMI, BF and WC percentile (Figure 3.3.2). Average salivary vitamin D levels were significantly lower in obese cohorts classified by body fat percentage ($p < 0.05$). Participants with a body fat percentage above the 95th centile had a -1.94ng/ml average decrease in salivary vitamin D compared to participants with a body fat percentage below the 95th centile (Table 3.3.2). Although salivary vitamin D levels were lower in participants with obesity as classified by BMI or waist circumference, these results were not statistically significant.

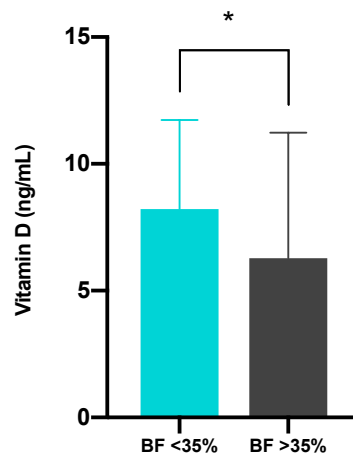
Table 3.3.2. Welch's t-test analysis of average salivary vitamin D between Non-obese and Obese cohorts classified by BMI, BF % and WC percentile.

<i>Vitamin D (ng/mL)</i>	Non-Obese BMI<91st	Obese BMI>98th	Non-Obese BF%<95th	Obese BF%>95th	Non-Obese WC<98th	Obese WC>98th
<i>n</i>	57	49	62	48	55	51
<i>Average ± SD</i>	8.056 ± 3.54	6.79 ± 4.96	8.214 ± 3.522	6.276 ± 4.952	8.256 ± 3.969	6.624 ± 4.477
<i>Difference in means</i>	-1.266		-1.938		-1.632	
<i>p value</i>	0.1397		0.024		0.0505	
<i>Significance</i>	ns		*		ns	

a)



b)



c)

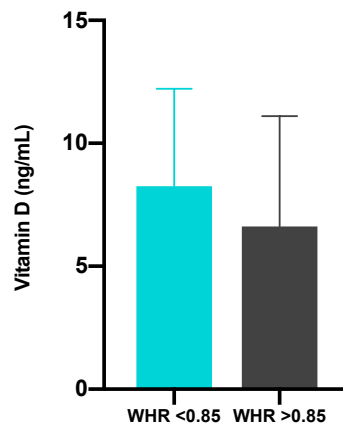


Figure 3.3.2 Welch's t-test of average vitamin D (VD) levels. a) average VD is not significantly lower in the obese (n=49) cohort when compared to the non-obese cohort (n=57) when participants are classified by **BMI percentiles** ($p>0.05$), b) average VD is significantly lower in the obese cohort (n=48) when compared to the non-obese cohort (n=62) classified by **body fat percentiles** ($*p<0.05$), c) average VD is not significantly lower in the obese cohort (n=51) when compared to the non-obese cohort (n=55) classified by **waist circumference percentiles** ($p>0.05$).

3.4 Urinary 8-OHdG Analysis

For the purpose of assessing levels of oxidative DNA damage in obesity, 8-OHdG was measured in urine samples. Across all 112 participants, urinary 8-OHdG was detected in a range of 18.8ng/ml – 545.99ng/ml.

Spearman rank correlation analysis was applied between multiple anthropometric indices and urinary 8-OHdG (Figure 3.4.1). The analysis revealed that urinary 8-OHdG was significantly and positively correlated with waist circumference ($p < 0.05$) and waist to hip ratio ($p < 0.01$), but not with BMI or body fat percentage. Based on an analysis of R-values, urinary 8-OHdG levels were most strongly correlated with WHR (Table 3.4.1).

Table 3.4.1. Spearman rank correlation analysis between urinary 8-OHdG and anthropometric assessments (n=112).

8-OHdG (ng/mL Creatinine)	BMI Z score	Body fat (%)	WC (mm)	WHR
<i>R value</i>	0.1633	0.1459	0.3100	0.2720
<i>95% CI</i>	-0.029 - 0.344	-0.046 - 0.328	0.473 - 0.127	0.0856 - 0.44
<i>p value</i>	0.0854	0.1248	0.0287	0.0037
<i>Significance</i>	ns	ns	*	**

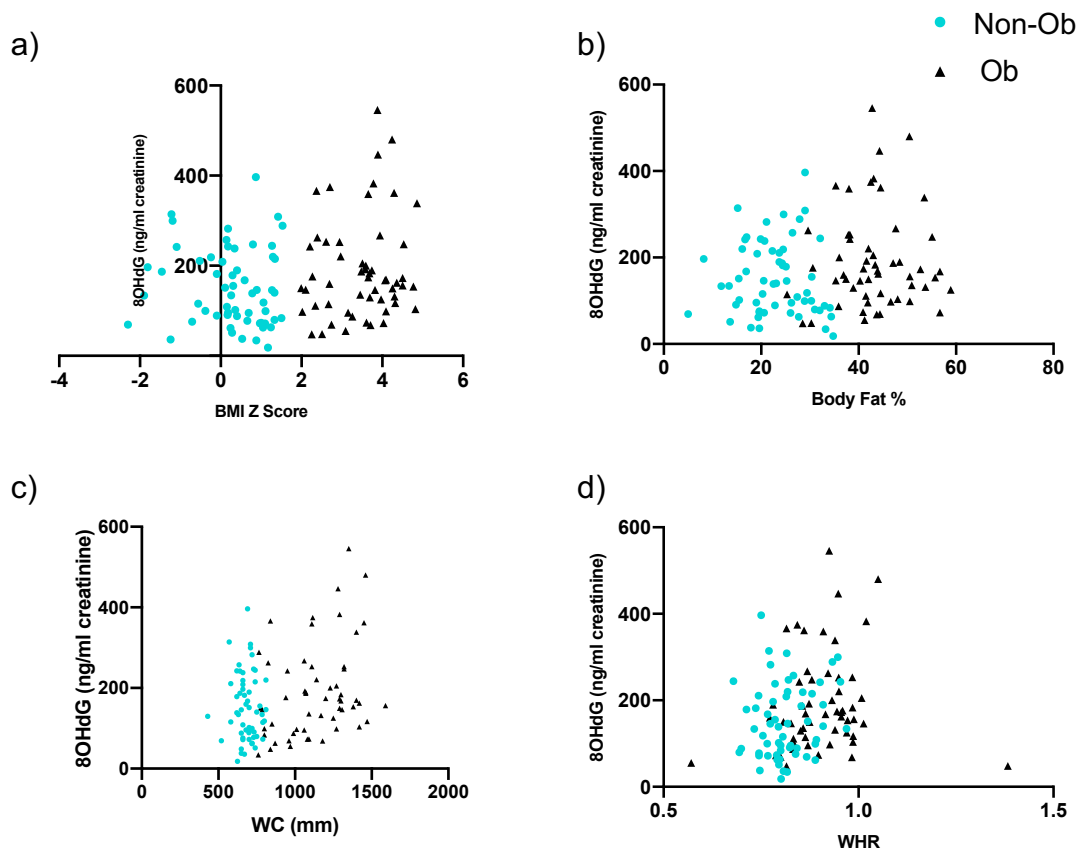


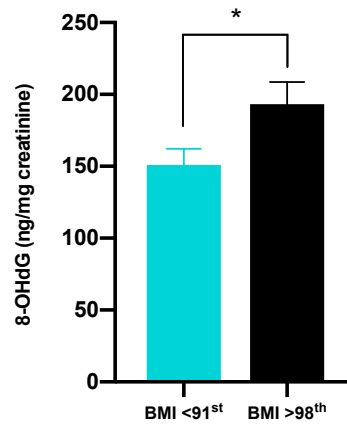
Figure 3.4.1 Spearman rank correlation analysis between urinary 8-OHdG and anthropometric indices across all 112 participants coded as obese or non-obese via BMI percentile. a) there is no significant correlation between BMI Z-score and 8-OHdG ($R=0.163$, $p>0.05$), b) there is no significant between body fat (%) and 8-OHdG ($R=0.146$, $p>0.05$), c) there is a significant inverse correlation between WC (mm) and 8-OHdG ($R=0.31$, $p<0.05$), d) there is a significant inverse correlation between WHR and 8-OHdG ($R=0.27$, $p<0.01$).

Furthermore, the Welch's t-test was applied to the average value of urinary 8-OHdG when participants were divided based on their BMI, BF and WC percentile (Figure 3.4.2). Obesity, when classified by BMI and waist circumference was associated with increased urinary 8-OHdG ($p < 0.05$). No significant difference was identified in average 8-OHdG levels when participants were classified by their body fat percentage. An analysis of the difference in means indicates that classification by BMI gave the largest and most significant difference in mean 8-OHdG levels. The obese participants had on average a x1.28 greater level of urinary 8-OHdG compared to non-obese participants.

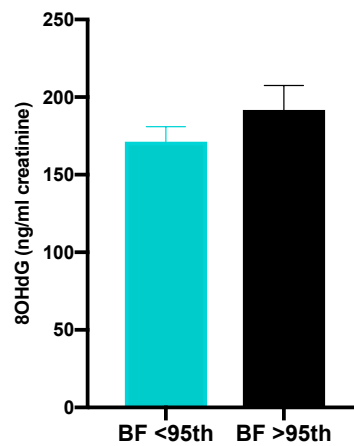
Table 3.4.2. Welch's t-test analysis of average urinary 8-OHdG between Non-obese and Obese cohorts classified by BMI, BF % and WC percentiles.

8-OHdG (ng/mL Creatinine)	Non- Obese BMI<91st	Obese BMI>98th	Non-Obese BF%<95th	Obese BF%>95th	Non-Obese WC<98th	Obese WC>98th
<i>n</i>	58	54	49	63	57	55
<i>Average ± SD</i>	150.9 ± 85.58	193.2 ± 114.0	171.3 ± 102.0	191.8 ± 116.1	151.5 ± 82.62	191.8 ± 116.1
<i>Difference in means</i>	42.27		20.48		40.25	
<i>p value</i>	0.0296		0.2681		0.0377	
<i>Significance</i>	*		ns		*	

a)



b)



c)

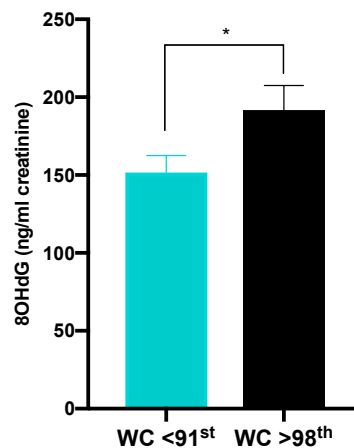


Figure 3.4.2 Welch's t-test of average 8-OHdG levels. a) average 8-OHdG is higher in the obese (n=54) cohort when compared to the non-obese cohort (n=58) when participants are classified by **BMI percentiles** (*p<0.05), b) average 8-OHdG is not significantly higher in the obese cohort (n=49) when compared to the non-obese cohort (n=63) classified by **body fat percentiles** (p>0.05), c) average 8-OHdG is higher in the obese cohort (n=55) when compared to the non-obese cohort (n=57) classified by **waist circumference percentiles** (*p<0.05).

Finally, to assess whether urinary 8-OHdG levels of each participant may be linked with their status of inflammation or salivary vitamin D, the Spearman rank correlation test was applied (Figure 3.4.3). The analysis revealed no correlation between salivary CRP and urinary 8-OHdG ($p=0.85$). There was a weak inverse correlation between urinary 8-OHdG and salivary vitamin D levels, but this was not statistically significant ($p=0.07$).

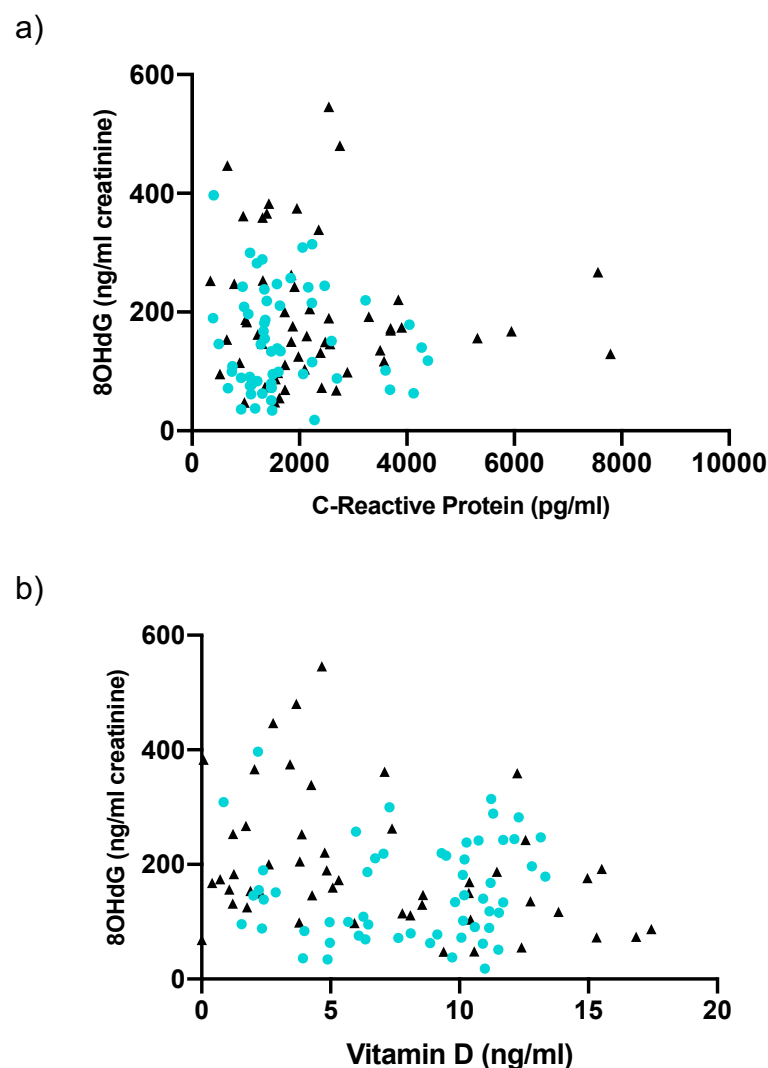


Figure 3.4.3 Spearman rank analysis indicates a) urinary 8-OHdG is not correlated with salivary CRP levels ($R=0.02$, $p>0.05$, $n=112$), b) urinary 8-OHdG is inversely but not significantly correlated with salivary vitamin D ($R= -0.17$, $p>0.05$, $n=106$).

3.5 Analysis of DNA damage in the buccal mucosa

In order to assess chromosomal instability and cytokinesis defects, 1000 differentiated cells from the buccal mucosa of each participant were scored for the frequency of cells with micronuclei (MNi), multiple nuclei (MNCs) and nuclear buds or bridges (NBuds/Bridges) (Figure 3.5.1). Overall, micronuclei frequency was in the range of 0-7 cells per 1000 buccal epithelial cells, multi-nucleated cell frequency was 2-24 cells per 1000 buccal epithelial cells and the frequency of nuclear buds/bridges was 0-10 cells per 1000 buccal epithelial cells. Overall, the most frequently noted abnormal cell morphology in the buccal mucosa was the retention of multiple nuclei.

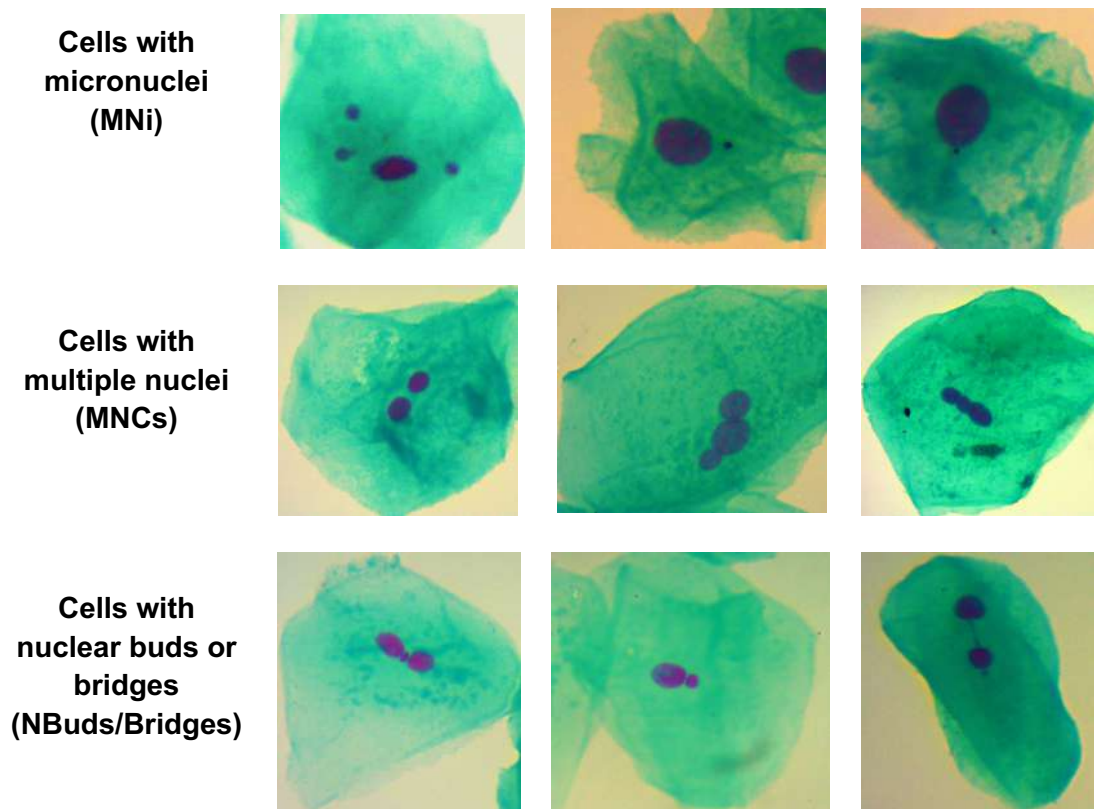


Figure 3.5.1 Abnormal nuclear morphologies (DNA damage markers) scored in buccal epithelial cells.

The frequency of each cell type was used to calculate a total DNA damage frequency for each participant. Spearman rank correlation analysis was applied between multiple anthropometric indices and the total DNA damage frequency in the buccal mucosa (Figure 3.5.2). The analysis indicated the total frequency of DNA damage markers in the buccal mucosa to be strongly and positively correlated with all markers of adiposity ($p < 0.001$). An analysis of the R-values revealed that the strongest correlation was between body fat percentage and total DNA damage (Table 3.5.1).

Table 3.5.1. Spearman rank correlation analysis between total DNA damage (%) in the buccal mucosa (BM) and anthropometric assessments (n=112).

Total DNA damage (%) in buccal mucosa	BMI Z score	Body fat (%)	WC (mm)	WHR
<i>R value</i>	0.4550	0.4940	0.4185	0.4085
<i>95% CI</i>	0.289 - 0.594	0.335 – 0.626	0.247 – 0.564	0.2359 - 0.556
<i>p value</i>	<0.0001	<0.0001	<0.0001	<0.0001
<i>Significance</i>	****	****	****	****

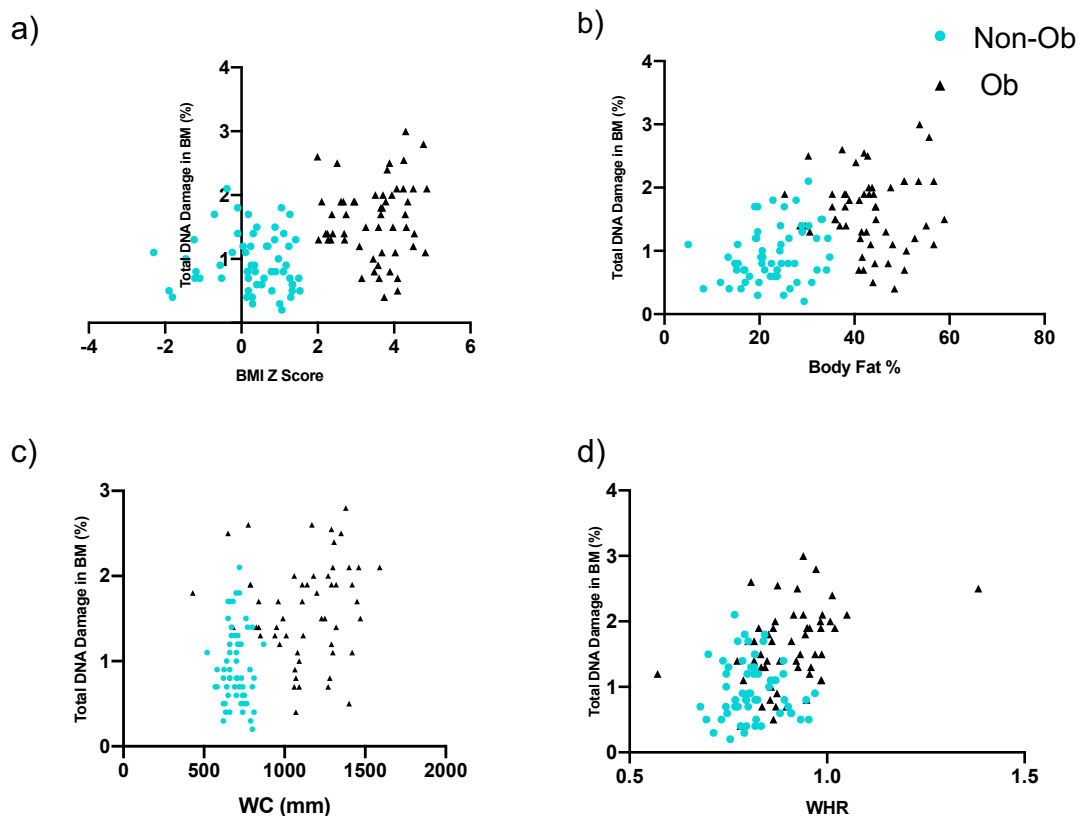


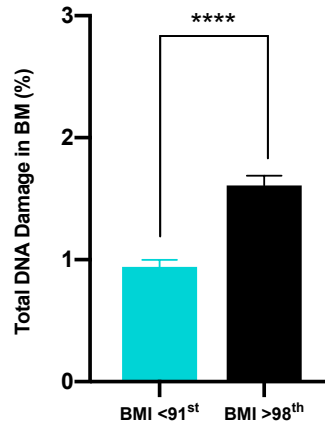
Figure 3.5.2 Spearman rank correlation analysis between anthropometric indices and total frequency of DNA damage in the buccal mucosa (%) across all 112 participants coded as obese or non-obese via BMI percentile. a) there is a significant and strong positive correlation between BMI Z-score and total DNA damage ($R=0.46$, $p<0.0001$), b) there is a significant and strong positive correlation between body fat (%) and total DNA damage ($R=0.49$, $p<0.0001$), c) there is a significant and strong correlation between WC (mm) and total DNA damage ($R=0.42$, $p<0.0001$), d) there is a significant and strong correlation between WHR and total DNA damage ($R=0.41$, $p<0.0001$).

Furthermore, the Welch's t-test was applied to the average total frequency of DNA damage in the buccal mucosa, when participants were divided based on their BMI, BF and WC percentile (Figure 3.5.3). Total DNA damage in the buccal mucosa was significantly higher in obesity categorised by BMI, body fat percentage and waist circumference percentiles. However, the largest difference was seen amongst the group classified by BMI percentiles (Table 3.5.2). Further analysis of this cohort revealed that the individual frequency of cells with micronuclei, multiple-nuclei, buds/bridges were all significantly higher in the obese cohort (Figure 3.5.4), with the largest difference being in the number of multi-nucleated cells (Table 3.5.3).

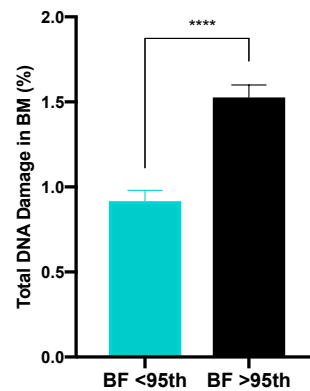
Table 3.5.2. Welch's t-test analysis of average total DNA damage (%) in the buccal mucosa between Non-obese and Obese cohorts classified by BMI Z score, BF % and WC.

Total DNA damage (%) in buccal mucosa	Non-Obese BMI<91st	Obese BMI>98th	Non-Obese BF%<95th	Obese BF%>95th	Non-Obese WC<98th	Obese WC>98th
<i>n</i>	58	54	49	63	57	55
<i>Average ± SD</i>	0.9414 ± 0.442	1.608 ± 0.594	0.9163 ± 0.445	1.526 ± 0.586	0.9982 ± 0.507	1.53 ± 0.588
<i>Difference in means</i>	0.667		0.6099		0.532	
<i>p value</i>	<0.0001		<0.0001		<0.0001	
<i>Significance</i>	****		****		****	

a)



b)



c)

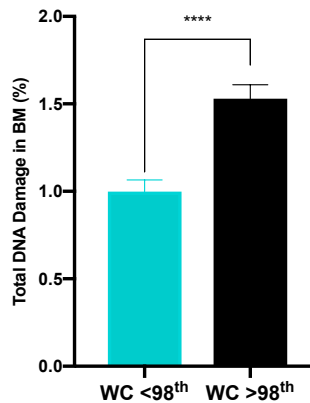


Figure 3.5.3 Welch's t-test of average total frequency of DNA damage in the buccal mucosa. a) the average frequency is higher in the obese (n=54) cohort when compared to the non-obese cohort (n=58) when participants are classified by **BMI percentiles** (****p<0.0001), b) average frequency is significantly higher in the obese cohort (n=49) when compared to the non-obese cohort (n=63) classified by **body fat percentiles** (****p<0.0001), c) average frequency is higher in the obese cohort (n=55) when compared to the non-obese cohort (n=57) classified by **waist circumference percentiles** (****p<0.0001).

Table 3.5.3. Welch's t-test analysis between frequency of individual DNA damage markers in the buccal mucosa (BM) per 1000 cells, across non-obese and obese cohorts, classified by BMI Z-Score

<i>DNA Damage marker in BM</i>	Non-obese (BMI <91st)	Obese (BMI >98th)	Difference in means	p-value
<i>MNi</i>	1.092 ± 1.042	2.030 ± 1.614	0.8778	***0.0003
<i>MNCs</i>	7.185 ± 3.477	10.23 ± 4.295	3.047	****<0.0001
<i>NBuds/Bridges</i>	1.077 ± 1.384	2.948 ± 2.691	1.871	****<0.0001

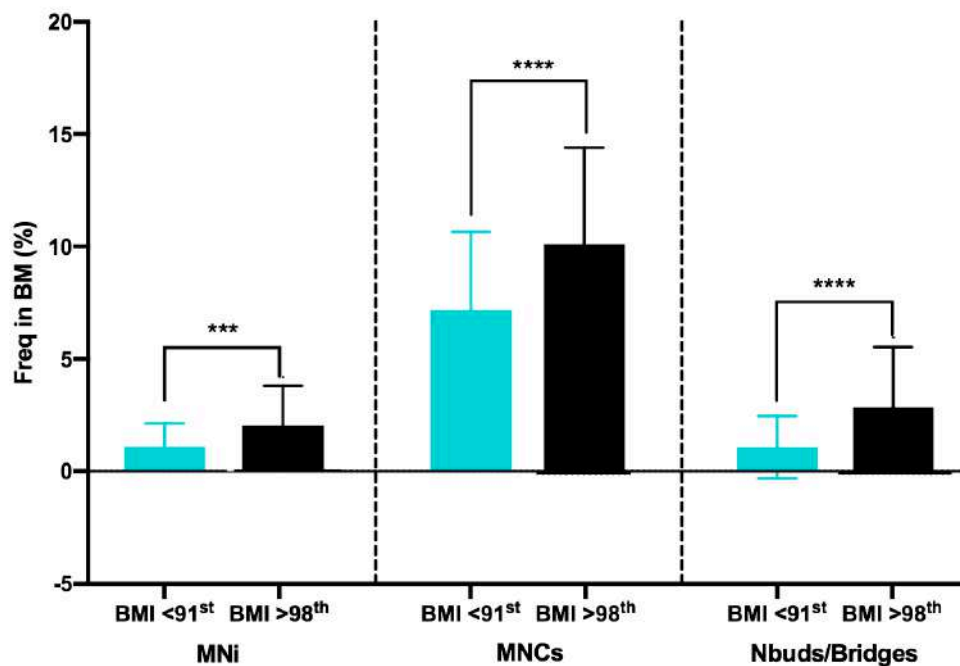


Figure 3.5.4. The average frequency of cells with micronuclei (MNi), multiple-nuclei (MNCs) and nuclear buds and bridges (NBuds/Bridges) is significantly greater in the buccal mucosa of the obese cohort when compared to non-obese (classified by BMI percentiles). Analysis using t-test with Welch's correction ***p=0.0003, ****p<0.0001, n=112, error bars are standard deviation.

To assess whether the total frequency of DNA damage in the buccal mucosa of each participant may be linked with their status of inflammation or salivary vitamin D, the Spearman rank correlation test was applied (Figure 3.5.5). The analysis indicated that the total frequency of DNA damage in the buccal mucosa was not associated with levels of salivary CRP ($p=0.45$). However, the total frequency of DNA damage in the buccal mucosa was strongly and inversely correlated with levels of salivary vitamin D ($p<0.0001$).

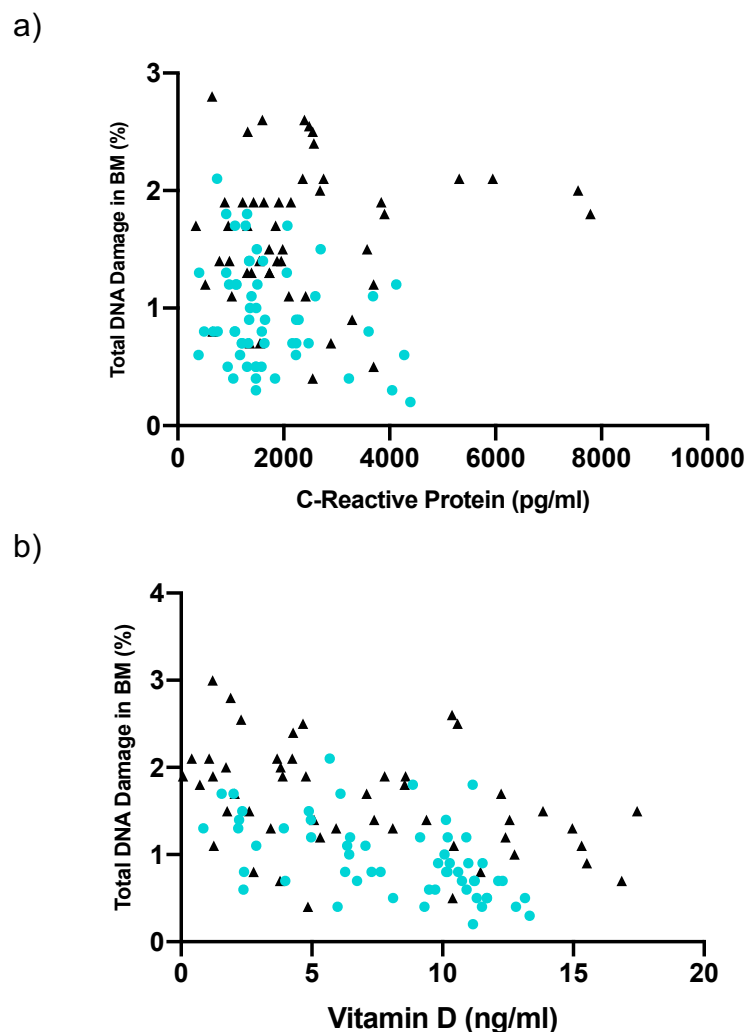


Figure 3.5.5 Spearman rank analysis indicates a) total DNA damage frequency in the buccal mucosa is not correlated with salivary CRP levels ($R=0.07$, $p>0.05$, $n=112$), b) total DNA damage frequency in the buccal mucosa is inversely and strongly correlated with salivary vitamin D ($R= -0.49$, $p<0.0001$, $n=106$).

In addition, multiple regression analysis was employed to create a prediction model for total DNA damage in the mucosa, based on the variables that were significantly correlated with this criterion. A significant model emerged: $F(3,108) = 17.38$, $p < 0.001$. The model explains 31% of the variance in the frequency of total DNA damage in the buccal mucosa. Table 3.5.4 gives information about regression coefficients for the predictor variables entered into the model. Body fat percentage is a significant predictor with a positive relationship to total DNA damage. Salivary vitamin D is also a significant predictor but has a negative relationship to total DNA damage. Based on this model, a one unit increase in body fat % would predict a 0.02 increase total nuclear anomalies, per 1000 cells in the buccal mucosa.. A one unit increase in vitamin D would predict a 0.04 decrease in total nuclear anomalies, per 1000 cells in the buccal mucosa.

Table 3.5.4 Multiple Regression Analysis best-fit model (adjusted $R^2=0.31$) for predicting total DNA damage in the buccal mucosa (%), $n=106$.

Model	Unstandardized Coefficients		t	Sig.
	B	Std. Error		
constant	1.19	0.3344	3.581	0.0005
Body Fat (%)	0.02	0.004235	4.866	<0.0001
Vitamin D (ng/mL)	-0.04	0.01152	3.425	0.0009

Finally, in order to predict the odds ratio of acquiring DNA damage in the buccal mucosa, each risk factor was analysed independently using the Fisher's exact test (Table 3.5.5). Five risk factors were established based on recommended cut-offs or the upper 95%CI of the average value for each biomarker assessed (Table 2.15). Odds ratio was calculated using the Baptisa-Pike method and the Fisher's exact test was used to assess significance at a confidence level of 95%. The results indicate that obesity, whether classified by BMI, body fat or waist circumference percentile, may increase the risk of total DNA damage in the buccal mucosa. Most significantly, a BMI above the 98th centile presented the largest odds ratio (OR=8.89, $p<0.0001$). Furthermore, a level of CRP in saliva above 2248pg/mL may independently increase the odds of DNA damage in the buccal mucosa by almost x9 fold, whereas vitamin D deficiency (<6.229 ng/mL) may increase the odds x7.5 fold ($p<0.0001$).

Table 3.5.5 Retrospective analysis of Odds Ratio using the Fisher's exact test, n=112.

<i>Risk Factor</i>	% children with elevated DNA damage in BM and risk factor present	% children with elevated DNA damage and risk factor absent	Odds ratio	95% CI	p-value
<i>BMI >98th centile</i>	74.07	24.14	8.980	3.75 – 20.52	<0.0001
<i>Body Fat >95th centile</i>	71.43	19	5.789	2.55 – 12.82	<0.0001
<i>Waist circumference >95th centile</i>	67.27	28.07	5.267	2.37 – 11.95	<0.0001
<i>CRP >2248 pg/mL</i>	71.19	22.64	8.441	3.53 – 19.41	<0.0001
<i>Vitamin D <6.229 ng/mL</i>	74.00	27.42	7.534	3.21 – 16.72	<0.0001

3.6 Analysis of salivary telomere length

Telomere length in saliva was assessed via qPCR as a marker of genome instability. Salivary telomere length across all 112 participants was in the range of 24.26 to 482.119 kb/diploid genome.

Spearman rank correlation analysis was applied between multiple anthropometric indices and salivary telomere length (Figure 3.6.1). The analysis indicated that telomere length in saliva was positively correlated with all anthropometric markers except WHR ($p < 0.01$). The most significant correlation existed between waist circumference and telomere length ($p < 0.0001$) (Table 3.6.1).

Table 3.6.1. Spearman rank correlation analysis between salivary telomere length (sTL) and anthropometric assessments (n=112).

<i>sTL</i> <i>(kb/diploid genome)</i>	BMI Z score	Body fat (%)	WC (mm)	WHR
<i>R value</i>	0.3695	0.3331	0.3932	0.1803
<i>95% CI</i>	0.192 - 0.5234	0.152 - 0.493	0.219 - 0.543	-0.014 - 0.356
<i>p value</i>	<0.0001	0.0003	<0.0001	0.0571
<i>Significance</i>	****	***	****	ns

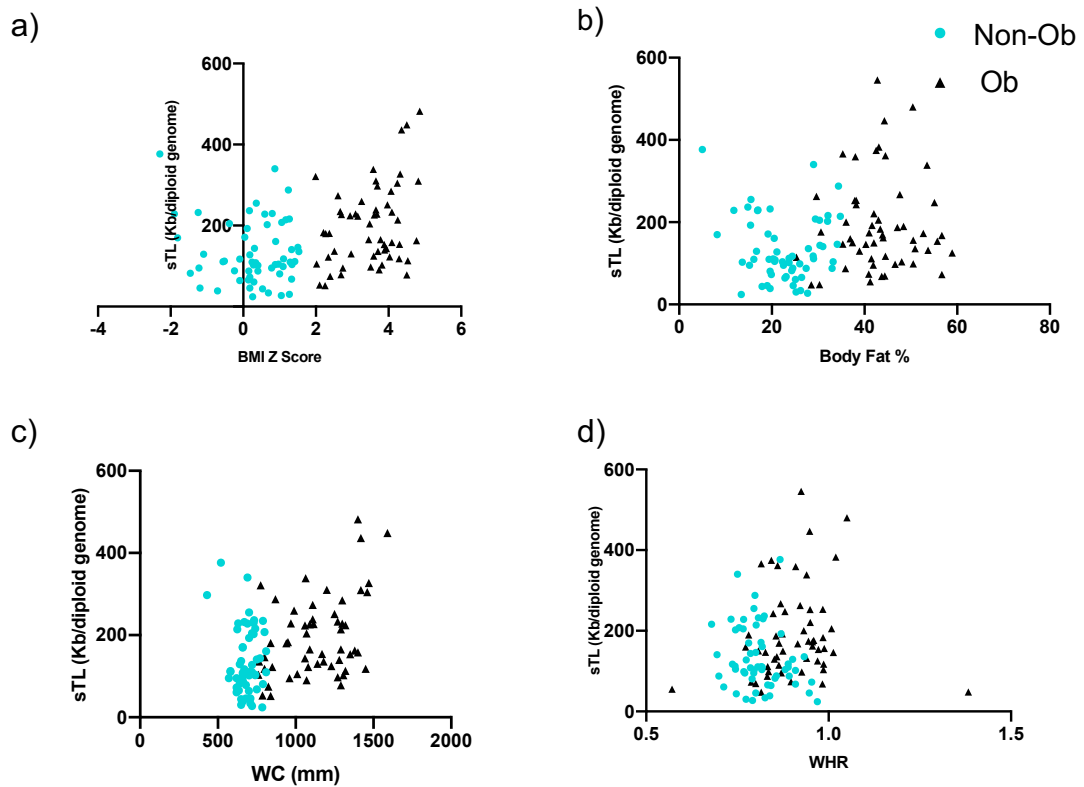


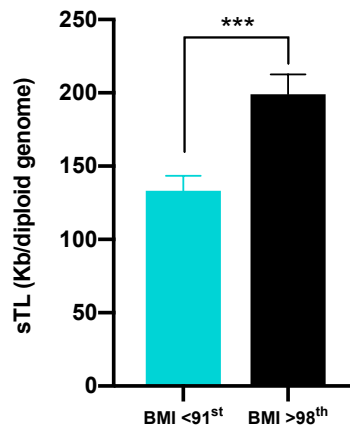
Figure 3.6.1 Spearman rank correlation analysis between anthropometric indices and salivary telomere length (sTL kb/diploid genome) across all 112 participants coded as obese or non-obese via BMI percentile indicates a) there is a significant, moderate positive correlation between BMI Z-score sTL ($R=0.37$, $p<0.0001$), b) there is a significant, moderate positive correlation between body fat (%) and sTL ($R=0.33$, $p<0.001$), c) there is a significant, moderate positive correlation between WC (mm) and sTL ($R=0.39$, $p<0.0001$) and d) there is no significant correlation between WHR and total DNA damage ($R=0.18$, $p>0.05$).

Furthermore, the Welch's t-test was applied to the average salivary telomere length when participants were divided based on their BMI, BF and WC percentile (Figure 3.6.2). The analysis indicated that salivary telomere length was significantly greater in childhood obesity when classified by all anthropometric markers ($p < 0.001$). The largest and most significant difference in means between non-obese and obese participants was seen when participants were classified by body fat percentage (Table 3.6.2).

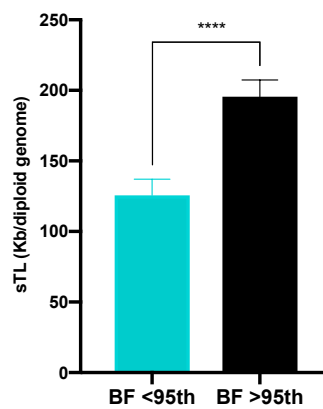
Table 3.6.2. Welch's t-test analysis of average salivary telomere length (sTL) between Non-obese and Obese cohorts classified by BMI Z score, BF % and WC.

Total DNA damage (%) in buccal mucosa	Non-Obese BMI < 91st	Obese BMI > 98th	Non-Obese BF% < 95th	Obese BF% > 95th	Non-Obese WC < 98th	Obese WC > 98th
<i>n</i>	58	54	49	63	57	55
<i>Average ± SD</i>	133.2 ± 78.03	199.0 ± 99.26	125.6 ± 80.4	195.6 ± 93.77	134.8 ± 80.43	196.2 ± 98.38
<i>Difference in means</i>	65.80		69.93		61.38	
<i>p value</i>	0.0002		<0.0001		0.0005	
<i>Significance</i>	***		****		***	

a)



b)



c)

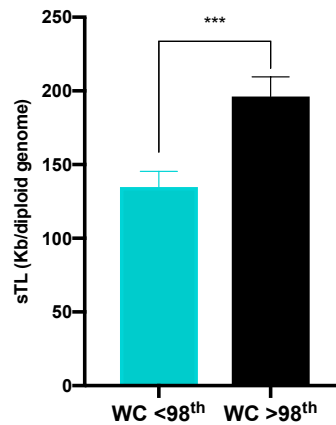


Figure 3.6.2 Welch's t-test of average salivary telomere length indicates a) the average frequency is higher in the obese (n=54) cohort when compared to the non-obese cohort (n=58) when participants are classified by **BMI percentiles** (** $p < 0.001$), b) average frequency is significantly higher in the obese cohort (n=49) when compared to the non-obese cohort (n=63) classified by **body fat percentiles** (** $p < 0.001$) and c) average frequency is higher in the obese cohort (n=55) when compared to the non-obese cohort (n=57) classified by **waist circumference percentiles** (** $p < 0.001$).

