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DNA damage in obesity: initiator, promoter and predictor of cancer

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Abstract

Epidemiological evidence linking obesity with increased risk of cancer is steadily growing, although the causative aspects underpinning this association are only partially understood. Obesity leads to a physiological imbalance in the regulation of adipose tissue and its normal functioning, resulting in hyperglycaemia, dyslipidaemia and inflammation. These states promote the generation of oxidative stress, which is exacerbated in obesity by a decline in anti-oxidant defence systems. Oxidative stress can have a marked impact on DNA, producing mutagenic lesions that could prove carcinogenic. Here we review the current evidence for genomic instability, sustained DNA damage and accelerated genome ageing in obesity. We explore the notion of genotoxicity, ensuing from systemic oxidative stress, as a key oncogenic factor in obesity. Finally, we advocate for early, pre-malignant assessment of genome integrity and stability to inform surveillance strategies and interventions.

Key words: DNA Damage, Obesity, Inflammation, Oxidative Stress, Cancer

1. Connecting obesity and cancer via oxidative stress and DNA damage

The association of obesity with cancer is naturally one of the most concerning aspects and the object of intense scrutiny. A number of prospective and case-control studies have recognised an epidemiological correlation between obesity and cancer in a sex and site-specific manner. Published evidence has been extensively reviewed [1, 2]. A recent paper by Kyrgiou and colleagues [3], in which over two-hundred meta-analyses of observational studies linking adiposity to cancer risk were systematically evaluated, has confirmed definitive evidential association between obesity and eleven types of cancer, including oesophageal adenocarcinoma, multiple myeloma, and cancers of the gastric cardia, colon, rectum, biliary tract system, pancreas, breast, endometrium, ovary and kidney. Obesity manifests with inflammation, hyperglycaemia and dyslipidaemia, maintained to be drivers of carcinogenesis as they may endorse the biological mechanisms associated with tumour development, sustainability and metastasis [4]. These pathological states in obesity may be have a key role in the initiation of cancer as chronic inflammation and the metabolic disturbances in obesity have a common pathological consequence termed oxidative stress, a long-recognised threat for genome stability and integrity [5]. Cellular function, viability and fate are predominantly determined by the genomic stability and integrity of the cell. Conversely, cancer genomes are highly rearranged and genomic instability has been described as an enabling characteristic of cancer [6]. Oxidative stress has a physiological relevance as the excessive generation of reactive oxygen species (ROS) may lead to oxidative DNA damage and induce tumorigenic alterations [7], as well as drive genomic instability by mimicking replication stress [8].

ROS are produced via many fundamental cellular processes. Endogenous sources of ROS include mitochondrial respiration and enzymatic reactions within phagocytes and peroxisomes. ROS are

stimulated by growth factors and serve as necessary signalling molecules [9] . Concentrations of ROS are tightly regulated under a redox (reduction oxidation) system to prevent toxicity. Obesity is known to disrupt the redox system by elevating the production of ROS, and decreasing the availability of anti-oxidant defence enzymes [10]. A calorie-rich diet and an abundance of macronutrients in obesity, calls for continuity in the Krebs cycle that consequently leads 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 more ROS. However, the over-generation of ROS in obese individuals is brought about by more than just a continuum in the Krebs cycle.

2. The oxidative burden of inflammation

Chronic inflammation is a well-known etiological factor for DNA damage and promotor of neoplastic transformations in cells [11]. In a state of obesity, the release of pro-inflammatory molecules including CRP, TNF-alpha and IL-8 from adipose tissue is well documented [12–15]. Adipocytokines particularly TNF-alpha, IL-6 and IL-1 promote the recruitment of various cells including neutrophils, macrophages and dendritic cells, indicating the beginning of an inflammatory process [16]. These cells not only generate more pro-inflammatory cytokines but also encourage production of ROS/RNS via NADPH oxidases, particularly the NOX1, NOX2 and NOX4 isoforms [17]. Further production of ROS/RNS is generated as obesity develops with hypertrophy and hyperplasia of adipocytes, resulting in tissue hypoxia and cell necrosis [10].

Cell necrosis causes altered adipokine expression, including reduced levels of the anti-inflammatory hormone adiponectin [18]. Adiponectin is secreted by adipose tissue and may inhibit the actions of TNF-alpha and prevent recruitment of inflammatory cells [19]. Adiponectin deficiency may play a pathological role further than inflammation as it has been associated with obesity and cancers of the breast [20], GI tract [21,22], blood [23] and prostate [24].

Increased death of adipose cells has also been indicated as a path to release cell-free DNA into systemic circulation [25]. Nishimoto and collaborators [26] have recently identified that 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. Macrophage infiltration into adipose tissue is characteristic of obesity and may have further pathological consequences when they secrete high concentrations of nitrous oxide (NO) [27]. In high concentration, NO can cause deamination, oxidation and strand breakages in DNA [28].

Chronic inflammation and its ability to inflict DNA damage has been demonstrated in models of *H.pylori* associated gastrointestinal cancer [29] and ulcerative colitis associated colon cancer [30], as well as HCV mediated liver cancer [31]. Unresolved inflammation has also been reported to cause pre-malignant lesions in the mouth [32]. Izano and colleagues [33] 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 findings evoke the causative relationship between chronic inflammation and colon cancer in obese adults, but evidence for other obesity related cancers is non-conclusive. Overall, there is substantial evidence that chronic inflammation is a causative factor for DNA damage. This phenomenon should be further explored in obesity to elucidate the risks of carcinogenesis.

3. Dyslipidaemia and Lipid Peroxidation

Obesity coincides with an increased circulation of free fatty acids (FFA) and the deposition of excess fat in white adipose tissue (WAT), marked as dyslipidaemia [34]. Excessive plasma lipids are susceptible to oxidative modification which generates more ROS, and can also activate a protein kinase C pathway resulting in elevated production of nitroxide – a potent ROS [35]. The deposition of FFA in WAT also contributes to ROS generation by attracting leukocytes and causing inflammation [35].

Dyslipidemia - mainly the elevation in low density lipoproteins and fall in high-density lipoproteins, disrupts the redox system and leads to lipid peroxidation in obesity [36]. Lipid peroxidation is a mechanism for generating malondialdehyde (MDA) that can react with DNA to produce mutagenic MDA-DNA adducts such as MDA-deoxyguanosine [36]. MDA has the potential to cause inter-strand cross links with DNA [37] and point mutations [38]. The first reaction can be highly cytotoxic, whereas the latter could prove carcinogenic [39]. Elevated levels of MDA have been identified in both adult [40] and child [41] models of obesity. As MDA levels can also be a remarkable finding in breast and lung cancer patients [42], a potential causative association between lipid peroxidation, DNA damage and cancer risk in obesity can be argued.

4. Hyperglycaemia and ROS generation

In obese individuals, insulin signalling can be significantly impaired. Glucose transport, protein C kinase and other enzymatic activity can be defective, resulting in increased plasma glucose levels (hyperglycaemia) [43]. FFA circulation and uptake in the liver can further contribute towards hyperglycaemia when FFA is selected as an energy source over glucose [44]. Hyperglycaemia has been associated with increasing ROS generation four-fold in adipocytes and thus promoting inflammation [45]. These factors also contribute towards hyperinsulinemia and insulin resistance in obesity [46]. Hyperinsulinemia correlates with decreased levels of IGF binding protein 1 and IGF binding protein 2, thereby readily causing an increase in the availability of IGF-1 [46].

Excess free circulating IGF-1 has been associated with an increased risk of breast cancer [47] and colorectal cancer [48]. Although, IGF-1 was found to have a role in activating DNA repair pathways against oxidative DNA damage [49], elevated levels of IGF-1 in obesity may be a physiological response to oxidative stress [50] but this avenue is open to question.