In addition, the Spearman rank correlation test was applied to assess whether excess telomere length was possibly linked with salivary inflammation or vitamin D status (Figure 3.6.3). The analysis indicated that salivary telomere length was not associated with inflammation or vitamin D levels in the oral cavity.

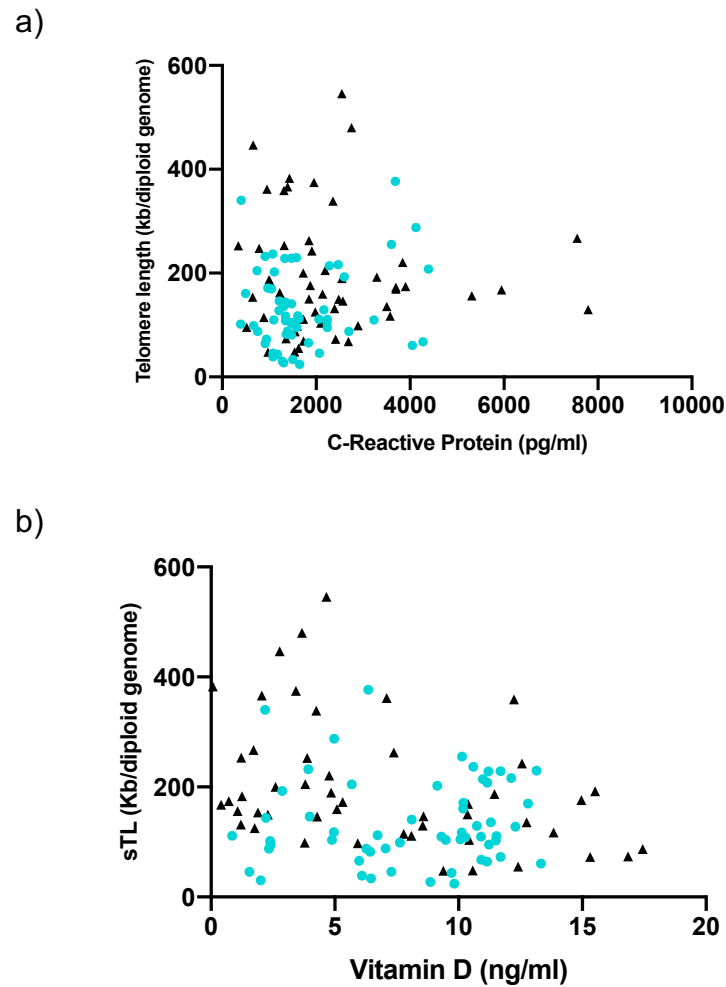


Figure 3.6.3 Spearman rank analysis indicates a) salivary telomere length is not significantly correlated with salivary CRP levels ($R=0.16$, $p>0.05$, $n=112$), b) salivary telomere length is not correlated with salivary vitamin D ($R=0.023$, $p>0.05$, $n=106$).

Finally, the Spearman rank correlation test was applied between salivary telomere length and the other DNA damage markers studied in this thesis (Figure 3.6.4). There was no correlation between salivary telomere length and urinary 8-OHdG. However, there was a weak, positive correlation between salivary telomere length and the total frequency of nuclear anomalies in the buccal mucosa ($R=0.21$, $p=0.03$).

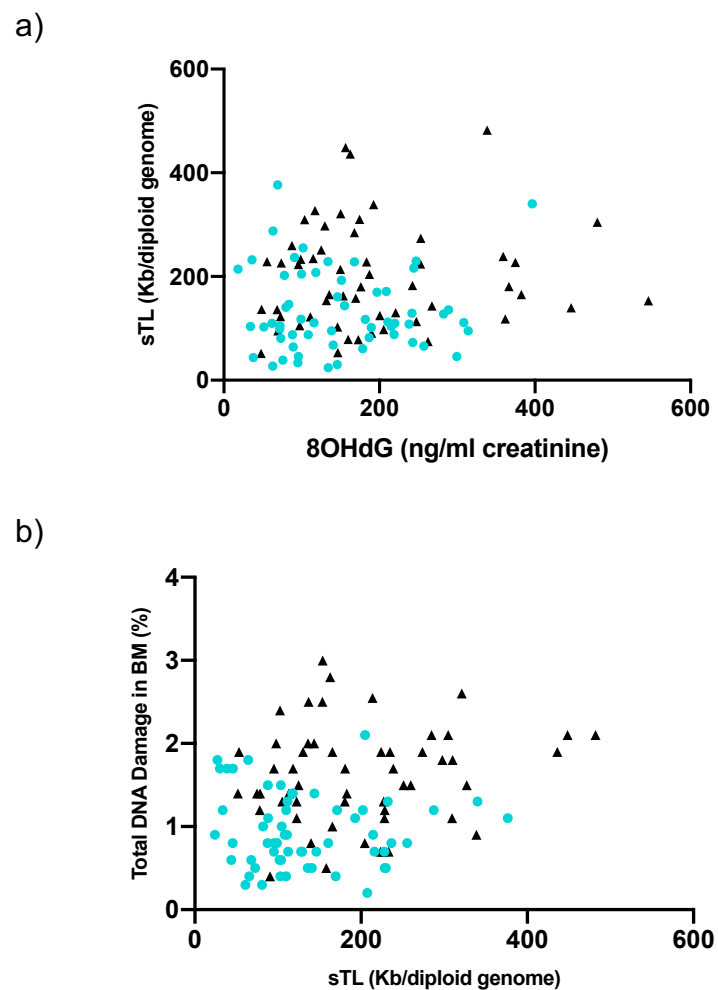


Figure 3.6.4 Spearman rank analysis across 112 participants indicates a) salivary telomere length is not significantly correlated with urinary 8-OHdG ($R=0.16$, $p>0.05$), b) salivary telomere length is positively correlated with the total frequency of DNA damage in the buccal mucosa ($R=0.21$, $p<0.05$).

3.7 Combined analysis of inflammation, micro-nutritional deficiency and DNA damage markers

One of the aims of this research was to establish which anthropometric markers may have potential clinical use in the assessment and monitoring of inflammation, vitamin D status and DNA damage. Table 3.7.1 presents a summary of the correlations between anthropometric markers and the biomarkers assessed in saliva and urine samples. BMI Z-score and body fat percentage were both significantly correlated with inflammation, vitamin D, DNA damage in the buccal mucosa, and telomere length. However, these two anthropometric markers were not correlated with levels of oxidative DNA damage in urine samples. Instead, central adiposity, assessed via waist circumference and waist to hip ratio was significantly correlated with urinary 8-OHdG.

Table 3.7.1. Summary of Spearman rank correlation coefficients of all anthropometric biomarkers with inflammation, vitamin D, DNA damage and telomere length markers (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Biomarker	BMI (Z-score)	Body fat (%)	WC (mm)	WHR
<i>CRP (pg/ml)</i>	**0.292	**0.262	0.178	0.108
<i>Vitamin D (ng/mL)</i>	** -0.251	** -0.321	** -0.252	** -0.262
<i>8OHdG (ng/ml creatinine)</i>	0.1633	0.1459	*0.3100	**0.2720
<i>Total DNA Damage in BM (%)</i>	****0.4550	****0.4940	****0.4185	****0.4085
<i>sTL (Kb/diploid genome)</i>	****0.3695	***0.331	****0.3932	0.1773

Furthermore, there were differences in the average values of biological markers when childhood obesity was classed via multiple classification systems. Table 3.7.2 presents a summary of the average percentage increase or decrease in inflammation, vitamin D and genome instability parameters when childhood obesity was classed in three different ways. Classification of childhood obesity via BMI percentiles was associated with increased inflammation, lower vitamin D and elevation of all genome instability markers. Classification via body fat percentiles presented with a greater difference and statistical significance in vitamin D status and DNA damage in the buccal mucosa, but this was not applicable for urinary 8-OHdG levels. Instead, classification by waist circumference percentiles presented a statistically significant difference in 8-OHdG levels between obese and non-obese participants. The greatest difference between obese and non-obese participants was the level of total DNA damage in the oral mucosa. Participants with obesity had on average a 70% increase in DNA damage events compared to non-obese participants, when classified by BMI Z-score. Interestingly, when participants are classified by BMI Z-score, around a 30% increase in CRP can be seen in participants with obesity which is coupled with almost a 30% increase in oxidative DNA damage. Overall, salivary telomere length was up to 55.7% greater in participants classified as obese and vitamin D levels were up to 23.6% lower.

Table 3.7.2. Summary of Welch’s t-test analysis, reported as percentage difference in means between non-obese and obese participants classified by different anthropometric markers (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

Biomarker	BMI	Body fat	WC
<i>CRP (pg/ml)</i>	*33.4% higher in obesity	**37.9% higher in obesity	24.4% higher in obesity
<i>Vitamin D (ng/mL)</i>	15.7% lower in obesity	*23.6% lower in obesity	19.8% lower in obesity
<i>8OHdG (ng/ml creatinine)</i>	*28% greater in obesity	12% greater in obesity	*26.6% greater in obesity
<i>Total DNA Damage in BM (%)</i>	****70% greater in obesity	****65% greater in obesity	**** 53.3% greater in obesity
<i>sTL</i>	***49.4% greater in obesity	****55.7% greater in obesity	***45.5% greater in obesity

In order to assess whether DNA damage was correlated with inflammation and vitamin D status, and whether there was agreement between markers of DNA damage, Spearman rank correlation analysis was applied. A summary of the results in Table 3.7.3 indicates that only the frequency of nuclear anomalies in the oral cavity was inversely correlated with levels of vitamin D. Furthermore, there was a significant positive correlation between DNA damage markers assessed in the oral cavity – salivary telomere length and nuclear anomalies in the buccal mucosa.

Table 3.7.3. Summary of Spearman rank correlation coefficients of inflammation, vitamin D, DNA damage and telomere length biomarkers (*p<0.05, ****p<0.0001).

Biomarker	<i>CRP</i> (pg/ml)	<i>Vitamin D</i> (ng/mL)	<i>8OHdG</i> (ng/ml creatinine)	<i>Total DNA</i> <i>Damage in</i> <i>BM (%)</i>	<i>sTL</i> (Kb/diploid genome)
<i>CRP (pg/mL)</i>		ns -(0.035)	ns (0.018)	ns (0.072)	ns (0.1635)
<i>Vitamin D</i> (ng/mL)	ns -(0.035)		ns (-0.174)	**** (-0.49)	ns (0.023)
<i>8OHdG</i> (ng/ml creatinine)	ns (0.018)	ns (-0.174)		ns (0.027)	ns (0.0934)
<i>Total DNA</i> <i>Damage in</i> <i>BM (%)</i>	ns (0.072)	**** (-0.49)	ns (0.027)		* (0.2053)
<i>sTL</i> (Kb/diploid genome)	ns (0.1635)	ns (0.23)	ns (0.0934)	* (0.2053)	

4. Discussion

4.1 Excess CRP and chronic inflammation in childhood obesity

The findings of this research are that salivary CRP is elevated in children with obesity when compared to healthy weight controls, and that CRP in saliva is positively correlated with BMI Z-score and body fat percentage. Two classification systems of obesity may differentiate between the status of inflammation in children – the RCPCH classification of obesity via BMI percentiles, and the Child Growth Foundation classification via bioelectrical impedance. Furthermore, this study identified no significant correlation between waist circumference or waist to hip ratio and salivary C-reactive protein in the cohort of children.

To date, several studies have assessed CRP levels systemically and drawn a link between childhood obesity and inflammation. Cook and colleagues, (2000) first reported an association between adiposity and serum CRP across 699 children aged between 10 and 11 years. Serum CRP was 250% higher in children from the top fifth Ponderal index (weight/height³) compared to children in the lower fifth of the index. These results were supported by Rönnekaa and colleagues (2006) who identified a positive correlation between BMI and serum CRP across 1617 participants aged 3-18 years. This group also identified obesity in childhood as an independent risk factor for increasing CRP in adulthood. A number of investigations that followed this research also confirmed that childhood obesity is associated with a systemic increase in CRP (Cohen, *et al.*, 2012; Schipper, *et al.*, 2012; Carmona-Montesinos, *et al.*, 2015; Nishide, *et al.*, 2015; Chang, *et al.*, 2015; Kitsios, *et al.*, 2013; Rowicka, *et al.*, 2017). The relationship between adiposity and serum CRP goes beyond that of

BMI and waist circumference as increased truncal-to-leg fat ratio assessed via DEXA, is also positively correlated with serum CRP (Cioffi, *et al.*, 2019). It is also interesting that CRP in serum is consistently raised in childhood obesity, unlike other inflammatory cytokines such as TNF-alpha and IL-6 (Cohen, *et al.*, 2012). This suggests it could be the marker of choice for monitoring inflammation status in obese children. Monitoring inflammatory status might be useful because a systemic increase in CRP is associated with an increased risk for metabolic syndrome. Nishide and colleagues (2015) identified dyslipidaemia and raised high sensitivity CRP (hs-CRP) across 1072 Japanese children. Other researchers have also found that hs-CRP levels are higher in children with obesity and non-alcoholic fatty liver disease (Kitsios, *et al.*, 2013). As discussed, there is strong evidence that childhood obesity is a state of chronic, low-grade inflammation.

In order to monitor inflammation status in children with obesity, it would be useful to assess CRP levels via non-invasive techniques. Excess CRP in saliva may reliably reflect systemic, low-grade, chronic inflammation. CRP is produced in serum by the liver and cells of the immune system during the acute phase response (Medzhitov, 2008). It has been demonstrated that serum proteins can enter saliva via passive diffusion, or through ultrafiltration between cellular tight junctions (Desai, & Mathews, 2014). Salivary CRP levels correlate strongly with serum CRP levels in infants ($R=0.62$), adolescents ($R=0.42$) and adults ($R=0.72$) (Lyengar, *et al.*, 2014; Ouellet-Morin, *et al.*, 2011; Byrne, *et al.*, 2013). Ouellet-Morin and colleagues (2011), conducted a study with 61 adults to validate the prediction of serum CRP from salivary CRP. They reported that the correlation between salivary CRP and serum CRP is not affected by sex, age or salivary flow rate. They also identified that the prediction of serum CRP levels is

more accurate at higher levels of salivary CRP. A later study, conducted in 259 adults also found a strong correlation between CRP in saliva and serum ($R=0.73$) (Labat, *et al.*, 2013). In addition, the researchers found that salivary CRP is also correlated with BMI, waist to hip ratio, as well as markers of metabolic syndrome and cardiovascular disease. A moderate correlation between salivary and serum CRP levels is also reported in adolescents with raised salivary CRP ($R=0.42$) (Byrne, *et al.*, 2013). In essence, the evidence suggests that saliva may be a suitable alternative biological tool to detect or monitor systemic inflammation in vulnerable populations including children. It is likely that an excess production of serum protein may drive more proteins to become incorporated into saliva.

The research findings in this thesis concur with other investigations that report higher salivary CRP in obese children, and support the potential use of salivary CRP to monitor inflammation in children with obesity. In 2012, Naidoo and colleagues first reported that salivary CRP is elevated in children with obesity. The researchers associated a BMI above 85th centile with increased inflammation across 170 South African children. Although children with obesity had increased body fat percentage, this study was unable to show an association between increased body fat percentage and salivary CRP. This may be because the researcher's assessed body fat percentage via skinfold thickness, which can in some cases overpredict body fat (Freedman, *et al.*, 2013). In 2014, Goodson and colleagues identified that salivary CRP is six times higher in 11-year old children with a BMI above the 95th centile. Across 744 saliva samples, the researchers also associated childhood obesity with reduced adiponectin – an anti-inflammatory cytokine secreted directly from adipose tissue. More recently, Janem and colleagues (2017) reported that there

is no significant difference in salivary CRP between children with and without obesity, as well as in children with type 2 diabetes and obesity. These discrepancies may owe to the limited size of the study population as only 19 normal weight and 14 obese participants were analysed. Furthermore, the levels of CRP in saliva varied largely in the healthy cohort, which is a trend also observed in this investigation and suggests that a large sample size is required for a meaningful statistical analysis. Also, in this investigation and contrary to the literature, there is no significant association between waist circumference or waist to hip ratio and inflammation assessed by salivary CRP in children. This discrepancy may owe to differences in the technique of measurement amongst researchers. Importantly, the obese and healthy-weight study cohorts in our study and the studies above screened and excluded all participants with signs of oral infection or inflammation. Overall, the research so far indicates that average salivary CRP levels are higher in children with increased adiposity. The mechanisms that drive inflammation in obesity may also explain the link between obesity and co-morbidities in children, as well as potential later life consequences.

There are multiple mechanisms that may contribute to increased systemic CRP and inflammation in childhood obesity. Firstly, obesity in children is associated with increased CD14⁺⁺ monocytes (Schipper, *et al.*, 2012). These cells can secrete cytokines which lead to the recruitment of inflammatory cells into adipose tissue or vascular lesions. The gut microbiome as well as dietary factors including hyperglycaemia and increased fish oils may also enhance myelopoiesis and thus increase the systemic release of leukocytes from the bone marrow (Singer, & Lumeng, 2017). Secondly, Landgraf and colleagues (2015) report that childhood obesity is associated with altered adipose tissue

biology - including hypertrophy and hyperplasia. They also describe a positive correlation between the size of adipocytes and the infiltration of inflammation promoting macrophages (CD68+). Furthermore, it has been suggested that as adipose tissue expands to contain stores of fat, the microcirculation is disrupted, which leads to adipose tissue hypoxia (Barbarroja, *et al.*, 2010).

Increased adipocyte death has been associated with a release of cell-free DNA into the systemic circulation (Haghiaci, *et al.*, 2012). Nishimoto and collaborators (2016) have recently identified that the release of cell-free DNA caused by adipocytes' degeneration promotes macrophages accumulation in adipose tissue via Toll-like Receptor 9 (TLR9), originally known as a sensor of exogenous DNA fragments. Adipose tissue necrosis further attracts inflammatory cells and leads to the secretion of pro-inflammatory cytokines such as TNF- α (Bhattacharya, *et al.*, 2015). Moreover, adipocyte cell necrosis can also cause altered adipokine expression, including reduced levels of the anti-inflammatory hormone adiponectin (Kern, *et al.*, 2003). Adiponectin is secreted by adipose tissue and may inhibit the actions of TNF- α and prevent recruitment of inflammatory cells (Ouchi, *et al.*, 1999).

A systemic increase of neutrophils, macrophages and dendritic cells encourages production of ROS/RNS via NADPH oxidases, particularly the NOX1, NOX2 and NOX4 isoforms (Mittal, *et al.*, 2014). Ultimately, the disrupted ratio of pro-inflammatory and anti-inflammatory cytokines, as well as excess circulation of free fatty acids, triggers the production of CRP from the liver and further promotes inflammation as well as oxidative stress (Codoñer-Franch, *et al.*, 2011). Overall, raised levels of CRP in childhood obesity can be explained by changes in adipose tissue biology and the increased production of

leukocytes. Inflammation may underlie the multiple co-morbidities that are associated with childhood obesity but also importantly, chronic inflammation may present consequences for later life health.

Chronic inflammation is a well-known etiological factor for genetic instability and neoplastic transformations in cells (Colotta, *et al.*, 2009). Chronic inflammation and its ability to inflict DNA damage has been demonstrated in models of *H.pylori* associated gastrointestinal cancer (Baik, *et al.*, 1996) and ulcerative colitis associated colon cancer (Bernstein, *et al.*, 2001) as well as HCV mediated liver cancer (Shawki, *et al.*, 2014).

Evidence is building which describes a causative role played by inflammation in malignancy. Firstly, chronic inflammation can drive the production of excess reactive oxygen and nitrogen species which may cause deamination, oxidation and strand breakages in DNA (Weisberg, *et al.*, 2003; Tamir, *et al.*, 1996). Secondly, the excess production of ROS/RNS can contribute to microsatellite instabilities and the formation of pre-malignant lesions (Hofseth, *et al.*, 2003). In fact, a profile of circulating pro-inflammatory cytokines has been associated with pre-malignant lesions of the oral mucosa, gastric mucosa and prostate in the absence of tissue infection (Di Silverio, *et al.*, 2003; van der Woude, *et al.*, 2004; Woodford, *et al.*, 2014; Tezal, *et al.*, 2005). Izano and colleagues (2016) investigated the risk of increased IL-6, CRP and TNF-alpha with colon cancer and other obesity related cancers in a follow-up study of 2490 participants. Their hazard ratio analysis, based on participants within the third tertile for CRP levels, indicated that a one-unit increase in CRP is associated with a 2.29 fold increase in the risk of colon cancer. Overall, these findings represent a possible

causative relationship between chronic inflammation and colon cancer in obese adults.

Finally, chronic inflammation may inactivate p53, leading to a loss of mitotic arrest following DNA damage (Cobbs, *et al.*, 2003). Thereby, enabling the accumulation of random mutations that may contribute to the genetic heterogeneity seen in cancer cells (Colotta, *et al.*, 2009). Other transcription factors that may be activated include NF- κ B, STAT-3, which can cause suppression of anti-tumour defence mechanisms (Grivennikov, & Karin, 2010). Overall, there is substantial evidence that chronic inflammation is a causative factor for DNA damage. Therefore, inflammation status should be an important consideration when assessing the risks of malignancy.

4.2 Hypovitaminosis D in childhood obesity

This research study is the first to identify lower vitamin D levels in saliva from children with obesity when compared to saliva from normal-weight controls. This association is significant when obesity is defined by body fat percentage but not statistically significant when participants are defined by BMI or waist percentiles. However, all markers of adiposity correlate with the levels of vitamin D in an inverse proportion.

Over the last five years a large proportion of studies have identified obesity in children as a state of hypovitaminosis D by assessing vitamin D levels in serum and plasma (Bellone, *et al.*, 2014; Ekblom, & Marcus, 2016; Wakayo, *et al.*, 2016; Kumaratne, *et al.*, 2017; Alyahya, 2017; Erol, *et al.*, 2017; Plesner, *et al.*, 2018). In 2014, Bellone and colleagues, reported lower average levels of vitamin D in serum from obese children compared to healthy-weight controls across a total of 557 participants classified by BMI percentiles. They also found that puberty status did not affect vitamin D levels in children. A meta-analysis further indicated that there are seasonal and ethnic factors that can modulate the level of vitamin D deficiency in obesity (Williams, *et al.*, 2014). Vitamin D deficiency is more prevalent during the winter season and in the African ethnic group. Therefore, study participants should be matched for ethnicity to reduce the possible effect of this confounding factor. Recently, Plesner and colleagues (2018) reported a greater prevalence of vitamin D deficiency in obese children and serum vitamin D status to be inversely correlated with BMI Z-score across a total of 3627 participants. In addition to the winter season being a risk factor for vitamin D deficiency, they also found screen-time over four hours to be another risk factor. Whilst our research project was able to achieve matching of ethnic groups, the timing of sampling was more difficult to control. Overall, the

identification of vitamin D in non-invasively obtained tissues such as saliva could enable the long-term monitoring of vitamin D status in vulnerable groups.

No evidence has been identified so far for differences in salivary vitamin D levels in obese children. One reason for this could be that the measurement of vitamin D in saliva is a challenge. Saliva is a dilute biological fluid, with concentrations of proteins lower than detected in serum. Therefore, the detection of vitamin D in saliva, particularly in patients with deficient serum levels, requires an assay with high sensitivity. For utilisation in this study, the commercially available immunoassay kit with the highest analytical sensitivity was selected. Furthermore, saliva samples were obtained via the stimulated method of saliva collection as this has been demonstrated to increase levels of salivary vitamin D (Higashi, *et al.*, 2013). However, there were six samples that were below the detection limit of the assay, and it is important to note that five samples were from participants with obesity (classified by BMI Z score). The removal of these data points reduced the sample size for statistical analysis, which could explain the lack of statistical significance associated with the lower vitamin D result when participants were classified by BMI and WC percentiles. It is likely that the use of a more sensitive tool would have enabled a more precise indication of hypovitaminosis D in obesity compared to healthy-weight controls.

A more sensitive technique that can be utilised for future work is the sensitive liquid chromatography–electrospray ionization–tandem mass spectrometric (LC–ESI–MS/MS) method. This method was used to detect salivary vitamin D in adults with a sensitivity of 0.002ng/ml and was also used to demonstrate a strong correlation between salivary vitamin D and serum vitamin D ($r=0.83$) (Higashi, *et al.*, 2008). There is a need for the development of high-sensitive

and high-throughput assays that can quantify vitamin D in saliva and provide a better understanding of the relationship between serum and salivary vitamin D levels in children. This is especially important since the diagnosis of hypovitaminosis D is enacted increasingly in children within the UK (Basatemur, *et al.*, 2017). Furthermore, the multiple studies that report serum vitamin D levels to be inadequate in children with obesity, reflect the coming together of two epidemics. Whether this hypovitaminosis is a cause or consequence of obesity is a question of interest.

It has been debated whether the accumulation of excess fat lowers vitamin D levels or whether low vitamin D drives the accumulation of excess fat. In 2009, Foss hypothesised that obesity may be treated by restoring vitamin D status. This theory described the onset of obesity to be an adaptive response to being in a cold climate, triggered by the decline in vitamin D synthesis due to inadequate UV light. It was suggested that a drop in calcidiol concentration is detected by the hypothalamus which then alters appetite and the body-weight set-point. Interestingly, Daraki and colleagues also found an association between reduced in utero exposure to vitamin D and an increased risk of adiposity in early childhood (Daraki, *et al.*, 2018). Children from mothers with vitamin D deficiency had a higher BMI Z-score and waist circumference at age 6 compared to children from mothers with higher levels of vitamin D during pregnancy. It has also been suggested that vitamin D deficiency in children may exacerbate the risk of obesity in those who are also carriers of the FTO rs9939609 allele (Lourenco, *et al.*, 2014). However, there is limited evidence that vitamin D supplementation can reverse adiposity.

Furthermore, Vimalleswaran and colleagues (2013) conducted a bi-directional Mendelian randomization analysis across 42024 participants and demonstrated that variations in vitamin D alleles have little effect on adiposity and instead obesity is a causative factor for vitamin D deficiency. They found that BMI related gene variants were significantly associated with vitamin D levels. More specifically, the researchers identified that a 10% increase in BMI may reduce levels of vitamin D by 4.2%. A number of mechanisms can be proposed for a causative association in obese children.

Firstly, studies have demonstrated that children with obesity can have altered behaviour including a lack of physical activity and reduced exposure to sunlight (Al-Othman, *et al.*, 2012). Reduced physical activity may be an important risk factor for hypovitaminosis D, as increased physical activity has been demonstrated to increase vitamin D levels without supplementation in obese children (Hossain, *et al.*, 2018). Secondly, genetic mechanisms such as allelic variation in the vitamin D receptor (VDR) gene have also been associated with obesity in children (Ferrarezi, *et al.*, 2012). Moreover, as adipose tissue is one of the storage sites for vitamin D (Rosenstreich, *et al.*, 1971), it has been suggested that Vitamin D may be increasingly sequestered into adipose tissue and released more slowly into plasma in a state of obesity (Vanlint, 2013). Vanlint (2013) also suggests that reduced activation of vitamin D or increased catabolism may be a pathway to vitamin D deficiency in obesity. This is because a lack of the CYP2J2 enzyme, required for 25-hydroxylation of vitamin D, has been reported in subcutaneous adipose tissue obtained from participants with obesity. A more direct explanation for the differences in vitamin D levels between obese and non-obese participants could be the effect of increased volume and therefore greater dilution of circulating vitamin D (Drincic,

et al., 2012). Ultimately, this presents considerations for the dosage of vitamin D during the monitoring and treatment of hypovitaminosis D which should be adjusted for obese patients. Previously, a large dose of vitamin D supplementation in obese adolescents failed to increase vitamin D levels or alter markers of cardiovascular risk (Shah, *et al.*, 2015). Therefore, defining adequate vitamin D supplementation for obese patients should be a priority, as it may prevent further adverse health outcomes.

Vitamin D deficiency is a concern in paediatric obesity because it is a risk factor for multiple co-morbidities. A number of studies report increased features of the metabolic syndrome to be associated with vitamin D deficiency in obese children. These features include insulin resistance and pre-diabetes (Reyman, *et al.*, 2014; Miraglia del Giudice, *et al.*, 2015; Ekbom, & Marcus, 2016; Gul, *et al.*, 2017), elevated blood pressure (Kao, *et al.*, 2015), and dyslipidaemia (Kumaratne, *et al.*, 2017; Erol, *et al.*, 2017). It is of interest that vitamin D deficiency in childhood obesity also coincides with increased hs-CRP levels (Rodriguez-Rodriguez, *et al.*, 2014). Vitamin D has been described as a hormone with anti-inflammatory properties by a number of studies (Abbas, 2017; Ding, *et al.*, 2013; Zhang, *et al.*, 2012). Studies in rodents imply that vitamin D deficiency can escalate the infiltration of macrophages into adipose tissue and increase the secretion of inflammatory cytokines (Chang, & Kim, 2017; Karkeni, *et al.*, 2015). In human adipocytes, vitamin D has been demonstrated to suppress the expression of three microRNAs that are regulated by TNF-alpha (Karkeni, *et al.*, 2018) and reduce the levels of inflammatory proteins including interleukin-6, monocyte chemoattractant protein-1 and interleukin-1 β (Abbas, 2017). Furthermore, vitamin D deficiency is a co-feature in common childhood inflammatory disorders such as asthma as

well as inflammatory bowel syndrome (IBS) (Nwosu, *et al.*, 2017; Vo, *et al.*, 2015). Therefore, there is a likely role for vitamin D deficiency in exacerbation of inflammation and increasing the risk of inflammation-associated disorders.

Vitamin D deficiency may also have implications for tumorigenesis. An inverse association has been reported between levels of vitamin D and cancers of the lung, breast, GI tract and prostate (L. Zhang, *et al.*, 2015; McNamara, & Rosenberger, 2019; Hossain, *et al.*, 2019; Yuan, *et al.*, 2019). Earlier studies report that vitamin D supplementation can reduce all-cancer risk in post-menopausal women (Lappe, *et al.*, 2007). Similarly, vitamin D supplementation was associated with a reduction in risk of post-menopausal breast cancer (O'Brien, *et al.*, 2017). Furthermore, lower levels of Vitamin D in serum have been linked with a poorer response to treatment in haematological malignancies (X. Thomas, *et al.*, 2011). A meta-analysis of 64 studies concluded that higher levels of vitamin D indicate a more positive prognosis in cancer patients (Vaughan-Shaw, *et al.*, 2017).

The possible anti-cancer effects of vitamin D can be explained by its role in the maintenance of genomic integrity and cell-cycle progression. It has been demonstrated that DNA damage can activate Vitamin D receptors (VDR) via p73 which is inhibited in cancer cells by mutant p53 (Kommagani, *et al.*, 2007). VDR activation by 1-25OHD has anti-proliferative effects and may lead to cell-cycle arrest in cancer cells. This process was first unveiled by Colston and colleagues in cultured melanoma cells (1981) and later described in cancerous cells of the breast, colon, prostate and liver (Lointier, *et al.*, 1987; Gross, *et al.*, 1986; Skowronski, *et al.*, 1993; Caputo, *et al.*, 2003). There is also evidence to suggest that treatment of prostate cancer cells with biologically active vitamin D

can lead to a suppression of CDK1 mRNA, which encodes the protein that is crucial for cell cycle progression (Kovalenko, *et al.*, 2010).

Furthermore, it has been demonstrated in a breast cancer cell line that analogues of vitamin D (EB1089 and CB1093) may enhance apoptosis by inhibiting proliferative growth factors like IGF-1 (Colston, *et al.*, 1998). Fleet and colleagues (2012), have reviewed the multiple instances whereby tumour cell line treatment with vitamin D analogues have promoted the expression of TGF β , another anti-proliferative factor. They also review other anti-proliferative mechanisms induced by vitamin D to include disruption of Wnt signalling pathways and upregulation of PTEN (a tumour suppressor gene).

Vitamin D also appears to play a role in the protection from oxidative DNA damage and initiation of DNA repair. It is interesting that vitamin D treatment has been demonstrated to reduce levels of oxidative DNA damage in human colon epithelial cells (Fedirko, *et al.*, 2010). Furthermore, Vitamin D treatment of prostate cancer upregulate mRNA expression of anti-oxidant enzymes such as SOD1 and SOD2 (Peehl, *et al.*, 2004). Treatment of human head and neck squamous carcinoma cells with a vitamin D analogue can also increase the expression of the DNA repair regulating protein GADD45 α (Akutsu, *et al.*, 2001). This can be a crucial tumour protective role for vitamin D, as there is consistent evidence linking increased oxidative DNA damage with tumorigenesis (Usman and Volpi, 2018).

However, the associations between vitamin D and cancer risk are not void of discrepancies. Jiang and colleagues (2018) conducted a Mendelian randomization study with data from a total of 275,824 genome-wide association studies. They assessed for a causal relation between vitamin D concentrations

and risk of breast and prostate cancer, but were unable to confirm this. Recent evidence that metastatic cancer cells can downregulate the vitamin D receptor may explain this discrepancy (Lopes, *et al.*, 2010). It is likely that the protective effects of vitamin D are not exhibited during all stages of cancer. As cancer cells can become capable of evading cell cycle checkpoints, they may also develop a protective mechanism against the effects of vitamin D by downregulating the VDR.

Polymorphisms in the VDR may alter the metabolism and cellular actions of vitamin D. A number of polymorphisms in the VDR have been identified which can alter the risk and prognosis of breast, colorectal and prostate cancer (Rai, *et al.*, 2017). Whilst some VDR polymorphisms can enhance the actions of vitamin D and have anti-cancer effects, others can reduce the efficacy of vitamin D and promote or aggregate carcinogenesis. Wactawski-Wende and colleagues (2006) conducted a randomised control trial (RCT) in a total of 36282 women and found that vitamin D supplementation with calcium did not reduce the risk of colorectal cancer. However, VDR polymorphisms such as CYP27B2 and CYP24A1 have been associated with the suppression of vitamin D and increased risk of colorectal cancer (Vidigal, *et al.*, 2017). Therefore, RCTs of vitamin D supplementation for cancer prevention should consider VDR polymorphisms in participants.

Furthermore, an analysis of 18 RCTs identified a lack of consistent evidence to support the supplementation of vitamin D for cancer prevention in adults (Bjelakovic, *et al.*, 2014). Differences in the duration of supplementation and compliance with supplementation are other factors that could explain the discrepancies amongst studies. Nevertheless, a longitudinal investigation of

vitamin D supplementation in childhood and the incidence of morbidity and cancer in adulthood, may uncover potential long-term benefits of possessing adequate vitamin D levels, particularly in patients with elevated DNA damage.

4.3 Increased oxidative DNA damage in childhood obesity

This thesis demonstrates that oxidative DNA damage assessed via urinary 8-OHdG levels is greater in children with obesity compared to healthy weight controls, when obesity is classified by BMI and WC percentiles. Furthermore, urinary 8-OHdG levels in children correlate with BMI and waist circumference.

To date, there are only three other investigations that have been conducted in children to assess the association between adiposity and levels of urinary 8-OHdG. In 2008, a Šebeková and colleagues (2009) demonstrated that children with obesity have higher levels of 8-OHdG in urine. Later in 2014, Protano and collaborators conducted a study across 159 healthy Italian children aged 5-11 years and assessed levels of 8-OHdG, 8-OHG and 8-OHGua in urine. They reported an inverse association between BMI and urinary 8-OHdG whilst no significant associations were reported for 8-OHG and 8-OHGua. On the other hand, Ramachandra and colleagues (2015) report higher levels of urinary 8-OHdG in children with obesity, corroborating the findings of Šebeková and colleagues but this cohort of children also present with insulin resistance. Furthermore, the number of obese participants in all of these studies is relatively small. Therefore, the indications between urinary 8-OHdG and adiposity in children have so far been inconclusive. The findings of this investigation present no statistically significant correlation between urinary 8-OHdG and BMI or body fat percentage, but a significant positive correlation with WC and WHR. This indicates that levels of oxidative DNA damage increases with central adiposity in children. These findings also match the observations recorded in children via serum concentration of 8-OHdG (El Wakkad, *et al.*, 2011). Across 103 adolescents (aged 13-18 years), a BMI greater than the 95th

percentile has been associated with an increased concentration of 8-OHdG. However, the researchers also demonstrated that body fat percentage assessed via bioelectrical impedance and BMI is positively correlated with serum 8-OHdG.

Similarly discordant are the findings of 8-OHdG levels in adult obesity. de la Maza and colleagues (2006) were the first to report an association between increased body fat and oxidative DNA damage by assessing 8-OHdG in skeletal muscle. These associations were based upon self-reported weight gain over the last 10 years by patients undergoing a hernia operation and coincided with increased TNF-alpha. Later, another study assessed 8-OHdG concentrations in lymphocytes and was unable to identify a correlation with BMI in young adults (Hofer, *et al.*, 2006). In contrast, a longitudinal study indicated a correlation between leanness and increased oxidative stress reporting a one-point reduction in BMI to coincide with a 2.7% increase in urinary 8-OHdG (Mizoue, *et al.*, 2007). Similar were the findings from Donmez-Altuntas and collaborators (2014) who reported decreased levels of 8-OHdG in plasma of obese adults compared to adults of healthy weight, although other biomarkers of DNA damage were elevated. More recently, a study conducted in over 100 obese and healthy weight men concluded that there were no significant differences in urinary 8-OHdG (Cejvanovic, *et al.*, 2016). Overall, there are more reports of an inverse association between adiposity and 8-OHdG levels in adults rather than a positive association. Whereas in children, there is now more evidence for a positive association between adiposity and urinary 8-OHdG.

The discrepancies in results may be explained in a number of ways. Firstly, there are some reports that individuals with a lower BMI have an increased metabolic rate and may therefore exhibit higher levels of oxidative stress (Tamae, *et al.*, 2009). However, in obesity, a calorie-rich diet and an abundance of macronutrients may call for continuity in the Krebs cycle and consequently lead to a leak of more electrons from the mitochondrial electron transport chain. These electrons promote a reduction of oxygen molecules – a process that will generate ROS. Obesity in children who are independent from other co-morbidities is associated with a higher total oxidant status (Kilic, *et al.*, 2016; Mohn, *et al.*, 2005). The over-generation of ROS in obese individuals can be brought about by more than just a continuum in the Krebs cycle.

As discussed in the previous chapter, there are numerous compelling findings that indicate obesity in children to be a state of chronic, low-grade inflammation. The excess production of ROS during inflammation in obesity is sourced from monocytes and neutrophils that are recruited via CRP (Vincent, & Taylor, 2006). In this process, monocytes can produce free oxygen radicals, hydroxyl radicals, hypochlorous acid, hydrogen peroxide and myeloperoxidase whereas neutrophils can generate oxygen radicals via NADPH oxidase (Garg, *et al.*, 2000). Inflammation also promotes the generation of nitric oxide – a neutrophil activator and contributor to oxidative stress and DNA damage (Guzik, *et al.*, 2003). Therefore, increased oxidative DNA damage may be caused by increased inflammation in obesity. In this study, obesity was independently associated with inflammation and oxidative DNA damage as the levels of both CRP and 8-OHdG were increased in the same cohort of obese participants grouped by BMI Z score. However, no significant correlation was noted between CRP and 8-OHdG markers. This may be because the markers were assessed

in different tissues or because of other pathological states that may predominate the production of ROS and perhaps are more directly associated with the increased urinary excretion of 8OHdG in children with obesity.

Other pathological mechanisms that may drive ROS production in obesity and promote oxidative DNA damage include hyperglycaemia, hyperleptinaemia and dyslipidaemia. Excess glucose in plasma can lead to auto-oxidation of glucose, activation of the polyol pathway to convert glucose into sorbitol, and increased production of Advanced Glycation End-Products (AGE) (Vincent, & Taylor, 2006). Excess oxidative stress has been attributed to increased levels of sorbitol in a mouse model but evidence of this mechanism is unknown in childhood obesity (Chung, *et al.*, 2003). AGE can bind to cell surface receptors (RAGE) and increase the production of ROS via intracellular signalling pathways (Evans, *et al.*, 2002). This receptor-ligand binding can be blocked via soluble RAGE receptors (sRAGE) acting as a decoy and may therefore act as a risk marker for cardiovascular disease (Hudson, *et al.*, 2005). It has been recently demonstrated that adiposity in adolescents is inversely correlated with plasma levels of sRAGE (He, *et al.*, 2014). Therefore, increased binding of AGEs to RAGE in obese children with hyperglycaemia may increase the production of ROS and promote oxidative DNA damage.

Furthermore, the excess production of the leptin hormone also coincides with increases ROS production. Levels of leptin are positively correlated with childhood obesity (Venner, *et al.*, 2006). The increased production of ROS via leptin has been demonstrated in cultured human endothelial cells (Bouloumié, *et al.*, 1999). Levels of leptin also correlate with levels of CRP and inflammation in paediatric obesity (Pires, *et al.*, 2014). Moreover, excess plasma leptin has

also been associated with an abnormal lipoprotein profile in children (Wu, *et al.*, 2001). Low levels of high density lipoproteins in obesity and the consequent excess circulation of free fatty acids can trigger the production of ROS by disrupting the mitochondrial adenine nucleotide transporter and leading to increased accumulation of electrons and their reaction with oxygen to form free radicals (Bakker, *et al.*, 2000). Overall, hyperglycaemia, hyperleptinaemia and dyslipidaemia are all etiological factors for oxidative stress in obesity. One of the limitations of this study is the lack of plasma glucose, lipid or hormonal status of the participants. This information could potentially explain the findings of increased oxidative DNA damage in the obese participants of this study.

Increased ROS production and the disparity amongst studies about 8-OHdG levels in obesity may also be explained by the fluctuating status of anti-oxidant defence mechanisms. Anti-oxidant enzymes such as Superoxide Dismutase (SOD) and glutathione peroxidase (GPO) can scavenge ROS from the system. Both a decrease and increase in anti-oxidant mechanisms have been reported in childhood obesity. Reports of reduced anti-oxidant defence mechanisms in obesity can suggest that the production of free radicals is thriving. On the other hand, if obesity coincides with increased defence mechanisms then this may be seen as a homeostatic mechanism to clear excess production of free radicals.

Firstly, dietary anti-oxidants that are essential for optimal activity of SOD including copper, zinc and magnesium levels are negatively correlated with BMI and reportedly lower in serum from obese adolescents (Lee, 2007).