Moreover, IGF-1 has been linked with down-regulation of sex hormone binding globulin (SHBG) [51], which could result in an increase in the availability of free oestrogen [52]. The association between obesity and increased risk of endometrial and breast cancer may owe to increased oestrogen

levels in obese individuals [53,54]. Traditionally, sustained exposure to oestrogen has been associated with upregulation of genes that control cell proliferation and cell cycle progression, marking an important role in tumorigenesis [55]. In addition, oestrogen may endogenously induce DNA modifications, create oestrogen-DNA adducts and generate oxidative DNA damage [56]. Oxygen radicals can also be generated as a result of the redox cycling of oestrogen metabolites [56]. Therefore, it can be inferred that hyperglycaemia in obese subjects may promote ROS generation via IGF-1, insulin and oestrogen, thus increasing the likelihood of DNA damage and cancer initiation.

5. Inadequate Anti-oxidant Defence

Increased BMI has been associated with a decline over time in essential regulators of the redox system. Ozata and collaborators [57] identified decreased activity of SOD and GPx in erythrocytes of 76 male obese subjects, in relation to age-matched healthy weight controls. Similarly, recent investigations have confirmed lower activities of SOD and GPx in obese women, although no significant difference in CAT activity was observed [58]. Interestingly, Erdeve and collaborators [59] reported the SOD enzyme to be increasingly active in a cohort of obese children. However, this finding was in parallel to identification of increased ROS production in the same cohort, suggesting that obesity may trigger an early physiological response to oxidative stress in children by increasing the activity of anti-oxidant enzymes to scavenge the ROS. Similarly, total oxidant status (TOS) and total anti-oxidant status (TAS) were both reported to be higher in a cohort of 37 obese children compared to health weight controls [60]. Other findings suggest that the implications of oxidative stress worsen with the severity of obesity, placing obese children at higher risk of oxidative stress. Albuali and colleagues [61] identified elevated activity of SOD and CAT in overweight children but found that this level of functioning did not exist in the obese cohort. Anti-oxidant status in obesity appears to be an interesting paradox whereby anti-oxidant defence mechanisms may be maximised in order to counteract oxidative stress [62], but whether this effect is substantial in controlling ROS and

preventing pathological consequences is unknown. Overall, there are substantial indications of a weakened anti-oxidant defence mechanism in both adult and childhood obesity [57,58,63].

A decrease in anti-oxidant activity has been linked with DNA damage and acute lymphoblastic leukaemia in children, although it is complicated to establish whether this association is causative [64]. A study conducted on mice deficient in SOD activity presented them to have a decreased lifespan and increased likelihood of liver malignancies, [65]. A latter review by Asaduzzaman and colleagues [66] explored the paradoxical roles of anti-oxidant enzymes in carcinogenesis. They highlighted that CAT, SOD and GPx activity is heterogeneous in a variety of cancers. Recent studies in oral [67] and breast cancer [68] have given a deeper insight into these roles and suggest that elevated activity of some of these enzymes may also be a cause for concern. Overall, it is plausible that inadequate anti-oxidant mechanisms may predispose to malignancy, possibly via increased DNA damage.

It is important to consider that obesity often coincides with a nutrient-poor diet and a lack of dietary intake of anti-oxidants [69]. Low levels of particular plasma micronutrients, including folate, vitamin B, vitamin C, iron and zinc, may imitate the effects of radiation on DNA, potentially resulting in DNA strand breaks or the production of oxidative lesions [70]. It has also recently emerged that obesity may cause vitamin D deficiency [71]. Vitamin D may play a significant role in managing the redox system, as it was found to upregulate the anti-oxidant enzyme G6PD in human prostate epithelial cells [72] and is associated with reducing the incidence of prostate cancer [73]. Thus, low levels of anti-oxidant enzymes in obesity may also be attributed to micro-nutritional deficiencies. Collectively these disturbances in the redox system and antioxidant defences may increase the potential for DNA damage and risk of cancer, although further research is required to confirm any causative association.