Furthermore, carnosine, a potent scavenger for reactive oxygen species was significantly decreased in urine from obese adolescents compared to healthy-weight controls (Cho, *et al.*, 2017). An extensive review also reported lower anti-

oxidant enzyme activity across multiple studies conducted in adults (Vincent, & Taylor, 2006).

On the other hand, obesity has been described as a state concurrent with increased anti-oxidant defence mechanisms. SOD activity was reported to be higher in a cohort of obese children compared to healthy-weight controls (Erdeve, *et al.*, 2004). Similar findings have been reported by other researchers (Codoñer-Franch, *et al.*, 2010; Sfar, *et al.*, 2013). Krzystek-Korpacka and colleagues (2013), reported a positive correlation between BMI and SOD activity in girls but not boys. Along with increased serum 8-OHdG, El-Wakkad and colleagues (2011) also reported elevated activity of SOD and GPO in the same cohort of children with obesity. Therefore, it is likely that increased anti-oxidant enzyme activity is a homeostatic phenomenon to counteract increasing levels of oxidative stress in obesity. In some individuals, the increased enzymatic activity may control levels of ROS and lower the production of oxidative DNA damage. Whereas in others, as demonstrated by El-Wakkad and colleagues in a cohort of children with obesity, increased enzymatic activity of antioxidants can still lead to an increase in oxidative DNA damage. This may also be because anti-oxidant enzyme activity can be affected by the severity of adiposity in children.

In one investigation, overweightness has been associated with increased SOD activity whereas obesity in the same study is associated with reduced activity of SOD (Albuali, 2014). This suggests that the implications of oxidative stress worsen with the severity of obesity, placing obese children at higher risk of oxidative stress than overweight or healthy-weight children. Thereby, fluctuating levels of anti-oxidant enzyme activity in obesity may lead to a fluctuating status

of 8-OHdG. It may be worthwhile to also investigate levels of DNA repair enzymes: DNA glycosylase and 8-oxoguanine-DNA glycosylase, in conjunction with 8-OHdG levels to better predict the consequences of increased oxidative stress.

Furthermore, an analysis of the literature suggests that it is unlikely for increased anti-oxidant enzyme activity to substantially control ROS production and prevent pathological consequences in obesity. This is because 8-OHdG is not the only oxidised product identified increasingly in obese patients. Increased BMI and waist circumference were positively correlated with levels of lipid peroxidation products in adults (Furukawa, *et al.*, 2017). Malondialdehyde levels are reportedly doubled in obese children compared to healthy weight children and correlate with BMI and waist to hip ratio (Mohn, *et al.*, 2005). Furthermore, a study conducted in obese children identified higher levels of oxidative stress coupled with increased inflammation, insulin resistance, and reduced estimated glomerular filtration rate (Correia-Costa, *et al.*, 2016). Oxidative stress and the consequent elevation in 8-OHdG has been previously described as a biological factor for acquiring cancer in adult obesity (Cerdá, *et al.*, 2014).

In 2006, Cooke and colleagues conducted a literature review and brought to light that 8-OHdG is a well-established risk marker for age-related pathologies including cancer. It is not surprising that elevated serum or urinary 8-OHdG lesions are undisputedly associated with malignant tumours at multiple sites. An increased concentration of 8-OHdG in urine has been identified in patients with cancers of the buccal mucosa, breast, colo-rectum, prostate and bladder (Murugaiyan, *et al.*, 2015; Kuo, *et al.*, 2007; Guo, *et al.*, 2016; Miyake, *et al.*, 2004; Chiou, *et al.*, 2003). In serum, increased levels of 8-OHdG have also

been reported in patients with cancer of the breast and GI tract, but also of the ovaries (Himmetoglu, *et al.*, 2009; Chang, *et al.*, 2008; Diakowska, *et al.*, 2007; Pylvas, *et al.*, 2011). Therefore, it is likely that the detection of 8-OHdG in serum or urine may be a clinically useful tool. Although, the identification of 8-OHdG in urine also makes this marker of oxidative DNA damage suitable for screening vulnerable populations.

There is a potential for 8-OHdG to be utilised as a clinical risk marker to detect early pre-cancerous processes. Salivary 8-OHdG levels are significantly higher in patients with precancerous lesions of the buccal mucosa (Kaur, *et al.*, 2016). The detection of 8-OHdG has also been utilised as part of a routine cervical cancer screening programme and successfully differentiated between normal tissue and high-grade squamous intraepithelial lesions (Romano, *et al.*, 2000). In addition, the measurement of 8-OHdG in hepatocarcinomatous tissue may possess prognostic value (Li, *et al.*, 2012). Higher levels of 8-OHdG in tumour tissue is related to poorer outcomes following a three year period. The researchers also noted that 8-OHdG levels may have a role in tumour development as the levels of this lesion correlated positively with tumour size, tumour quantity, clinical stage, portal vein thrombosis and ascites.

Moreover, the heterogenous activity of anti-oxidant enzymes in obesity is also commonly described in cancer. A decrease in anti-oxidant activity has been linked with DNA damage and acute lymphoblastic leukaemia in children (Sentürker, *et al.*, 1997). Whereas upregulated expression of SOD has been identified in patients with breast cancer (Er, *et al.*, 2004). Furthermore, increased activity of GPO but reduced activity of SOD has been reported in patients with oral cancer (Srivastava, *et al.*, 2016). Overall, it is plausible that

inadequate anti-oxidant mechanisms may predispose to malignancy, by enabling ROS to persist and react with DNA.

Presence of the 8-OHdG lesion in DNA during replication can lead to mutations. The G→T transversion is the most commonly described mutational consequence of 8-OHdG lesions (Suzuki, & Kamiya, 2017). G→T transversion mutations in the p53 gene have been documented in cancers of the breast, lung and bladder (Coles, *et al.*, 1992; Pfeifer, & Hainaut, 2003; Schroeder, *et al.*, 2003).

Furthermore, the G→T transversion has also been noted in the KRAS gene where it is associated with pancreatic, lung and myeloid cancer (Smit, *et al.*, 1988; Noda, *et al.*, 2001; Bowen, *et al.*, 2005). Other mutational consequences of the 8-OHdG adduct include G→A and G→C mutations (Suzuki, & Kamiya, 2017). Additionally, 8-OHdG can also inhibit methylation of adjacent cytosines (Wu, & Ni, 2015). This could be a cancer causing mechanism if hypomethylation occurs in the region of a tumour suppressor gene.

The etiological role of 8-OHdG in cancer can be explained by more than its increased persistence in DNA. The DNA repair enzyme 8-oxoguanine DNA glycosylase 1 (OGG1) enables the removal of 8-OHdG lesions to prevent tumorigenesis via the base excision repair (BER) pathway. Reduced activity of OGG1 in human peripheral blood mononuclear cells has been associated with increased risk of carcinogenesis in the lungs, head and neck (Paz-Elizur, *et al.*, 2006). In addition, a recent review has highlighted that OGG1 may also play a role in carcinogenesis by recruiting 8-OHdG as a ligand for gene regulation and activation of the Ras pathway (Ba, & Boldogh, 2018). The Ras pathway is frequently activated in human cancers as Ras proteins control cell shape, survival and progression through the cell cycle by responding to growth factors

(Rajalingam, *et al.*, 2007). Mechanisms of 8-OHdG interference with gene transcription were first identified by Ramon and colleagues (1999). They demonstrated that 8-OHdG alters binding of the transcription factor Sp1 when located at promoter regions in HeLa cells. Black and colleagues (2001) have reviewed the various roles that Sp1 can have in human and animal cancer lines. These include, maintaining cell growth, proliferation, angiogenesis and apoptosis. Overall, there are implications that 8-OHdG presents with remarkable oncogenic signatures, and is therefore a well-established marker for genomic instability.

Moreover, 8-OHdG levels can be elevated in cancerous and pre-cancerous pathologies as well as paediatric obesity. This evidence advocates the clinical detection of this lesion more widely to monitor genomic instability in children with increased adiposity.

4.4 Increased nuclear anomalies in the buccal mucosa of children with obesity

Following a detailed evaluation of nuclear morphology in buccal epithelial cells, this research has identified obesity in children to be associated with up to a 1.7 fold increase in the frequency of genome damage events. The statistically significant difference is evident whether obesity is defined by BMI Z-score, body fat percentiles or waist circumference percentiles. Furthermore, BMI Z-score, body fat percentage via bioelectrical impedance, waist circumference and waist to hip ratio are all strongly correlated with the total frequency of nuclear anomalies in the upper aerodigestive tract.

In 2009, the first investigation of anthropometric markers of adiposity and nuclear anomalies in the buccal mucosa was conducted on a small cohort of Mexican children aged 7-11 years by Flores-García and colleagues. This study found no association between DNA damage and overweightness. The confounding findings may be due to the researchers choice of orcein to stain the cells instead of a DNA specific dye. This may have led to cytoplasmic genetic material being rejected as non-nuclear bodies such as debris or artefacts from microorganisms. More recently, Idolo and colleagues (2018) investigated the impact of various lifestyle factors on nuclear anomalies in the buccal mucosa of 6-8 year old Italians using the Feulgen stain. Despite the small number of obese participants in this study as well, the researchers concluded obesity in children to be an independent risk factor for increased micronuclei frequency. Therefore, the results predating this research were so far inconclusive. This thesis has identified five independent risk factors for an increased total frequency of nuclear anomalies in the buccal mucosa. Namely, excess salivary CRP, low salivary vitamin D, a BMI above the 98th centile, body

fat percentage above the 95th centile and waist circumference above the 95th centile.

Other associations between adiposity and micronuclei frequency in children have been conducted on peripheral blood lymphocytes (PBLs). In 2011, Scarpato and colleagues first reported a 2.7 fold higher frequency of micronuclei in PBLs of 60 obese children compared to 38 age-matched healthy weight controls. In addition, they demonstrated a raised frequency of γ -H2AX foci, TNF- α , CRP and IL-6 in the same cohort of children, implying a causative association between inflammation and chromosomal aberrations. Recently, positive associations between weight and length at birth, and nuclear anomalies including the frequency of MNi, NBuds/Bridges have been reported in a longitudinal study conducted on 87 neonates (Dass Singh, *et al.*, 2017). The researchers also noted a high maternal BMI to be associated with increased nuclear anomalies in PBLs of their offspring. This suggests that possible genotoxic effects of adiposity may be transgenerational. Finally, studies conducted in adults have also demonstrated positive associations between chromosomal aberrations and weight status (Donmez-Altuntas, *et al.*, 2014).

Excess body fat has also been proposed as a pathological factor for chromosomal breaks following investigations with other biomarkers. In a cohort of adolescents, an almost 2 fold increase was found in mitomycin C induced DSBs when compared to healthy controls, measured by γ -H2AX foci (Azzarà, *et al.*, 2016). Intriguingly, the DNA damage in the obese cohort was repaired faster and more efficiently than in the healthy weight counterparts, indicating that there may be a potential for DNA repair mechanisms to be able to cope with the emerging accounts of DNA damage in obesity, but this warrants further

investigations. So far, more studies have evaluated chromosomal aberrations in adult obesity rather than childhood obesity. A correlation study first indicated a positive association between BMI in 99 females and oxidative DNA damage via the comet assay (Hofer, *et al.*, 2006). Later, A positive correlation between increased chromosomal damage and BMI (>30) was demonstrated by the comet assay in pregnant women of a Pakistani population (Bukhari, *et al.*, 2010) and was subsequently confirmed in an Indian population too (Gandhi, & Kaur, 2012). Remarkably, DNA damage was also identified via the comet assay in women with a normal BMI but elevated fat mass (Tomasello, *et al.*, 2011). This 'normal-weight-obese' cohort also had higher levels of oxidative DNA damage, measured by 8-OHdG, than the obese cohort of women. These findings support the need of multiple measures of adiposity to be considered for the assessment of genomic instability. The results in this thesis also demonstrate a stronger correlation between body fat percentage and nuclear anomalies compared with BMI. Overall, determining the causes and consequences of chromosomal instability in obesity should be a priority for the prevention of further morbidity.

Firstly, we have identified increased levels of CRP in saliva in the same cohort of obese patients that also present with excess nuclear anomalies. However, there is a lack of statistically significant correlation between salivary CRP and nuclear anomalies in the buccal mucosa. This means that there are other factors that also condition the levels of DNA damage in the buccal mucosa and may perhaps modulate levels of inflammation. In chapter 4.1, it was concluded that inflammation in obesity may cause DNA damage by promoting the production of ROS. Therefore, it is also likely that levels of anti-oxidant enzymes can modulate the response to inflammation and DNA damage in obesity.

Our results demonstrate that the total frequency of nuclear anomalies in the buccal mucosa is correlated with levels of vitamin D in saliva, and that body fat percentage can be combined with levels of salivary vitamin D to predict DNA damage in the buccal mucosa. It is likely that deficient levels of salivary vitamin D in obesity may exacerbate the effects of adipose tissue dysfunction and consequent DNA damage. This is because vitamin D has been described as an anti-inflammatory and anti-oxidant that may also have a role in halting the cell cycle following DNA damage (Abbas, 2017; Fedirko, *et al.*, 2010; Kovalenko, *et al.*, 2010). Previous studies have not assessed vitamin D status and DNA damage simultaneously in childhood obesity. However, excess DNA damage in sperm cells has been related to vitamin D deficiency and excess adiposity in a rat model (Merino, *et al.*, 2018).

There is also some evidence to suggest that vitamin D deficiency, independent of adiposity, is associated with increased chromosomal aberrations. A recent study demonstrated that a sufficient serum vitamin D status may modulate the effects of UV-light induced micronuclei formation in human lymphocytes (Nair-Shalliker, *et al.*, 2012). Furthermore, vitamin D treatment has also demonstrated a reduction in micronuclei frequency of rat hepatocytes and in a model of murine lymphoma (Chatterjee, 2001). Overall, it can be postulated that vitamin D deficiency is a modifiable risk factor for chromosomal aberrations in children with obesity. It is also likely that diminishing the extent of nuclear anomalies in early-onset obesity may have remarkable effects on the likelihood of acquiring co-morbidities later in life.

There is evidence that excess micronuclei frequency can be a predictor and driver of malignancy. Prospective research approaches have already

associated micronuclei occurrence in PBLs with an increased risk of developing cancer. Early research by Bonassi and colleagues (2006) followed up 6718 participants across 20 sites after their participation in the Human Micronucleus (HUMN) project and reported a statistically significant risk of stomach and urogenital cancer to be associated with a high baseline frequency of micronuclei in lymphocytes. Another follow-up study reported remarkable evidence to support the link between micronuclei and risk of cancer (Murgia, et al., 2008). This research followed 1650 initially disease free adults for up to 14 years. Participants that developed cancer had, on average, a three-fold increase in micronucleus frequency at baseline. There are multiple reports of micronuclei frequency being up to three folds higher in patients with cancers of the brain, bladder, breast, cervix, GI tract, lungs and pancreas (Appendix XIII). Whilst the long-term effects of micronuclei frequency in buccal cells have not been demonstrated to date, there has been a report that MNi frequency is well correlated between PBL and buccal cells in cancer patients (Podrimaj-Bytyqi, et al., 2018). This indicates the an increased frequency of micronuclei in buccal epithelial cells may also have systemic relevance.

Firstly, an increased frequency of micronuclei in buccal epithelial cells has been consistently identified in patients with cancers of the aerodigestive tract including squamous cell carcinoma (Mandard, et al., 1987; Bloching, et al., 2000; Ramirez, & Saldanha, 2002; Saran, et al., 2008). Furthermore, the buccal micronucleus assay has also been used to screen pre-malignant oral lesions such as oral submucous fibrosis and leucoplakia. Findings from such research initiatives have indicated that pre-malignancy in the oral tract is also associated with a higher frequency of MNi (Desai, et al., 1996; Saran, et al., 2008; Bloching, et al., 2000). Recently, Katarkar and colleagues (2014) demonstrated

that excess MNi in pre-malignant oral lesions is strongly correlated with chromosomal breakages (assessed via comet tail length) in PBLs. These studies clarify that an excess MNi frequency in buccal epithelial cells has a relevance in oral malignancies. Evidence is building that MNi frequency in buccal cells may also relate to cancer at other sites.

In 2004, a higher frequency of micronuclei in the oral mucosa was identified in patients with breast and uterine cancer by Nersesyan and Adamyan. These results were substantiated by subsequent research in a cohort of 21 breast cancer patients (Flores-García, *et al.*, 2014). The researchers also identified an increased frequency of other nuclear anomalies in the buccal mucosa including bi-nucleated cells and nuclear buds. Other evidence demonstrated that patients with lung, stomach or colorectal cancer can also present with an increased occurrence of MNi in the oral mucosa (Yildirim, *et al.*, 2006). Moreover, micronuclei frequency in urothelial cells, buccal epithelial cells and peripheral blood lymphocytes is higher in non-smoking patients with urothelial cell carcinoma compared to cancer-free controls (Podrimaj-Bytyqi, *et al.*, 2018). Interestingly, these researchers also demonstrated a moderate correlation between lymphocyte and buccal MNi frequency, suggesting that the buccal micronucleus assay may be a useful non-invasive tool for predicting cancer risk. Finally, a recent systematic meta-analysis of forty-two studies has concluded that the prevalence of micronuclei in the oral mucosa may be reflective of chromosomal instability occurring in other tissues (Feki-Tounsi, *et al.*, 2014). Overall, a higher incidence of micronuclei in the oral cavity has been associated with cancer at eight different sites; head and neck, oral, lung, breast, stomach, colon, bladder and the uterus. Longitudinal studies in large cohorts may

consolidate the use of the buccal cytome assay as a tool for cancer risk prediction, particularly in the case for obese children.

The increased associations between chromosomal aberrations and cancer can be explained by the phenomenal role of micronuclei as potential drivers of malignancy. There are indications that micronuclei containing whole chromosomes can proceed into several cell generations and be reincorporated into the genome following further mitotic divisions (Shimizu, 2011). Micronuclei division cycles can lead to catastrophic genetic re-arrangements in a single or few chromosomes – a newly described mutational process called chromothripsis (C.-Z. Zhang, *et al.*, 2015). Such localized chromosomal re-arrangements may be transferred to daughter nuclei in subsequent mitotic cycles and play a role in generating a pre-cancerous genome. This is because the insertion of damaged DNA and consequent end-joining base repair mechanisms may amplify oncogenes and lead to a loss of tumour suppressor gene function (Rode, *et al.*, 2016). Chromothripsis and catastrophic DNA rearrangements have been described in aggressive tumours of the brain, blood and skin (Rausch, *et al.*, 2012; Magrangeas, *et al.*, 2011; Hirsch, *et al.*, 2013). Furthermore, MNi can display a lack of nuclear envelope integrity when occurring in cancer cells (Hatch, *et al.*, 2013). Firstly, impaired nuclear envelope function has been related to an increase in DNA damage in MNi within cancer cells – a process that may also promote chromothripsis (Hatch, *et al.*, 2013).

Secondly, it is also likely that the nuclear envelope of a micronucleus is more likely to rupture, causing exposure of self-DNA to the cytosol. Possible immunostimulatory consequences of this event have recently been reported in a mouse model and human cancer cells (Mackenzie, *et al.*, 2017). This means that the

occurrence of micronuclei may also drive carcinogenesis by triggering inflammation. Inflammation can play an important role in the carcinogenic transformation of cells by causing DNA alterations and by supporting the tumour microenvironment with chemokines for cell proliferation, survival, metastasis, and angiogenesis (Multhoff, *et al.*, 2012).

Interestingly, chromatids in micronuclei can also demonstrate a lack of important kinetochore proteins which may prevent them from being re-incorporated into the main nucleus (Soto, *et al.*, 2018). This phenomenon has been proposed as a protective mechanism of removing damaged DNA from the cell. However, the complete removal of a micronucleus from a cell may also have carcinogenic implications if the micronucleus contained a tumour suppressor gene. Early research has demonstrated the accumulation of p53 in micronuclei within cultured mammalian cells (Granetto, *et al.*, 1996). A higher frequency of micronuclei has also been detected in pre-cancerous cells of the oral mucosa that are p53 deficient (Abbondandolo, *et al.*, 2002). Overall, micronuclei are not only markers of genome damage but can also contribute to chromosomal instability and malignancy.

The increased frequency of nuclear buds and nucleoplasmic bridges may also have a relevance in cancer risk. Nuclear buds and nucleoplasmic bridges have been described as a consequence of unrepaired DNA damage or gene amplification and associated with BFB cycles. It is important to emphasise that the addition of this biomarker to the micronucleus assay was only considered in the year 2000, and therefore, a limited number of studies have taken on board the 'cytome approach' (Heddle, *et al.*, 2010). Secondly, these DNA damage events are scored in one category despite having their own aetiologies

(Thomas, *et al.*, 2009). Nonetheless, an increased frequency of NBUDs and Bridges in the buccal mucosa has been identified in breast cancer patients (Flores-García, *et al.*, 2014). And recently, an increased frequency of NBuds and Bridges was documented in PBLs from cancer patients compared to healthy controls (Podrimaj-Bytyqi, *et al.*, 2018).

Furthermore, NBridges may drive malignancy as BFB cycles can lead to the accumulation of DNA amplifications and chromosomal rearrangements seen in tumour genomes (Zakov, *et al.*, 2013). DNA amplification and chromosomal rearrangements have been noted in cancers of the lung, breast, prostate, GI tract and skin (Fenech, 2002). Chromosomal instability has also been suggested as a potential prognostic marker for colorectal cancer, due to its association with poorer outcomes (Walther, *et al.*, 2008). Chromosomal instability can lead to aneuploidy via malsegregation of chromosomes (McGranahan, *et al.*, 2012). The resulting heterozygosity may lead to a loss of wild-type tumour suppressor alleles and thereby favour malignancy (Ryland, *et al.*, 2015). Overall, it is undisputed that NBuds and Bridges are markers of early events in chromosomal instability - a feature associated with aggressive malignancies. However, more research is required to confirm the clinical potential of an increased frequency of NBuds and Bridges in the buccal mucosa to predict pre-cancerous changes.

In this investigation, the largest difference in means amongst nuclear anomalies between obese and non-obese children was observed in the frequency of multi-nucleated cells. Within this category, a large proportion of cells were identified to be bi-nucleated although cells with three or more nuclei were also observed.

Therefore, it is vital to deduce the possible clinical significance of such increased cytokinesis failure in the buccal mucosa.

Recently, an increased frequency of binucleate cells has been reported in the buccal mucosa of breast cancer patients undergoing chemotherapy, compared to cancer free controls (Paz, *et al.*, 2018). Moreover, the occurrence of binucleated cells in the buccal mucosa can also be 1.8x higher in patients with head and neck cancer (Khlifi, *et al.*, 2013). In 2000, Morin and colleagues described an increased frequency of binucleate cells in lesions of the cervix. Later, researchers explored the independent use of cytological features to diagnose HPV associated endometrial cancer (Bollmann, *et al.*, 2005). Multinucleated cell frequency correctly diagnosed 90.78% of cases and appeared to be the most sensitive cytological feature for detecting HPV infection. Furthermore, increased multinucleated cell frequency has been associated with mesothelioma across a cohort of 42 patients (Kimura, *et al.*, 2009). In this study, 82% of cells from patients with malignant mesothelioma had more than eight nuclei, compared to just 15% of cells obtained from patients with benign tumours.

An increased frequency of binucleated cells has also been observed in cultured cancer cells. It is of interest that nutrient starvation of HeLa cells – a cervical cancer cell line, led to a significant increase in binucleated cells of which some were also capable of proliferating (Nishimura, *et al.*, 2016). From this research, it can be postulated that despite acquiring cytokinesis failure, cancer cells are able to proliferate with possibly more mutagenic complications.

In a recent review, tetraploidy and cytokinesis failure have been evaluated as mechanisms for aneuploidy in subsequent mitotic cycles. It has been suggested

that these events can lead to genetic diversification in cancer cells that possibly gives them an advantage in development (Lens, & Medema, 2019).

Centrosomes are not just important for the appropriate segregation of chromosomes but also play a role in marking the cleavage plane for cytokinesis (Hurtley, 2001). Centrosome abnormalities are associated with cancers of the breast, prostate, bladder, pancreas, head and neck, oral cavity, and the nervous system (Weber, *et al.*, 1998; Lingle, *et al.*, 1998; Sato, *et al.*, 1999; Mark Gustafson, *et al.*, 2000; Pihan, *et al.*, 2001; Thirthagiri, *et al.*, 2007; Yamamoto, *et al.*, 2009; Lingle, *et al.*, 2002). Therefore, increased association between binucleated cells and carcinogenesis can be related to dysfunctional centrosomes.

Furthermore, a number of different mitotic and cell cycle checkpoint proteins that regulate cytokinesis can be mutated in cancer (Sagona, & Stenmark, 2010). The inactivation of BRCA2 has also been associated with carcinogenesis (Daniels, *et al.*, 2004). This leads to the speculation that cytokinesis failure may be an early event in tumorigenesis. However, polyploidy in some types of cancer may not just be due to cytokinesis failure but can also be attributed to cell fusion or cell cannibalism, a phenomenon described in breast cancer (Krajcovic, *et al.*, 2011; Krajcovic, & Overholtzer, 2012). To conclude, there is some evidence that the frequency of tetraploid cells is associated with malignancy. The biological mechanisms that underpin the formation of bi or multinucleated cells lead to the postulation that cytokinesis failure may lead to genetic alterations that are favourable for cancer cells.

Together, these findings warrant further research into the optimisation of the buccal cytome assay as a potential non-invasive, clinical tool for monitoring

prepathological disorders in vulnerable cohorts. The molecular mechanisms that underpin nuclear anomalies with carcinogenesis further substantiate the need for this tool.

4.5 Salivary telomere length is greater in children with obesity

This research has identified obesity in children to be associated with up to a x1.5 fold increase in salivary telomere length (sTL). The classification of obesity via BMI percentiles, body fat percentage and waist circumference are all associated with a significant increase in sTL. Furthermore, sTL in children is positively correlated with BMI Z-score, body fat percentage, waist circumference but not with WHR. This research also finds a weak, yet statistically significant correlation between sTL and nuclear anomalies in the buccal mucosa.

To date, investigations into the relationship between adiposity and telomere length have assessed the former in peripheral blood lymphocytes, other leukocytes or adipocytes. The outcomes of these investigations in children present with discrepancies. Firstly, a study that established a link between obesity and telomere shortening in adults did not verify this phenomenon in a cohort of 53 Caucasian children using TRF analysis (Zannolli, *et al.*, 2008). However, later studies confirmed that childhood obesity can be associated with a state of telomere attrition. Via qPCR analysis, Al-Attas and collaborators (2010) reported an inverse correlation between obesity and telomere length in boys but not girls. A large case-control investigation conducted in 793 French children also using qPCR techniques, reported leukocyte telomere length (LTL) to be 23.% shorter in children with obesity, defined via BMI percentiles (Buxton, *et al.*, 2011). It is interesting that a subsequent study conducted in a large cohort of adolescents using the same methods to assess LTL reported no association between BMI, WHR or body fat percentage and LTL (Haidong Zhu, *et al.*, 2011). Furthermore, in the same cohort, there was no association between the adipokines leptin, and adiponectin and LTL. However, the

investigators reported ethnic and sex differences such that LTL was longer in black African and female adolescents compared to Caucasians and males. The discrepancies amongst LTL and adiposity in childhood highlight the complexity of telomere length dynamics.

Similar are the discrepancies between telomere length and adult adiposity. Initial reports of a negative correlation between BMI and telomere shortening were presented in a study conducted on 561 female twins (Valdes, *et al.*, 2005). Interestingly, a subsequent study published by Nordfjäll and colleagues (2008) confirmed these findings with a variety of obesity parameters including BMI, weight, and waist and hip circumference. Similar to the results in adolescents, this study also reported a sex bias as the correlation is only statistically significant in women. An extensive study conducted by Kim and collaborators (2009), also reported an inverse correlation between weight gain and telomere length in 647 women. A number of subsequent studies have built evidence to support the hypothesis that increased body fat in adults is an independent causative factor for accelerated ageing (Appendix XIV). However, some studies have also found little or no association between multiple measures of adiposity and telomere length (Diaz, *et al.*, 2010). In adults, harbouring the metabolic syndrome can alter telomere length dynamics. Obese women without metabolic syndrome have longer telomeres than obese women with an abnormal insulin, lipid and inflammatory profile (Iglesias Molli, *et al.*, 2017).

It is also likely that the dynamics of telomere length are tissue-specific, as telomere length results appear to contrast with those reported in peripheral blood. Shorter telomere length has been reported in cells of subcutaneous adipose tissue extracted from obese men and women (Moreno-Navarrete, *et*

al., 2010). However, studies conducted in saliva present conflicting results. Our results are the first to demonstrate excess salivary telomere length in childhood obesity compared to healthy weight controls. Similar findings have been reported in the only direct investigation of the effects of adult adiposity on salivary telomere length (An, & Yan, 2017). An and Yan (2017) have conducted qPCR analysis of saliva samples obtained from 2749 adults 16 years after recording height and weight, and conclude BMI and obesity to positively predict telomere length. Overall, further research is needed to confirm the contrary associations between greater telomere length in saliva and obesity status.

The contrasting results between studies of adiposity and telomere length analysis across leukocytes and saliva suggests possible differences in the regulation of telomere length across tissues. Recent research has pointed out an inverse correlation between age and telomere length in lymphocytes in contrast to a positive correlation between age and telomere length in buccal cells (O'Callaghan, *et al.*, 2008). Buccal epithelial cells contribute a large (75%) proportion of genomic DNA in saliva (Garbieri, *et al.*, 2017). This is due to the shedding of the epithelial layer of the buccal mucosa that takes place every 2.7 hours (Dawes, 2003). This means that only a small proportion of salivary DNA is from leukocytes. Furthermore, although there is a moderate correlation between sTL in samples obtained via the passive-drool technique and TL from PBLs in female adults (Goldman, *et al.*, 2018), it is unknown whether this correlation is prevalent in children or may be modified by the effects of obesity.

Nonetheless, the findings of excess telomere length in saliva samples from this cohort of obese children may be explained by the evidence of excess oxidative DNA damage in the same cohort. Recent research has uncovered a paradox whereby oxidative DNA damage lesions such as 8-OHdG can induce both telomere shortening or lengthening (Fouquerel, *et al.*, 2016). The researchers demonstrated that the presence of the oxidised guanine base in single strand telomeric DNA may trigger upregulation of telomerase in a human cell line. The telomerase enzyme has been detected in normal buccal epithelial tissue with low activity (Rai, *et al.*, 2016). An investigation in mice also supports these findings whereby strains with a deletion in the repair enzyme OGG1 had longer telomeres compared to the wild-type mice (Wang, *et al.*, 2010). This is an interesting result because other research has demonstrated the incorporation of oxidised guanine to trigger chain termination, inhibition of telomerase and consequently, telomere shortening (Hukezalie, *et al.*, 2012). One explanation for this paradox is that that low levels of the oxidised guanine base may cause telomere lengthening whereas higher levels may lead to telomere attrition (Barnes, *et al.*, 2019).

Another possible mechanism that may explain telomere lengthening in our cohort of obese participants is the lack of vitamin D when compared to the healthy-weight cohort. Treatment of an ovarian cancer cell line with 1,25-dihydroxyvitamin D can down-regulate telomerase activity (Jiang, *et al.*, 2004). Recent research has substantiated these findings again in malignant ovarian tumours and describe a microRNA-498 pathway may mediate this process (Kasiappan, *et al.*, 2012). On the other hand, vitamin D supplementation can also upregulate telomerase expression and length telomeres in PBLs (H Zhu, *et al.*, 2011). Therefore, there is a possible role for vitamin D in modulating the

expression of telomerase and maintaining telomere length. However, the cellular conditions and mechanisms that may drive telomere lengthening or shortening in the presence of vitamin D are unclear and warrant further research.

Traditionally, greater telomere length is associated with positive health outcomes, whilst telomere attrition is associated with morbidity and age related disorders. In 1973, Olovnikov first proposed telomere shortening to be associated with cellular ageing and the Hayflick constant, followed by Harley and colleagues (1992) who further report telomere shortening as a phenomenon in ageing human fibroblasts. Since then, telomere shortening has been discussed as a biomarker for detecting the onset of multiple age related disorders including cardiovascular disease and diabetes, Alzheimer's, Parkinson's and arthritis (Wang, *et al.*, 2008; Hochstrasser, *et al.*, 2012; Xi, *et al.*, 2013; Steer, *et al.*, 2007). Telomere length has also been assessed in disease models of accelerated ageing including Werner's syndrome and Hutchinson-Gilford Progeria (HGPS). Ishikawa and colleagues (2011) identified shorter telomeres *in vivo* from skin cells that were cultured from patients with Werner syndrome compared to those cultured from healthy controls. Similar findings were identified in patients with HGPS (Decker, *et al.*, 2009). It is of interest that despite telomere shortening being a phenomenon common to both disorders, patients with Werner's syndrome have an increased susceptibility to acquiring cancer, whereas HGPS is associated with a resistance to oncogenic transformations (Fernandez, *et al.*, 2014). Although HGPS is associated with high levels of DNA damage, this research indicated that the expression of progerin can override carcinogenic processes through the cell cycle, particularly the inhibition of p53 and pRB. Recent evidence has shown that telomere

shortening may not be the primary cancer driving mechanism in Werner's syndrome (Tokita, *et al.*, 2016), but other mechanisms such as an upregulation of genes associated with cellular senescence may be more important (Tang, *et al.*, 2016). Interestingly, a recently conducted population based follow-up study of 598 adults concluded that telomere length in PBLs is not associated with ageing or morbidity over the age of 85 years, possibly due to instabilities in telomere length during old age (Martin-Ruiz, *et al.*, 2005). These findings indicate that telomere length maintenance is a more complex phenomenon, likely to modulate with normal, physiological aging as well as disease. Nonetheless, telomere shortening has become a well-known, undisputed biomarker for ageing, but its role as a biomarker for detecting early pathological changes in cancer, requires clarification.

There is a heterogenous relationship across telomere length and different types of cancer. A lower mean buccal cell telomere length is seen patients with bladder cancer compared to cancer-free controls (Broberg, *et al.*, 2005). A recent comprehensive review of over 23,000 cases has unveiled a significant correlation between telomere attrition in PBLs and increased risk of GI tract, head and neck cancers, but not skin cancers (Zhu, *et al.*, 2016). The researchers have brought to light that instead, longer telomere length is a risk factor for acquiring skin cancer. Similarly, other studies have noted a non-linear relationship for cancers of the breast, pancreas and oesophagus (Risques, *et al.*, 2007; Qu, *et al.*, 2013; Skinner, *et al.*, 2012). In the case of lung cancer, both telomere shortening and lengthening have been described as risk factors (Jang, *et al.*, 2008; Lan, *et al.*, 2013). Furthermore, genetic variants associated with longer telomere length are also associated with an increased risk of acquiring renal cell carcinoma (Machiela, *et al.*, 2017). These associations

between lengthened telomeres and cancer risk are not surprising because 80-90% of cancer can exhibit increased telomerase activity (Stewart, & Weinberg, 2006). Whilst research assessing the significance of telomere length in buccal cells is limited, it is clearer that telomere length in PBLs is an important marker of genomic instability where excessive lengthening may be just as pathological as telomere shortening for carcinogenesis.

Telomere attrition may promote generation of cancer stem cells by causing a series of chromosomal fusions, anaphase bridge formations and breakage-fusion-breakage cycles that enable DNA damage to accumulate (De Lange, 1995). Whereas lengthened telomeres may enable the cell to enter into more division cycles, thus increasing the susceptibility of the genome to abnormalities, lethal mutations as well as immortality (Hahn, *et al.*, 1999). For this reason, telomeres are considered to be targets for anti-cancer therapies. Recently, Bejarano and colleagues (2019) demonstrated that deletion of TRF1 shelterin protein can impair the growth of tumours in aggressive lung and glioblastoma mouse models. Interestingly, this induction of telomere damage was independent to telomere length.

There is also evidence that initial telomere lengthening may lead to telomere attrition, due to telomeres being long but dysfunctional (Bull, *et al.*, 2014). Bull and colleagues (2014) report a positive correlation between the frequency of nuclear anomalies in cultured human lymphocytes and telomere length. They concluded this to be an indicator of long, dysfunctional telomeres. Similarly, our research reports a positive correlation between nuclear anomalies in the buccal mucosa and salivary telomere length. There is evidence linking telomere dysfunction with increased cytokinesis failure and potentially mutagenic

consequences (Pampalona, *et al.*, 2012). Considering this evidence in light of excess inflammation, vitamin D deficiency and oxidative DNA damage in the same obese cohort, leads to the speculation that the telomeres are not just greater in length but possibly also dysfunctional. To confirm these findings, further research in salivary DNA to assess levels of oxidised guanine within the telomeric overhang region may be required.

Overall, the findings presented by this study are in contrast with other associations described between adiposity and telomere length dynamics. Further research is recommended to first confirm that obesity in childhood is associated with excess salivary telomere length. This research is limited in that the quantification of salivary DNA was conducted using a spectrophotometer, which has been described as an unreliable technique to quantify salivary DNA due to possible bacterial contamination of samples (Quinque, *et al.*, 2006). Although the contamination of bacteria in samples was limited by rinsing the mouth prior to sample collection and by employing a commercially available kit to inhibit bacterial growth, there is still a chance that bacterial DNA could affect the quantification of salivary DNA samples. Secondly, further research is needed to confirm the clinical relevance of the potentially excess telomere length in saliva and whether this phenomenon may give rise to protective or pathological consequences.

4.6 Integrating DNA damage assessments in the clinical management of childhood obesity

Overall, the results of this research confirm that obesity in childhood, when defined by one or more indices of adiposity, is concurrent with excess inflammation, lower vitamin D and increased genomic instability. The non-invasive sampling employed as part of this research verifies that these pathological states can be detected without blood sampling. Therefore, this research recommends the development of a clinical algorithm to guide monitoring of inflammation, micronutrient status and DNA damage in children with obesity.

Assessing inflammation, micronutrient status and DNA damage in severe childhood obesity may assist clinicians with selecting interventions and determining adequate weight-loss to prevent carcinogenesis. The resolution of an excess BMI to normality can take several months or years depending on the severity of obesity and the selected intervention. In the interim, genomic health could be monitored to inform progress and adherence with interventions, and escalate approaches where necessary.

The findings of this thesis indicate that BMI Z score correlates most strongly with all the other markers of adiposity that include body fat percentage, waist circumference, and waist to hip ratio. This indicates that BMI percentiles could be selected as a primary method of classifying obesity, as it is in agreement with other markers of adiposity. Grouping participants based on their body fat percentage generates a greater significant difference in biomarkers. However, none of the differences between biomarkers are present when adiposity is defined by body fat percentage alone. Classification of participants via BMI Z-

score led to a statistically significant difference across all biomarkers that were assessed. This means that BMI Z-score may be a more adequate predictor of co-morbidities compared to body fat percentage. Bohn and colleagues (2015) have conducted an assessment of BMI Z-score, body fat percentage via bioelectrical impedance, and cardiovascular disease risk markers in a cohort of 3,327 children. They conclude that the assessment of body fat percentage is not superior to assessment of BMI Z-score when determining the risk of cardiovascular markers in children and adolescents with obesity. Therefore, based on the findings in this thesis, the assessment of co-morbidities should be recommended for patients with a BMI above the 98th centile.

However, BMI Z-score like body fat percentage, is not correlated with the level of oxidative DNA damage assessed in urine. Whereas a moderate, positive correlation exists between WC, WHR and urinary 8-OHdG. In addition, waist circumference and waist to hip ratio are well correlated. The assessment of hip circumference in addition to waist circumference may introduce a higher risk of methodological error. Secondly, the agreement between WHR and other anthropometric markers in this research is poor. Finally, conducting multiple anthropometric assessments may be a time-consuming task in clinic. Based on the moderate correlation between central adiposity and oxidative DNA damage, this research recommends utilising waist circumference in addition to BMI Z-score to inform the assessment and monitoring of inflammation, vitamin D status and genomic instability in children with obesity.

It is interesting that despite obesity, assessed via BMI Z-score, being concurrent with increased salivary CRP, the levels of salivary CRP are not correlated with either of the DNA damage markers. Although, odds ratio analysis between

inflammation and DNA damage in the buccal mucosa does indicate that these two events may be linked. Whilst a causative association can neither be approved nor rejected, the analysis of salivary CRP could still be a component of the monitoring protocol. This is because inflammation is a well-known etiological risk factor for cancer and changes in salivary CRP levels could potentially inform this risk.

In addition, other possible markers that could be assessed include total ROS production, although this too may be modulated by anti-oxidant function. Other inflammatory cytokines that have been monitored in obese children include TNF-alpha and IL-6 (Halle, *et al.*, 2004). It is also recommended that in order to fully understand the risk of co-morbidities and cancer, other investigations at baseline may also include markers that have been mechanistically linked with inflammation and DNA damage. These include plasma fasting glucose, insulin, lipid and oestrogen levels (Olusi, 2002; Roy, & Liehr, 1999; Lin, *et al.*, 2005; Lee, & Chan, 2015). Furthermore, hyperpigmentation around the neck, axilla, knuckles and popliteal fossa can also signify insulin resistance and is noted increasingly in adolescents with obesity (Ng, 2016). Therefore, this dermatological condition known as Acanthosis Nigricans, may be a part of the initial physical work-up. Similarly, as oestrogen levels increase with puberty, pubertal status should also be recorded. We struggled with determining pubertal status in children using a self-reported questionnaire to recall the date of menses or state whether puberty has been achieved. For future research and clinical assessments, the Tanner stages should be used to determine pubertal status as they correlate well with sex hormone production (Rapkin, *et al.*, 2006). The initial work-up should also include an evaluation of pain and skeletal injury

as these states may also mark inflammation. Overall, a baseline assessment of all these parameters is essential for informing the type of intervention that will need to be administered.

Furthermore, this research has noted significant differences between salivary vitamin D levels in children with and without obesity, defined by percentage of body fat. However, a causative link between salivary vitamin D status and adiposity cannot be confirmed by this study. One of the limitations of this study is that although the Fitzpatrick scoring method and travel history were incorporated into the screening questionnaire to rule out sun exposure and skin type as causative factors for low vitamin D levels, we noted differences in interpretation of the questions by participants. Recording travel history depended on recall of memory, which proved to be more challenging for the younger participants. Therefore data from these questions was excluded from the analysis. Furthermore, the assessment of vitamin D status in saliva remains a challenge because of lower levels in saliva compared to serum, and a lack of studies confirming a correlation between the two fluids. Therefore, vitamin D analysis requires a more sensitive analytical tool for determining cut-offs in saliva before vitamin D supplementation can be administered. In the interim, the initial assessment and work-up of childhood obesity should include assessment of plasma vitamin D to adequately diagnose and treat a deficiency.

Nonetheless, it is of interest that salivary vitamin D levels were inversely correlated with the level of total nuclear anomalies in the buccal mucosa. The multiple regression analysis in this thesis presents a model to predict the level of DNA damage events in the buccal mucosa based on salivary vitamin D and body fat percentage. In light of this result, the monitoring of vitamin D in saliva

should be considered to predict possible implications on chromosomal integrity. Furthermore, deducing accurate cut-offs for vitamin D supplementation based on salivary levels could not be more urgent.

However, salivary vitamin D levels do not correlate with the other two markers of 'genome health' – urinary 8-OHdG and salivary telomere length. It may be that there are other micronutrients that when abundant or lacking in obesity, also play a role in triggering DNA damage or protecting from it. For example, obesity has been linked with an increased intake of omega-6 (Muhlhausler, & Ailhaud, 2013). A diet rich in omega-6 has been associated with increased DNA strand breaks (Bishop, *et al.*, 2015). Furthermore, excess fructose consumption has also been associated with obesity, insulin resistance as well as carcinogenesis (Laguna, *et al.*, 2014). Micronutrients where deficiencies are associated with DNA damage in children include vitamin B12, folate and α -tocopherol (Thomas, *et al.*, 2009; Milne, *et al.*, 2015). It is unfortunate that the dietary intake of participants could not be recorded as part of this investigation. Having this information could have also possibly strengthened the regression model presented here to predict levels of nuclear anomalies in the buccal mucosa. Overall, the monitoring of vitamin D is recommended in combination with a dietary assessment to establish overall micronutrient status.

This research has combined the assessment of three, well-established markers of genomic instability. Whilst it has been established that increased nuclear anomalies in the buccal mucosa and excess urinary 8-OHdG can be seen in patients with various types of cancer, the significance of possessing longer telomeres in saliva are less clear. Therefore, non-invasive monitoring of DNA

damage in children with obesity is recommended via the buccal micronucleus assay and combining this with urinary 8-OHdG could also be explored.

With respect to tools for intervention, a combined approach with dietary modifications, increments in the level of physical activity and reduction in screen time are useful for weight-loss (Summerbell, *et al.*, 2010). Indeed, such interventions should be implemented alongside motivational interviews, stage based goal-setting and parental training for adequate weight maintenance (Summerbell, *et al.*, 2010). Yet, achieving and maintaining weight-loss in extreme obesity can be more challenging.

The FDA approved drug Orlistat can be used to treat adolescent obesity in combination with lifestyle modifications. However, side-effects such as malabsorption of essential fat soluble vitamins from the gut is a cause of concern (Kanekar, & Sharma, 2010). When conventional methods of treatment fail, most often due to non-compliance, surgical interventions for extreme childhood obesity may be required (Widhalm, & Helk, 2015). More recently, gastric bypass for adolescents with severe obesity and co-morbidities has been acknowledged as an effective method for weight-loss surgery to improve quality of life (Widhalm, & Helk, 2015). It has been suggested that bariatric surgery as an early weight loss intervention can be more beneficial for reducing obesity related co-morbidities when implemented in adolescence rather than adulthood (Inge, *et al.*, 2007). Since bariatric surgery prior to a progression of severe co-morbidities has positive implications on markers of chronic diseases such as CVD, obstructive sleep apnoea, diabetes and NAFLD (Inge, *et al.*, 2007), it would be useful to explore the impact of bariatric surgery on markers of systemic inflammation and DNA damage.

There is evidence that bariatric surgery may improve genomic stability in obese adults, but this phenomenon is yet to be studied in adolescents. Laparoscopic gastric band application has shown a reversal in oxidative DNA damage, as reduced levels of urinary 8-OHdG were identified 6 months after this surgical procedure in a group of morbidly obese patients (Kocael, *et al.*, 2014). The same surgical procedure has demonstrated a reduction in DNA double strand breaks with decreased levels of γ -H2AX positive cells up to 7 years after surgery and restoration of telomere length in obese men (Mitterberger, *et al.*, 2014; O'Callaghan, *et al.*, 2009). Recently, Bankoglu and colleagues (2017) reported a reduction in DNA breaks assessed by the comet assay in 56 blood samples 12 months after surgery. Although, there are also some discrepancies amongst studies. In a study conducted on 107 obese subjects with and without metabolic syndrome, no extension in PBL telomere length was evident after a 12 month follow up of bariatric surgery (Formichi, *et al.*, 2014). However, it is unknown whether the obese adults in these studies were obese since childhood. It is therefore also unknown whether genome damaging effects of childhood obesity are reversible if interventions are conducted too far later in life. Therefore, interventions should be prioritised to correct pathological states associated with early-onset obesity.

Whilst there is currently no evidence for the effects of bariatric surgery on genome health in adolescents, other interventions do suggest genome instability may be a reversible phenomenon in young age. A calorie-restriction intervention coupled with prescriptive physical activity guidelines over a two-month period has demonstrated an increase in PBL telomere length after six months in a cohort of 74 adolescents (García-Calzón, *et al.*, 2014). Similarly, in

a mouse model, calorie restriction was also associated with weight loss and a decrease in DNA damaged demonstrated by reduced comet formation (Setayesh, *et al.*, 2019). Not just calories restriction but an increase in dietary anti-oxidants may also have the potential to reverse DNA damage. Recently, a two-month dietary intervention that incorporated hazelnut consumption resulted in improved lipid profiles as well as reduced oxidative DNA damage assessed via the comet assay in PBLs from adolescents (Guaraldi, *et al.*, 2018). However, in severe obesity, lifestyle changes alone may be inadequate for achieving substantial or sustainable weight-loss (Durkin, & Desai, 2017). Whilst bariatric surgery appears to be propitious for improving DNA integrity and stability in obese adults, whether such is the case in severe adolescent obesity should also be explored.

5. Conclusion

To conclude, excess adiposity in childhood is associated with an increase in acquired DNA damage and genomic instability. More specifically, a BMI above the 98th centile is concurrent with excess oxidative DNA damage in urine and excess chromosomal aberrations and cytokinesis defects in the buccal mucosa. Evidence supporting the use of salivary telomere length to monitor genomic instability in obesity requires clarification.

There is evidence that micro-nutritional deficiency and chronic inflammation are detrimental for optimal 'genome health'. This is the first research study to report hypovitaminosis D in saliva from children with obesity and correlate it with increased nuclear anomalies in the buccal mucosa. Secondly, the same cohort of children with obesity further present with increased levels of salivary CRP. Recent research confirms that salivary CRP is a reliable indicator of systemic inflammation. It is also well-established that inflammation is a causative factor for cancer whilst vitamin D may have anti-cancer properties.

Overall, this research supports the development of a clinical algorithm to guide the assessment, intervention and monitoring of adiposity, genome health and pre-pathological markers of malignancy in children with obesity. It is postulated that such an algorithm may be used to assess the success of weight-loss interventions and ultimately prevent co-morbidities in childhood obesity, including cancer.

6. Future Work

Future work involves developing a clinical algorithm for detecting pre-cancerous changes in children with obesity, and exploring the effect of weight-loss interventions on 'genome health' markers.

Firstly, there is a need to establish adequate cut-offs for salivary vitamin D and salivary CRP at which there is an increased risk of oxidative DNA damage and nuclear anomalies. Secondly, there is a need to define reference values for 8-OHdG in urine and nuclear anomalies in the buccal mucosa whereby increased risk of malignancy, pre-malignancy and malignancy can be reported.

Furthermore, the excess telomere length identified in the obese cohort requires corroboration. It is recommended that DNA quantification in saliva be performed via a fluorescent based technique that is specific for human DNA, rather than a spectrophotometer (Quinque, *et al.*, 2006). Secondly, this experiment could be coupled with quantifying the expression of telomerase in RNA. Other enzymes that can modulate telomere length such as TEN1 (Kasbek, *et al.*, 2013).

Furthermore, as 8-OHdG may also play a role in the activation of telomerase, quantification of 8-OHdG in saliva may explain the findings of excess telomere length (Fouquerel, *et al.*, 2016). Finally, whilst the significance of telomere length in PBLs is clearer, it is also important to establish the relevance of excess salivary telomere length in cancer.

In addition, the buccal cytome assay protocol can also make use of molecular probes for DNA adduct, aneuploidy and chromosome break assessment within the nuclei of buccal cells (Ramirez, *et al.*, 1999). This analysis could provide

more specific detail of the contents of micronuclei and thus establish stronger mechanistic links with cancer.

There is also a need to confirm the reversibility of DNA damage in children with obesity via weight-loss interventions and vitamin D supplementation. Such research could help establish which interventions models are more relevant for children that present with obesity and increased DNA damage. Overall, further work is need to define a clinical algorithm that would be useful for preventing co-morbidities in childhood obesity and optimising genome health.

7. References

- Aaltonen, L.A., Peltomaki, P., Leach, F.S., Sistonen, P., Pylkkanen, L., Mecklin, J.P., Jarvinen, H., Powell, S.M., Jen, J. and Hamilton, S.R., (1993). Clues to the pathogenesis of familial colorectal cancer. *Science (New York, N.Y.)*, **260**(5109), pp.812–816.
- Abbas, M.A., (2017). Physiological functions of Vitamin D in adipose tissue. *The Journal of steroid biochemistry and molecular biology*, **165**(Pt B), pp.369–381.
- Abbondandolo, A., Garzoglio, B., Casartelli, G., Margarino, G., Scala, M., Mereu, P., Bonatti, S. and Delfino, V., (2002). Micronuclei and p53 accumulation in preneoplastic and malignant lesions of the head and neck. *Mutagenesis*, **17**(1), pp.73–77. Available from: <<https://doi.org/10.1093/mutage/17.1.73>>.
- Aggarwal, B. and Jain, V., (2018). Obesity in Children : Definition , Etiology and Approach. , **85**(June), pp.463–471.
- Ahima, R.S., Saper, C.B., Flier, J.S. and Elmquist, J.K., (2000). Leptin regulation of neuroendocrine systems. *Frontiers in neuroendocrinology*, **21**(3), pp.263–307.
- Ahima, R.S. and Antwi, D.A., (2008). Brain regulation of appetite and satiety. *Endocrinology and metabolism clinics of North America*, **37**(4), pp.811–823. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/19026933>>.
- Aires, G.M.A., Meireles, J.R.C., Oliveira, P.C., Oliveira, J.L., Araújo, E.L., Pires, B.C., Cruz, E.S.A., Jesus, N.F., Pereira, C.A.B. and Cerqueira, E.M.M., (2011). Micronuclei as biomarkers for evaluating the risk of malignant transformation in the uterine cervix. *Genetics and Molecular Research*, **10**(3), pp.1558–1564.
- Ajala, O., Mold, F., Boughton, C., Cooke, D. and Whyte, M., (2017). Childhood predictors of cardiovascular disease in adulthood. A systematic review and meta-analysis. *Obesity reviews : an official journal of the International Association for the Study of Obesity*, **18**(9), pp.1061–1070.
- Akutsu, N., Lin, R., Bastien, Y., Bestawros, A., Enepekides, D.J., Black, M.J. and White, J.H., (2001). Regulation of gene Expression by 1alpha,25-dihydroxyvitamin D3 and Its analog EB1089 under growth-inhibitory conditions in squamous carcinoma Cells. *Molecular endocrinology (Baltimore, Md.)*, **15**(7), pp.1127–1139.
- Al-Attas, O.S., Al-Daghri, N., Bamakhramah, A., Shaun Sabico, S., McTernan, P. and Huang, T.-K., (2010). Telomere length in relation to insulin resistance, inflammation and obesity among Arab youth. *Acta Pædiatrica*, **99**(6), pp.896–899. Available from: <<http://dx.doi.org/10.1111/j.1651-2227.2010.01720.x>>.
- Al-Othman, A., Almusharaf, S., Al-Daghri, N., Krishnaswamy, S., Yusuf, D., M Alkharfy, K., Al-Saleh, Y., Al-Attas, O., S Alokail, M., Moharram, O., Sabico, S. and Chrousos, G., (2012). *Effect of physical activity and sun exposure on vitamin D status of Saudi children and adolescents*,
- Albuali, W.H., (2014). Evaluation of oxidant-antioxidant status in overweight and morbidly obese Saudi children. *World Journal of Clinical Pediatrics*, **3**(1), pp.6–13. Available from: <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4145643/>>.
- Allsopp, R.C., Vaziri, H., Patterson, C., Goldstein, S., Younglai, E. V, Futcher, A.B., Greider, C.W. and Harley, C.B., (1992). Telomere length predicts replicative capacity of human fibroblasts. *Proceedings of the National*

- Academy of Sciences of the United States of America*, **89**(21), pp.10114–10118. Available from:
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC50288/>.
- Alyahya, K.O., (2017). Vitamin D levels in schoolchildren: a cross-sectional study in Kuwait. *BMC pediatrics*, **17**(1), p.213.
- Amb, S., Bennett, W.P., Merriam, W.G., Ogunfusika, M.O., Oser, S.M., Harrington, A.M., Shields, P.G., Felley-Bosco, E., Hussain, S.P. and Harris, C.C., (1999). Relationship between p53 mutations and inducible nitric oxide synthase expression in human colorectal cancer. *Journal of the National Cancer Institute*, **91**(1), pp.86–88.
- An, H.J., Kim, K. II, Kim, J.Y., Shim, J.Y., Kang, H., Kim, T.H., Kim, J.K., Jeong, J.K., Lee, S.Y. and Kim, S.J., (2007). Microsatellite instability in endometrioid type endometrial adenocarcinoma is associated with poor prognostic indicators. *The American journal of surgical pathology*, **31**(6), pp.846–853.
- An, R. and Yan, H., (2017). Body weight status and telomere length in U.S. middle-aged and older adults. *Obesity Research & Clinical Practice*, **11**(1), pp.51–62. Available from:
<http://www.sciencedirect.com/science/article/pii/S1871403X16000053>.
- Anderson, E.L., Howe, L.D., Jones, H.E., Higgins, J.P.T., Lawlor, D.A. and Fraser, A., (2015). The Prevalence of Non-Alcoholic Fatty Liver Disease in Children and Adolescents: A Systematic Review and Meta-Analysis. *PLOS ONE*, **10**(10), p.e0140908. Available from:
<https://doi.org/10.1371/journal.pone.0140908>.
- Apostolou, P., Toloudi, M., Kourtidou, E., Mimikakou, G., Vlachou, I., Chatziioannou, M. and Papisotiriou, I., (2014). Use of the comet assay technique for quick and reliable prediction of in vitro response to chemotherapeutics in breast and colon cancer. *Journal of Biological Research*, **21**(1), p.14. Available from:
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4389674/>.
- Arens, R. and Muzumdar, H., (2010). Childhood obesity and obstructive sleep apnea syndrome. *Journal of applied physiology (Bethesda, Md. : 1985)*, **108**(2), pp.436–444. Available from:
<https://www.ncbi.nlm.nih.gov/pubmed/19875714>.
- Aucouturier, J., Meyer, M., Thivel, D., Taillardat, M. and Duché, P., (2009). Effect of Android to Gynoid Fat Ratio on Insulin Resistance in Obese Youth Android to Gynoid Fat Ratio and Insulin Resistance. *Archives of Pediatrics & Adolescent Medicine*, **163**(9), pp.826–831. Available from:
<https://dx.doi.org/10.1001/archpediatrics.2009.148>.
- Azzarà, A., Pirillo, C., Giovannini, C., Federico, G. and Scarpato, R., (2016). Different repair kinetic of DSBs induced by mitomycin C in peripheral lymphocytes of obese and normal weight adolescents. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **789**, pp.9–14. Available from:
<http://www.sciencedirect.com/science/article/pii/S0027510716300562>.
- Ba, X. and Boldogh, I., (2018). 8-Oxoguanine DNA glycosylase 1: Beyond repair of the oxidatively modified base lesions. *Redox biology*, **14**, pp.669–678.
- Bacher, J.W., Clipson, L., Steffen, L.S. and Halberg, R.B., (2016). Microsatellite Instability and its Significance to Hereditary and Sporadic Cancer. *Microsatellite Markers*, p.187.
- Baik, S.-C., Youn, H.-S., Chung, M.-H., Lee, W.-K., Cho, M.-J., Ko, G.-H., Park,

- C.-K., Kasai, H. and Rhee, K.-H., (1996). Increased Oxidative DNA Damage in Helicobacter pylori-infected Human Gastric Mucosa. *Cancer Research*, **56**(6), pp.1279–1282. Available from: <<http://cancerres.aacrjournals.org/content/56/6/1279.abstract>>.
- Bakhoun, S.F., Danilova, O. V, Kaur, P., Levy, N.B. and Compton, D.A., (2011). Chromosomal instability substantiates poor prognosis in patients with diffuse large B-cell lymphoma. *Clinical cancer research : an official journal of the American Association for Cancer Research*, **17**(24), pp.7704–7711. Available from: <<https://pubmed.ncbi.nlm.nih.gov/22184286>>.
- Bakhoun, S.F. and Cantley, L.C., (2018). The Multifaceted Role of Chromosomal Instability in Cancer and Its Microenvironment. *Cell*, **174**(6), pp.1347–1360. Available from: <<https://pubmed.ncbi.nlm.nih.gov/30193109>>.
- Bakhoun, S.F., Genovese, G. and Compton, D.A., (2009). Deviant kinetochore microtubule dynamics underlie chromosomal instability. *Current Biology*, **19**(22), pp.1937–1942.
- Bakker, S.J.L., IJzerman, R.G., Teerlink, T., Westerhoff, H. V, Gans, R.O.B. and Heine, R.J., (2000). Cytosolic triglycerides and oxidative stress in central obesity: the missing link between excessive atherosclerosis, endothelial dysfunction, and β -cell failure? *Atherosclerosis*, **148**(1), pp.17–21.
- Bakthavatchalu, V., Dey, S., Xu, Y., Noel, T., Jungsuwadee, P., Holley, A.K., Dhar, S.K., Batinic-Haberle, I. and St Clair, D.K., (2012). Manganese superoxide dismutase is a mitochondrial fidelity protein that protects Poly against UV-induced inactivation. *Oncogene*, **31**(17), pp.2129–2139. Available from: <<https://doi.org/10.1038/onc.2011.407>>.
- Bandyopadhyay, U., Das, D. and Banerjee, R.K., (1999). Reactive oxygen species: oxidative damage and pathogenesis. *Current Science-Bangalore*, **77**, pp.658–666.
- Bankoglu, E.E., Seyfried, F., Arnold, C., Soliman, A., Jurowich, C., Germer, C.T., Otto, C. and Stopper, H., (2017). Reduction of DNA damage in peripheral lymphocytes of obese patients after bariatric surgery-mediated weight loss. *Mutagenesis*.
- Bar-Shavit, Z., (2007). The osteoclast: A multinucleated, hematopoietic-origin, bone-resorbing osteoimmune cell. *Journal of Cellular Biochemistry*, **102**(5), pp.1130–1139. Available from: <<https://doi.org/10.1002/jcb.21553>>.
- Barbarroja, N., López-Pedreira, R., Mayas, M.D., Garcia-Fuentes, E., Garrido-Sánchez, L., Macías-González, M., El Bekay, R., Vidal-Puig, A. and Tinahones, F.J., (2010). The obese healthy paradox: is inflammation the answer? *Biochemical Journal*, **430**(1), pp.141–149.
- Barnes, R.P., Fouquerel, E. and Opresko, P.L., (2019). The impact of oxidative DNA damage and stress on telomere homeostasis. *Mechanisms of ageing and development*, **177**, pp.37–45.
- Barregard, L., Møller, P., Henriksen, T., Mistry, V., Koppen, G., Rossner Jr, P., Sram, R.J., Weimann, A., Poulsen, H.E., Nataf, R., Andreoli, R., Manini, P., Marczylo, T., Lam, P., Evans, M.D., Kasai, H., Kawai, K., Li, Y.-S., Sakai, K., et al., (2013). Human and methodological sources of variability in the measurement of urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine. *Antioxidants & redox signaling*, **18**(18), pp.2377–2391. Available from: <<https://pubmed.ncbi.nlm.nih.gov/23198723>>.
- Basatemur, E., Horsfall, L., Marston, L., Rait, G. and Sutcliffe, A., (2017). Trends in the Diagnosis of Vitamin D Deficiency. *Pediatrics*, **139**(3), p.e20162748. Available from:

- <<https://www.ncbi.nlm.nih.gov/pubmed/28159871>>.
- Beedanagari, S., Vulimiri, S. V, Bhatia, S. and Mahadevan, B., (2014). Chapter 43 - Genotoxicity biomarkers: Molecular basis of genetic variability and susceptibility. In R. C. B. T.-B. in T. Gupta, ed. Boston: Academic Press, pp. 729–742. Available from: <<http://www.sciencedirect.com/science/article/pii/B9780124046306000439>>.
- Bejarano, L., Bosso, G., Louzame, J., Serrano, R., Gómez-Casero, E., Martínez-Torrecedrada, J., Martínez, S., Blanco-Aparicio, C., Pastor, J. and Blasco, M.A., (2019). Multiple cancer pathways regulate telomere protection. *EMBO molecular medicine*, p.e10292.
- Bellone, S., Esposito, S., Giglione, E., Genoni, G., Fiorito, C., Petri, A., Bona, G. and Prodam, F., (2014). Vitamin D levels in a paediatric population of normal weight and obese subjects. *Journal of endocrinological investigation*, **37**(9), pp.805–809.
- Berger, A., (2002). How does it work?: Magnetic resonance imaging. *BMJ: British Medical Journal*, **324**(7328), p.35.
- Berger, N.A., (2018). Young Adult Cancer: Influence of the Obesity Pandemic. *Obesity*, **26**(4), pp.641–650. Available from: <<https://doi.org/10.1002/oby.22137>>.
- Berkey, C.S., Rockett, H.R.H., Field, A.E., Gillman, M.W. and Colditz, G.A., (2004). Sugar-added beverages and adolescent weight change. *Obesity research*, **12**(5), pp.778–788.
- Bernstein, C.N., Blanchard, J.F., Kliewer, E. and Wajda, A., (2001). Cancer risk in patients with inflammatory bowel disease. *Cancer*, **91**(4), pp.854–862. Available from: <[http://dx.doi.org/10.1002/1097-0142\(20010215\)91:4%3C854::AID-CNCR1073%3E3.0.CO](http://dx.doi.org/10.1002/1097-0142(20010215)91:4%3C854::AID-CNCR1073%3E3.0.CO)>.
- Bester, A.C., Roniger, M., Oren, Y.S., Im, M.M., Sarni, D., Chaoat, M., Bensimon, A., Zamir, G., Shewach, D.S. and Kerem, B., (2011). Nucleotide deficiency promotes genomic instability in early stages of cancer development. *Cell*, **145**(3), pp.435–446.
- Bhattacharya, I., Domínguez, A.P., Dräger, K., Humar, R., Haas, E. and Battegay, E.J., (2015). Hypoxia potentiates tumor necrosis factor- α induced expression of inducible nitric oxide synthase and cyclooxygenase-2 in white and brown adipocytes. *Biochemical and Biophysical Research Communications*, **461**(2), pp.287–292. Available from: <<http://www.sciencedirect.com/science/article/pii/S0006291X15006865>>.
- Bikle, D.D., (2014). Vitamin D metabolism, mechanism of action, and clinical applications. *Chemistry & biology*, **21**(3), pp.319–329.
- Bishop, K., Erdreich, S., Karunasinghe, N., Han, D., Zhu, S., Jesuthasan, A. and Ferguson, L., (2015). An investigation into the association between DNA damage and dietary fatty acid in men with prostate cancer. *Nutrients*, **7**(1), pp.405–422.
- Bjelakovic, G., Gluud, L.L., Nikolova, D., Whitfield, K., Krstic, G., Wetterslev, J. and Gluud, C., (2014). Vitamin D supplementation for prevention of cancer in adults. *The Cochrane database of systematic reviews*, (6), p.CD007469.
- Bjørge, T., Engeland, A., Tverdal, A. and Smith, G.D., (2008). Body Mass Index in Adolescence in Relation to Cause-specific Mortality: A Follow-up of 230,000 Norwegian Adolescents. *American Journal of Epidemiology*, **168**(1), pp.30–37. Available from: <<https://dx.doi.org/10.1093/aje/kwn096>>.
- Black, A.R., Black, J.D. and Azizkhan-Clifford, J., (2001). Sp1 and krüppel-like factor family of transcription factors in cell growth regulation and cancer.

- Journal of Cellular Physiology*, **188**(2), pp.143–160. Available from: <<http://dx.doi.org/10.1002/jcp.1111>>.
- Blackburn, E.H. and Gall, J.G., (1978). A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in *Tetrahymena*. *Journal of molecular biology*, **120**(1), pp.33–53.
- Bloching, M., Hofmann, A., Lautenschläger, C., Berghaus, A. and Grummt, T., (2000). Exfoliative cytology of normal buccal mucosa to predict the relative risk of cancer in the upper aerodigestive tract using the MN-assay. *Oral oncology*, **36**(6), pp.550–555.
- Bohn, B., Müller, M.J., Simic-Schleicher, G., Kiess, W., Siegfried, W., Oelert, M., Tuschy, S., Berghem, S. and Holl, R.W., (2015). BMI or BIA: Is Body Mass Index or Body Fat Mass a Better Predictor of Cardiovascular Risk in Overweight or Obese Children and Adolescents? *Obesity Facts*, **8**(2), pp.156–165. Available from: <<https://www.karger.com/DOI/10.1159/000381227>>.
- Bollmann, M., Bánkfalvi, A., Trosic, A., Speich, N., Schmitt, C. and Bollmann, R., (2005). Can we detect cervical human papillomavirus (HPV) infection by cytomorphology alone? Diagnostic value of non-classic cytological signs of HPV effect in minimally abnormal Pap tests. *Cytopathology*, **16**(1), pp.13–21.
- Bolognesi, C., Bruzzi, P., Gismondi, V., Volpi, S., Viassolo, V., Pedemonte, S. and Varesco, L., (2014). Clinical Application of Micronucleus Test: A Case-Control Study on the Prediction of Breast Cancer Risk/Susceptibility M. R. Scarfi, ed. *PLoS ONE*, **9**(11), p.e112354. Available from: <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4240584/>>.
- Bolognesi, C., Knasmueller, S., Nersesyan, A., Thomas, P. and Fenech, M., (2013). The HUMNxl scoring criteria for different cell types and nuclear anomalies in the buccal micronucleus cytome assay – An update and expanded photogallery. *Mutation Research/Reviews in Mutation Research*, **753**(2), pp.100–113. Available from: <<http://www.sciencedirect.com/science/article/pii/S1383574213000495>>.
- Bonassi, S., Znaor, A., Ceppi, M., Lando, C., Chang, W.P., Holland, N., Kirsch-Volders, M., Zeiger, E., Ban, S., Barale, R., Bigatti, M.P., Bolognesi, C., Cebulska-Wasilewska, A., Fabianova, E., Fucic, A., Hagmar, L., Joksic, G., Martelli, A., Migliore, L., et al., (2007). An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis*, **28**(3), pp.625–631.
- Bonassi, S., Znaor, A., Ceppi, M., Lando, C., Chang, W.P., Holland, N., Kirsch-Volders, M., Zeiger, E., Ban, S., Barale, R., Bigatti, M.P., Bolognesi, C., Cebulska-Wasilewska, A., Fabianova, E., Fucic, A., Hagmar, L., Joksic, G., Martelli, A., Migliore, L., et al., (2006). An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis*, **28**(3), pp.625–631. Available from: <<http://carcin.oxfordjournals.org/content/28/3/625.abstract>>.
- Bouloumié, A., Marumo, T., Lafontan, M. and Busse, R., (1999). Leptin induces oxidative stress in human endothelial cells. *The FASEB Journal*, **13**(10), pp.1231–1238.
- Bowen, D.T., Frew, M.E., Hills, R., Gale, R.E., Wheatley, K., Groves, M.J., Langabeer, S.E., Kottaridis, P.D., Moorman, A. V and Burnett, A.K., (2005). RAS mutation in acute myeloid leukemia is associated with distinct cytogenetic subgroups but does not influence outcome in patients younger than 60 years. *Blood*, **106**(6), pp.2113–2119.

- Broberg, K., Björk, J., Paulsson, K., Höglund, M. and Albin, M., (2005). Constitutional short telomeres are strong genetic susceptibility markers for bladder cancer. *Carcinogenesis*, **26**(7), pp.1263–71. Available from: <<http://carcin.oxfordjournals.org/cgi/content/long/26/7/1263>> [Accessed May 29, 2016].
- Brookes, P. and Lawley, P.D., (1960). The reaction of mustard gas with nucleic acids in vitro and in vivo. *Biochemical Journal*, **77**(3), pp.478 LP – 484. Available from: <<http://www.biochemj.org/content/77/3/478.abstract>>.
- Brown, K.F., Rungay, H., Dunlop, C., Ryan, M., Quartly, F., Cox, A., Deas, A., Elliss-brookes, L., Gavin, A., Hounsoms, L., Huws, D., Ormiston-smith, N., Shelton, J., White, C. and Parkin, D.M., (2018). The fraction of cancer attributable to modifiable risk factors in England, Wales, Scotland, Northern Ireland, and the United Kingdom in 2015. *British Journal of Cancer*, (2). Available from: <<http://dx.doi.org/10.1038/s41416-018-0029-6>>.
- Bruss, M.B., Morris, J.R., Dannison, L.L., Orbe, M.P., Quitugua, J.A. and Palacios, R.T., (2005). Food, culture, and family: exploring the coordinated management of meaning regarding childhood obesity. *Health communication*, **18**(2), pp.155–175.
- Bukhari, S.A., Rajoka, M.I., Nagra, S.A. and Rehman, Z.U., (2010). Plasma homocysteine and DNA damage profiles in normal and obese subjects in the Pakistani population. *Molecular Biology Reports*, **37**(1), pp.289–295. Available from: <<http://dx.doi.org/10.1007/s11033-009-9686-0>>.
- Bull, C.F., Mayrhofer, G., O’Callaghan, N.J., Au, A.Y., Pickett, H.A., Low, G.K.M., Zeegers, D., Hande, M.P. and Fenech, M.F., (2014). Folate Deficiency Induces Dysfunctional Long and Short Telomeres; Both States Are Associated with Hypomethylation and DNA Damage in Human WIL2-NS Cells. *Cancer Prevention Research*, **7**(1), pp.128 LP – 138. Available from: <<http://cancerpreventionresearch.aacrjournals.org/content/7/1/128.abstract>>.
- Bull, C.F., Mayrhofer, G., Zeegers, D., Mun, G.L.K., Hande, M.P. and Fenech, M.F., (2012). Folate deficiency is associated with the formation of complex nuclear anomalies in the cytokinesis-block micronucleus cytome assay. *Environmental and molecular mutagenesis*, **53**(4), pp.311–323.
- Buxton, J.L., Walters, R.G., Visvikis-Siest, S., Meyre, D., Froguel, P. and Blakemore, A.I.F., (2011). Childhood obesity is associated with shorter leukocyte telomere length. *The Journal of Clinical Endocrinology & Metabolism*, **96**(5), pp.1500–1505.
- Byrne, M.L., O’Brien-Simpson, N.M., Reynolds, E.C., Walsh, K.A., Laughton, K., Waloszek, J.M., Woods, M.J., Trinder, J. and Allen, N.B., (2013). Acute phase protein and cytokine levels in serum and saliva: A comparison of detectable levels and correlations in a depressed and healthy adolescent sample. *Brain, Behavior, and Immunity*, **34**, pp.164–175. Available from: <<http://www.sciencedirect.com/science/article/pii/S0889159113004212>>.
- Cadet, J. and Wagner, J.R., (2013). DNA base damage by reactive oxygen species, oxidizing agents, and UV radiation. *Cold Spring Harbor perspectives in biology*, **5**(2), p.a012559. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/23378590>>.
- Calle, E.E. and Kaaks, R., (2004). Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nature Reviews Cancer*, **4**(8), pp.579–591. Available from:

- <<https://doi.org/10.1038/nrc1408>>.
- Caputo, A., Pourgholami, M.H., Akhter, J. and Morris, D.L., (2003). 1,25-Dihydroxyvitamin D3 induced cell cycle arrest in the human primary liver cancer cell line HepG2. *Hepatology Research*, **26**(1), pp.34–39. Available from:
<<http://www.sciencedirect.com/science/article/pii/S1386634602003285>>.
- Carmona-Montesinos, E., Ruiz-Fragoso, Z., Ponce-Hinojosa, G. and Rivas-Arancibia, S., (2015). CHANGES IN C-REACTIVE PROTEIN AND BIOCHEMICAL PROFILE IN PRESCHOOL CHILDREN WITH OBESITY. *Nutricion hospitalaria*, **32**(4), pp.1548–1553.
- Cassidy, A., De Vivo, I., Liu, Y., Han, J., Prescott, J., Hunter, D.J. and Rimm, E.B., (2010). Associations between diet, lifestyle factors, and telomere length in women. *The American Journal of Clinical Nutrition*, **91**(5), pp.1273–1280. Available from:
<<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2854902/>>.
- Cecil, J.E., Tavendale, R., Watt, P., Hetherington, M.M. and Palmer, C.N.A., (2008). An Obesity-Associated FTO Gene Variant and Increased Energy Intake in Children. *New England Journal of Medicine*, **359**(24), pp.2558–2566. Available from: <<https://doi.org/10.1056/NEJMoa0803839>>.
- Cejvanovic, V., Asferg, C., Kjær, L.K., Andersen, U.B., Linneberg, A., Frystyk, J., Henriksen, T., Flyvbjerg, A., Christiansen, M., Weimann, A., Jeppesen, J. and Poulsen, H.E., (2016). Markers of oxidative stress in obese men with and without hypertension. *Scandinavian Journal of Clinical and Laboratory Investigation*, **76**(8), pp.620–625. Available from:
<<https://doi.org/10.1080/00365513.2016.1230776>>.
- Cerdá, C., Sánchez, C., Climent, B., Vázquez, A., Iradi, A., El Amrani, F., Bediaga, A. and Sáez, G.T., (2014). Oxidative stress and DNA damage in obesity-related tumorigenesis. In *Oxidative Stress and Inflammation in Non-communicable Diseases-Molecular Mechanisms and Perspectives in Therapeutics*. Springer, pp. 5–17.
- Chairman, M.P., Tucker, K., Beaumont, R., Young, E., Forsyth, O. and Swanson, M., (2012). National Joint Registry for England and Wales. *9th Annual Report 2012*, **50**(data to December 2011).
- Challis, B.G., Yeo, G.S.H., Farooqi, I.S. and O’Rahilly, S., (2003). Minireview: Human Obesity—Lessons from Monogenic Disorders. *Endocrinology*, **144**(9), pp.3757–3764. Available from: <<https://dx.doi.org/10.1210/en.2003-0373>>.
- Chan, Y.L., Leung, S.S.F., Lam, W.W.M., Peng, X.H. and Metrewelli, C., (1998). Body fat estimation in children by magnetic resonance imaging, bioelectrical impedance, skinfold and body mass index: A pilot study. *Journal of Paediatrics and Child Health*, **34**(1), pp.22–28. Available from:
<<https://doi.org/10.1046/j.1440-1754.1998.00147.x>>.
- Chang, C.-J., Jian, D.-Y., Lin, M.-W., Zhao, J.-Z., Ho, L.-T. and Juan, C.-C., (2015). Evidence in obese children: contribution of hyperlipidemia, obesity-inflammation, and insulin sensitivity. *PloS one*, **10**(5), pp.e0125935–e0125935. Available from:
<<https://www.ncbi.nlm.nih.gov/pubmed/26011530>>.
- Chang, D., Wang, F.A.N., Zhao, Y.-S. and Pan, H.-Z., (2008). Evaluation of oxidative stress in colorectal cancer patients. *Biomedical and Environmental Sciences*, **21**(4), pp.286–289.
- Chang, E. and Kim, Y., (2017). Vitamin D Insufficiency Exacerbates Adipose Tissue Macrophage Infiltration and Decreases AMPK/SIRT1 Activity in

- Obese Rats. *Nutrients*, **9**(4).
- Chang, P., Li, Y. and Li, D., (2011). Micronuclei levels in peripheral blood lymphocytes as a potential biomarker for pancreatic cancer risk. *Carcinogenesis*, **32**(2), pp.210–215. Available from: <<http://carcin.oxfordjournals.org/content/32/2/210.abstract>>.
- Chaput, J.-P., Katzmarzyk, P.T., Barnes, J.D., Fogelholm, M., Hu, G., Kuriyan, R., Kurpad, A., Lambert, E. V, Maher, C., Maia, J., Matsudo, V., Olds, T., Onywera, V., Sarmiento, O.L., Standage, M., Tudor-Locke, C., Zhao, P. and Tremblay, M.S., (2017). Mid-upper arm circumference as a screening tool for identifying children with obesity: a 12-country study. *Pediatric obesity*, **12**(6), pp.439–445.
- Chatterjee, M., (2001). Vitamin D and genomic stability. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **475**(1), pp.69–87. Available from: <<http://www.sciencedirect.com/science/article/pii/S002751070100080X>>.
- Cheadle, J.P. and Sampson, J.R., (2007). MUTYH-associated polyposis—from defect in base excision repair to clinical genetic testing. *DNA repair*, **6**(3), pp.274–279.
- Cheong, H.S.J., Seth, I., Tucker, J.D. and Joiner, M.C., (2013). Relationships among micronuclei, nucleoplasmic bridges and nuclear buds within individual cells in the cytokinesis-block micronucleus assay. *Mutagenesis*, **28**(4), pp.433–440. Available from: <<https://doi.org/10.1093/mutage/get020>>.
- Chiou, C.-C., Chang, P.-Y., Chan, E.-C., Wu, T.-L., Tsao, K.-C. and Wu, J.T., (2003). Urinary 8-hydroxydeoxyguanosine and its analogs as DNA marker of oxidative stress: development of an ELISA and measurement in both bladder and prostate cancers. *Clinica Chimica Acta*, **334**(1), pp.87–94. Available from: <<http://www.sciencedirect.com/science/article/pii/S0009898103001918>>.
- Cho, K., Moon, J.S., Kang, J., Jang, H.B., Lee, H., Park, S.I., Yu, K. and Cho, J., (2017). Combined untargeted and targeted metabolomic profiling reveals urinary biomarkers for discriminating obese from normal-weight adolescents. *Pediatric obesity*, **12**(2), pp.93–101.
- Chung, S.S.M., Ho, E.C.M., Lam, K.S.L. and Chung, S.K., (2003). Contribution of polyol pathway to diabetes-induced oxidative stress. *Journal of the American Society of Nephrology*, **14**(suppl 3), pp.S233–S236.
- Cioffi, C.E., Alvarez, J.A., Welsh, J.A. and Vos, M.B., (2019). Truncal-to-leg fat ratio and cardiometabolic disease risk factors in US adolescents: NHANES 2003-2006. *Pediatric obesity*, p.e12509.
- Cobbs, C.S., Whisenhunt, T.R., Wesemann, D.R., Harkins, L.E., Van Meir, E.G. and Samanta, M., (2003). Inactivation of wild-type p53 protein function by reactive oxygen and nitrogen species in malignant glioma cells. *Cancer research*, **63**(24), pp.8670–8673.
- Codoñer-Franch, P., Boix-García, L., Simó-Jordá, R., del Castillo-Villaescusa, C., Maset-Maldonado, J. and Valls-Bellés, V., (2010). Is obesity associated with oxidative stress in children? *International Journal of Pediatric Obesity*, **5**(1), pp.56–63.
- Codoñer-Franch, P., Valls-Bellés, V., Arilla-Codoñer, A. and Alonso-Iglesias, E., (2011). Oxidant mechanisms in childhood obesity: the link between inflammation and oxidative stress. *Translational research : the journal of laboratory and clinical medicine*, **158**(6), pp.369–84. Available from: <<http://www.sciencedirect.com/science/article/pii/S1931524411002647>>

- [Accessed February 26, 2015].
- Cohen, J.I., Maayan, L. and Convit, A., (2012). Preliminary evidence for obesity-associated insulin resistance in adolescents without elevations of inflammatory cytokines. *Diabetology & metabolic syndrome*, **4**(1), p.26. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/22682228>>.
- Cole, T.J., Bellizzi, M.C., Flegal, K.M. and Dietz, W.H., (2000). Establishing a standard definition for child overweight and obesity worldwide: international survey. , **320**, pp.1–6.
- Coles, C., Condie, A., Chetty, U., Steel, C.M., Evans, H.J. and Prosser, J., (1992). p53 mutations in breast cancer. *Cancer research*, **52**(19), pp.5291–5298.
- Collins, A.R., Oscoz, A.A., Brunborg, G., Gaivao, I., Giovannelli, L., Kruszewski, M., Smith, C.C. and Štětina, R., (2008). The comet assay: topical issues. *Mutagenesis*, **23**(3), pp.143–151.
- Colotta, F., Garlanda, C., Allavena, P., Sica, A. and Mantovani, A., (2009). Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis*, **30**(7), pp.1073–1081. Available from: <<https://doi.org/10.1093/carcin/bgp127>>.
- COLSTON, K.A.Y., COLSTON, M.J. and FELDMAN, D., (1981). 1, 25-dihydroxyvitamin D3 and malignant melanoma: the presence of receptors and inhibition of cell growth in culture. *Endocrinology*, **108**(3), pp.1083–1086.
- Colston, K.W., Perks, C.M., Xie, S.P. and Holly, J.M.P., (1998). Growth inhibition of both MCF-7 and Hs578T human breast cancer cell lines by vitamin D analogues is associated with increased expression of insulin-like growth factor binding protein-3. *Journal of Molecular Endocrinology*, **20**(1), p.157.
- Cook, D.G., Mendall, M.A., Whincup, P.H., Carey, I.M., Ballam, L., Morris, J.E., Miller, G.J. and Strachan, D.P., (2000). C-reactive protein concentration in children: relationship to adiposity and other cardiovascular risk factors. *Atherosclerosis*, **149**(1), pp.139–150. Available from: <<http://www.sciencedirect.com/science/article/pii/S0021915099003123>>.
- Cooke, M.S., Olinski, R. and Evans, M.D., (2006). Does measurement of oxidative damage to DNA have clinical significance? *Clinica Chimica Acta*, **365**(1–2), pp.30–49. Available from: <<http://www.sciencedirect.com/science/article/pii/S0009898105005577>>.
- Coombs, N., Shelton, N., Rowlands, A. and Stamatakis, E., (2013). Children and adolescents sedentary behaviour in relation to socioeconomic position. *Journal of Epidemiology and Community Health*, **67**(10), pp.868 LP – 874. Available from: <<http://jech.bmj.com/content/67/10/868.abstract>>.
- Correia-Costa, L., Sousa, T., Morato, M., Cosme, D., Afonso, J., Areias, J.C., Schaefer, F., Guerra, A., Afonso, A.C., Azevedo, A. and Albino-Teixeira, A., (2016). Oxidative stress and nitric oxide are increased in obese children and correlate with cardiometabolic risk and renal function. *British Journal of Nutrition*, **116**(5), pp.805–815. Available from: <<https://www.cambridge.org/core/article/oxidative-stress-and-nitric-oxide-are-increased-in-obese-children-and-correlate-with-cardiometabolic-risk-and-renal-function/544F777BF1B70F16C6084295FB39C655>>.
- da Costa, K.-A., Niculescu, M.D., Craciunescu, C.N., Fischer, L.M. and Zeisel, S.H., (2006). Choline deficiency increases lymphocyte apoptosis and DNA damage in humans. *The American journal of clinical nutrition*, **84**(1), pp.88–94.