6. Markers of DNA damage in obesity

6.1 Micronuclei

Micronuclei (MNi) are well-recognised markers of genotoxic stress and genomic instability. By definition, a micronucleus is an extra-nuclear body within the cell cytoplasm containing chromosomal fragments or whole chromosomes, resulting either form *clastogenic* events, such as DNA double strand breaks (DSBs), or *aneugenic* events, such as segregation defects giving rise to abnormal numbers of chromosomes. There are indications that retention of micronuclei containing whole chromosomes can proceed into several cell generations [74]. Recently, the relevance of micronuclei as potential drivers of malignant transformation has come to the fore in relation to the phenomenon of chromothripsis, a newly described mutational process whereby micronuclei division cycles enable accumulation of mutagenic re-arrangements in a single or few chromosomes [75]. These localized chromosomal re-arrangements may be transferred to daughter nuclei in subsequent mitotic cycles and play a role in generating a pre-cancerous genome. Micronuclei may display a lack of nuclear membrane integrity when occurring in normal or cancer cells. Therefore, the nuclear envelope of a micronucleus is more likely to rupture, causing exposure of self-DNA to the cytosol. Possible immuno-stimulatory consequences of this event have recently been reported in a mouse model and human cancer cells [76].

Evidence for micronuclei frequency as a possible predictor of cancer is being explored. A series of observational and follow-up studies have consistently demonstrated raised incidence of micronuclei in 10 different types of cancer (examples in Table 1.). Most relevantly, prospective research approaches have associated an elevated frequency of micronuclei with an increased risk of developing cancer. Early research by Bonassi and colleagues 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 [77]. Another longitudinal study reported evidence to support a possible link between micronuclei and risk of cancer. This research followed 1650 initially disease free adults

for up to 14 years. Retrospective analysis indicates that participants that developed cancer had, on average, a three-fold increase in micronucleus frequency at baseline [78].

Ageing appears to be a major contributing factor to chromosomal instability and micronuclei formation. The influence of age on micronuclei frequency has been quite extensively researched. In an initial study on a large population of 791 participants, micronuclei frequency was found to increase with age and was higher in women than men [79]. Subsequent investigations have detected a biphasic relationship between age and induction of micronuclei in lymphocytes, with a positive correlation between micronuclei and age up to 50 years, but an inverted association beyond that age threshold [80,81]. This biphasic response may owe to the decreased proliferative capacity of cells in older individuals, that may exist as a mechanism to limit possible genomic instabilities [81]. Moreover, the frequency of micronuclei was reported as at least three times higher in the buccal mucosa of adults aged over 60, compared to cells from a cohort of 19-29 year olds [82]. More recent research draws out that micronuclei frequency in lymphocytes can be an indicator of frailty in an ageing cohort, however, whether micronuclei frequency in buccal epithelial cells is positively associated with age is yet to be confirmed [83] .

There is increasing evidence to suggest a link between adiposity and micronuclei frequency (Table 2.). The first indication of a positive correlation between micronuclei frequency and BMI was provided by a research on polycystic ovarian syndrome (PCOS) [84]. Further evidence was provided by Andreassi *et al.,* [85], highlighting the correlations between BMI, DNA damage and insulin resistance in women with PCOS. Later, BMI was related with an almost four-fold increase in micronuclei frequency within the PBLs of 83 obese adults, when compared with normal-BMI controls [86]. A positive correlation between BMI and the number of apoptotic and necrotic cells in plasma was identified suggesting the elevated frequency of dying cells to be a result of them possessing a damaged and unstable genomic state. Furthermore, an increased association between BMI and micronuclei frequency was observed in a cohort of obese Italian children [87]. This was the

first study in which the occurrence of genome damage has been investigated in childhood obesity. Compelling evidence on positive associations between weight and length at birth, and three markers of chromosomal instability (MN frequency, nucleoplasmic bridges and nuclear buds) has been reported [88].

Some studies in human and animal models have highlighted discrepancies in terms of influence of adiposity on micronuclei frequency. Although MN frequency was positively associated with age in 300 Korean adults, no correlations were drawn with BMI [89]. An in-depth meta-analysis by Croco and collaborators [90] discovered body mass to be a determining factor for genomic stability, demonstrated by erythrocyte micronucleus frequencies across 47 mammalian species. However, more recently a, research on a diet-induced mouse model of obesity did not find a significant difference in the levels of micronuclei in erythrocytes or reticulocytes, albeit the obese mice sustained a greater frequency of *Pig-a* mutant erythrocytes, a newly described biomarker for somatic mutations [91].

The origins of micronuclei formation in obesity need to be extensively researched. Although, chronic inflammation is a possible pathological factor that may underpin the pathway to micronuclei formation, as identified in patients with cirrhotic liver as a result of hepatitis C infection [92]. Whether this concept can be applied to low-grade, systemic inflammation identified in obesity is an important, unanswered question.

6.2 8-OH-2-deoxy Guanosine (8-OHdG)

8-oxo-deoxy-guanosine (8-OHdG) is the product of free-radical induced oxidation of guanosine. 8-OHdG can be detected in serum, saliva and urine, making it an ideal DNA damage biomarker for large population based studies. The possible mutational consequences of this lesion, including mis-pairing with adenosine, inhibiting methylation of adjacent cytosines and deleterious GàT transversion mutations have been recently reviewed [93]. Ramon and colleagues [94] first identified that 8-OHdG may also directly interfere with the gene transcription process when found at promoter

regions, by demonstrating that 8-OHdG alters binding of the transcription factor Sp1. The role of Sp1 transcription factors in cell growth, development, and death have been extensively reviewed concluding Sp1 as key players in carcinogenesis [95]. In addition, a recent review highlighted that 8-Oxoguanine DNA Glycosylase 1 (OGG1) – a key repair enzyme, may also play a role in carcinogenesis by recruiting 8-OHdG as a ligand for gene regulation and activation of the Ras pathway [96].

The clinical significance of 8-OHdG has been extensively discussed by Cooke and colleagues, bringing to light that 8-OHdG is a well-established risk marker for age-related pathologies such as cancer [97]. The GT to AC substitution is a commonly described mutation that is derived from the oxidation of guanosine and is also prominent in gastrointestinal tumours [98]. It is not surprising that elevated serum or urinary 8-OHdG lesions are undisputedly associated with malignant tumours at multiple sites (Table 3.).

Interestingly, levels of 8-OHdG are also significantly higher in patients with pre-cancerous lesions of buccal mucosa [99] and dysplastic cervical cells [100]. Therefore, there may be a potential for 8-OHdG to be utilised as a clinical risk marker to detect early carcinogenic processes.

For long, the phenomenon of ageing has been described as a process that incorporates free-radical mediated attacks on nuclear DNA, leading to oxidative stress, telomere attrition and a halt in cell division and proliferation [101]. 8-OHdG lesions may have implications in cellular senescence and ageing caused by oxidative stress. This is because early research recognised 8-OHdG to emerge from senescing human fibroblasts in higher concentrations than their younger counterparts [102]. More recent studies have recognized increasing concentrations of 8-OHdG to be associated with age-related cataracts [103], Alzheimer's [104], Parkinson's [105], and vascular complications in type 2 diabetes [106]. This suggests that 8-OHdG is a specific biomarker for oxidative stress that is consistent with ageing.

Multiple studies have assessed the correlation of 8-OHdG with adiposity with emerging findings not devoid of discrepancies (Table 4.). de la Maza and colleagues were the first to report an association between increased body fat and oxidative DNA damage by assessing 8-OHdG in skeletal muscle [107]. These associations were based upon self-reported weight gain over the last 10 years by patients undergoing a hernia operation. It is intriguing that increased 8-OHdG in these weight gainers coincided with increased TNF-alpha – an undisputed marker of inflammation. Another study assessed 8-OHdG concentrations in lymphocytes and was unable to identify a correlation with BMI in adults [108]. In contrast, there are findings that indicate a correlation between leanness and increased oxidative stress. A one-point reduction in BMI was reported to coincide with a 2.7% increase in urinary 8-OHdG [109]. Similarly, Donmez-Altuntas and collaborators reported decreased levels of 8-OHdG in plasma of obese adults compared to adults of healthy weight, although other biomarkers of DNA damage including micronuclei frequency was elevated [86]. More recently, a study conducted in over 100 obese and healthy weight men concluded that there were no significant differences in urinary 8-OHdG [110]. In summary, the evidence for 8-OHdG as a marker of oxidative stress in adult obesity is inconclusive, this outcome likely due to discrepancies arising from differences in the degree of obesity in participants between studies.