- Creemers, J.W.M., Choquet, H., Stijnen, P., Vatin, V., Pigeyre, M., Beckers, S., Meulemans, S., Than, M.E., Yengo, L., Tauber, M., Balkau, B., Elliott, P., Jarvelin, M.-R., Van Hul, W., Van Gaal, L., Horber, F., Pattou, F., Froguel, P. and Meyre, D., (2012). Heterozygous Mutations Causing Partial Prohormone Convertase 1 Deficiency Contribute to Human Obesity. *Diabetes*, **61**(2), pp.383 LP – 390. Available from: <<http://diabetes.diabetesjournals.org/content/61/2/383.abstract>>.
- CRUK, (2019). Obese people outnumber smokers two to one. *cr*. Available from: <<https://www.cancerresearchuk.org/about-us/cancer-news/press-release/2019-07-03-obese-people-outnumber-smokers-two-to-one>>.
- Cui, Y., Gao, Y.-T., Cai, Q., Qu, S., Cai, H., Li, H.-L., Wu, J., Ji, B.-T., Yang, G., Chow, W.-H., Shu, X.-O. and Zheng, W., (2013). Associations of leukocyte telomere length with body anthropometric indices and weight change in Chinese women. *Obesity (Silver Spring, Md.)*, **21**(12), pp.2582–2588. Available from: <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3676725/>>.
- Dalamaga, M., Diakopoulos, K.N. and Mantzoros, C.S., (2012). The role of adiponectin in cancer: a review of current evidence. *Endocrine reviews*, **33**(4), pp.547–594. Available from: <<https://pubmed.ncbi.nlm.nih.gov/22547160>>.
- van Dam, R.M., Willett, W.C., Manson, J.E. and Hu, F.B., (2006). The relationship between overweight in adolescence and premature death in women. *Annals of internal medicine*, **145**(2), pp.91–97.
- Dandona, P., Aljada, A. and Bandyopadhyay, A., (2004). Inflammation: the link between insulin resistance, obesity and diabetes. *Trends in Immunology*, **25**(1), pp.4–7. Available from: <<http://www.sciencedirect.com/science/article/pii/S1471490603003363>>.
- Daniels, M.J., Wang, Y., Lee, M. and Venkitaraman, A.R., (2004). Abnormal cytokinesis in cells deficient in the breast cancer susceptibility protein BRCA2. *Science*, **306**(5697), pp.876–879.
- Daraki, V., Roumeliotaki, T., Chalkiadaki, G., Katrinaki, M., Karachaliou, M., Leventakou, V., Vafeiadi, M., Sarri, K., Vassilaki, M., Papavasiliou, S., Kogevinas, M. and Chatzi, L., (2018). Low maternal vitamin D status in pregnancy increases the risk of childhood obesity. *Pediatric obesity*, **13**(8), pp.467–475.
- Dass Singh, M., Thomas, P., Hor, M., Almond, T., Owens, J., Hague, W. and Fenech, M., (2017). Infant birth outcomes are associated with DNA damage biomarkers as measured by the cytokinesis block micronucleus cytome assay: the DADHI study. *Mutagenesis*, **32**(3), pp.355–370. Available from: <<http://dx.doi.org/10.1093/mutage/gex001>>.
- Dawes, C., (2003). Estimates, from salivary analyses, of the turnover time of the oral mucosal epithelium in humans and the number of bacteria in an edentulous mouth. *Archives of oral biology*, **48**(5), pp.329–336.
- Decker, M.L., Chavez, E., Vulto, I. and Lansdorp, P.M., (2009). Telomere length in Hutchinson-Gilford Progeria Syndrome. *Mechanisms of Ageing and Development*, **130**(6), pp.377–383. Available from: <<http://www.sciencedirect.com/science/article/pii/S0047637409000396>>.
- Deeb, A., Attia, S., Mahmoud, S., Elhaj, G. and Elfatih, A., (2018). Dyslipidemia and Fatty Liver Disease in Overweight and Obese Children. *Journal of obesity*, **2018**, p.8626818. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/30009050>>.
- Deng, T., Lyon, C.J., Bergin, S., Caligiuri, M.A. and Hsueh, W.A., (2016). Obesity, Inflammation, and Cancer. *Annual Review of Pathology*:

- Mechanisms of Disease*, **11**(1), pp.421–449. Available from: <<https://doi.org/10.1146/annurev-pathol-012615-044359>>.
- Deng, Y. and Chang, S., (2007). Role of telomeres and telomerase in genomic instability, senescence and cancer. *Laboratory investigation*, **87**(11), p.1071.
- Denkins, Y., Kempf, D., Ferniz, M., Nileshwar, S. and Marchetti, D., (2005). Role of ω -3 polyunsaturated fatty acids on cyclooxygenase-2 metabolism in brain-metastatic melanoma. *Journal of lipid research*, **46**(6), pp.1278–1284.
- Desai, G.S. and Mathews, S.T., (2014). Saliva as a non-invasive diagnostic tool for inflammation and insulin-resistance. *World journal of diabetes*, **5**(6), pp.730–8. Available from: <<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4265859&tool=pmcentrez&rendertype=abstract>> [Accessed December 19, 2014].
- Desai, S.S., Ghaisas, S.D., Jakhi, S.D. and Bhide, S. V, (1996). Cytogenetic damage in exfoliated oral mucosal cells and circulating lymphocytes of patients suffering from precancerous oral lesions. *Cancer letters*, **109**(1–2), pp.9–14.
- Diakowska, D., Lewandowski, A., Kopeć, W., Diakowski, W. and Chrzanowska, T., (2007). Oxidative DNA damage and total antioxidant status in serum of patients with esophageal squamous cell carcinoma. *Hepato-gastroenterology*, **54**(78), pp.1701–1704.
- Dias, K.A., Ramos, J.S., Wallen, M.P., Davies, P.S.W., Cain, P.A., Leong, G.M., Ingul, C.B., Coombes, J.S. and Keating, S.E., (2019). Accuracy of Longitudinal Assessment of Visceral Adipose Tissue by Dual-Energy X-Ray Absorptiometry in Children with Obesity C. I. Ardern, ed. *Journal of Obesity*, **2019**, p.2193723. Available from: <<https://doi.org/10.1155/2019/2193723>>.
- Diaz, V.A., Mainous, a G., Player, M.S. and Everett, C.J., (2010). Telomere length and adiposity in a racially diverse sample. *International Journal of Obesity (London)*, **34**(2), pp.261–265. Available from: <<http://dx.doi.org/10.1038/ijo.2009.198>>.
- Ding, C., Wilding, J.P.H. and Bing, C., (2013). 1,25-dihydroxyvitamin D3 protects against macrophage-induced activation of NFkappaB and MAPK signalling and chemokine release in human adipocytes. *PloS one*, **8**(4), p.e61707.
- Dong, S.-S., Zhang, Y.-J., Chen, Y.-X., Yao, S., Hao, R.-H., Rong, Y., Niu, H.-M., Chen, J.-B., Guo, Y. and Yang, T.-L., (2018). Comprehensive review and annotation of susceptibility SNPs associated with obesity-related traits. *Obesity Reviews*, **19**(7), pp.917–930. Available from: <<https://doi.org/10.1111/obr.12677>>.
- Donmez-Altuntas, H., Sahin, F., Bayram, F., Bitgen, N., Mert, M., Guclu, K., Hamurcu, Z., Aribas, S., Gundogan, K. and Diri, H., (2014). Evaluation of chromosomal damage, cytostasis, cytotoxicity, oxidative DNA damage and their association with body-mass index in obese subjects. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, **771**, pp.30–36. Available from: <<http://www.sciencedirect.com/science/article/pii/S138357181400179X>>.
- Drincic, A.T., Armas, L.A.G., Van Diest, E.E. and Heaney, R.P., (2012). Volumetric dilution, rather than sequestration best explains the low vitamin D status of obesity. *Obesity (Silver Spring, Md.)*, **20**(7), pp.1444–1448.
- Durkin, N. and Desai, A.P., (2017). What Is the Evidence for

- Paediatric/Adolescent Bariatric Surgery? *Current obesity reports*, **6**(3), pp.278–285. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/28815416>>.
- Dutta, D., Ghosh, S., Pandit, K., Mukhopadhyay, P. and Chowdhury, S., (2012). Leptin and cancer: Pathogenesis and modulation. *Indian journal of endocrinology and metabolism*, **16**(Suppl 3), pp.S596–S600. Available from: <<https://pubmed.ncbi.nlm.nih.gov/23565495>>.
- Ehtisham, S., Barrett, T.G. and Shaw, N.J., (2008). Type 2 diabetes mellitus in UK children – an emerging problem. *Diabetic Medicine*, **17**(12), pp.867–871. Available from: <<https://doi.org/10.1046/j.1464-5491.2000.00409.x>>.
- Eisenberg, M.E., Neumark-Sztainer, D. and Story, M., (2003). Associations of weight-based teasing and emotional well-being among adolescents. *Archives of pediatrics & adolescent medicine*, **157**(8), pp.733–738.
- Ekbom, K. and Marcus, C., (2016). Vitamin D deficiency is associated with prediabetes in obese Swedish children. *Acta paediatrica (Oslo, Norway : 1992)*, **105**(10), pp.1192–1197.
- van Emmerik, N.M.A., Renders, C.M., van de Veer, M., van Buuren, S., van der Baan-Slootweg, O.H., Kist-van Holthe, J.E. and HiraSing, R.A., (2012). High cardiovascular risk in severely obese young children and adolescents. *Archives of Disease in Childhood*, **97**(9), pp.818 LP – 821. Available from: <<http://adc.bmj.com/content/97/9/818.abstract>>.
- Er, T.-K., Hou, M.-F., Tsa, E.-M., Lee, J.-N. and Tsai, L.-Y., (2004). Differential expression of manganese containing superoxide dismutase in patients with breast cancer in Taiwan. *Annals of Clinical & Laboratory Science*, **34**(2), pp.159–164.
- Erdeve, O., Siklar, Z., Kocaturk, P.A., Dallar, Y. and Kavas, G.O., (2004). Antioxidant superoxide dismutase activity in obese children. *Biological trace element research*, **98**(3), pp.219–227.
- Erol, M., Bostan Gayret, O., Hamilcikan, S., Can, E. and Yigit, O.L., (2017). Vitamin D deficiency and insulin resistance as risk factors for dyslipidemia in obese children. *Archivos argentinos de pediatria*, **115**(2), pp.133–139.
- Eshleman, J.R. and Markowitz, S.D., (1996). Mismatch repair defects in human carcinogenesis. *Human molecular genetics*, **5**(Supplement_1), pp.1489–1494.
- Evans, J.L., Goldfine, I.D., Maddux, B.A. and Grodsky, G.M., (2002). Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocrine reviews*, **23**(5), pp.599–622.
- Falck, J., Petrini, J.H.J., Williams, B.R., Lukas, J. and Bartek, J., (2002). The DNA damage-dependent intra-S phase checkpoint is regulated by parallel pathways. *Nature genetics*, **30**(3), p.290.
- Fang, F.C. and Vazquez-Torres, A., (2002). Nitric oxide production by human macrophages: there's NO doubt about it. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, **282**(5), pp.L941–L943.
- Farooq, M.A., Parkinson, K.N., Adamson, A.J., Pearce, M.S., Reilly, J.K., Hughes, A.R., Janssen, X., Basterfield, L. and Reilly, J.J., (2018). Timing of the decline in physical activity in childhood and adolescence: Gateshead Millennium Cohort Study. *British journal of sports medicine*, **52**(15), pp.1002–1006.
- Farooqi, I.S., Matarese, G., Lord, G.M., Keogh, J.M., Lawrence, E., Agwu, C., Sanna, V., Jebb, S.A., Perna, F., Fontana, S., Lechler, R.I., DePaoli, A.M. and O'Rahilly, S., (2002). Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human

- congenital leptin deficiency. *The Journal of Clinical Investigation*, **110**(8), pp.1093–1103. Available from: <<https://doi.org/10.1172/JCI15693>>.
- Farooqi, I.S., Keogh, J.M., Yeo, G.S.H., Lank, E.J., Cheetham, T. and O’rahilly, S., (2003). Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. *New England Journal of Medicine*, **348**(12), pp.1085–1095.
- Fedewa, A.L. and Davis, M.C., (2015). How Food as a Reward Is Detrimental to Children’s Health, Learning, and Behavior. *The Journal of school health*, **85**(9), pp.648–658.
- Fedirko, V., Bostick, R.M., Long, Q., Flanders, W.D., McCullough, M.L., Sidelnikov, E., Daniel, C.R., Rutherford, R.E. and Shaukat, A., (2010). Effects of supplemental vitamin D and calcium on oxidative DNA damage marker in normal colorectal mucosa: a randomized clinical trial. *Cancer Epidemiology and Prevention Biomarkers*, **19**(1), pp.280–291.
- Feki-Tounsi, M., Khlifi, R., Mhiri, M.-N., Rebai, A. and Hamza-Chaffai, A., (2014). Cytogenetic damage in the oral mucosa cells of bladder cancer patients exposed to tobacco in Southern Tunisia. *Environmental Science and Pollution Research*, **21**(22), pp.12922–12927.
- Fenech, M., (2002). Chromosomal biomarkers of genomic instability relevant to cancer. *Drug discovery today*, **7**(22), pp.1128–1137.
- Fenech, M., Baghurst, P., Luderer, W., Turner, J., Record, S., Ceppi, M. and Bonassi, S., (2005). Low intake of calcium, folate, nicotinic acid, vitamin E, retinol, β -carotene and high intake of pantothenic acid, biotin and riboflavin are significantly associated with increased genome instability—results from a dietary intake and micronucleus index study. *Carcinogenesis*, **26**(5), pp.991–999.
- Fenech, M., (2003). Nutritional treatment of genome instability: a paradigm shift in disease prevention and in the setting of recommended dietary allowances. *Nutrition Research Reviews*, **16**(1), pp.109–122. Available from: <<https://www.cambridge.org/core/article/nutritional-treatment-of-genome-instability-a-paradigm-shift-in-disease-prevention-and-in-the-setting-of-recommended-dietary-allowances/89AB2DB018FB484472364A1C018E1B57>>.
- Fenech, M., (2001). The role of folic acid and vitamin B12 in genomic stability of human cells. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **475**(1–2), pp.57–67.
- Fernandez, P., Scaffidi, P., Markert, E., Lee, J.-H., Rane, S. and Misteli, T., (2014). Transformation Resistance in a Premature Aging Disorder Identifies a Tumor-Protective Function of BRD4. *Cell Reports*, **9**(1), pp.248–260. Available from: <<http://www.sciencedirect.com/science/article/pii/S221112471400761X>>.
- Ferrarezi, D.A.F., Bellili-Munoz, N., Nicolau, C., Cheurfa, N., Guazzelli, I.C., Frazzatto, E., Velho, G. and Villares, S.M., (2012). Allelic variations in the vitamin D receptor gene, insulin secretion and parents’ heights are independently associated with height in obese children and adolescents. *Metabolism: clinical and experimental*, **61**(10), pp.1413–1421.
- Fields, D.A., Goran, M.I. and McCrory, M.A., (2002). Body-composition assessment via air-displacement plethysmography in adults and children: a review. *The American journal of clinical nutrition*, **75**(3), pp.453–467.
- Fisher, J.O. and Kral, T.V.E., (2008). Super-size me: Portion size effects on young children’s eating. *Physiology & Behavior*, **94**(1), pp.39–47. Available from: <<http://www.sciencedirect.com/science/article/pii/S0031938407004568>>.

- Fleet, J.C., DeSmet, M., Johnson, R. and Li, Y., (2012). Vitamin D and cancer: a review of molecular mechanisms. *Biochemical Journal*, **441**(1), pp.61–76.
- Flores-García, A., Torres-Bugarín, O., Velarde-Félix, J.S., Rangel-Villalobos, H., Zepeda-Carrillo, E.A., Rodríguez-Trejo, A., Aguiar-García, P. and Nersesyan, A., (2014). Micronuclei and other nuclear anomalies in exfoliated buccal mucosa cells of Mexican women with breast cancer. *J BUON*, **19**(4), pp.895–899.
- Formichi, C., Cantara, S., Ciuli, C., Neri, O., Chiofalo, F., Selmi, F., Tirone, A., Colasanto, G., Di Cosmo, L. and Vuolo, G., (2014). Weight loss associated with bariatric surgery does not restore short telomere length of severe obese patients after 1 year. *Obesity surgery*, **24**(12), pp.2089–2093.
- Foss, Y.J., (2009). Vitamin D deficiency is the cause of common obesity. *Medical Hypotheses*, **72**(3), pp.314–321. Available from: <<http://www.sciencedirect.com/science/article/pii/S0306987708005288>>.
- Fouquerel, E., Lormand, J., Bose, A., Lee, H.-T., Kim, G.S., Li, J., Sobol, R.W., Freudenthal, B.D., Myong, S. and Opresko, P.L., (2016). Oxidative guanine base damage regulates human telomerase activity. *Nature Structural & Molecular Biology*, **23**, p.1092. Available from: <<https://doi.org/10.1038/nsmb.3319>>.
- Freedman, D.S., Horlick, M. and Berenson, G.S., (2013). A comparison of the Slaughter skinfold-thickness equations and BMI in predicting body fatness and cardiovascular disease risk factor levels in children. *The American journal of clinical nutrition*, **98**(6), pp.1417–1424.
- Friedman, J.E., Dohm, G.L., Leggett-Frazier, N., Elton, C.W., Tapscott, E.B., Pories, W.P. and Caro, J.F., (1992). Restoration of insulin responsiveness in skeletal muscle of morbidly obese patients after weight loss. Effect on muscle glucose transport and glucose transporter GLUT4. *Journal of clinical investigation*, **89**(2), p.701.
- Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., Nakayama, O., Makishima, M., Matsuda, M. and Shimomura, I., (2017). Increased oxidative stress in obesity and its impact on metabolic syndrome. *The Journal of clinical investigation*, **114**(12), pp.1752–1761.
- Gagos, S. and Irminger-Finger, I., (2005). Chromosome instability in neoplasia: chaotic roots to continuous growth. *The international journal of biochemistry & cell biology*, **37**(5), pp.1014–1033.
- Gandhi, G. and Kaur, G., (2012). Assessment of DNA Damage in Obese Individuals. *Research Journal of Biology*, **02**(02), pp.37–44.
- Garbieri, T.F., Brozoski, D.T., Dionísio, T.J., Santos, C.F. and Neves, L.T. das, (2017). Human DNA extraction from whole saliva that was fresh or stored for 3, 6 or 12 months using five different protocols. *Journal of applied oral science : revista FOB*, **25**(2), pp.147–158. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/28403355>>.
- García-Calzón, S., Molerés, A., Marcos, A., Campoy, C., Moreno, L.A., Azcona-Sanjulián, M.C., Martínez-González, M.A., Martínez, J.A., Zalba, G., Marti, A. and Group, on behalf of the E.S., (2014). Telomere Length as a Biomarker for Adiposity Changes after a Multidisciplinary Intervention in Overweight/Obese Adolescents: The EVASYON Study. *PLOS ONE*, **9**(2), p.e89828. Available from: <<https://doi.org/10.1371/journal.pone.0089828>>.
- Garg, R., Kumbkarni, Y., Aljada, A., Mohanty, P., Ghanim, H., Hamouda, W. and Dandona, P., (2000). Troglitazone reduces reactive oxygen species generation by leukocytes and lipid peroxidation and improves flow-mediated vasodilatation in obese subjects. *Hypertension*, **36**(3), pp.430–

- Garnis, C., Chari, R., Buys, T.P.H., Zhang, L., Ng, R.T., Rosin, M.P. and Lam, W.L., (2009). Genomic imbalances in precancerous tissues signal oral cancer risk. *Molecular cancer*, **8**(1), p.50.
- Giehl, M., Fabarius, A., Frank, O., Hochhaus, A., Hafner, M., Hehlmann, R. and Seifarth, W., (2005). Centrosome aberrations in chronic myeloid leukemia correlate with stage of disease and chromosomal instability. *Leukemia*, **19**(7), p.1192.
- Giovannucci, E., (2007). Metabolic syndrome, hyperinsulinemia, and colon cancer: a review. *The American Journal of Clinical Nutrition*, **86**(3), pp.836S-842S. Available from: <<https://doi.org/10.1093/ajcn/86.3.836S>>.
- Glasser, N., Zellner, K. and Kromeyer-Hauschild, K., (2011). Validity of body mass index and waist circumference to detect excess fat mass in children aged 7-14 years. *European journal of clinical nutrition*, **65**(2), pp.151–159.
- Goldman, E.A., Eick, G.N., Compton, D., Kowal, P., Snodgrass, J.J., Eisenberg, D.T.A. and Sterner, K.N., (2018). Evaluating minimally invasive sample collection methods for telomere length measurement. *American journal of human biology: the official journal of the Human Biology Council*, **30**(1).
- Goodman, E. and Whitaker, R.C., (2002). A Prospective Study of the Role of Depression in the Development and Persistence of Adolescent Obesity. *Pediatrics*, **110**(3), pp.497 LP – 504. Available from: <<http://pediatrics.aappublications.org/content/110/3/497.abstract>>.
- Goulding, A., Jones, I.E., Williams, S.M. and Taylor, R.W., (2000). Evaluation of waist circumference, waist-to-hip ratio, and the conicity index as screening tools for high trunk fat mass, as measured by dual-energy X-ray absorptiometry, in children aged 3–19 y. *The American Journal of Clinical Nutrition*, **72**(2), pp.490–495. Available from: <<https://dx.doi.org/10.1093/ajcn/72.2.490>>.
- Granetto, C., Ottaggio, L., Abbondandolo, A. and Bonatti, S., (1996). p53 accumulates in micronuclei after treatment with a DNA breaking chemical, methylnitrosourea, and with the spindle poison, vinblastine. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **352**(1), pp.61–64. Available from: <<http://www.sciencedirect.com/science/article/pii/0027510795002359>>.
- Grant, W.B., (2018). A review of the evidence supporting the vitamin D-cancer prevention hypothesis in 2017. *Anticancer research*, **38**(2), pp.1121–1136.
- Green, N.S., (2002). Folic acid supplementation and prevention of birth defects. *The Journal of nutrition*, **132**(8), pp.2356S-2360S.
- Grivennikov, S.I. and Karin, M., (2010). Inflammation and oncogenesis: a vicious connection. *Current opinion in genetics & development*, **20**(1), pp.65–71. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/20036794>>.
- Gross, M., Kost, S.B., Ennis, B., Stumpf, W. and Kumar, R., (1986). Effect of 1, 25-dihydroxyvitamin D3 on mouse mammary tumor (GR) cells: Evidence for receptors, cellular uptake, inhibition of growth and alteration in morphology at physiologic concentrations of hormone. *Journal of Bone and Mineral Research*, **1**(5), pp.457–467.
- Gruys, E., Toussaint, M.J.M., Niewold, T.A. and Koopmans, S.J., (2005). Acute phase reaction and acute phase proteins. *Journal of Zhejiang University. Science. B*, **6**(11), pp.1045–1056. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/16252337>>.
- Guaraldi, F., Deon, V., Del Bo', C., Vendrame, S., Porrini, M., Riso, P. and

- Guardamagna, O., (2018). Effect of short-term hazelnut consumption on DNA damage and oxidized LDL in children and adolescents with primary hyperlipidemia: a randomized controlled trial. *The Journal of Nutritional Biochemistry*, **57**, pp.206–211. Available from: <<http://www.sciencedirect.com/science/article/pii/S0955286317307179>>.
- Gul, A., Ozer, S., Yilmaz, R., Sonmezgoz, E., Kasap, T., Takci, S. and Demir, O., (2017). Association between vitamin D levels and cardiovascular risk factors in obese children and adolescents. *Nutricion hospitalaria*, **34**(2), pp.323–329.
- Guo, C., Li, X., Wang, R., Yu, J., Ye, M., Mao, L., Zhang, S. and Zheng, S., (2016). Association between Oxidative DNA Damage and Risk of Colorectal Cancer: Sensitive Determination of Urinary 8-Hydroxy-2'-deoxyguanosine by UPLC-MS/MS Analysis. *Scientific Reports*, **6**, p.32581. Available from: <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5009303/>>.
- Guo, X., Ni, J., Liang, Z., Xue, J., Fenech, M.F. and Wang, X., (2019). The molecular origins and pathophysiological consequences of micronuclei: New insights into an age-old problem. *Mutation Research/Reviews in Mutation Research*, **779**, pp.1–35. Available from: <<http://www.sciencedirect.com/science/article/pii/S1383574218300620>>.
- Guzik, T., Korbout, R. and Adamek-Guzik, T., (2003). Nitric oxide and superoxide in inflammation. *Journal of physiology and pharmacology*, **54**, pp.469–487.
- Hageman, G.J. and Stierum, R.H., (2001). Niacin, poly (ADP-ribose) polymerase-1 and genomic stability. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **475**(1–2), pp.45–56.
- Haghiac, M., Vora, N.L., Basu, S., Johnson, K.L., Presley, L., Bianchi, D.W. and Mouzon, S.H., (2012). Increased Death of Adipose Cells, a Path to Release Cell-Free DNA Into Systemic Circulation of Obese Women. *Obesity*, **20**(11), pp.2213–2219.
- Hahn, W.C., Stewart, S.A., Brooks, M.W., York, S.G., Eaton, E., Kurachi, A., Beijersbergen, R.L., Knoll, J.H.M., Meyerson, M. and Weinberg, R.A., (1999). Inhibition of telomerase limits the growth of human cancer cells. *Nature Medicine*, **5**(10), pp.1164–1170. Available from: <<https://doi.org/10.1038/13495>>.
- Halle, M., Korsten-Reck, U., Wolfarth, B. and Berg, A., (2004). Low-grade systemic inflammation in overweight children: impact of physical fitness. *Exerc Immunol Rev*, **10**(10), pp.66–74.
- Hanahan, D. and Weinberg, R.A., (2017). Biological Hallmarks of Cancer. *Holland-Frei Cancer Medicine*, pp.1–10. Available from: <<https://doi.org/10.1002/9781119000822.hfcm002>>.
- Hanahan, D. and Weinberg, R.A., (2016). Hallmarks of Cancer: The Next Generation. *Cell*, **144**(5), pp.646–674. Available from: <<http://dx.doi.org/10.1016/j.cell.2011.02.013>>.
- Hanahan, D., Weinberg, R.A. and Francisco, S., (2000). The Hallmarks of Cancer Review. *Cell*, **100**, pp.57–70.
- Hardwick, J.C., Van Den Brink, G.R., Offerhaus, G.J., Van Deventer, S.J. and Peppelenbosch, M.P., (2001). Leptin is a growth factor for colonic epithelial cells. *Gastroenterology*, **121**(1), pp.79–90.
- Hartlerode, A.J. and Scully, R., (2009). Mechanisms of double-strand break repair in somatic mammalian cells. *Biochemical Journal*, **423**(2), pp.157–168.
- Hartwell, L., (1992). Defects in a cell cycle checkpoint may be responsible for the genomic instability of cancer cells. *Cell*, **71**(4), pp.543–546.

- Hartwell, L.H. and Weinert, T.A., (1989). Checkpoints: controls that ensure the order of cell cycle events. *Science*, **246**(4930), pp.629–634.
- Hartwig, A., (2001). Role of magnesium in genomic stability. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **475**(1–2), pp.113–121.
- Hatch, E.M., Fischer, A.H., Deerinck, T.J. and Hetzer, M.W., (2013). Catastrophic Nuclear Envelope Collapse in Cancer Cell Micronuclei. *Cell*, **154**(1), pp.47–60. Available from: <<http://dx.doi.org/10.1016/j.cell.2013.06.007>>.
- Hatipoglu, N., Mazicioglu, M.M., Kurtoglu, S. and Kendirci, M., (2010). Neck circumference: an additional tool of screening overweight and obesity in childhood. *European Journal of Pediatrics*, **169**(6), pp.733–739. Available from: <<https://doi.org/10.1007/s00431-009-1104-z>>.
- Hayashi, M., (2016). The micronucleus test-most widely used in vivo genotoxicity test. *Genes and environment : the official journal of the Japanese Environmental Mutagen Society*, **38**, p.18. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/27733885>>.
- He, C.-T., Lee, C.-H., Hsieh, C.-H., Hsiao, F.-C., Kuo, P., Chu, N.-F. and Hung, Y.-J., (2014). Soluble form of receptor for advanced glycation end products is associated with obesity and metabolic syndrome in adolescents. *International journal of endocrinology*, **2014**.
- Heddle, J.A., Fenech, M., Hayashi, M. and MacGregor, J.T., (2010). Reflections on the development of micronucleus assays. *Mutagenesis*, **26**(1), pp.3–10. Available from: <<https://doi.org/10.1093/mutage/geq085>>.
- Vander Heiden, M.G., Cantley, L.C. and Thompson, C.B., (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science (New York, N.Y.)*, **324**(5930), pp.1029–1033. Available from: <<https://pubmed.ncbi.nlm.nih.gov/19460998>>.
- Heng, H.H.Q., Liu, G., Stevens, J.B., Abdallah, B.Y., Horne, S.D., Ye, K.J., Bremer, S.W., Chowdhury, S.K. and Ye, C.J., (2013). Karyotype heterogeneity and unclassified chromosomal abnormalities. *Cytogenetic and genome research*, **139**(3), pp.144–157.
- Heng, H.H.Q., Regan, S.M., Liu, G. and Ye, C.J., (2016). Why it is crucial to analyze non clonal chromosome aberrations or NCCAs? *Molecular cytogenetics*, **9**, p.15. Available from: <<https://pubmed.ncbi.nlm.nih.gov/26877768>>.
- Higashi, T., Shibayama, Y., Fuji, M. and Shimada, K., (2008). Liquid chromatography–tandem mass spectrometric method for the determination of salivary 25-hydroxyvitamin D3: a noninvasive tool for the assessment of vitamin D status. *Analytical and Bioanalytical Chemistry*, **391**(1), pp.229–238. Available from: <<https://doi.org/10.1007/s00216-007-1780-3>>.
- Higashi, T., Hijikuro, M., Yamagata, K. and Ogawa, S., (2013). Overestimation of salivary 25-hydroxyvitamin D3 level when using stimulated saliva with gum-chewing. *Steroids*, **78**(9), pp.884–887. Available from: <<http://www.sciencedirect.com/science/article/pii/S0039128X13001104>>.
- Himmetoglu, S., Dincer, Y., Ersoy, Y.E., Bayraktar, B., Celik, V. and Akcay, T., (2009). DNA oxidation and antioxidant status in breast cancer. *Journal of Investigative Medicine*, **57**(6), pp.720–723.
- Hirsch, D., Kemmerling, R., Davis, S., Camps, J., Meltzer, P.S., Ried, T. and Gaiser, T., (2013). Chromothripsis and focal copy number alterations determine poor outcome in malignant melanoma. *Cancer research*, **73**(5), pp.1454–1460. Available from:

- <<https://www.ncbi.nlm.nih.gov/pubmed/23271725>>.
- Ho, E. and Ames, B.N., (2002). Low intracellular zinc induces oxidative DNA damage, disrupts p53, NFκB, and AP1 DNA binding, and affects DNA repair in a rat glioma cell line. *Proceedings of the National Academy of Sciences*, **99**(26), pp.16770–16775.
- Hochstrasser, T., Marksteiner, J. and Humpel, C., (2012). Telomere length is age-dependent and reduced in monocytes of Alzheimer patients. *Experimental Gerontology*, **47**(2), pp.160–163. Available from: <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3278593/>>.
- Hofer, T., Karlsson, H.L. and Möller, L., (2006). DNA oxidative damage and strand breaks in young healthy individuals: A gender difference and the role of life style factors. *Free Radical Research*, **40**(7), pp.707–714. Available from: <<https://doi.org/10.1080/10715760500525807>>.
- Hofseth, L.J., Khan, M.A., Ambrose, M., Nikolayeva, O., Xu-Welliver, M., Kartalou, M., Hussain, S.P., Roth, R.B., Zhou, X., Mechanic, L.E., Zurer, I., Rotter, V., Samson, L.D. and Harris, C.C., (2003). The adaptive imbalance in base excision-repair enzymes generates microsatellite instability in chronic inflammation. *The Journal of clinical investigation*, **112**(12), pp.1887–1894. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/14679184>>.
- Holick, M.F., (2007). Vitamin D deficiency. *New England Journal of Medicine*, **357**(3), pp.266–281.
- Holland, N., Bolognesi, C., Kirsch-Volders, M., Bonassi, S., Zeiger, E., Knasmueller, S. and Fenech, M., (2008). The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: The HUMN project perspective on current status and knowledge gaps. *Mutation Research/Reviews in Mutation Research*, **659**(1), pp.93–108. Available from: <<http://www.sciencedirect.com/science/article/pii/S1383574208000410>>.
- Horan, M., Gibney, E., Molloy, E. and McAuliffe, F., (2015). Methodologies to assess paediatric adiposity. *Irish Journal of Medical Science (1971 -)*, **184**(1), pp.53–68. Available from: <<https://doi.org/10.1007/s11845-014-1124-1>>.
- Hossain, S., Beydoun, M.A., Beydoun, H.A., Chen, X., Zonderman, A.B. and Wood, R.J., (2019). Vitamin D and breast cancer: A systematic review and meta-analysis of observational studies. *Clinical nutrition ESPEN*, **30**, pp.170–184.
- Hudda, M.T., Nightingale, C.M., Donin, A.S., Owen, C.G., Rudnicka, A.R., Wells, J.C.K., Rutter, H., Cook, D.G. and Whincup, P.H., (2018). Reassessing Ethnic Differences in Mean BMI and Changes Between 2007 and 2013 in English Children. *Obesity (Silver Spring, Md.)*, **26**(2), pp.412–419.
- Hudson, B.I., Harja, E., Moser, B. and Schmidt, A.M., (2005). Soluble levels of receptor for advanced glycation endproducts (sRAGE) and coronary artery disease: the next C-reactive protein?
- Hukezalie, K.R., Thumati, N.R., Coˆte, H.C.F. and Wong, J.M.Y., (2012). In vitro and ex vivo inhibition of human telomerase by anti-HIV nucleoside reverse transcriptase inhibitors (NRTIs) but not by non-NRTIs. *PLoS One*, **7**(11), p.e47505.
- Hurtley, S.M., (2001). Centrosomes and Cytokinesis. *Science*, **292**(5516), pp.401 LP – 401. Available from: <<http://science.sciencemag.org/content/292/5516/401.2.abstract>>.

- Ichijo, H., Nishida, E., Irie, K., ten Dijke, P., Saitoh, M., Moriguchi, T., Takagi, M., Matsumoto, K., Miyazono, K. and Gotoh, Y., (1997). Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science*, **275**(5296), pp.90–94.
- Idolo, A., Grassi, T., Bagordo, F., Panico, A., De Giorgi, M., Serio, F., Guido, M., Piscitelli, P., De Filippis, G., Raho, A. and De Donno, A., (2018). Micronuclei in Exfoliated Buccal Cells of Children Living in a Cluster Area of Salento (Southern Italy) with a High Incidence of Lung Cancer: The IMP.AIR Study. *International journal of environmental research and public health*, **15**(8), p.1659. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/30081588>>.
- Iglesias Molli, A.E., Panero, J., Dos Santos, P.C., González, C.D., Vilariño, J., Serey, M., Cerrone, G.E., Slavutsky, I. and Frechtel, G.D., (2017). Metabolically healthy obese women have longer telomere length than obese women with metabolic syndrome. *PloS one*, **12**(4), pp.e0174945–e0174945. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/28384193>>.
- Inge, T.H., King, W.C., Jenkins, T.M., Courcoulas, A.P., Mitsnefes, M., Flum, D.R., Wolfe, B.M., Pomp, A., Dakin, G.F., Khandelwal, S., Zeller, M.H., Horlick, M., Pender, J.R., Chen, J.-Y. and Daniels, S.R., (2013). The effect of obesity in adolescence on adult health status. *Pediatrics*, **132**(6), pp.1098–1104.
- Inge, T.H., Xanthakos, S.A. and Zeller, M.H., (2007). Bariatric surgery for pediatric extreme obesity: now or later? *International journal of obesity*, **31**(1), pp.1–14.
- Ishikawa, N., Nakamura, K.-I., Izumiyama-Shimomura, N., Aida, J., Ishii, A., Goto, M., Ishikawa, Y., Asaka, R., Matsuura, M., Hatamochi, A., Kuroiwa, M. and Takubo, K., (2011). Accelerated in vivo epidermal telomere loss in Werner syndrome. *Aging (Albany NY)*, **3**(4), pp.417–429. Available from: <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3117457/>>.
- Iyengar, A., Paulus, J.K., Gerlanc, D.J. and Maron, J.L., (2014). Detection and potential utility of C-reactive protein in saliva of neonates. *Frontiers in pediatrics*, **2**, p.131. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/25485262>>.
- Izano, M., Wei, E.K., Tai, C., Swede, H., Gregorich, S., Harris, T.B., Klepin, H., Satterfield, S., Murphy, R. and Newman, A.B., (2016). Chronic inflammation and risk of colorectal and other obesity-related cancers: The health, aging and body composition study. *International Journal of Cancer*, **138**(5), pp.1118–1128.
- Jacociunas, L.V., de Andrade, H.H.R., Lehmann, M., de Abreu, B.R.R., Ferraz, A. de B.F., da Silva, J., Grivicich, I. and Dihi, R.R., (2013). Artichoke induces genetic toxicity in the cytokinesis-block micronucleus (CBMN) cytome assay. *Food and chemical toxicology*, **55**, pp.56–59.
- Jaiswal, M., LaRusso, N.F., Nishioka, N., Nakabeppu, Y. and Gores, G.J., (2001). Human Ogg1, a protein involved in the repair of 8-oxoguanine, is inhibited by nitric oxide. *Cancer research*, **61**(17), pp.6388–6393.
- Janem, W.F., Scannapieco, F.A., Sabharwal, A., Tsompana, M., Berman, H.A., Haase, E.M., Miecznikowski, J.C. and Mastrandrea, L.D., (2017). Salivary inflammatory markers and microbiome in normoglycemic lean and obese children compared to obese children with type 2 diabetes. *PloS one*, **12**(3), pp.e0172647–e0172647. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/28253297>>.

- Jang, J.S., Choi, Y.Y., Lee, W.K., Choi, J.E., Cha, S.I., Kim, Y.J., Kim, C.H., Kam, S., Jung, T.H. and Park, J.Y., (2008). Telomere length and the risk of lung cancer. *Cancer Science*, **99**(7), pp.1385–1389. Available from: <<http://dx.doi.org/10.1111/j.1349-7006.2008.00831.x>>.
- Jiang, F., Bao, J., Li, P., Nicosia, S. V and Bai, W., (2004). Induction of ovarian cancer cell apoptosis by 1, 25-dihydroxyvitamin D3 through the down-regulation of telomerase. *Journal of Biological Chemistry*, **279**(51), pp.53213–53221.
- Joerger, A.C. and Fersht, A.R., (2016). The p53 pathway: origins, inactivation in cancer, and emerging therapeutic approaches. *Annual review of biochemistry*, **85**, pp.375–404.
- Kanaar, R., Wyman, C. and Rothstein, R., (2008). Quality control of DNA break metabolism: in the 'end', it's a good thing. *The EMBO journal*, **27**(4), pp.581–588.
- Kanekar, A. and Sharma, M., (2010). Pharmacological approaches for management of child and adolescent obesity. *Journal of clinical medicine research*, **2**(3), pp.105–111.
- Kao, K.-T., Abidi, N., Ranasinha, S., Brown, J., Rodda, C., McCallum, Z., Zacharin, M., Simm, P.J., Magnussen, C.G. and Sabin, M.A., (2015). Low vitamin D is associated with hypertension in paediatric obesity. *Journal of paediatrics and child health*, **51**(12), pp.1207–1213.
- Karkeni, E., Marcotorchino, J., Tourniaire, F., Astier, J., Peiretti, F., Darmon, P. and Landrier, J.-F., (2015). Vitamin D limits chemokine expression in adipocytes and macrophage migration in vitro and in male mice. *Endocrinology*, **156**(5), pp.1782–1793.
- Karkeni, E., Bonnet, L., Marcotorchino, J., Tourniaire, F., Astier, J., Ye, J. and Landrier, J.-F., (2018). Vitamin D limits inflammation-linked microRNA expression in adipocytes in vitro and in vivo: A new mechanism for the regulation of inflammation by vitamin D. *Epigenetics*, **13**(2), pp.156–162.
- Karpouzi, C., Nikolaidis, S., Kabasakalis, A., Tsalis, G. and Mougios, V., (2016). Exercise-induced oxidatively damaged DNA in humans: evaluation in plasma or urine? *Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals*, **21**(3), pp.204–207.
- Kasai, H. and Nishimura, S., (1984). Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. *Nucleic acids research*, **12**(4), pp.2137–2145. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/6701097>>.
- Kasbek, C., Wang, F. and Price, C.M., (2013). Human TEN1 Maintains Telomere Integrity and Functions in Genome-wide Replication Restart. *Journal of Biological Chemistry*, **288**(42), pp.30139–30150. Available from: <<http://www.jbc.org/content/288/42/30139.abstract>>.
- Kasiappan, R., Shen, Z., Anfernee, K.W., Jinwal, U., Tang, J., Lungchukiet, P., Sun, Y., Kruk, P., Nicosia, S. V and Zhang, X., (2012). 1, 25-Dihydroxyvitamin D3 suppresses telomerase expression and human cancer growth through microRNA-498. *Journal of Biological Chemistry*, **287**(49), pp.41297–41309.
- Kass, L.B. and Chomet, P., (2009). Barbara McClintock. In *Handbook of Maize*. Springer, pp. 17–52.
- Katarkar, A., Mukherjee, S., Khan, M.H., Ray, J.G. and Chaudhuri, K., (2014). Comparative evaluation of genotoxicity by micronucleus assay in the buccal mucosa over comet assay in peripheral blood in oral precancer and cancer patients. *Mutagenesis*, **29**(5), pp.325–334. Available from:

- <<http://mutage.oxfordjournals.org/content/29/5/325.abstract>>.
- Kaufmann, W.K. and Paules, R.S., (1996). DNA damage and cell cycle checkpoints. *The FASEB Journal*, **10**(2), pp.238–247.
- Kaur, J., Politis, C. and Jacobs, R., (2016). Salivary 8-hydroxy-2-deoxyguanosine, malondialdehyde, vitamin C, and vitamin E in oral pre-cancer and cancer: diagnostic value and free radical mechanism of action. *Clinical oral investigations*, **20**(2), pp.315–319.
- Kawakami, H., Zaanan, A. and Sinicrope, F.A., (2015). Microsatellite instability testing and its role in the management of colorectal cancer. *Current treatment options in oncology*, **16**(7), p.30. Available from: <<https://pubmed.ncbi.nlm.nih.gov/26031544>>.
- Kelishadi, R., Heidari-Beni, M., Qorbani, M., Motamed-Gorji, N., Motlagh, M.E., Ziaodini, H., Taheri, M., Ahadi, Z., Aminae, T. and Heshmat, R., (2017). Association between neck and wrist circumferences and cardiometabolic risk in children and adolescents: The CASPIAN-V study. *Nutrition*, **43–44**, pp.32–38. Available from: <<http://www.sciencedirect.com/science/article/pii/S089990071730120X>>.
- Kern, P.A., Di Gregorio, G.B., Lu, T., Rassouli, N. and Ranganathan, G., (2003). Adiponectin Expression From Human Adipose Tissue. *Diabetes*, **52**(7), pp.1779–1785. Available from: <<http://diabetes.diabetesjournals.org/content/52/7/1779.abstract>>.
- Khelifi, R., Trabelsi-Ksibi, F., Chakroun, A., Rebai, A. and Hamza-Chaffai, A., (2013). Cytogenetic abnormality in exfoliated cells of buccal mucosa in head and neck cancer patients in the Tunisian population: impact of different exposure sources. *BioMed research international*, **2013**, p.905252. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/23957010>>.
- Kilic, E., Özer, Ö.F., Erek, A.T., Erman, H., Torun, E., Ayhan, S.K., Caglar, H.G., Selek, S. and Kocyigit, A., (2016). Oxidative Stress Status in Childhood Obesity: A Potential Risk Predictor. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*, **22**, pp.3673–3679.
- Kim, S.-J., Jung, H.-J. and Lim, C.-J., (2013). Reactive Oxygen Species-Dependent Down-Regulation of Tumor Suppressor Genes PTEN, USP28, DRAM, TIGAR, and CYLD Under Oxidative Stress. *Biochemical Genetics*, **51**(11), pp.901–915. Available from: <<https://doi.org/10.1007/s10528-013-9616-7>>.
- Kim, S., Parks, C.G., DeRoo, L.A., Chen, H., Taylor, J.A., Cawthon, R.M. and Sandler, D.P., (2009). Obesity and Weight Gain in Adulthood and Telomere Length. *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*, **18**(3), p.816. Available from: <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2805851/>>.
- Kimura, N., Dota, K., Araya, Y., Ishidate, T. and Ishizaka, M., (2009). Scoring system for differential diagnosis of malignant mesothelioma and reactive mesothelial cells on cytology specimens. *Diagnostic cytopathology*, **37**(12), pp.885–890.
- Kiraz, A., Açmaz, G., Uysal, G., Unal, D. and Dönmez-Altuntas, H., (2016). Micronucleus testing as a cancer detector: endometrial hyperplasia to carcinoma. *Archives of Gynecology and Obstetrics*, **293**(5), pp.1065–1071.
- Kirsch-Volders, M., Plas, G., Elhajouji, A., Lukamowicz, M., Gonzalez, L., Looock, K. Vande and Decordier, I., (2011). The in vitro MN assay in 2011:

- origin and fate, biological significance, protocols, high throughput methodologies and toxicological relevance. *Archives of toxicology*, **85**(8), pp.873–899.
- Kitsios, K., Papadopoulou, M., Kosta, K., Kadoglou, N., Papagianni, M. and Tsiroukidou, K., (2013). High-sensitivity C-reactive protein levels and metabolic disorders in obese and overweight children and adolescents. *Journal of clinical research in pediatric endocrinology*, **5**(1), p.44.
- Kobayashi, J., (2004). Molecular mechanism of the recruitment of NBS1/hMRE11/hRAD50 complex to DNA double-strand breaks: NBS1 binds to gamma-H2AX through FHA/BRCT domain. *Journal of radiation research*, **45**(4), pp.473–8. Available from: <<http://www.ncbi.nlm.nih.gov/pubmed/15635255>>.
- Kocael, A., Erman, H., Zengin, K., Kocael, P.C.A., Korkmaz, G.G., Gelisgen, R., Taskin, M., Ersan, Y. and Uzun, H., (2014). The effects on oxidative DNA damage of laparoscopic gastric band applications in morbidly obese patients. *Canadian Journal of Surgery*, **57**(3), p.183.
- Kohler, M.J. and van den Heuvel, C.J., (2008). Is there a clear link between overweight/obesity and sleep disordered breathing in children? *Sleep Medicine Reviews*, **12**(5), pp.347–361. Available from: <<http://www.sciencedirect.com/science/article/pii/S1087079208000476>>.
- Kolb, R., Sutterwala, F.S. and Zhang, W., (2016). Obesity and cancer: inflammation bridges the two. *Current opinion in pharmacology*, **29**, pp.77–89. Available from: <<https://pubmed.ncbi.nlm.nih.gov/27429211>>.
- Kommagani, R., Payal, V. and Kadakia, M.P., (2007). Differential regulation of vitamin D receptor (VDR) by the p53 Family: p73-dependent induction of VDR upon DNA damage. *The Journal of biological chemistry*, **282**(41), pp.29847–29854.
- de Koning, L., Denhoff, E., Kellogg, M.D. and de Ferranti, S.D., (2015). Associations of total and abdominal adiposity with risk marker patterns in children at high-risk for cardiovascular disease. *BMC Obesity*, **2**(1), p.15. Available from: <<https://doi.org/10.1186/s40608-015-0043-7>>.
- Korkmaz, K.S., Butuner, B.D. and Roggenbuck, D., (2018). Detection of 8-OHdG as a diagnostic biomarker. *Journal of Laboratory and Precision Medicine; November 2018*. Available from: <<http://jlp.m.amegroups.com/article/view/4605>>.
- Kovalenko, P.L., Zhang, Z., Cui, M., Clinton, S.K. and Fleet, J.C., (2010). 1, 25 dihydroxyvitamin D-mediated orchestration of anticancer, transcript-level effects in the immortalized, non-transformed prostate epithelial cell line, RWPE1. *BMC genomics*, **11**(1), p.26.
- Krajcovic, M., Johnson, N.B., Sun, Q., Normand, G., Hoover, N., Yao, E., Richardson, A.L., King, R.W., Cibas, E.S. and Schnitt, S.J., (2011). A non-genetic route to aneuploidy in human cancers. *Nature cell biology*, **13**(3), p.324.
- Krajcovic, M. and Overholtzer, M., (2012). Mechanisms of ploidy increase in human cancers: a new role for cell cannibalism. *Cancer research*, **72**(7), pp.1596–1601.
- Krajewska, M., Fehrmann, R.S.N., de Vries, E.G.E. and van Vugt, M.A.T.M., (2015). Regulators of homologous recombination repair as novel targets for cancer treatment. *Frontiers in Genetics*, **6**, p.96. Available from: <<https://www.frontiersin.org/article/10.3389/fgene.2015.00096>>.
- Kral, T.V.E. and Faith, M.S., (2008). Influences on Child Eating and Weight Development from a Behavioral Genetics Perspective. *Journal of Pediatric*

- Psychology*, **34**(6), pp.596–605. Available from: <<https://doi.org/10.1093/jpepsy/jsn037>>.
- Kreutz, C., MacNelly, S., Follo, M., Wäldin, A., Binninger-Lacour, P., Timmer, J. and Bartolomé-Rodríguez, M.M., (2017). Hepatocyte Ploidy Is a Diversity Factor for Liver Homeostasis. *Frontiers in physiology*, **8**, p.862. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/29163206>>.
- Krishnan, A. V, Trump, D.L., Johnson, C.S. and Feldman, D., (2012). The Role of Vitamin D in Cancer Prevention and Treatment. *Rheumatic Disease Clinics*, **38**(1), pp.161–178. Available from: <<https://doi.org/10.1016/j.rdc.2012.03.014>>.
- Krokan, H.E. and Bjørås, M., (2013). Base excision repair. *Cold Spring Harbor perspectives in biology*, **5**(4), pp.a012583–a012583. Available from: <<https://pubmed.ncbi.nlm.nih.gov/23545420>>.
- Krude, H., Biebermann, H., Schnabel, D., Tansek, M.Z., Theunissen, P., Mullis, P.E. and Grüters, A., (2003). Obesity due to proopiomelanocortin deficiency: three new cases and treatment trials with thyroid hormone and ACTH4–10. *The Journal of Clinical Endocrinology & Metabolism*, **88**(10), pp.4633–4640.
- Krul, M., Van Der Wouden, J.C., Schellevis, F.G., Van Suijlekom-Smit, L.W.A. and Koes, B.W., (2009). Musculoskeletal problems in overweight and obese children. *Annals of Family Medicine*, **7**(4), pp.352–356.
- Krzystek-Korpaczka, M., Patryn, E., Hotowy, K., Czapinska, E., Majda, J. and Kustrzeba, W.I., (2013). Paraoxonase-1 activity in overweight and obese children and adolescents: association with obesity-related inflammation and oxidative stress. *Adv Clin Exp Med*, **22**(2), pp.229–236.
- Kumaratne, M., Early, G. and Cisneros, J., (2017). Vitamin D Deficiency and Association With Body Mass Index and Lipid Levels in Hispanic American Adolescents. *Global pediatric health*, **4**, p.2333794X17744141.
- Kuo, H.-W., Chou, S.-Y., Hu, T.-W., Wu, F.-Y. and Chen, D.-J., (2007). Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) and genetic polymorphisms in breast cancer patients. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, **631**(1), pp.62–68.
- de la Maza, M.-P., Olivares, D., Hirsch, S., Sierralta, W., Gattas, V., Barrera, G., Bunout, D., Leiva, L. and Fernandez, M., (2006). Weight increase and overweight are associated with DNA oxidative damage in skeletal muscle. *Clinical nutrition (Edinburgh, Scotland)*, **25**(6), pp.968–976.
- Labat, C., Temmar, M., Nagy, E., Bean, K., Brink, C., Benetos, A. and Bäck, M., (2013). Inflammatory mediators in saliva associated with arterial stiffness and subclinical atherosclerosis. *Journal of Hypertension*, **31**(11). Available from: <https://journals.lww.com/jhypertension/Fulltext/2013/11000/Inflammatory_mediators_in_saliva_associated_with.23.aspx>.
- Laguna, J., Alegret, M. and Roglans, N., (2014). Simple sugar intake and hepatocellular carcinoma: epidemiological and mechanistic insight. *Nutrients*, **6**(12), pp.5933–5954.
- Lan, Q., Cawthon, R., Gao, Y., Hu, W., Hosgood III, H.D., Barone-Adesi, F., Ji, B.-T., Bassig, B., Chow, W.-H., Shu, X., Cai, Q., Xiang, Y., Berndt, S., Kim, C., Chanock, S., Zheng, W. and Rothman, N., (2013). Longer Telomere Length in Peripheral White Blood Cells Is Associated with Risk of Lung Cancer and the rs2736100 (CLPTM1L-TERT) Polymorphism in a Prospective Cohort Study among Women in China. *PLOS ONE*, **8**(3), p.e59230. Available from: <<https://doi.org/10.1371/journal.pone.0059230>>.

- Landgraf, K., Rockstroh, D., Wagner, I. V, Weise, S., Tauscher, R., Schwartz, J.T., Loffler, D., Buhligen, U., Wojan, M., Till, H., Kratzsch, J., Kiess, W., Bluher, M. and Korner, A., (2015). Evidence of early alterations in adipose tissue biology and function and its association with obesity-related inflammation and insulin resistance in children. *Diabetes*, **64**(4), pp.1249–1261.
- De Lange, T., (1995). 10 Telomere Dynamics and Genome Instability in Human Cancer. *Cold Spring Harbor Monograph Archive*, **29**, pp.265–293.
- Lappe, J.M., Travers-Gustafson, D., Davies, K.M., Recker, R.R. and Heaney, R.P., (2007). Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. *The American journal of clinical nutrition*, **85**(6), pp.1586–1591.
- Lawes, D.A., SenGupta, S. and Boulous, P.B., (2003). The clinical importance and prognostic implications of microsatellite instability in sporadic cancer. *European Journal of Surgical Oncology (EJSO)*, **29**(3), pp.201–212. Available from: <<http://www.sciencedirect.com/science/article/pii/S0748798302913995>>.
- Lee, D.-H., (2007). A study on SOD activity and serum antioxidant mineral concentrations in obese adolescents. *Journal of Nutrition and Health*, **40**(1), pp.41–48.
- LEE, M., MARTIN, H., FIRPO, M.A. and DEMERATH, E.W., (2011). Inverse Association Between Adiposity and Telomere Length: The Fels Longitudinal Study. *American journal of human biology : the official journal of the Human Biology Council*, **23**(1), pp.100–106. Available from: <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3245638/>>.
- Lee, S.C. and Chan, J.C.N., (2015). Evidence for DNA damage as a biological link between diabetes and cancer. *Chinese medical journal*, **128**(11), pp.1543–1548. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/26021514>>.
- Lehmann, A.R., (2003). DNA repair-deficient diseases, xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy. *Biochimie*, **85**(11), pp.1101–1111. Available from: <<http://www.sciencedirect.com/science/article/pii/S0300908403001603>>.
- Lens, S.M.A. and Medema, R.H., (2019). Cytokinesis defects and cancer. *Nature Reviews Cancer*, **19**(1), pp.32–45. Available from: <<https://doi.org/10.1038/s41568-018-0084-6>>.
- Li, G.-M., (2008). Mechanisms and functions of DNA mismatch repair. *Cell Research*, **18**(1), pp.85–98. Available from: <<https://doi.org/10.1038/cr.2007.115>>.
- Li, S., Wang, X., Wu, Y., Zhang, H., Zhang, L., Wang, C., Zhang, R. and Guo, Z., (2012). 8-Hydroxy-2'-deoxyguanosine expression predicts hepatocellular carcinoma outcome. *Oncology letters*, **3**(2), pp.338–342.
- Li, W., Zhang, X., Sang, H., Zhou, Y., Shang, C., Wang, Y. and Zhu, H., (2019). Effects of hyperglycemia on the progression of tumor diseases. *Journal of Experimental & Clinical Cancer Research*, **38**(1), p.327. Available from: <<https://doi.org/10.1186/s13046-019-1309-6>>.
- Lin, Y., Berg, A.H., Iyengar, P., Lam, T.K.T., Giacca, A., Combs, T.P., Rajala, M.W., Du, X., Rollman, B. and Li, W., (2005). The hyperglycemia-induced inflammatory response in adipocytes the role of reactive oxygen species. *Journal of Biological Chemistry*, **280**(6), pp.4617–4626.
- Lindberg, H.K., Wang, X., Järventaus, H., Falck, G.C.-M., Norppa, H. and Fenech, M., (2007). Origin of nuclear buds and micronuclei in normal and

- folate-deprived human lymphocytes. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **617**(1), pp.33–45. Available from: <<http://www.sciencedirect.com/science/article/pii/S0027510706003484>>.
- Lingle, W.L., Barrett, S.L., Negrón, V.C., D'Assoro, A.B., Boeneman, K., Liu, W., Whitehead, C.M., Reynolds, C. and Salisbury, J.L., (2002). Centrosome amplification drives chromosomal instability in breast tumor development. *Proceedings of the National Academy of Sciences*, **99**(4), pp.1978–1983.
- Lingle, W.L., Lutz, W.H., Ingle, J.N., Maihle, N.J. and Salisbury, J.L., (1998). Centrosome hypertrophy in human breast tumors: implications for genomic stability and cell polarity. *Proceedings of the National Academy of Sciences*, **95**(6), pp.2950–2955.
- Lips, P., (2006). Vitamin D physiology. *Progress in Biophysics and Molecular Biology*, **92**(1), pp.4–8. Available from: <<http://www.sciencedirect.com/science/article/pii/S0079610706000083>>.
- Liu, B., Nicolaides, N.C., Markowitz, S., Willson, J.K. V, Parsons, R.E., Jen, J., Papadopolous, N., Peltomäki, P., de la Chapelle, A., Hamilton, S.R., Kinzler, K.W. and Vogelstein, B., (1995). Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. *Nature Genetics*, **9**(1), pp.48–55. Available from: <<https://doi.org/10.1038/ng0195-48>>.
- Llewellyn, A., Simmonds, M., Owen, C.G. and Woolacott, N., (2016). Childhood obesity as a predictor of morbidity in adulthood: a systematic review and meta-analysis. *Obesity reviews: an official journal of the International Association for the Study of Obesity*, **17**(1), pp.56–67.
- Locke, A.E., Kahali, B., Berndt, S.I., Justice, A.E., Pers, T.H., Day, F.R., Powell, C., Vedantam, S., Buchkovich, M.L. and Yang, J., (2015). Genetic studies of body mass index yield new insights for obesity biology. *Nature*, **518**(7538), p.197.
- Lodish, M.B., Keil, M.F. and Stratakis, C.A., (2018). Cushing's Syndrome in Pediatrics: An Update. *Endocrinology and Metabolism Clinics of North America*, **47**(2), pp.451–462. Available from: <<http://www.sciencedirect.com/science/article/pii/S0889852918300161>>.
- Lointier, P., Wargovich, M.J., Saez, S., Levin, B., Wildrick, D.M. and Boman, B.M., (1987). The role of vitamin D3 in the proliferation of a human colon cancer cell line in vitro. *Anticancer research*, **7**(4B), pp.817–821.
- Lopes, N., Sousa, B., Martins, D., Gomes, M., Vieira, D., Veronese, L.A., Milanezi, F., Paredes, J., Costa, J.L. and Schmitt, F., (2010). Alterations in Vitamin D signalling and metabolic pathways in breast cancer progression: a study of VDR, CYP27B1 and CYP24A1 expression in benign and malignant breast lesions. *BMC cancer*, **10**, p.483. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/20831823>>.
- Lourenco, B.H., Qi, L., Willett, W.C. and Cardoso, M.A., (2014). FTO genotype, vitamin D status, and weight gain during childhood. *Diabetes*, **63**(2), pp.808–814.
- Ludwig, D.S., Peterson, K.E. and Gortmaker, S.L., (2001). Relation between consumption of sugar-sweetened drinks and childhood obesity: a prospective, observational analysis. *Lancet (London, England)*, **357**(9255), pp.505–508.
- Lukaski, H.C., Johnson, P.E., Bolonchuk, W.W. and Lykken, G.I., (1985). Assessment of fat-free mass using bioelectrical impedance measurements of the human body. *The American journal of clinical nutrition*, **41**(4), pp.810–817.
- Machiela, M.J., Hofmann, J.N., Carreras-Torres, R., Brown, K.M., Johansson,

- M., Wang, Z., Foll, M., Li, P., Rothman, N., Savage, S.A., Gaborieau, V., McKay, J.D., Ye, Y., Henrion, M., Bruinsma, F., Jordan, S., Severi, G., Hveem, K., Vatten, L.J., et al., (2017). Genetic Variants Related to Longer Telomere Length are Associated with Increased Risk of Renal Cell Carcinoma. *European Urology*, **72**(5), pp.747–754. Available from: <<https://doi.org/10.1016/j.eururo.2017.07.015>>.
- Mackenzie, K.J., Carroll, P., Martin, C.-A., Murina, O., Fluteau, A., Simpson, D.J., Olova, N., Sutcliffe, H., Rainger, J.K., Leitch, A., Osborn, R.T., Wheeler, A.P., Nowotny, M., Gilbert, N., Chandra, T., Reijns, M.A.M. and Jackson, A.P., (2017). cGAS surveillance of micronuclei links genome instability to innate immunity. *Nature*, **548**(7668), pp.461–465.
- Maffei, F., Zolezzi Moraga, J.M., Angelini, S., Zenesini, C., Musti, M., Festi, D., Cantelli-Forti, G. and Hrelia, P., (2014). Micronucleus frequency in human peripheral blood lymphocytes as a biomarker for the early detection of colorectal cancer risk. *Mutagenesis*, **29**(3), pp.221–225. Available from: <<http://mutage.oxfordjournals.org/content/29/3/221.abstract>>.
- Mager, D.R., Yap, J., Rodriguez-Dimitrescu, C., Mazurak, V., Ball, G. and Gilmour, S., (2013). Anthropometric measures of visceral and subcutaneous fat are important in the determination of metabolic dysregulation in boys and girls at risk for nonalcoholic fatty liver disease. *Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition*, **28**(1), pp.101–111.
- Magrangeas, F., Avet-Loiseau, H., Munshi, N.C. and Minvielle, S., (2011). Chromothripsis identifies a rare and aggressive entity among newly diagnosed multiple myeloma patients. *Blood*, **118**(3), pp.675–678.
- Malumbres, M. and Barbacid, M., (2009). Cell cycle, CDKs and cancer: a changing paradigm. *Nature Reviews Cancer*, **9**, p.153. Available from: <<https://doi.org/10.1038/nrc2602>>.
- Mandard, A.M., Duigou, F., Marnay, J., Barrellier, P. and Lebigot, G., (1987). Analysis of the results of the micronucleus test in patients presenting upper digestive tract cancers and in non-cancerous subjects. *International journal of cancer*, **39**(4), pp.442–444.
- Marion, A.W., Baker, A.J. and Dhawan, A., (2004). Fatty liver disease in children. *Archives of Disease in Childhood*, **89**(7), pp.648–652.
- Mark Gustafson, L., Gleich, L.L., Fukasawa, K., Chadwell, J., Ann Miller, M., Stambrook, P.J. and Gluckman, J.L., (2000). Centrosome hyperamplification in head and neck squamous cell carcinoma: a potential phenotypic marker of tumor aggressiveness. *The Laryngoscope*, **110**(11), pp.1798–1801.
- Martin-Ruiz, C.M., Gussekloo, J., van Heemst, D., von Zglinicki, T. and Westendorp, R.G.J., (2005). Telomere length in white blood cells is not associated with morbidity or mortality in the oldest old: a population-based study. *Aging Cell*, **4**(6), pp.287–290. Available from: <<https://doi.org/10.1111/j.1474-9726.2005.00171.x>>.
- Mazıcıoğlu, M.M., Hatipoğlu, N., Oztürk, A., Çiçek, B., Ustünbaş, H.B. and Kurtoğlu, S., (2010). Waist circumference and mid-upper arm circumference in evaluation of obesity in children aged between 6 and 17 years. *Journal of clinical research in pediatric endocrinology*, **2**(4), pp.144–150. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/21274313>>.
- McCarthy, H.D., (2006). Body fat measurements in children as predictors for the metabolic syndrome: focus on waist circumference. *Proceedings of the Nutrition Society*, **65**(4), pp.385–392. Available from:

- <<https://www.cambridge.org/core/article/body-fat-measurements-in-children-as-predictors-for-the-metabolic-syndrome-focus-on-waist-circumference/085644290E014A54C1F1F4C7C421F089>>.
- McCarthy, H.D., Cole, T.J., Fry, T., Jebb, S.A. and Prentice, A.M., (2006). Body fat reference curves for children. *International Journal Of Obesity*, **30**, p.598. Available from: <<https://doi.org/10.1038/sj.ijo.0803232>>.
- McCarthy, H.D., Jarrett, K. V and Crawley, H.F., (2001). The development of waist circumference percentiles in British children aged 5.0-16.9 y. *European journal of clinical nutrition*, **55**(10), pp.902–907.
- McGranahan, N., Burrell, R.A., Endesfelder, D., Novelli, M.R. and Swanton, C., (2012). Cancer chromosomal instability: therapeutic and diagnostic challenges. *EMBO reports*, **13**(6), pp.528 LP – 538. Available from: <<http://embor.embopress.org/content/13/6/528.abstract>>.
- McNamara, M. and Rosenberger, K.D., (2019). The Significance of Vitamin D Status in Breast Cancer: A State of the Science Review. *Journal of midwifery & women's health*.
- Medzhitov, R., (2008). Origin and physiological roles of inflammation. *Nature*, **454**, p.428. Available from: <<https://doi.org/10.1038/nature07201>>.
- Melbye, H., Hvidsten, D., Holm, A., Nordbø, S.A. and Brox, J., (2004). The course of C-reactive protein response in untreated upper respiratory tract infection. *The British journal of general practice : the journal of the Royal College of General Practitioners*, **54**(506), pp.653–658. Available from: <<https://pubmed.ncbi.nlm.nih.gov/15353049>>.
- Meredith-Jones, K.A., Williams, S.M. and Taylor, R.W., (2015). Bioelectrical impedance as a measure of change in body composition in young children. *Pediatric obesity*, **10**(4), pp.252–259.
- Merino, O., Sánchez, R., Gregorio, B.M., Sampaio, F.J. and Risopatrón, J., (2018). Effects of Diet-Induced Obesity and Deficient in Vitamin D on Spermatozoa Function and DNA Integrity in Sprague-Dawley Rats. *BioMed research international*, **2018**.
- Milne, E., Greenop, K.R., Ramankutty, P., Miller, M., de Klerk, N.H., Armstrong, B.K., Almond, T., O'Callaghan, N.J. and Fenech, M., (2015). Blood micronutrients and DNA damage in children. *Molecular Nutrition & Food Research*, **59**(10), pp.2057–2065. Available from: <<https://doi.org/10.1002/mnfr.201500110>>.
- Miraglia del Giudice, E., Grandone, A., Cirillo, G., Capristo, C., Marzuillo, P., Di Sessa, A., Umamo, G.R., Ruggiero, L. and Perrone, L., (2015). Bioavailable Vitamin D in Obese Children: The Role of Insulin Resistance. *The Journal of clinical endocrinology and metabolism*, **100**(10), pp.3949–3955.
- Mittal, M., Siddiqui, M.R., Tran, K., Reddy, S.P. and Malik, A.B., (2014). Reactive oxygen species in inflammation and tissue injury. *Antioxidants & redox signaling*, **20**(7), pp.1126–1167.
- Mitterberger, M.C., Mattesich, M. and Zwerschke, W., (2014). Bariatric surgery and diet-induced long-term caloric restriction protect subcutaneous adipose-derived stromal/progenitor cells and prolong their life span in formerly obese humans. *Experimental gerontology*, **56**, pp.106–113.
- Miyake, H., Hara, I., Kamidono, S. and Eto, H., (2004). Oxidative Dna Damage in Patients With Prostate Cancer and Its Response To Treatment. *The Journal of Urology*, **171**(4), pp.1533–1536. Available from: <<http://linkinghub.elsevier.com/retrieve/pii/S0022534705623361>>.
- Mizoue, T., Tokunaga, S., Kasai, H., Kawai, K., Sato, M. and Kubo, T., (2007). Body mass index and oxidative DNA damage: A longitudinal study. *Cancer*

- Science*, **98**(8), pp.1254–1258. Available from:
<<http://dx.doi.org/10.1111/j.1349-7006.2007.00511.x>>.
- Mohn, A., Catino, M., Capanna, R., Giannini, C., Marcovecchio, M. and Chiarelli, F., (2005). Increased oxidative stress in prepubertal severely obese children: effect of a dietary restriction-weight loss program. *The Journal of Clinical Endocrinology & Metabolism*, **90**(5), pp.2653–2658.
- Monasta, L., Batty, G.D., Cattaneo, A., Lutje, V., Ronfani, L., Van Lenthe, F.J. and Brug, J., (2010). Early-life determinants of overweight and obesity: a review of systematic reviews. *Obesity reviews : an official journal of the International Association for the Study of Obesity*, **11**(10), pp.695–708.
- Montpetit, A.J., Alhareeri, A.A., Montpetit, M., Starkweather, A.R., Elmore, L.W., Filler, K., Mohanraj, L., Burton, C.W., Menzies, V.S. and Lyon, D.E., (2014). Telomere length: a review of methods for measurement. *Nursing research*, **63**(4), p.289.
- Mook-Kanamori, D.O., Holzhauer, S., Hollestein, L.M., Durmus, B., Manniesing, R., Koek, M., Boehm, G., van der Beek, E.M., Hofman, A., Witteman, J.C.M., Lequin, M.H. and Jaddoe, V.W. V., (2009). Abdominal fat in children measured by ultrasound and computed tomography. *Ultrasound in medicine & biology*, **35**(12), pp.1938–1946.
- Moon, D.-O., Kim, M.-O., Choi, Y.H., Hyun, J.W., Chang, W.Y. and Kim, G.-Y., (2010). Butein induces G2/M phase arrest and apoptosis in human hepatoma cancer cells through ROS generation. *Cancer letters*, **288**(2), pp.204–213.
- Moore, L.M., Fals, A.M., Jennelle, P.J., Green, J.F., Pepe, J. and Richard, T., (2015). Analysis of Pediatric Waist to Hip Ratio Relationship to Metabolic Syndrome Markers. *Journal of Pediatric Health Care*, **29**(4), pp.319–324. Available from: <<https://doi.org/10.1016/j.pedhc.2014.12.003>>.
- Moreno-Navarrete, J.M., Ortega, F., Sabater, M., Ricart, W., Fernández-Real, J.M. and Fernandez-Real, J.M., (2010). Telomere length of subcutaneous adipose tissue cells is shorter in obese and formerly obese subjects. *International Journal of Obesity (London)*, **34**(8), pp.1345–1348.
- Morin, C., Bairati, I., Bouchard, C., Fortier, M., Roy, M., Moore, L. and Meisels, A., (2000). Cytologic predictors of cervical intraepithelial neoplasia in women with an ASCUS Pap smear. *Acta cytologica*, **44**(4), pp.576–586.
- Motulsky, H.J. and Brown, R.E., (2006). Detecting outliers when fitting data with nonlinear regression -- a new method based on robust nonlinear regression and the false discovery rate. *BMC Bioinformatics*, **7**(1), p.123. Available from: <<https://doi.org/10.1186/1471-2105-7-123>>.
- Muhlhausler, B.S. and Ailhaud, G.P., (2013). Omega-6 polyunsaturated fatty acids and the early origins of obesity. *Current Opinion in Endocrinology, Diabetes and Obesity*, **20**(1). Available from: <https://journals.lww.com/co-endocrinology/Fulltext/2013/02000/Omega_6_polyunsaturated_fatty_acids_and_the_early.12.aspx>.
- Mukherjee, S., Leong, H.F. and Wong, X.X., (2016). Waist circumference percentiles for Singaporean children and adolescents aged 6-17 years. *Obesity research & clinical practice*, **10 Suppl 1**, pp.S17–S25.
- Multhoff, G., Molls, M. and Radons, J., (2012). Chronic inflammation in cancer development. *Frontiers in immunology*, **2**, p.98. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/22566887>>.
- Murnane, J.P., (2006). Telomeres and chromosome instability. *DNA Repair*, **5**(9), pp.1082–1092. Available from: <<http://www.sciencedirect.com/science/article/pii/S1568786406001704>>.

- Murugaiyan, S.B., Ramasamy, R., Nakkeeran, M., Rangdhol, V., Srinivasan, A.R. and Niranjana, G., (2015). Urinary 8-hydroxydeoxyguanosine as a marker of oxidative stress induced genetic toxicity in oral cancer patients. *Indian Journal of Dental Research*, **26**(3), p.226.
- Must, A., Phillips, S.M. and Naumova, E.N., (2012). Occurrence and timing of childhood overweight and mortality: findings from the Third Harvard Growth Study. *The Journal of pediatrics*, **160**(5), pp.743–750.
- Naidoo, T., Konkol, K., Biccand, B., Dudose, K. and McKune, A.J., (2012). Elevated salivary C-reactive protein predicted by low cardio-respiratory fitness and being overweight in African children. *Cardiovascular journal of Africa*, **23**(9), pp.501–506. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/23108518>>.
- Nair-Shalliker, V., Armstrong, B.K. and Fenech, M., (2012). Does vitamin D protect against DNA damage? *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **733**(1), pp.50–57. Available from: <<http://www.sciencedirect.com/science/article/pii/S0027510712000450>>.
- Narang, I. and Mathew, J.L., (2012). Childhood obesity and obstructive sleep apnea. *Journal of nutrition and metabolism*, **2012**.
- National Child Measurement Programme, (2018). National Statistics. Available from: <<https://digital.nhs.uk/data-and-information/publications/statistical/national-child-measurement-programme/2017-18-school-year>>.
- NCMP, (2018). *No Title*, Available from: <<https://app.box.com/s/og3q86aqejc99okxe9xyvpfvo21xai21/file/311350142247>>.
- Negrini, S., Gorgoulis, V.G. and Halazonetis, T.D., (2010). Genomic instability — an evolving hallmark of cancer. *Nature Reviews Molecular Cell Biology*, **11**, p.220. Available from: <<https://doi.org/10.1038/nrm2858>>.
- Nersesyan, A., Muradyan, R., Kundi, M. and Knasmueller, S., (2010). Impact of smoking on the frequencies of micronuclei and other nuclear abnormalities in exfoliated oral cells: a comparative study with different cigarette types. *Mutagenesis*, **26**(2), pp.295–301. Available from: <<https://doi.org/10.1093/mutage/geq092>>.
- Nersesyan, A.K. and Adamyan, R.T., (2004). Micronuclei level in exfoliated buccal mucosa cells of patients with benign and malignant tumors of female reproductive organs and breasts. *CYTOLOGY AND GENETICS C/C OF TSITOLOGIIA I GENETIKA*, **38**(3), p.69.
- Ng, H.Y., (2016). Acanthosis nigricans in obese adolescents: prevalence, impact, and management challenges. *Adolescent health, medicine and therapeutics*, **8**, pp.1–10. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/28031729>>.
- NHS, (2011). *Start Active, Stay Active: A report on physical activity for health from the four home countries*, Available from: <https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/216370/dh_128210.pdf>.
- Nishide, R., Ando, M., Funabashi, H., Yoda, Y., Nakano, M. and Shima, M., (2015). Association of serum hs-CRP and lipids with obesity in school children in a 12-month follow-up study in Japan. *Environmental health and preventive medicine*, **20**(2), pp.116–122. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/25511645>>.
- Nishimoto, S., Fukuda, D., Higashikuni, Y., Tanaka, K., Hirata, Y., Murata, C., Kim-Kaneyama, J., Sato, F., Bando, M. and Yagi, S., (2016). Obesity-

- induced DNA released from adipocytes stimulates chronic adipose tissue inflammation and insulin resistance. *Science advances*, **2**(3), p.e1501332.
- Nishimura, K., Watanabe, S., Hayashida, R., Sugishima, S., Iwasaka, T. and Kaku, T., (2016). Binucleated HeLa cells are formed by cytokinesis failure in starvation and keep the potential of proliferation. *Cytotechnology*, **68**(4), pp.1123–1130. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/25894790>>.
- Njajou, O.T., Cawthon, R.M., Blackburn, E.H., Harris, T.B., Li, R., Sanders, J.L., Newman, A.B., Nalls, M., Cummings, S.R. and Hsueh, W.-C., (2012). Shorter telomeres are associated with obesity and weight gain in the elderly. *Int J Obes*, **36**(9), pp.1176–1179. Available from: <<http://dx.doi.org/10.1038/ijo.2011.196>>.
- Noda, N., Matsuzoe, D., Konno, T., Kawahara, K., Yamashita, Y. and Shirakusa, T., (2001). K-ras gene mutations in non-small cell lung cancer in Japanese. *Oncology reports*, **8**(4), pp.889–892.
- Nordfjäll, K., Eliasson, M., Stegmayr, B., Melander, O., Nilsson, P. and Roos, G., (2008). Telomere Length Is Associated With Obesity Parameters but With a Gender Difference. *Obesity*, **16**(12), pp.2682–2689. Available from: <<http://dx.doi.org/10.1038/oby.2008.413>>.
- Normand, G. and King, R.W., (2010). Understanding cytokinesis failure. *Advances in experimental medicine and biology*, **676**, pp.27–55. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/20687468>>.
- Nwosu, B.U., Maranda, L. and Candela, N., (2017). Vitamin D status in pediatric irritable bowel syndrome. *PLoS one*, **12**(2), p.e0172183.
- O'Brien, K.M., Sandler, D.P., Taylor, J.A. and Weinberg, C.R., (2017). Serum Vitamin D and Risk of Breast Cancer within Five Years. *Environmental health perspectives*, **125**(7), p.77004.
- O'Callaghan, N., Bull, C., Palmer, L., Lyons, G., Graham, R. and Fenech, M., (2008). Buccal cells: a non-invasive measurement of selenium, zinc and magnesium status, and telomere length. *Asia Pacific Journal of Clinical Nutrition*, **17**(Suppl 3), p.S129. Available from: <https://assets.publishing.service.gov.uk/media/57a08bcd40f0b652dd000ec6/Vol17_S3-0139.pdf>.
- O'Callaghan, N.J., Clifton, P.M., Noakes, M. and Fenech, M., (2009). Weight loss in obese men is associated with increased telomere length and decreased abasic sites in rectal mucosa. *Rejuvenation research*, **12**(3), pp.169–176.
- O'Callaghan, N.J. and Fenech, M., (2011). A quantitative PCR method for measuring absolute telomere length. *Biological procedures online*, **13**, p.3.
- O'sullivan, R.J. and Karlseder, J., (2010). Telomeres: protecting chromosomes against genome instability. *Nature reviews Molecular cell biology*, **11**(3), p.171.
- Olive, P.L. and Banath, J.P., (2006). The comet assay: a method to measure DNA damage in individual cells. *Nat. Protocols*, **1**(1), pp.23–29. Available from: <<http://dx.doi.org/10.1038/nprot.2006.5>>.
- Olovnikov, A.M., (1973). A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. *Journal of theoretical biology*, **41**(1), pp.181–190.
- Olusi, S.O., (2002). Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotective enzymes in humans. *International Journal of Obesity & Related Metabolic Disorders*, **26**(9).

- Ostling, O. and Johanson, K.J., (1984). Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. *Biochemical and biophysical research communications*, **123**(1), pp.291–298.
- Ouchi, N., Kihara, S., Arita, Y., Maeda, K., Kuriyama, H., Okamoto, Y., Hotta, K., Nishida, M., Takahashi, M. and Nakamura, T., (1999). Novel modulator for endothelial adhesion molecules adipocyte-derived plasma protein adiponectin. *Circulation*, **100**(25), pp.2473–2476.
- Ouellet-Morin, I., Danese, A., Williams, B. and Arseneault, L., (2011). Validation of a high-sensitivity assay for C-reactive protein in human saliva. *Brain, Behavior, and Immunity*, **25**(4), pp.640–646. Available from: <<http://www.sciencedirect.com/science/article/pii/S0889159111000055>>.
- Ozuysal, Y. and Baccus, S.A., (2012). The computational structure of variance adaptation. *Neuron*, **103**(10), pp.1332–1338.
- Palla, V.-V., Karaolanis, G., Katafigiotis, I., Anastasiou, I., Patapis, P., Dimitroulis, D. and Perrea, D., (2017). gamma-H2AX: Can it be established as a classical cancer prognostic factor? *Tumor Biology*, **39**(3), p.1010428317695931. Available from: <<https://doi.org/10.1177/1010428317695931>>.
- Pampalona, J., Frías, C., Genescà, A. and Tusell, L., (2012). Progressive telomere dysfunction causes cytokinesis failure and leads to the accumulation of polyploid cells. *PLoS genetics*, **8**(4), p.e1002679.
- Paradis, A.N., Gay, M.S. and Zhang, L., (2014). Binucleation of cardiomyocytes: the transition from a proliferative to a terminally differentiated state. *Drug discovery today*, **19**(5), pp.602–609. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/24184431>>.
- Pardini, B., Viberti, C., Naccarati, A., Allione, A., Oderda, M., Critelli, R., Preto, M., Zijno, A., Cucchiareale, G., Gontero, P., Vineis, P., Sacerdote, C. and Matullo, G., (2017). Increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of bladder cancer. *British Journal of Cancer*, **116**(2), pp.202–210. Available from: <<http://dx.doi.org/10.1038/bjc.2016.411>>.
- Park, M.H., Falconer, C., Viner, R.M. and Kinra, S., (2012). The impact of childhood obesity on morbidity and mortality in adulthood: a systematic review. *Obesity Reviews*, **13**(11), pp.985–1000.
- Parry, J.M., Parry, E.M., Boumer, R., Doherty, A., Ellard, S., O'Donovan, J., Hoebee, B., de Stoppelaar, J.M., Mohn, G.R., Önfelt, A., Renglin, A., Schultz, N., Soderpalm-Bemdes, C., Jensen, K.G., Kirsch-Volders, M., Elhajouji, A., Van Hummelen, P., Degrassi, F., Antoccia, A., et al., (1996). The detection and evaluation of aneugenic chemicals. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **353**(1), pp.11–46. Available from: <<http://www.sciencedirect.com/science/article/pii/0027510795002421>>.
- Pastucha, D. and Horak, V., (2014). *Experimental & Clinical Cardiology*, (October).
- Patnaik, L., Pattnaik, S., Rao, E.V. and Sahu, T., (2017). Validating Neck Circumference and Waist Circumference as Anthropometric Measures of Overweight/Obesity in Adolescents. *Indian pediatrics*, **54**(5), pp.377–380.
- Paz-Elizur, T., Ben-Yosef, R., Elinger, D., Vexler, A., Krupsky, M., Berrebi, A., Shani, A., Schechtman, E., Freedman, L. and Livneh, Z., (2006). Reduced repair of the oxidative 8-oxoguanine DNA damage and risk of head and neck cancer. *Cancer research*, **66**(24), pp.11683–11689.
- Paz, M.F.C.J., Sobral, A.L.P., Picada, J.N., Grivicich, I., Júnior, A.L.G., da Mata,

- A.M.O.F., de Alencar, M.V.O.B., de Carvalho, R.M., da Conceição Machado, K., Islam, M.T., de Carvalho Melo Cavalcante, A.A. and da Silva, J., (2018). Persistent Increased Frequency of Genomic Instability in Women Diagnosed with Breast Cancer: Before, during, and after Treatments. *Oxidative medicine and cellular longevity*, **2018**, p.2846819. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/30013718>>.
- Pecoraro, P., Guida, B., Caroli, M., Trio, R., Falconi, C., Principato, S. and Pietrobelli, A., (2003). Body mass index and skinfoldthickness versus bioimpedance analysis: fat mass prediction in children. *Acta Diabetologica*, **40**(1), pp.s278–s281. Available from: <<https://doi.org/10.1007/s00592-003-0086-y>>.
- Peehl, D.M., Shinghal, R., Nonn, L., Seto, E., Krishnan, A. V, Brooks, J.D. and Feldman, D., (2004). Molecular activity of 1, 25-dihydroxyvitamin D3 in primary cultures of human prostatic epithelial cells revealed by cDNA microarray analysis. *The Journal of steroid biochemistry and molecular biology*, **92**(3), pp.131–141.
- Pepys, M.B. and Hirschfield, G.M., (2003). C-reactive protein: a critical update. *The Journal of Clinical Investigation*, **111**(12), pp.1805–1812. Available from: <<https://doi.org/10.1172/JCI18921>>.
- Perry, D.C., Metcalfe, D., Lane, S. and Turner, S., (2018). Childhood Obesity and Slipped Capital Femoral Epiphysis. *Pediatrics*, **142**(5), p.e20181067.
- Pfeifer, G.P. and Hainaut, P., (2003). On the origin of G → T transversions in lung cancer. *Mutation research*, **526**(1–2), pp.39–43.
- PHE, (2019). Cancer registration statistics, England Statistical bulletins. Available from: <<https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/bulletins/cancerregistrationstatisticsengland/previousReleases>> [Accessed April 8, 2020].
- Phengchat, R., Takata, H., Morii, K., Inada, N., Murakoshi, H., Uchiyama, S. and Fukui, K., (2016). Calcium ions function as a booster of chromosome condensation. *Scientific Reports*, **6**, p.38281. Available from: <<https://doi.org/10.1038/srep38281>>.
- Pietrobelli, A., Faith, M.S., Allison, D.B., Gallagher, D., Chiumello, G. and Heymsfield, S.B., (1998). Body mass index as a measure of adiposity among children and adolescents: a validation study. *The Journal of pediatrics*, **132**(2), pp.204–210.
- Pihan, G.A., Purohit, A., Wallace, J., Malhotra, R., Liotta, L. and Doxsey, S.J., (2001). Centrosome defects can account for cellular and genetic changes that characterize prostate cancer progression. *Cancer research*, **61**(5), pp.2212–2219.
- Pinhal, D., Gontijo, Á.M. de M.C., Reyes, V.A.V. and Salvadori, D.M.F., (2006). Viable human buccal mucosa cells do not yield typical nucleoids: Impacts on the single-cell gel electrophoresis/comet assay. *Environmental and molecular mutagenesis*, **47**(2), pp.117–126.
- Pires, A., Martins, P., Pereira, A.M., Marinho, J., Silva, P.V., Marques, M., Castela, E., Sena, C. and Seça, R., (2014). Pro-inflammatory triggers in childhood obesity: correlation between leptin, adiponectin and high-sensitivity C-reactive protein in a group of obese Portuguese children. *Revista Portuguesa de Cardiologia*, **33**(11), pp.691–697.
- Pirgon, Ö. and Aslan, N., (2015). The Role of Urbanization in Childhood Obesity. , **7**(3), pp.163–167.
- Plesner, J.L., Dahl, M., Fonvig, C.E., Nielsen, T.R.H., Kloppenborg, J.T.,

- Pedersen, O., Hansen, T. and Holm, J.-C., (2018). Obesity is associated with vitamin D deficiency in Danish children and adolescents. *Journal of pediatric endocrinology & metabolism : JPEM*, **31**(1), pp.53–61.
- Podrimaj-Bytyqi, A., Borovečki, A., Selimi, Q., Manxhuka-Kerliu, S., Gashi, G. and Elezaj, I.R., (2018). The frequencies of micronuclei, nucleoplasmic bridges and nuclear buds as biomarkers of genomic instability in patients with urothelial cell carcinoma. *Scientific reports*, **8**(1), p.17873.
- Protano, C., Andreoli, R., Mutti, A., Petti, S. and Vitali, M., (2014). Biomarkers of oxidative stress to nucleic acids: Background levels and effects of body mass index and life-style factors in an urban paediatric population. *Science of The Total Environment*, **500–501**, pp.44–51. Available from: <<http://www.sciencedirect.com/science/article/pii/S0048969714012741>>.
- Pylvas, M., Puistola, U., Laatio, L., Kauppila, S. and Karihtala, P., (2011). Elevated Serum 8-OHdG Is Associated with Poor Prognosis in Epithelial Ovarian Cancer. *Anticancer Research*, **31**(4), pp.1411–1415. Available from: <<http://ar.iijournals.org/content/31/4/1411.abstract>>.
- Qu, S., Wen, W., Shu, X.-O., Chow, W.-H., Xiang, Y.-B., Wu, J., Ji, B.-T., Rothman, N., Yang, G., Cai, Q., Gao, Y.-T. and Zheng, W., (2013). Association of Leukocyte Telomere Length With Breast Cancer Risk: Nested Case-Control Findings From the Shanghai Women's Health Study. *American Journal of Epidemiology*, **177**(7), pp.617–624. Available from: <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3657533/>>.
- Quinque, D., Kittler, R., Kayser, M., Stoneking, M. and Nasidze, I., (2006). Evaluation of saliva as a source of human DNA for population and association studies. *Analytical Biochemistry*, **353**(2), pp.272–277. Available from: <<http://www.sciencedirect.com/science/article/pii/S0003269706001862>>.
- Rai, A., Naikmasur, V.G. and Sattur, A., (2016). Quantification of telomerase activity in normal oral mucosal tissue and oral squamous cell carcinoma. *Indian journal of medical and paediatric oncology : official journal of Indian Society of Medical & Paediatric Oncology*, **37**(3), pp.183–188. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/27688612>>.
- Rai, V., Abdo, J.O.E., Agrawal, S. and Agrawal, D.K., (2017). Vitamin D receptor polymorphism and cancer: an update. *Anticancer Research*, **37**(8), pp.3991–4003.
- Rajalingam, K., Schreck, R., Rapp, U.R. and Albert, Š., (2007). Ras oncogenes and their downstream targets. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, **1773**(8), pp.1177–1195. Available from: <<http://www.sciencedirect.com/science/article/pii/S0167488907000286>>.
- Ramachandra, N., Rodriguez, L. and Devaraj, S., (2015). Urinary Biomarkers of Oxidative Stress and Insulin Resistance in Childhood Obesity. *The FASEB Journal*, **29**(1 Supplement). Available from: <http://www.fasebj.org/content/29/1_Supplement/927.2.abstract>.
- Ramirez, A. and Saldanha, P.H., (2002). Micronucleus investigation of alcoholic patients with oral carcinomas. *Genet Mol Res*, **1**(3), pp.246–260.
- Ramirez, M.J., Surralles, J., Galofre, P., Creus, A. and Marcos, R., (1999). FISH analysis of 1cen-1q12 breakage, chromosome 1 numerical abnormalities and centromeric content of micronuclei in buccal cells from thyroid cancer and hyperthyroidism patients treated with radioactive iodine. *Mutagenesis*, **14**(1), pp.121–127.
- Ramon, O., Sauvaigo, S., Gasparutto, D., Faure, P., Favier, A. and Cadet, J., (1999). Effects of 8-oxo-7, 8-dihydro-2'-deoxyguanosine on the binding of

- the transcription factor Sp1 to its cognate target DNA sequence (GC box). *Free radical research*, **31**(3), pp.217–229.
- Ramos-Nino, M.E., (2013). The Role of Chronic Inflammation in Obesity-Associated Cancers B. Luber et al., eds. *ISRN Oncology*, **2013**, p.697521. Available from: <<https://doi.org/10.1155/2013/697521>>.
- Rapkin, A.J., Tsao, J.C.I., Turk, N., Anderson, M. and Zeltzer, L.K., (2006). Relationships among self-rated tanner staging, hormones, and psychosocial factors in healthy female adolescents. *Journal of pediatric and adolescent gynecology*, **19**(3), pp.181–187.
- Rausch, T., Jones, D.T.W., Zapatka, M., Stütz, A.M., Zichner, T., Weischenfeldt, J., Jäger, N., Remke, M., Shih, D. and Northcott, P.A., (2012). Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations. *Cell*, **148**(1–2), pp.59–71.
- RCPCH, (2020). About childhood obesity. Available from: <https://www.rcpch.ac.uk/key-topics/nutrition-obesity/about-childhood-obesity>. [Accessed 16.04.20].
- RCPCH, (2019). State of Child Health. Available from: <https://stateofchildhealth.rcpch.ac.uk/>. [Accessed 04.04.20].
- Rearson, J.T. and Sancar, A., (2001). Excision Repair. In S. Brenner & J. H. B. T.-E. of G. Miller, eds. New York: Academic Press, pp. 673–675. Available from: <<http://www.sciencedirect.com/science/article/pii/B0122270800004377>>.
- Reddig, A., Roggenbuck, D. and Reinhold, D., (2018). Comparison of different immunoassays for γ H2AX quantification. *Journal of Laboratory and Precision Medicine*, **3**.
- Redon, C.E., Nakamura, A.J., Martin, O.A., Parekh, P.R., Weyemi, U.S. and Bonner, W.M., (2011). Recent developments in the use of γ -H2AX as a quantitative DNA double-strand break biomarker. *Aging (Albany NY)*, **3**(2), pp.168–174. Available from: <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3082012/>>.
- Rehkopf, D.H., Needham, B.L., Lin, J., Blackburn, E.H., Zota, A.R., Wojcicki, J.M. and Epel, E.S., (2016). Leukocyte Telomere Length in Relation to 17 Biomarkers of Cardiovascular Disease Risk: A Cross-Sectional Study of US Adults. *PLOS Medicine*, **13**(11), p.e1002188. Available from: <<https://doi.org/10.1371/journal.pmed.1002188>>.
- Renehan, A.G., Tyson, M., Egger, M., Heller, R.F. and Zwahlen, M., (2008). Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *The Lancet*, **371**(9612), pp.569–578.
- Reuter, S., Gupta, S.C., Chaturvedi, M.M. and Aggarwal, B.B., (2010). Oxidative stress, inflammation, and cancer: how are they linked? *Free radical biology & medicine*, **49**(11), pp.1603–1616. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/20840865>>.
- Reyman, M., Verrijn Stuart, A.A., van Summeren, M., Rakhshandehroo, M., Nuboer, R., de Boer, F.K., van den Ham, H.J., Kalkhoven, E., Prakken, B. and Schipper, H.S., (2014). Vitamin D deficiency in childhood obesity is associated with high levels of circulating inflammatory mediators, and low insulin sensitivity. *International journal of obesity (2005)*, **38**(1), pp.46–52.
- Risques, R.A., Vaughan, T.L., Li, X., Odze, R.D., Blount, P.L., Ayub, K., Gallaher, J.L., Reid, B.J. and Rabinovitch, P.S., (2007). Leukocyte telomere length predicts cancer risk in Barrett's esophagus. *Cancer*

- Epidemiology Biomarkers & Prevention*, **16**(12), pp.2649–2655.
- Rode, A., Maass, K.K., Willmund, K.V., Lichter, P. and Ernst, A., (2016). Chromothripsis in cancer cells: an update. *International journal of cancer*, **138**(10), pp.2322–2333.
- Rodriguez-Rodriguez, E., Aparicio, A., Andres, P. and Ortega, R.M., (2014). Moderate vitamin D deficiency and inflammation related markers in overweight/obese schoolchildren. *International journal for vitamin and nutrition research. Internationale Zeitschrift fur Vitamin- und Ernährungsforschung. Journal international de vitaminologie et de nutrition*, **84**(1–2), pp.98–107.
- Romano, G., Sgambato, A., Mancini, R., Capelli, G., Giovagnoli, M.R., Flamini, G., Boninsegna, A., Vecchione, A. and Cittadini, A., (2000). 8-Hydroxy-2'-deoxyguanosine in cervical cells: correlation with grade of dysplasia and human papillomavirus infection. *Carcinogenesis*, **21**(6), pp.1143–1147. Available from: <<http://dx.doi.org/10.1093/carcin/21.5.143>>.
- Romualdo, M.C. dos S., de Nóbrega, F.J. and Escrivão, M.A.M.S., (2014). Insulin resistance in obese children and adolescents. *Jornal de Pediatria*, **90**(6), pp.600–607. Available from: <<http://www.sciencedirect.com/science/article/pii/S0021755714000977>>.
- Rönnemaa, T., Juonala, M., Marniemi, J., Raitakari, O.T., Taittonen, L. and Viikari, J.S.A., (2006). Childhood C-Reactive Protein in Predicting CRP and Carotid Intima-Media Thickness in Adulthood. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **26**(8), pp.1883–1888.
- Rosenbaum, M., Hirsch, J., Murphy, E. and Leibel, R.L., (2000). Effects of changes in body weight on carbohydrate metabolism, catecholamine excretion, and thyroid function. *The American journal of clinical nutrition*, **71**(6), pp.1421–1432.
- Rosenstreich, S.J., Rich, C. and Volwiler, W., (1971). Deposition in and release of vitamin D₃ from body fat: evidence for a storage site in the rat. *The Journal of clinical investigation*, **50**(3), pp.679–687.
- Rothkamm, K. and Löbrich, M., (2003). Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. *Proceedings of the National Academy of Sciences*, **100**(9), pp.5057–5062.
- Rowicka, G., Dyląg, H., Ambroszkiewicz, J., Riahi, A., Weker, H. and Chelchowska, M., (2017). Total Oxidant and Antioxidant Status in Prepubertal Children with Obesity. *Oxidative medicine and cellular longevity*, **2017**, p.5621989. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/28904738>>.
- Roy, D. and Liehr, J.G., (1999). Estrogen, DNA damage and mutations. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **424**(1), pp.107–115.
- Royal College Paediatrics and Child Health, (2016). Paediatric Diabetes Audit Report 2016-17. Available from: <https://www.rcpch.ac.uk/sites/default/files/2018-07/npda_annual_report_2016_-_2017_april_2018_final_updated_2.pdf>.
- Roycroft, G., (2015). *Food for thought: promoting healthy diets among children and young people*,
- Ryland, G.L., Doyle, M.A., Goode, D., Boyle, S.E., Choong, D.Y.H., Rowley, S.M., Li, J., Group, A.O.C.S., Bowtell, D.D.L., Tothill, R.W., Campbell, I.G. and Gorringer, K.L., (2015). Loss of heterozygosity: what is it good for? *BMC medical genomics*, **8**, p.45. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/26231170>>.

- Sagona, A.P. and Stenmark, H., (2010). Cytokinesis and cancer. *FEBS letters*, **584**(12), pp.2652–2661.
- Salomon-Perzyński, A., Salomon-Perzyńska, M., Michalski, B. and Skrzypulec-Plinta, V., (2017). High-grade serous ovarian cancer: the clone wars. *Archives of Gynecology and Obstetrics*, **295**(3), pp.569–576. Available from: <<https://doi.org/10.1007/s00404-017-4292-1>>.
- Samara, A., Ventura, E.E., Alfadda, A.A. and Goran, M.I., (2012). Use of MRI and CT for fat imaging in children and youth: what have we learned about obesity, fat distribution and metabolic disease risk? *Obesity reviews*, **13**(8), pp.723–732.
- Sanchez-Alarcon, J., Milić, M., Gomez-Arroyo, S., Montiel-Gonzalez, J.M.R. and Valencia-Quintana, R., (2016). Assessment of DNA damage by comet assay in buccal epithelial cells: problems, achievement, perspectives. In *Environmental Health Risk-Hazardous Factors to Living Species*. IntechOpen.
- Santos, R.A., Teixeira, A.C., Mayorano, M.B., Carrara, H.H.A., Andrade, J.M. and Takahashi, C.S., (2010). Basal levels of DNA damage detected by micronuclei and comet assays in untreated breast cancer patients and healthy women. *Clinical and Experimental Medicine*, **10**(2), pp.87–92.
- Saran, R., Tiwari, R.K., Reddy, P.P. and Ahuja, Y.R., (2008). Risk assessment of oral cancer in patients with pre-cancerous states of the oral cavity using micronucleus test and challenge assay. *Oral Oncology*, **44**(4), pp.354–360.
- Sato, N., Mizumoto, K., Nakamura, M., Nakamura, K., Kusumoto, M., Niiyama, H., Ogawa, T. and Tanaka, M., (1999). Centrosome abnormalities in pancreatic ductal carcinoma. *Clinical Cancer Research*, **5**(5), pp.963–970.
- Savva, S.C., Tornaritis, M., Savva, M.E., Kourides, Y., Panagi, A., Silikiotou, N., Georgiou, C. and Kafatos, A., (2000). Waist circumference and waist-to-height ratio are better predictors of cardiovascular disease risk factors in children than body mass index. *International journal of obesity*, **24**(11), p.1453.
- Scarpato, R., Verola, C., Fabiani, B., Bianchi, V., Saggese, G. and Federico, G., (2011). Nuclear damage in peripheral lymphocytes of obese and overweight Italian children as evaluated by the γ -H2AX focus assay and micronucleus test. *The FASEB Journal*, **25**(2), pp.685–693. Available from: <<http://www.fasebj.org/content/25/2/685.abstract>>.
- Schipper, H.S., Nuboer, R., Prop, S., Van Den Ham, H.J., De Boer, F.K., Kesmir, C., Mombers, I.M.H., van Bakkum, K.A., Woudstra, J. and Kieft, J.H., (2012). Systemic inflammation in childhood obesity: circulating inflammatory mediators and activated CD14⁺⁺ monocytes. *Diabetologia*, **55**(10), pp.2800–2810.
- Schroeder, J.C., Conway, K., Li, Y., Mistry, K., Bell, D.A. and Taylor, J.A., (2003). p53 mutations in bladder cancer: evidence for exogenous versus endogenous risk factors. *Cancer research*, **63**(21), pp.7530–7538.
- Schueler, M.G. and Sullivan, B.A., (2006). Structural and functional dynamics of human centromeric chromatin. *Annu. Rev. Genomics Hum. Genet.*, **7**, pp.301–313.
- Scientific Advisory Committee on Nutrition, (2011). *Dietary Reference Values for Energy*, London: The Stationery Office.
- Šebeková, K., Somoza, V., Jarčušková, M., Heidland, A. and Podracká, L., (2009). Plasma advanced glycation end products are decreased in obese children compared with lean controls. *International Journal of Pediatric Obesity*, **4**(2), pp.112–118.

- Setayesh, T., Mišik, M., Langie, S.A.S., Godschalk, R., Waldherr, M., Bauer, T., Leitner, S., Bichler, C., Prager, G. and Krupitza, G., (2019). Impact of Weight Loss Strategies on Obesity Induced DNA Damage. *Molecular nutrition & food research*, p.1900045.
- Sfar, S., Boussoffara, R., Sfar, M.T. and Kerkeni, A., (2013). Antioxidant enzymes activities in obese Tunisian children. *Nutrition Journal*, **12**, p.18.
- Shah, S., Wilson, D.M. and Bachrach, L.K., (2015). Large Doses of Vitamin D Fail to Increase 25-Hydroxyvitamin D Levels or to Alter Cardiovascular Risk Factors in Obese Adolescents: A Pilot Study. *The Journal of adolescent health : official publication of the Society for Adolescent Medicine*, **57**(1), pp.19–23.
- Shawki, S.M., Meshaal, S.S., El Dash, A.S., Zayed, N.A. and Hanna, M.O.F., (2014). Increased DNA damage in hepatitis C virus-related hepatocellular carcinoma. *DNA and cell biology*, **33**(12), pp.884–90. Available from: <<http://www.ncbi.nlm.nih.gov/pubmed/25211328>> [Accessed February 25, 2015].
- Shen, W., Chen, J., Gantz, M., Velasquez, G., Punyanitya, M. and Heymsfield, S.B., (2012). A single MRI slice does not accurately predict visceral and subcutaneous adipose tissue changes during weight loss. *Obesity (Silver Spring, Md.)*, **20**(12), pp.2458–2463.
- Shen, Z., (2011). Genomic instability and cancer: an introduction. *Journal of Molecular Cell Biology*, **3**(1), pp.1–3. Available from: <<https://doi.org/10.1093/jmcb/mjq057>>.
- Shi, Q. and King, R.W., (2005). Chromosome nondisjunction yields tetraploid rather than aneuploid cells in human cell lines. *Nature*, **437**(7061), p.1038.
- Shimada, M. and Nakanishi, M., (2006). DNA damage checkpoints and cancer. *Journal of molecular histology*, **37**(5–7), pp.253–260.
- Shimizu, N., (2011). Molecular mechanisms of the origin of micronuclei from extrachromosomal elements. *Mutagenesis*, **26**(1), pp.119–123.
- Shimizu, N., Kamezaki, F. and Shigematsu, S., (2005). Tracking of microinjected DNA in live cells reveals the intracellular behavior and elimination of extrachromosomal genetic material. *Nucleic acids research*, **33**(19), pp.6296–6307. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/16269822>>.
- Shook, R.P., Hand, G.A., Paluch, A.E., Drenowatz, C., Blair, S.N., Hebert, J.R., Blundell, J.E., Hill, J.O., Katzmarzyk, P.T. and Church, T.S., (2015). Low levels of physical activity are associated with dysregulation of energy intake and fat mass gain over 1 year. *The American Journal of Clinical Nutrition*, **102**(6), pp.1332–1338. Available from: <<https://dx.doi.org/10.3945/ajcn.115.115360>>.
- Di Silverio, F., Gentile, V., De Matteis, A., Mariotti, G., Giuseppe, V., Luigi, P.A. and Sciarra, A., (2003). Distribution of inflammation, pre-malignant lesions, incidental carcinoma in histologically confirmed benign prostatic hyperplasia: a retrospective analysis. *European urology*, **43**(2), pp.164–175.
- Simmonds, M., Llewellyn, A., Owen, C.G. and Woolacott, N., (2016). Predicting adult obesity from childhood obesity: A systematic review and meta-analysis. *Obesity Reviews*, **17**(2), pp.95–107.
- Simonetti, G., Bruno, S., Padella, A., Tenti, E. and Martinelli, G., (2019). Aneuploidy: Cancer strength or vulnerability? *International journal of cancer*, **144**(1), pp.8–25.
- Singer, K. and Lumeng, C.N., (2017). The initiation of metabolic inflammation in

- childhood obesity. *The Journal of Clinical Investigation*, **127**(1), pp.65–73. Available from: <<https://doi.org/10.1172/JCI88882>>.
- Skinner, H.G., Gangnon, R.E., Litzelman, K., Johnson, R.A., Chari, S.T., Petersen, G.M. and Boardman, L.A., (2012). Telomere length and pancreatic cancer: a case-control study. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*, **21**(11), pp.2095–2100. Available from: <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3493789/>>.
- Skowronski, R.J., Peehl, D.M. and Feldman, D., (1993). Vitamin D and prostate cancer: 1, 25 dihydroxyvitamin D₃ receptors and actions in human prostate cancer cell lines. *Endocrinology*, **132**(5), pp.1952–1960.
- Smit, V.T., Boot, A.J.M., Smits, A.M.M., Fleuren, G.J., Cornelisse, C.J. and Bos, J.L., (1988). KRAS codon 12 mutations occur very frequently in pancreatic adenocarcinomas. *Nucleic acids research*, **16**(16), pp.7773–7782.
- Soto, M., García-Santisteban, I., Krenning, L., Medema, R.H. and Raaijmakers, J.A., (2018). Chromosomes trapped in micronuclei are liable to segregation errors. *Journal of Cell Science*, **131**(13), p.jcs214742. Available from: <<http://jcs.biologists.org/content/131/13/jcs214742.abstract>>.
- Srivastava, K.C., Austin, R.D. and Shrivastava, D., (2016). Evaluation of oxidant-antioxidant status in tissue samples in oral cancer: A case control study. *Dental Research Journal*, **13**(2), pp.181–187. Available from: <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4810917/>>.
- Steer, S.E., Williams, F.M.K., Kato, B., Gardner, J.P., Norman, P.J., Hall, M.A., Kimura, M., Vaughan, R., Aviv, A. and Spector, T.D., (2007). Reduced telomere length in rheumatoid arthritis is independent of disease activity and duration. *Annals of the Rheumatic Diseases*, **66**(4), pp.476–480. Available from: <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1856061/>>.
- Stewart, S.A. and Weinberg, R.A., (2006). Telomeres: cancer to human aging. *Annu. Rev. Cell Dev. Biol.*, **22**, pp.531–557.
- Strauss, R.S., (2000). Childhood obesity and self-esteem. *Pediatrics*, **105**(1), pp.e15–e15.
- Summerbell, C., Ashton, V., Campbell, K., Edmunds, L., Kelly, S. and Waters, E., (2010). Interventions for treating obesity in children (a review)1. *Nederlands Tijdschrift voor Evidence Based Practice*, **5**(2), pp.45–46.
- Sun, S., Li, X., Ren, A., Du, M., Du, H., Shu, Y., Zhu, L. and Wang, W., (2016). Choline and betaine consumption lowers cancer risk: a meta-analysis of epidemiologic studies. *Scientific reports*, **6**, p.35547.
- Sung, P. and Klein, H., (2006). Mechanism of homologous recombination: mediators and helicases take on regulatory functions. *Nature reviews Molecular cell biology*, **7**(10), p.739.
- Sutaria, S., Devakumar, D., Yasuda, S.S., Das, S. and Saxena, S., (2019). Is obesity associated with depression in children? Systematic review and meta-analysis. *Archives of Disease in Childhood*, **104**(1), pp.64 LP – 74. Available from: <<http://adc.bmj.com/content/104/1/64.abstract>>.
- Suzuki, T., Fujii, M. and Ayusawa, D., (2002). Demethylation of classical satellite 2 and 3 DNA with chromosomal instability in senescent human fibroblasts. *Experimental gerontology*, **37**(8–9), pp.1005–1014.
- Suzuki, T. and Kamiya, H., (2017). Mutations induced by 8-hydroxyguanine (8-oxo-7, 8-dihydroguanine), a representative oxidized base, in mammalian cells. *Genes and Environment*, **39**(1), p.2.
- Takeshita, M. and Eisenberg, W., (1994). Mechanism of mutation on DNA

- templates containing synthetic abasic sites: study with a double strand vector. *Nucleic acids research*, **22**(10), pp.1897–1902.
- Talma, H., Chinapaw, M.J.M., Bakker, B., HiraSing, R.A., Terwee, C.B. and Altenburg, T.M., (2013). Bioelectrical impedance analysis to estimate body composition in children and adolescents: a systematic review and evidence appraisal of validity, responsiveness, reliability and measurement error. *Obesity reviews : an official journal of the International Association for the Study of Obesity*, **14**(11), pp.895–905.
- Tamae, K., Kawai, K., Yamasaki, S., Kawanami, K., Ikeda, M., Takahashi, K., Miyamoto, T., Kato, N. and Kasai, H., (2009). Effect of age, smoking and other lifestyle factors on urinary 7-methylguanine and 8-hydroxydeoxyguanosine. *Cancer science*, **100**(4), pp.715–721.
- Tamir, S., Burney, S. and Tannenbaum, S.R., (1996). DNA damage by nitric oxide. *Chemical research in toxicology*, **9**(5), pp.821–7. Available from: <<http://www.ncbi.nlm.nih.gov/pubmed/8828916>>.
- Tamura, K., (2015). Development of cell-cycle checkpoint therapy for solid tumors. *Japanese journal of clinical oncology*, **45**(12), pp.1097–1102.
- Tanaka, K. and Hirota, T., (2016). Chromosomal instability: A common feature and a therapeutic target of cancer. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, **1866**(1), pp.64–75. Available from: <<http://www.sciencedirect.com/science/article/pii/S0304419X16300427>>.
- Tang, W., Robles, A.I., Beyer, R.P., Gray, L.T., Nguyen, G.H., Oshima, J., Maizels, N., Harris, C.C. and Monnat, R.J.J., (2016). The Werner syndrome RECQ helicase targets G4 DNA in human cells to modulate transcription. *Human molecular genetics*, **25**(10), pp.2060–2069.
- Taylor, M., Couto-Silva, A.-C., Adan, L., Trivin, C., Sainte-Rose, C., Zerah, M., Valteau-Couanet, D., Doz, F., Chalumeau, M. and Brauner, R., (2012). Hypothalamic-Pituitary Lesions in Pediatric Patients: Endocrine Symptoms Often Precede Neuro-Ophthalmic Presenting Symptoms. *The Journal of Pediatrics*, **161**(5), pp.855-863.e3. Available from: <<http://www.sciencedirect.com/science/article/pii/S0022347612005318>>.
- Tezal, M., Grossi, S.G. and Genco, R.J., (2005). Is Periodontitis Associated With Oral Neoplasms? *Journal of Periodontology*, **76**(3), pp.406–410. Available from: <<http://dx.doi.org/10.1902/jop.2005.76.3.406>>.
- Thirthagiri, E., Robinson, C.M., Huntley, S., Davies, M., Yap, L.F., Prime, S.S. and Paterson, I.C., (2007). Spindle assembly checkpoint and centrosome abnormalities in oral cancer. *Cancer letters*, **258**(2), pp.276–285.
- Thomas, P., Holland, N., Bolognesi, C., Kirsch-Volders, M., Bonassi, S., Zeiger, E., Knasmueller, S. and Fenech, M., (2009). Buccal micronucleus cytome assay. *Nature protocols*, **4**(6), pp.825–837.
- Thomas, P., Fenech, M., Kirsch-Volders, M., Natarajan, A.T., Surralles, J., Crott, J.W., Parry, J., Norppa, H., Eastmond, D.A. and Tucker, J.D., (2011). Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. *Mutagenesis*, **26**(1), pp.125–132. Available from: <<https://doi.org/10.1093/mutage/geq052>>.
- Thomas, P., Harvey, S., Gruner, T. and Fenech, M., (2008). The buccal cytome and micronucleus frequency is substantially altered in Down's syndrome and normal ageing compared to young healthy controls. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **638**(1–2), pp.37–47.
- Thomas, P. and Fenech, M., (2011). Cytokinesis-block micronucleus cytome assay in lymphocytes. In *DNA Damage Detection In Situ, Ex Vivo, and In*

- Vivo. Springer, pp. 217–234.
- Thomas, X., Chelghoum, Y., Fanari, N. and Cannas, G., (2011). Serum 25-hydroxyvitamin D levels are associated with prognosis in hematological malignancies. *Hematology (Amsterdam, Netherlands)*, **16**(5), pp.278–283.
- Tokita, M., Kennedy, S.R., Risques, R.A., Chun, S.G., Pritchard, C., Oshima, J., Liu, Y., Bryant-Greenwood, P.K., Welcsh, P. and Monnat, R.J., (2016). Werner syndrome through the lens of tissue and tumour genomics. *Scientific Reports*, **6**, p.32038. Available from: <<http://dx.doi.org/10.1038/srep32038>>.
- Tomasello, B., Malfa, G., Galvano, F. and Renis, M., (2011). DNA damage in normal-weight obese syndrome measured by Comet assay. *Mediterranean Journal of Nutrition and Metabolism*, **4**(2), pp.99–104. Available from: <<http://dx.doi.org/10.1007/s12349-010-0035-6>>.
- Trapani, V., Wolf, F.I. and Scaldaferri, F., (2015). Dietary magnesium: The magic mineral that protects from colon cancer? *Magnesium research*, **28**(3), pp.108–111.
- Tu, W.Z., Li, B., Huang, B., Wang, Y., Liu, X.D., Guan, H., Zhang, S.M., Tang, Y., Rang, W.Q. and Zhou, P.K., (2013). h2AX foci formation in the absence of DNA damage: Mitotic H2AX phosphorylation is mediated by the DNA-PKcs/CHK2 pathway. *FEBS Letters*, **587**(21), pp.3437–3443. Available from: <<http://dx.doi.org/10.1016/j.febslet.2013.08.028>>.
- Tyson, N. and Frank, M., (2018). Childhood and adolescent obesity definitions as related to BMI, evaluation and management options. *Best Practice & Research Clinical Obstetrics & Gynaecology*, **48**, pp.158–164. Available from: <<http://www.sciencedirect.com/science/article/pii/S1521693417301207>>.
- Umegaki, K. and Fenech, M., (2000). Cytokinesis-block micronucleus assay in WIL2-NS cells: a sensitive system to detect chromosomal damage induced by reactive oxygen species and activated human neutrophils. *Mutagenesis*, **15**(3), pp.261–269. Available from: <<http://mutage.oxfordjournals.org/content/15/3/261.abstract>>.
- Usman, M. and Volpi, E.V., (2018). DNA damage in obesity: Initiator, promoter and predictor of cancer. *Mutation Research - Reviews in Mutation Research*, **778**.
- VAGUE, J., (1956). The Degree of Masculine Differentiation of Obesities: A FACTOR DETERMINING PREDISPOSITION TO DIABETES, ATHEROSCLEROSIS, GOUT, AND URIC CALCULOUS DISEASE. *The American Journal of Clinical Nutrition*, **4**(1), pp.20–34. Available from: <<https://doi.org/10.1093/ajcn/4.1.20>>.
- Vaisse, C., Lahlou, N., Cabrol, S., Pelloux, V., Cassuto, D., Goumelen, M., Dina, C., Chambaz, J., Lebouc, Y., Froguel, P. and Guy-grand, B., (1998). A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature*, **392**(March), pp.415–418.
- Valavanidis, A., Vlachogianni, T. and Fiotakis, C., (2009). 8-hydroxy-2'-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. *Journal of environmental science and health Part C*, **27**(2), pp.120–139.
- Valdes, A.M., Andrew, T., Gardner, J.P., Kimura, M., Oelsner, E., Cherkas, L.F., Aviv, A. and Spector, T.D., (2005). Obesity, cigarette smoking, and telomere length in women. *Lancet (London, England)*, **366**(9486), pp.662–664.
- Vanderwall, C., Randall Clark, R., Eickhoff, J. and Carrel, A.L., (2017). BMI is a

- poor predictor of adiposity in young overweight and obese children. *BMC pediatrics*, **17**(1), p.135. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/28577356>>.
- Vanlint, S., (2013). Vitamin D and obesity. *Nutrients*, **5**(3), pp.949–956. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/23519290>>.
- Varga, D., Hoegel, J., Maier, C., Jainta, S., Hoehne, M., Patino-Garcia, B., Michel, I., Schwarz-Boeger, U., Kiechle, M., Kreienberg, R. and Vogel, W., (2006). On the difference of micronucleus frequencies in peripheral blood lymphocytes between breast cancer patients and controls. *Mutagenesis*, **21**(5), pp.313–320.
- Vaughan-Shaw, P.G., O'Sullivan, F., Farrington, S.M., Theodoratou, E., Campbell, H., Dunlop, M.G. and Zgaga, L., (2017). The impact of vitamin D pathway genetic variation and circulating 25-hydroxyvitamin D on cancer outcome: systematic review and meta-analysis. *British journal of cancer*, **116**(8), pp.1092–1110.
- Venner, A.A., Lyon, M.E. and Doyle-Baker, P.K., (2006). Leptin: a potential biomarker for childhood obesity? *Clinical biochemistry*, **39**(11), pp.1047–1056.
- Verdun, R.E. and Karlseder, J., (2007). Replication and protection of telomeres. *Nature*, **447**(7147), p.924.
- Vidigal, V.M., Silva, T.D., de Oliveira, J., Pimenta, C.A.M., Felipe, A.V. and Forones, N.M., (2017). Genetic polymorphisms of vitamin D receptor (VDR), CYP27B1 and CYP24A1 genes and the risk of colorectal cancer. *The International journal of biological markers*, **32**(2), pp.224–230.
- Vijayakumar, A., Yakar, S. and Leroith, D., (2011). The intricate role of growth hormone in metabolism. *Frontiers in endocrinology*, **2**, p.32. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/22654802>>.
- Vilenchik, M.M. and Knudson, A.G., (2003). Endogenous DNA double-strand breaks: Production, fidelity of repair, and induction of cancer. *Proceedings of the National Academy of Sciences of the United States of America*, **100**(22), pp.12871–12876. Available from: <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC240711/>>.
- Vimaleswaran, K.S., Berry, D.J., Lu, C., Tikkanen, E., Pilz, S., Hiraki, L.T., Cooper, J.D., Dastani, Z., Li, R. and Houston, D.K., (2013). Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. *PLoS Med*, **10**(2), p.e1001383.
- Vincent, H.K. and Taylor, A.G., (2006). Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. *International journal of obesity*, **30**(3), pp.400–418.
- Viner, R.M., Kinra, S., Nicholls, D., Cole, T., Kessel, A., Christie, D., White, B., Croker, H., Wong, I.C.K. and Saxena, S., (2018). Burden of child and adolescent obesity on health services in England. *Archives of Disease in Childhood*, **103**(3), pp.247–254.
- Viner, R.M., Segal, T.Y., Lichtarowicz-Krynska, E. and Hindmarsh, P., (2005). Prevalence of the insulin resistance syndrome in obesity. *Archives of Disease in Childhood*, **90**(1), pp.10 LP – 14. Available from: <<http://adc.bmj.com/content/90/1/10.abstract>>.
- Vivante, A., Golan, E., Tzur, D., Leiba, A., Tirosh, A., Skorecki, K. and Calderon-Margalit, R., (2012). Body mass index in 1.2 million adolescents and risk for end-stage renal disease. *Archives of internal medicine*, **172**(21), pp.1644–1650.
- Vo, P., Bair-Merritt, M. and Camargo, C.A., (2015). The potential role of vitamin

- D in the link between obesity and asthma severity/control in children. *Expert review of respiratory medicine*, **9**(3), pp.309–325.
- Vral, A., Fenech, M. and Thierens, H., (2011). The micronucleus assay as a biological dosimeter of in vivo ionising radiation exposure. *Mutagenesis*, **26**(1), pp.11–17.
- Wactawski-Wende, J., Kotchen, J.M., Anderson, G.L., Assaf, A.R., Brunner, R.L., O'sullivan, M.J., Margolis, K.L., Ockene, J.K., Phillips, L. and Pottern, L., (2006). Calcium plus vitamin D supplementation and the risk of colorectal cancer. *New England Journal of Medicine*, **354**(7), pp.684–696.
- Wakayo, T., Whiting, S.J. and Belachew, T., (2016). Vitamin D Deficiency is Associated with Overweight and/or Obesity among Schoolchildren in Central Ethiopia: A Cross-Sectional Study. *Nutrients*, **8**(4), p.190.
- El Wakkad, A., Giza, A.O., Elwakkad, A., Hassan, N.E., Sibaii, H. and el Zayat, S., (2011). Relationship Between Obesity and 8—hydroxy—2—deoxy Guanosine as an Oxidative Marker in Obese. *J. Med. Sci*, **11**(5), pp.231–235.
- Walter, P.B., Knutson, M.D., Paler-Martinez, A., Lee, S., Xu, Y., Viteri, F.E. and Ames, B.N., (2002). Iron deficiency and iron excess damage mitochondria and mitochondrial DNA in rats. *Proceedings of the national academy of sciences*, **99**(4), pp.2264–2269.
- Walther, A., Houlston, R. and Tomlinson, I., (2008). Association between chromosomal instability and prognosis in colorectal cancer: a meta-analysis. *Gut*. Available from: <<http://gut.bmj.com/content/early/2008/03/27/gut.2007.135004.abstract>>.
- Wang, B., Li, F., Zhou, X., Ma, Y. and Fu, W., (2019). Is microsatellite instability-high really a favorable prognostic factor for advanced colorectal cancer? A meta-analysis. *World Journal of Surgical Oncology*, **17**(1), p.169. Available from: <<https://doi.org/10.1186/s12957-019-1706-5>>.
- Wang, C.-C., Chen, W.-L., Lin, C.-M., Lai, C.-H., Loh, C.-H., Chen, H.-I. and Liou, S.-H., (2016). The relationship between plasma and urinary 8-hydroxy-2-deoxyguanosine biomarkers measured by liquid chromatography tandem mass spectrometry. *Environmental science and pollution research international*, **23**(17), pp.17496–17502.
- Wang, H., Chen, H., Gao, X., McGrath, M., Deer, D., De Vivo, I., Schwarzschild, M.A. and Ascherio, A., (2008). Telomere length and risk of Parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society*, **23**(2), pp.302–305. Available from: <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2387100/>>.
- Wang, Z., Rhee, D.B., Lu, J., Bohr, C.T., Zhou, F., Vallabhaneni, H., de Souza-Pinto, N.C. and Liu, Y., (2010). Characterization of oxidative guanine damage and repair in mammalian telomeres. *PLoS genetics*, **6**(5), p.e1000951.
- Weber, R.G., Bridger, J.M., Benner, A., Weisenberger, D., Ehemann, V., Reifemberger, G. and Lichter, P., (1998). Centrosome amplification as a possible mechanism for numerical chromosome aberrations in cerebral primitive neuroectodermal tumors with TP53 mutations. *Cytogenetic and Genome Research*, **83**(3–4), pp.266–269.
- Weihrauch-Blüher, S., Schwarz, P. and Klusmann, J.-H., (2019). Childhood obesity: increased risk for cardiometabolic disease and cancer in adulthood. *Metabolism*, **92**, pp.147–152. Available from: <<http://www.sciencedirect.com/science/article/pii/S0026049518302555>>.
- Weisberg, S.P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R.L. and

- Ferrante, A.W., (2003). Obesity is associated with macrophage accumulation in adipose tissue. *Journal of Clinical Investigation*, **112**(12), pp.1796–1808. Available from: <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC296995/>>.
- Wells, J.C.K. and Fewtrell, M.S., (2006). Measuring body composition. *Archives of Disease in Childhood*, **91**(7), pp.612 LP – 617. Available from: <<http://adc.bmj.com/content/91/7/612.abstract>>.
- Weststrate, J.A. and Deurenberg, P., (1989). Body composition in children: proposal for a method for calculating body fat percentage from total body density or skinfold-thickness measurements. *The American Journal of Clinical Nutrition*, **50**(5), pp.1104–1115. Available from: <<https://dx.doi.org/10.1093/ajcn/50.5.1104>>.
- WHEELER, J.M.D., BODMER, W.F., WHEELER, J.M.D. and MORTENSEN, N.J.M., (2000). DNA mismatch repair genes and colorectal cancer. *Gut*, **47**(1), pp.148 LP – 153. Available from: <<http://gut.bmj.com/content/47/1/148.abstract>>.
- WHO, (2018). *Childhood obesity*. Available from: <<https://www.who.int/dietphysicalactivity/childhood/en/e>>. [Accessed 04.04.20].
- Widhalm, K. and Helk, O., (2015). Bariatric Surgery in Adolescents: Practical Guidelines from a Pediatrician's Point of View. In *Morbid Obesity in Adolescents*. Springer, pp. 75–81.
- Williams, R., Novick, M. and Lehman, E., (2014). Prevalence of hypovitaminosis D and its association with comorbidities of childhood obesity. *The Permanente journal*, **18**(4), pp.32–39.
- Wong, G., Gupta, R., Dixon, K.M., Deo, S.S., Choong, S.M., Halliday, G.M., Bishop, J.E., Ishizuka, S., Norman, A.W., Posner, G.H. and Mason, R.S., (2004). 1,25-Dihydroxyvitamin D and three low-calcemic analogs decrease UV-induced DNA damage via the rapid response pathway. *The Journal of Steroid Biochemistry and Molecular Biology*, **89–90**, pp.567–570. Available from: <<http://www.sciencedirect.com/science/article/pii/S096007600400127X>>.
- Woodford, D., Johnson, S.D., De Costa, A.-M.A. and Young, M.R.I., (2014). An Inflammatory Cytokine Milieu is Prominent in Premalignant Oral Lesions, but Subsides when Lesions Progress to Squamous Cell Carcinoma. *Journal of clinical & cellular immunology*, **5**(3).
- van der Woude, C.J., Kleibeuker, J.H., Jansen, P.L.M. and Moshage, H., (2004). Chronic inflammation, apoptosis and (pre-)malignant lesions in the gastro-intestinal tract. *Apoptosis*, **9**(2), pp.123–130. Available from: <<https://doi.org/10.1023/B:APPT.0000018794.26438.22>>.
- Wright, C.M., Booth, I.W., Buckler, J.M.H., Cameron, N., Cole, T.J., Healy, M.J.R., Hulse, J.A., Preece, M.A., Reilly, J.J. and Williams, A.F., (2002). Growth reference charts for use in the United Kingdom. *Archives of Disease in Childhood*, **86**(1), pp.11 LP – 14. Available from: <<http://adc.bmj.com/content/86/1/11.abstract>>.
- Wu, D.-M., Shen, M.-H. and Chu, N.-F., (2001). Relationship between plasma leptin levels and lipid profiles among school children in Taiwan—the Taipei Children Heart Study. *European journal of epidemiology*, **17**(10), pp.911–916.
- Wu, L.L., Chiou, C.-C., Chang, P.-Y. and Wu, J.T., (2004). Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. *Clinica Chimica Acta*, **339**(1–2), pp.1–9.

Available from:

- <<http://www.sciencedirect.com/science/article/pii/S0009898103004534>>.
- Wu, Q. and Ni, X., (2015). ROS-mediated DNA methylation pattern alterations in carcinogenesis. *Current drug targets*, **16**(1), pp.13–19.
- Xi, H., Li, C., Ren, F., Zhang, H. and Zhang, L., (2013). Telomere, aging and age-related diseases. *Aging Clinical and Experimental Research*, **25**(2), pp.139–146. Available from: <<https://doi.org/10.1007/s40520-013-0021-1>>.
- Yamamoto, Y., Eguchi, S., Junpei, A., Nagao, K., Sakano, S., Furuya, T., Oga, A., Kawachi, S., Sasaki, K. and Matsuyama, H., (2009). Intercellular centrosome number is correlated with the copy number of chromosomes in bladder cancer. *Cancer genetics and cytogenetics*, **191**(1), pp.38–42.
- Yang, H., Huynh, Q.L., Venn, A.J., Dwyer, T. and Marwick, T.H., (2017). Associations of childhood and adult obesity with left ventricular structure and function. *International journal of obesity (2005)*, **41**(4), pp.560–568.
- Yang, Y., Fruehauf, J., Xiang, S. and Li, C.J., (2006). Genomic instability in precancerous lesions before inactivation of tumor suppressors p53 and APC in patients. *Cell Cycle*, **5**(13), pp.1443–1447.
- Yano, K., Morotomi-Yano, K., Adachi, N. and Akiyama, H., (2009). Molecular mechanism of protein assembly on DNA double-strand breaks in the non-homologous end-joining pathway. *Journal of radiation research*, **50**(2), pp.97–108.
- Yasumoto, S., Kunimura, C., Kikuchi, K., Tahara, H., Ohji, H., Yamamoto, H., Ide, T. and Utakoji, T., (1996). Telomerase activity in normal human epithelial cells. *Oncogene*, **13**(2), pp.433–439.
- Yildirim, I.H., Yesilada, E. and Yologlu, S., (2006). Micronucleus frequency in peripheral blood lymphocytes and exfoliated buccal cells of untreated cancer patients. *Russian Journal of Genetics*, **42**(5), pp.573–577. Available from: <<https://doi.org/10.1134/S1022795406050152>>.
- Yuan, C., Shui, I.M., Wilson, K.M., Stampfer, M.J., Mucci, L.A. and Giovannucci, E.L., (2019). Circulating 25-hydroxyvitamin D, vitamin D binding protein and risk of advanced and lethal prostate cancer. *International journal of cancer*, **144**(10), pp.2401–2407.
- Zakov, S., Kinsella, M. and Bafna, V., (2013). An algorithmic approach for breakage-fusion-bridge detection in tumor genomes. *Proceedings of the National Academy of Sciences*, **110**(14), pp.5546–5551.
- Zannolli, R., Mohn, A., Buoni, S., Pietrobelli, A., Messina, M., Chiarelli, F. and Miracco, C., (2008). Telomere length and obesity. *Acta Paediatrica*, **97**(7), pp.952–954.
- Zgheib, N.K., Sleiman, F., Nasreddine, L., Nasrallah, M., Nakhoul, N., Isma'eel, H. and Tamim, H., (2018). Short Telomere Length is Associated with Aging, Central Obesity, Poor Sleep and Hypertension in Lebanese Individuals. *Aging and disease*, **9**(1), pp.77–89. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/29392083>>.
- Zhang, C.-Z., Spektor, A., Cornils, H., Francis, J.M., Jackson, E.K., Liu, S., Meyerson, M. and Pellman, D., (2015). Chromothripsis from DNA damage in micronuclei. *Nature*, **522**(7555), pp.179–184. Available from: <<http://dx.doi.org/10.1038/nature14493>>.
- Zhang, L., Wang, S., Che, X. and Li, X., (2015). Vitamin D and lung cancer risk: a comprehensive review and meta-analysis. *Cellular physiology and biochemistry: international journal of experimental cellular physiology, biochemistry, and pharmacology*, **36**(1), pp.299–305.
- Zhang, Y., Leung, D.Y.M., Richers, B.N., Liu, Y., Remigio, L.K., Riches, D.W.

- and Goleva, E., (2012). Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. *The Journal of Immunology*, **188**(5), pp.2127–2135.
- Zhu, H, Guo, D., Li, K., Pedersen-White, J., Stallmann-Jorgensen, I.S., Huang, Y., Parikh, S., Liu, K. and Dong, Y., (2011). Increased telomerase activity and vitamin D supplementation in overweight African Americans. *International Journal Of Obesity*, **36**, p.805. Available from: <<https://doi.org/10.1038/ijo.2011.197>>.
- Zhu, Haidong, Wang, X., Gutin, B., Davis, C.L., Keeton, D., Thomas, J., Stallmann-Jorgensen, I., Mookken, G., Bundy, V., Snieder, H., van der Harst, P. and Dong, Y., (2011). Leukocyte Telomere Length in Healthy White and Black Adolescents: Relations to Race, Sex, Adiposity, Adipokines and Physical Activity. *The Journal of pediatrics*, **158**(2), pp.215–220. Available from: <<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3010324/>>.
- Zhu, X., Han, W., Xue, W., Zou, Y., Xie, C., Du, J. and Jin, G., (2016). The association between telomere length and cancer risk in population studies. *Scientific reports*, **6**, p.22243. Available from: <<http://www.ncbi.nlm.nih.gov/pubmed/26915412>>.
- Zillikens, M.C., Uitterlinden, A.G., van Leeuwen, J.P.T.M., Berends, A.L., Henneman, P., van Dijk, K.W., Oostra, B.A., van Duijn, C.M., Pols, H.A.P. and Rivadeneira, F., (2010). The role of body mass index, insulin, and adiponectin in the relation between fat distribution and bone mineral density. *Calcified tissue international*, **86**(2), pp.116–125.
- Zoidis, E., Seremelis, I., Kontopoulos, N. and Danezis, G.P., (2018). Selenium-Dependent Antioxidant Enzymes: Actions and Properties of Selenoproteins. *Antioxidants (Basel, Switzerland)*, **7**(5), p.66. Available from: <<https://www.ncbi.nlm.nih.gov/pubmed/29758013>>.

8. Appendix

I. Participant Information Booklet for Parents (Clinic Version)



Part 2 continued.



10. Who is organising and funding the research?

The University of Westminster is funding this research and it is organised in collaboration with St George's Hospital and King's College NHS Trust.

11. Where can I get more information about the research?

If you have any further questions please do not hesitate to contact a member of the research team by phone or email using the contact details below:

Local Collaborator – Dr Martha Ford-Adams, martha.ford-adams@nhs.net

Chief Investigator - Dr Emanuela Volpi, e.Volpi@Westminster.ac.uk /020 79115000 ext. 64156

Doctoral Researcher - Mrs Moonisah Usman, m.Bajwa@my.Westminster.ac.uk

12. What if there is a problem?

If you have a concern about any aspect of this study, you should speak to the clinical team who will do their best to answer your questions. If the issue is unresolved, you may wish to seek advice from the Patient Advice and Liaison Service (PALS). You can contact the hospital's PALS office using the details below:

PALS, King's College Hospital

Ground Floor, [Hambleton Wing Central](#) (Open: 09.00 - 16.30 Monday – Friday)

E-mail kch-tr.pals@nhs.net Tel 0203 299 3601



Information for Parents (Clinic Version)

Version 9. 28.06.17 IRAS ID: 212869

UNIVERSITY OF
RIGOUR
RESEARCH
RESULTS
WESTMINSTER

115 New Cavendish Street
London W1W 6UW
T: +44 (0)20 7911 5000
F: +44 (0)20 7911 5079
E: stregistry@westminster.ac.uk
westminster.ac.uk/ft

A charity and company limited by guarantee
Registered in London 077018 England
Registered office 115 New Cavendish Street London W1W 6UW

Part 1 – What is involved in the study?**1. Why is this research taking place?**

You and your child are being invited to take part in a research study that is looking at the effects of body fat on DNA health. DNA is the material inside our cells that codes for how our bodies function. We would like to find out how excess body fat can have an effect on DNA which may have a further detrimental health impact later in life. The aim of the research is to use saliva and urine samples to look at DNA and levels of vitamin D and a marker of inflammation called C-Reactive Protein.

2. What would my child have to do?

If you give your consent the study will involve your child:

1. Completing a **questionnaire** asking about their overall health.
2. Having their height, weight, body fat percentage, hip and waist circumference **measured** using a set of scales.
3. Collecting a urine sample, two saliva samples and a cheek swab. This is done firstly by drinking water to rinse your child's mouth and then chewing a cotton swab for one minute and spitting it into a tube. The cheek swab will be collected by rotating a small headed toothbrush in both of your child's inner cheeks. Your child will be given a plastic tube to spit into and another plastic container for them to collect the urine sample themselves in private.

This process will take around **10 minutes** to be completed and will only be conducted on **one occasion** when your child attends their **usual appointment** at the clinic. Prior to sample collection, we request your child not to consume any food or drink within the last 30 minutes and not to participate in vigorous physical activity over the last hour. Potential participants will be screened against inclusion/exclusion criteria.

3. Is the research safe and are there any benefits from taking part?

This research project has been checked and approved by an independent group of people at the NHS Research Ethics Committee. **There are no risks or benefits for your child taking part in this study.**

4. Do we have to take part? Your child's participation in this research is entirely **voluntary** and will not affect any current or future treatment they may receive under the NHS.

If you think you and your child might like to take part in this study, please continue reading to Part 2.

Part 2 – More information about your child's participation.**5. Who will know about my child's participation in the research?**

Samples will **only** be collected by your child's existing **clinical team** and they may notify your **child's GP** that they are taking part in the study. The clinical team will keep all identifiable information **anonymous** from the researchers by using a confidential coding frame. This means no individuals will be identifiable from any collated data, written report of the research, or any publications arising from it. Our procedures for handling, processing, storage and destruction of data are compliant with the Data Protection Act 1998. It is possible that authorised persons from the regulatory authorities that monitor the quality of research may require access to view your medical records for the purposes of audit or monitoring.

6. Can I withdraw my child from the study after giving consent? You can withdraw your child from the research at any time without giving a reason. Withdrawing your child from the research **will not** affect any current or future treatment they receive under the NHS. You also have the right to ask for your child's data to be withdrawn as long as this is practical, and for personal information to be destroyed. If you would like to withdraw your child, please contact a member of the research team by email or phone.

7. Does my child have to answer all questions in the study? Your child does **not** have to answer particular questions on questionnaires if he/she does not wish to do so.

8. What happens with my child's samples after the study has ended? All samples will be securely destroyed after the research has ended.

9. What will happen to the results of the research study? Once the study has come to an end we will present the results to other doctors and scientists by putting them on websites and journals that they read. We would also like to put a brief summary on the hospital website <insert link> so that you will be able to read about our results too. We expect this summary to be available by July 2019. We will not be giving information about individual results of the tests because they are not clinically meaningful at this stage of the research. All the results will be anonymous, which means that your child will not be able to be identified from them.

II. Participant Information Booklet for 10-18 year olds (Clinic Version)



Part 2 continued...



9. What will happen with the results of the study?

We will present our findings to other scientists and we will put the results in medical magazines and websites that doctors and scientists read. The results will also be included as part of the Chief Investigator's educational qualification. The information will be **anonymous**, which means that you will not be able to be identified from the materials we publish.

10. Who has checked the study?

Before any research goes ahead it has to be checked by a Research Ethics Committee (REC). This is a group of people who make sure that the research is safe. This study has been looked at and approved by the NHS REC.

11. Where can I find out more?

If you have any more questions about the research, you can speak to your doctors or you can contact the researchers using the details below:

Your local Doctor: Dr Martha Ford-Adams, martha.ford-adams@nhs.net

Chief Investigator: Dr Emanuela Volpi e.Volpi@Westminster.ac.uk
020 79115000 ext. 64156

Doctoral Researcher: Mrs Moonisah Usman
m.Bajwa@my.Westminster.ac.uk



Thank you for reading this booklet!

Information for 11-15 year olds (Clinic Version)

Version 9 21.02.17 IRAS ID 212869

UNIVERSITY OF
RIGOUR
RESEARCH
RESULTS
WESTMINSTER[®]

115 New Cavendish Street
London W1W 6JW
T: +44 (0)20 7911 5000
F: +44 (0)20 7911 5079
E: stregisty@westminster.ac.uk
westminster.ac.uk/fit

Activity and economy limited by companies
Registration number 079104 England
Registered office 115 New Cavendish Street W1W 6JW

Part 1 Information for young people**1. What is the research about?**

We would like your help with a research study so we can find out more about body fat and DNA. DNA is the material inside living things that carries all the instructions for how they function.

We would like to collect some urine and saliva samples to find out more about what DNA looks like, and how some proteins function including C-reactive protein (CRP) and levels of vitamin D. CRP is a special type of protein that helps your body to fight infections.

2. What will I need to do if I take part?

If you take part in this study, your usual doctor or a nurse from their team will take you through these 3 steps:

<p>1. Complete a Questionnaire about your overall health and have your height, weight, waist, hips and body fat % measured.</p>	<p>2. The doctor will brush the inside of your cheeks and ask you to chew a clean cotton swab for 1 minute before spitting into a tube.</p>	<p>3. Go to the toilet and collect some urine into a small container in private.</p>
---	---	--

3. Is there any pain involved?

You should **not** feel any discomfort or pain because you will **not** be asked to give any blood samples for this study. To collect a cheek swab, the doctor will only use a soft, small headed toothbrush to rotate on the inside of your cheeks. This might tickle but won't hurt!

**Part 2 – More details if you are interested to take part!****4. Do I have to take part?**

You do **not** have to take part in this study and your choice will not affect any care you receive at hospital. If you decide to take part, the samples will only be collected **once** at the clinic you attend during your usual appointment. After this, you will not be required to do anything else.

5. Do I need to prepare to give samples?

We will need you not to eat or drink anything for at least **30 minutes** and not to take part in any vigorous physical activity **one hour** before sample collection. Before you start filling the questionnaire the doctor will ask you to rinse your mouth with a cup of clean water.

6. How will I be measured and would I need to undress?

You **will not** need to undress to be measured. You will only be asked to remove your shoes and socks to step on a set of scales. A standard measuring tape will be used to measure your waist and hips.

**7. Who will know that I have taken part in this research?**

Your doctor will assign a code to all the information and samples they collected from you, this means nobody will be able to tell that you have taken part in this study as your name and contact details will be removed before information is sent out of the hospital. Only your GP will be sent a letter to inform them about your participation.

8. Do I have to answer all the questions and take part in everything the doctor will ask me?

You do not have to answer particular questions, even on questionnaires if you don't want to. You can take part in as much or as little as you wish!

IV Assent Form (Clinic Version)



King's College Hospital 
NHS Foundation Trust



Assent Form for 11-15 year olds

Research study title: Genome Instability in Childhood Obesity

Please tick as appropriate:

- | | | |
|--|---------------------------------|--------------------------------|
| I have been given the 'Participation Information Booklet for 11-15 year olds', and had its contents explained to me. | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| I have had an opportunity to ask any questions and I am happy with the answers given to me. | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| I understand that I can leave the study at any time I want to. | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| I understand that if I leave the research, any information about me will also be removed from the study unless it is anonymous and therefore not possible. | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| I understand the study will be researching using my urine and saliva | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| I would like to know about the overall results from the study and I understand I will not be given individual results. | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| I confirm I am willing to be a participant in the above research study. | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |

Child's Full Name : _____ **Date:** _____

To be completed by researcher:

Parent/Guardian's full name: _____

Date of consent: _____

Principal Investigator: Dr Martha Ford-Adams, IRAS ID: 212869 Assent form v4.0 21.02.17

V. Participant Information Booklet for Parents (School Version)



Information for Parents

What happens next?

If you are happy for your child to participate in the research study then please complete the attached consent form and hand it back to the school reception using the envelope provided. We will then ask your child for their permission using an 'assent form'. A trained member of the research team will measure your child and collect samples in a designated room at your school, ensuring all needs of privacy and confidentiality are met.

Thank you for reading through this information!

If you have any further questions please do not hesitate to contact a member of the research team:

Chief Investigator: Mrs Moonisah Usman
m.Bajwa@my.Westminster.ac.uk

Principal Investigator: Dr Emanuela Volpi
e.Volpi@Westminster.ac.uk / 020 79115000 ext. 64156

FACULTY OF
SCIENCE & TECHNOLOGY



Information for Parents (School Version)

Version 3. 27.09.16

UNIVERSITY OF
RIGOUR
RESEARCH
RESULTS
WESTMINSTER

115 New Cavendish Street
London W1W 6LW
T: +44 (0)20 7911 5000
F: +44 (0)20 7911 5079
E: registry@westminster.ac.uk
westminster.ac.uk/fsi

A charity and a company limited by guarantee
Registration number 917818 England
Registered office: 359 Regent Street London W1B 3JW



Information for Parents

Why is this research taking place?

Your child is being invited to take part in a research study that is looking at the effects of body fat on DNA health. DNA is the material inside our cells, that codes for how our bodies function. There is evidence from many studies that excess fat can be harmful for DNA. The aim of the research is to test for this using saliva and urine samples to look at DNA and levels of vitamin D and a marker of inflammation called CRP.

What does my child have to do?

Following your consent, the study will involve your child:

1. Completing a questionnaire asking about your child's overall health and getting measured. A copy of this medical questionnaire has been included in this pack for your reference. We will record your child's height, weight and body fat percentage using a set of scales.
1. Collecting a urine sample, two saliva samples and a cheek swab. This is done firstly by drinking water to rinse your child's mouth and then chewing a cotton swab for one minute and spitting it into a tube. The cheek swab will be collected by rotating a small headed toothbrush in both of your child's inner cheeks. Your child will be asked to collect the urine sample themselves.

Please note, this process will take around **10 minutes** to be completed and will only be conducted on **one occasion**. Prior to sample collection, we require your child not to consume any food or drink within the last 30 minutes and not to participate in vigorous physical activity over the last hour.

Is the research safe?

This research project has been checked and approved by the Research Ethics Committee at the University of Westminster ref VRE1415-1348. **There are no risks or benefits from taking part in this research project.**



Information for Parents

Who will know about my child's participation in the research?

Your child's participation in this research is entirely **voluntary** and will be kept **anonymous** by using a confidential coding frame that will be kept securely. Your child's responses will also be made **anonymous**. All computer data files will be encrypted and password protected. The researcher will keep files in a secure place and will comply with the requirements of the Data Protection Act. This means no individuals will be identifiable from any collated data, written report of the research, or any publications arising from it.

Can I withdraw my child from the study after giving consent?

You have the right to withdraw your child at any time without giving a reason. Withdrawing your child from the research will not affect any current or future services they receive at The Harris Academy. You also have the right to ask for your child's data to be withdrawn as long as this is practical, and for personal information to be destroyed.

Does my child have to answer all questions in the study?

Your child does not have to answer particular questions on questionnaires if he/she does not wish to do so.

What happens with my child's saliva and urine samples after the study has ended?

There is a possibility that any remaining or left over samples may be used in future studies at the University of Westminster, but the researcher will ensure that any link to the donor is broken before samples are retained.

Can I receive information about the results of the study?

If you wish, you can receive information on the results of the research. Please indicate on the consent form if you would like to receive this information. However, this information will only be communicated in terms of trends or overall findings, and please take note that any results of the research can **NOT** be deemed clinically significant at this stage.

VI. Participant Information Booklet for 10-18 year olds (School Version)



Information for Children

FACULTY OF
SCIENCE & TECHNOLOGY

If you have any more questions, speak to your school teacher or you can contact a member from the research team:

Chief Investigator: Mrs Moonisah Usman

m.Bajwa@my.Westminster.ac.uk

Principal Investigator: Dr Emanuela Volpi

e.Volpi@Westminster.ac.uk

020 79115000 ext. 64156



Genome Instability in Childhood Obesity

Information for 11-15 year olds

Version 3. 30.09.16

UNIVERSITY OF
RIGOUR
RESEARCH
RESULTS
WESTMINSTER#

115 New Cavendish Street
London W1W 6UW
T: +44 (0)20 7911 5000
F: +44 (0)20 7911 5079
E: stregisty@westminster.ac.uk
westminster.ac.uk/fst

A charity and a company limited by guarantee
Registration number 972618 England
Registered office: 309 Regent Street London W1B 3UW



Information for Children

What is the research about?

You are being invited to take part in a research study about the effects of body fat on DNA. DNA is the material that carries all the information about how living things work. We will be collecting some urine and saliva samples to look at DNA, and some proteins including C-reactive protein (CRP) and levels of vitamin D. CRP is a type of protein that helps your body to fight infections.

Before you decide if you would like to part, have a read through this leaflet carefully.

What will I need to do?

The study will involve you:

- Completing a questionnaire about your overall health.
- Having some measurements taken including your height, weight and body fat percentage using a set of scales. You will **only** be requested to remove your shoes and socks to do this.
- Providing a urine sample, two saliva samples and a cheek swab. This is done firstly by drinking water to rinse your mouth and then spitting into a tube. You will also be asked to chew a clean cotton swab for one minute. The cheek swab will be collected by rotating a soft, small headed toothbrush inside your cheeks. We will give you a plastic container so you can collect your own urine sample in a toilet.

We will need you not to eat or drink anything for at least 30 minutes before we take the samples. We will also need you not to take part in any vigorous physical activity one hour before sample collection.

Thank you for reading through this information!
Please continue if you are interested in taking part.



Information for Children

Do I have to take part?

You do **not** have to take part in this study and your choice will not affect any activities you currently take part in. If you **decide** to take part, the samples will only be collected **once** at the school you attend, in privacy. After this, you will not be required to do anything else. You may also quit participation at any point you would like to without giving anybody a reason.

Who will know that I have taken part in this research?

We will assign a code to all the information we collect from you, this means nobody will be able to tell that you have taken part in this study. The researcher will safely store the consent form signed by your parents separately to any other data we collect from you. We will only send out information that has your name and contact details removed.

Do I have to answer all the questions the researcher will ask me?

You do not have to answer particular questions, even on questionnaires if you don't want to.

What will happen with the results of the study?

We will present our findings to other scientists and we will put the results in medical magazines and websites that doctors and scientists read. The results will also be included as part of the chief investigator's educational qualification. The information will be **anonymous**, which means that you will not be able to be identified from the materials we publish.

Who has checked the study?

Before any research goes ahead it has to be checked by a Research Ethics Committee. This is a group of people who make sure that the research is safe. This study has been looked at by The University of Westminster, Faculty of Science and Technology research ethics committee.

VII. Parental Consent Form (Schools Version)

FACULTY OF
SCIENCE & TECHNOLOGY



Consent Form

Research Title: Genome Instability in Childhood Obesity

Please tick as appropriate:

I have been given the Participation Information Booklet for Parents and/or had its contents explained to me.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I have had an opportunity to ask any questions and I am satisfied with the answers given.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I understand I have a right to withdraw my child from the research at any time and I do not have to provide a reason.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I understand that if I withdraw my child from the research, any data included in the results will be removed if that is practicable (I understand that once anonymised data has been collated into other datasets it may not be possible to remove that data).	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I understand the study includes undertaking research on my child's donated human tissue (urine and saliva).	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I would like to receive information relating to the results from this study.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I wish to receive a copy of this Consent form.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I confirm I am willing for my child to be a participant in the above research study.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I note the data collected may be retained in an archive and I am happy for my data to be reused as part of future research activities, in fully anonymised form.	Yes <input type="checkbox"/>	No <input type="checkbox"/>

UNIVERSITY OF
RIGOUR
RESEARCH
RESULTS
WESTMINSTER

115 New Cavendish Street
London W1W 6UW
T: +44 (0)20 7911 5000
F: +44 (0)20 7911 5079
E: stregistry@westminster.ac.uk
westminster.ac.uk/ist

A charity and a company limited by guarantee
Registration number 977818 England
Registered office 309 Regent Street London W1B 2UW

Chief Investigator: Moonisah Usman
Version 6. 03.01.17

Principal Researcher: Dr Emanuela Volpi

Child's Full Name : _____

Parent/Guardian's Full Name: _____

Parent/Guardian's Signature: _____ **Date:** _____

This consent form will be stored separately from any data you provide so that your child's responses remain anonymous.

I confirm I have provided a copy of the Participant Information Booklet approved by the relevant research ethics committees to the participant and their parent and fully explained its contents. I have given the participant and their parents the opportunity to ask questions, which have been answered.

Researcher's full name: _____

Signature: _____ **Date:** _____

Participant Code Assigned: _____

VIII. Assent Form (School Version)

FACULTY OF
SCIENCE & TECHNOLOGY



Assent Form for 11-15 year olds

Research study title: Genome Instability in Childhood Obesity

Please tick as appropriate:

- | | | |
|--|---------------------------------|--------------------------------|
| I have been given the Participation Information Booklet for children, and had its contents explained to me. | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| I have had an opportunity to ask any questions and I am happy with the answers given to me. | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| I understand that I can leave the study at any time I want to. | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| I understand that if I leave the research, any information about me will also be removed from the study unless it is anonymous and therefore not possible. | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| I understand the study will be researching using my urine and saliva | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| I would like to know about the overall results from the study and I understand I will not be given individual results. | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |
| I confirm I am willing to be a participant in the above research study. | Yes
<input type="checkbox"/> | No
<input type="checkbox"/> |

Child's Full Name : _____ **Date:** _____

To be completed by researcher:

Parent/Guardian's full name: _____ **Date of consent:** _____

UNIVERSITY OF
RIGOUR
RESEARCH
RESULTS
WESTMINSTER

115 New Cavendish Street
London W1W 6UW
T: +44 (0)20 7911 5000
F: +44 (0)20 7911 5079
E: stregistry@westminster.ac.uk
westminster.ac.uk/fst

A charity and a company limited by guarantee
Registration number 977818 England
Registered office 309 Regent Street London W1B 2UW

Chief Investigator: Moonisah Usman
Version 3. 03.01.17

Principal Researcher: Dr Emanuela Volpi

IX. Medical Questionnaire



Principal Investigator: Dr Murray Bain, IRAS ID 212869 V8.0 21.02.17

Participant Screening Form (Medical Questionnaire)

Study Title Genome Instability in Childhood Obesity (GICO)

Date of Consent:		Participant Code:	
Age (y):	Gender:	Ethnicity:	
Height (m):	Weight (kg):	BMI (Kg/m ²):	Body Fat (%)
Waist Circ. (mm):	Hip Circ. (mm):	Saliva Flow Rate (ml/min):	

Please answer the following questions, as honestly as possible.
All your answers will be kept strictly confidential and none of your data will be identifiable in this study.

Q1	Have you had previous exposure to X-rays? If yes, please provide details of date and type of scan:	
Q2	Have you had any dental treatment in the last 6 weeks ? If yes, please indicate briefly what treatment you had:	
Q3	Do you currently suffer from tooth decay, or sense any sort of swelling or pain in your mouth? If yes, please indicate briefly your condition:	
Q4	Have you consumed anything to eat or drink (including water), or brushed your teeth in the last 30 minutes ?	Please circle as appropriate: Yes No
Q5	Have you participated in any vigorous physical activity in the last one hour ?	Please circle as appropriate: Yes No
Q6	Do you have, or have suffered from any medical conditions? If yes, please explain briefly your condition:	
Q7	Are you currently taking any medications, including vitamin, mineral or other herbal supplementation? If yes, please provide brief details of what you take:	
Q8	Please indicate your travel history abroad, over the last 12 months. This is only to help us determine exposure to the sun. Destination:..... Duration of stay:..... Month of Return:..... Destination:..... Duration of stay:..... Month of Return:..... Destination:..... Duration of stay:..... Month of Return:.....	

Please turn over to complete the questionnaire.

Q9. Please complete the Fitzpatrick Skin Typing test* below by entering your total score into the allocated spaces. This will help us to determine your skin type:

1. Genetic Disposition					
Score	0	1	2	3	4
What are the color of your eyes?	Light Blue, Gray or Green	Blue, Gray or Green	Blue	Dark Brown	Brownish Black
What is the natural color of your hair?	Sandy Red	Blond	Chestnut/Dark Blond	Dark Brown	Black
What is the color of your skin (non exposed areas)?	Reddish	Very Pale	Pale with Beige Tint	Light Brown	Dark Brown
Do you have freckles on unexposed areas?	Many	Several	Few	Incidental	none
Total Score for Genetic Disposition:					
[]					
2. Reaction to Sun Exposure					
Score	0	1	2	3	4
What happens when you stay in the sun too long?	Painful redness, blistering, peeling	Blistering followed by peeling	Burns sometimes followed by peeling	Rare Burns	Never had Burns
To what degree do you turn brown?	Hardly or not at all	Light color tan	Reasonable tan	Tan very easy	Turn Dark Brown Quickly
How deeply do you tan?	Not at all or very little	Lightly	Moderately	Deeply	Very Deeply
How does your face react to the sun?	Very Sensitive	Sensitive	Normal	Very Resistant	Never had a Problem
Total Score for Reaction to Sun Exposure:					
[]					
Score	0	1	2	3	4
When did you last expose your body to sun (or artificial sunlamp)?	More than 3 months ago	2-3 months ago	1-2 months ago	Less than a month ago	Less than 2 weeks ago
Total Score for Tanning Habits:					
[]					
Skin Type Score	Fitzpatrick Skin Type				
0-7	I				
8-16	II				
17-25	III				
26-30	IV				
over 30	V-VI				

*(Fitzpatrick TB. Arch Dermatol. 1988;124(6):869-871)

Please turn over to complete the final part of the questionnaire.

Female participants please proceed to question 12. Male participants please continue to question 10.

Q10	Have you gone through puberty?	
Q11	When did you go through puberty?	
<i>End of questionnaire for male participants.</i>		
Q12	Have you started having periods?	Please circle as appropriate: Yes No If no, please go to the end of questionnaire.
Q13	Please record the date of your first period:	
Q14	Please record the date of your last period:	
Q15	How often do you have a period?	

End of Questionnaire - Thank you for your participation!

X. Invitation Letter (School Parents/Guardians)

FACULTY OF
SCIENCE & TECHNOLOGY



Dear Parent/Guardian,

Your child is being invited to take part in a research study titled: 'Genome Instability in Childhood Obesity – (GICO)', in collaboration with The Harris Academy Battersea and the University of Westminster.

The purpose of the research is to establish the different effects of body fat on health. It will require your child to be measured, provide a urine and some saliva samples, and complete a medical questionnaire.

Please note:

1. Participation in this study is **entirely voluntary**. If your child wishes to participate, all information collected will be kept **anonymous**.
2. If your child does, or does not wish to participate, it will not affect their activities or involvement at The Harris Academy.
3. All research activities will be carried out at The Harris Academy, and you or your child will not be required to carry out any travel.
4. This research study has been checked and approved by the relevant Research Ethics Committee.

For further information about the study, please refer to the attached *Participant Information Booklet for Parents*, and *Participant Information Booklet for 11-15 Year olds*.

If you and your child agree to take part in the study, you should carefully read and complete the attached *Consent form*. Please keep the Participant Information Booklets for your own reference, and return the consent form to the school reception **by <insert date>**. If you do not return these forms, your child will **not** be included in the study automatically.

Following your consent, we will start by taking your child through the questions on the 'medical questionnaire' before collecting any samples from them. A copy of this questionnaire is included for your reference only.

If you have any further questions, please contact **<NAME>** on **<insert number>** or the research team using the contact details at the back of the Information Booklet for Parents.

Kind regards,

<Organisation >

UNIVERSITY OF
RIGOUR
RESEARCH
RESULTS
WESTMINSTER

115 New Cavendish Street
London W1W 6LW
T: +44 (0)20 7911 5000
F: +44 (0)20 7911 5079
E: stregistry@westminster.ac.uk
westminster.ac.uk/fsi

A charity and a company limited by guarantee
Registration number 927618 England
Registered office 309 Regent Street London W1B 2UW

Draft Letter of Invitation for Parents V4 27/09/16

Chief Investigator: Moonisah Bajwa Principal Investigator: Dr Emanuela Volpi

XII. Normality test results

Assessment of data for Gaussian distribution via the D'Agostino-Pearson normality test ($p=0.05$, $n=112$).

	Mean \pm SD	Range	Passed normality test?
<i>Age (years)</i>	14.11 \pm 2.308	10.0 - 18.0	No
<i>BMI</i>	29.72 \pm 12.69	15.10 - 63.60	No
<i>BMI (Z-score)</i>	1.828 \pm 1.821	-2.3 - 4.86	No
<i>WHR</i>	0.858 \pm 0.0994	0.571 - 1.383	No
<i>Body Fat (%)</i>	32.66 \pm 12.44	5 – 58.9	No
<i>CRP (pg/mL)</i>	2002 \pm 1313	341.8 - 7789	No
<i>Vitamin D (ng/mL)</i>	7.070 \pm 4.493	0 – 17.44	No
<i>DNA Damage in BM (%)</i>	1.263 \pm 0.617	0.2 - 3	Yes
<i>8-OHdG (ng/mL creatinine)</i>	171.3 \pm 102.0	18.18 – 546	No
<i>Telomere Length (kb/diploid genome)</i>	165 \pm 94.45	24.26 - 482.1	No

XIII. Associations between micronuclei (MNi) frequency in PBLs and site-specific malignancy and pre-malignancy.

Cancer Site	Study Population	Key Findings	Authors, Date
Bladder	158 patients/ 158 controls (age matched)	Increase in MN frequency associated with increased risk of cancer	Pardini <i>et al.</i> , 2017 (Pardini, <i>et al.</i> , 2017)
Breast	91 patients/96 controls	Higher frequency of MNi in breast cancer patients	Varga <i>et al.</i> , 2006
	45 patients/ 85 controls (age matched)	Higher frequency of MNi in breast cancer patients	Santos <i>et al.</i> , 2010.
	220 patients/95 controls	No significant difference of MNi frequency between patients and controls	Bolognesi <i>et al.</i> , 2014
	Follow up of 1650 adults	Higher frequency of MNi at baseline in cancer patients.	Murgia <i>et al.</i> , 2008
Colorectal	25 cancer patients/ 26 polyp patients/ 31 controls	2.1x higher frequency of MNi in cancer patients than controls. Polyp patients had 1.5x higher frequency of MNi than controls.	Maffei <i>et al.</i> , 2014
Encephalon	Follow up of 1650 adults	Higher frequency of MNi at baseline in cancer patients.	Murgia <i>et al.</i> , 2008

Endometrial	59 patients/ 59 controls	20% higher frequency of MNi in patients.	Aires <i>et al.</i> , 2011
	20 endometrial hyperplasia patients/ 20 cancer patients/ 20 controls	2.9x higher frequency of MNi in women with endometrial cancer.	Kiraz <i>et al.</i> , 2016
Lung	216 small cell lung cancer patients/ 173 Non-small cell lung cancer patients/ 204 controls	Higher MNi frequency in lung cancer patients.	El-Zein <i>et al.</i> , 2006
Lymphoma	Follow up of 1650 adults	Higher frequency of MNi at baseline in cancer patients.	Murgia <i>et al.</i> , 2008
Oral	Follow up of 1650 adults	Higher frequency of MNi at baseline in cancer patients.	Murgia <i>et al.</i> , 2008
Pancreatic	346 patients/ 449 controls	1.6x higher MNi frequency in pancreatic cancer patients.	Chang, Li and Li, 2011
Stomach	6718 adult participants across 20 sites	Higher frequencies of MNi were associated with increased risk of stomach cancer.	Bonassi <i>et al.</i> , 2007
Urogenital	6718 adult participants across 20 sites	Higher frequencies of MNi were associated with increased risk of urogenital cancer.	Bonassi <i>et al.</i> , 2007

XIV Associations between adiposity and telomere length.

Date	Study type	Study population	Cell type & technique for telomere length test	Key findings	Reference
2005	Case control	561 female twins	PBLs via TRF	TRF length was lowest in the obese cohort and correlated with BMI and serum leptin.	(Valdes, <i>et al.</i> , 2005)
2008	Correlation	989 adults	Leucocytes via qPCR	Inverse correlation between BMI and TL in women.	(Nordfjäll, <i>et al.</i> , 2008)
2008	Case control	53 children/23 adults	PBLs via TRF	No difference in TL in children. Obese adults had shorter TL.	(Zannolli, <i>et al.</i> , 2008)
2009	Correlation follow-up	647 female adults	Leucocytes via qPCR	High BMI and hip circumference inversely correlated with TL. Obese females had shortest TL.	(Kim, <i>et al.</i> , 2009)
2010	Case control	51 obese/ 21 non-obese adults	Subcutaneous adipose tissue via Southern blotting	BMI inversely correlated with TL. Formerly obese patients had shorter TL than never-obese.	(Moreno-Navarrete, <i>et al.</i> , 2010)
2010	Correlation	317 adults (aged 40-64 years)	Leukocytes via qPCR	No significant correlations between BMI or visceral adipose tissue and TL.	(Diaz, <i>et al.</i> , 2010)
2010	Correlation	2284 females	Leukocytes via qPCR	Waist circumference was inversely correlated with TL.	(Cassidy, <i>et al.</i> , 2010)
2011	Correlation	309 non-Hispanic white participants aged 8 to 80 years	Leukocytes via qPCR	BMI, waist circumference, hip circumference, total body fat, and visceral adipose tissue volume inversely correlated with TL.	(Lee, <i>et al.</i> , 2011)

2011	Case control	793 children (aged 2-17 years)	Leukocytes via qPCR	23.9% shorter TL in obese children.	(Buxton, <i>et al.</i> , 2011)
2011	Case control	667 adolescents	Leukocytes via qPCR	Shorter TL is not associated with childhood obesity.	(Haidong Zhu, <i>et al.</i> , 2011)
2012	Correlation at baseline and 7yr follow up	2721 elderly subjects	Leukocytes via qPCR	BF% and subcutaneous fat inversely correlated with TL. No correlation between BMI and TL. 7 year follow up showed inverse correlation with BMI and BF%.	(Njajou, <i>et al.</i> , 2012)
2013	Correlation	2,912 females (aged 40-70 years)	Leukocytes via qPCR	TL is inversely correlated with BMI, waist circumference, waist-to-height ratio, weight, and hip circumference but not waist to hip ratio.	(Cui, <i>et al.</i> , 2013)
2016	Correlation	7527 adults (aged 20-84)	Leukocytes via qPCR	Telomere length is inversely correlated with BMI, waist circumference, BF% and C-reactive protein.	(Rehkopf, <i>et al.</i> , 2016)
2017	Correlation follow-up	2749 adults	Saliva via qPCR	BMI positively predicts salivary telomere length over 16 years.	(An, & Yan, 2017)
2018	Correlation	497 Lebanese adults	PBLs via qPCR	Telomere length is inversely associated with waist circumference but not with BMI.	(Zgheib, <i>et al.</i> , 2018)