Similarly, discordant are the results of investigations carried out across adolescent cohorts. The first evidence showing a positive association between BMI and oxidative DNA damage in obese adolescents, quantified 8-OHdG in serum via an ELISA method [111]. Across 103 adolescents, a BMI greater than the 95th percentile was associated with increased 8-OHdG. However, Protano and collaborators assessed 8-OHdG in urine across 159 children via liquid chromatography–tandem mass spectrometry and found an inverse association [112]. Yet more recent research has reported a higher concentration of 8-OHdG in urine from a much smaller cohort - 24 obese children [113]. However, the children in this cohort also presented with insulin resistance and metabolic syndrome (MS). The absence or presence of insulin resistance or MS may account for discrepancies in the measurement of

oxidative DNA damage, as MS has been suggested to disrupt anti-oxidant defences and encourage oxidative stress [114].

6.3 γ-H2AX foci

Induction of nuclear γ-H2AX foci or gamma foci signal the start of a crucial repair process that follows a potentially carcinogenic DNA lesion [115]. DNA double strand breaks (DSBs) are detected by the MRN repair complex consisting of NBS1, MRE11 and Rad50. This recognition process leads to activation and recruitment of ATM and a subsequent phosphorylation of γ-H2AX and other targets including BRCA1. As reviewed by Khanna & Jackson, DSBs can have lethal consequences for a cell as they can drive the cell into apoptosis or towards carcinogenesis [116]. Although the suitability of γ-H2AX as a DNA damage marker has been questioned as its presence has been observed in the absence of recognisable DNA damage [117], evidence against the use of γ-H2AX as a marker of DSBs remains limited. Recent research indicates the induction of γ-H2AX to coincide with unrepaired DSBs as well as blocked replication forks [118,119]. Indeed, the suitability of gamma foci continues to be explored as a diagnostic and prognostic marker for cancer (Table 5).

A higher frequency of γ-H2AX foci have been found expressed in breast cancer cells from the more aggressive triple negative breast cancer (TNBC) when compared to cells from non-TNBC [120]. Gamma foci have also been suggested as an appropriate diagnostic and prognostic marker for colon cancer [121] and more recently, epithelial ovarian cancer [122]. The detection of γ-H2AX in pre-cancerous lesions of the bladder *in vivo* [121] and pre-cancerous lesions of the lung and skin *in vitro* gives reason to explore the use of γ-H2AX as a possible predictor of cancer [123].

The DNA damage response to DSBs has been described as a major pathway to cellular senescence in mouse models [124,125]. Sedelnikova and collaborators discovered γ-H2AX foci at sites of persistent DSBs that led to cellular senescence in ageing mice and human cell lines, indicating that y-H2AX foci may be a marker of un-repairable DNA damage and ageing in mammals [124]. They also

tested this association in cell lines from patients with Werner syndrome – a disorder characterised with premature ageing, and identified a comparable association, although recruitment of DNA proteins was slower to γ-H2AX foci. A more recent study also documented a linear correlation between γ-H2AX foci and ageing in human cell lines [126]. Schurman and colleagues further studied the association of γ-H2AX foci with age related disorders and found a positive correlation with cataracts and hypertension, but not with cancer [126]. This indicates the need for further research identifying whether there is a causative role for γ-H2AX foci in ageing and cancer.

Redon and colleagues reviewed the practical applications of H2AX foci in population studies, and identified their extensive use to draw associations between chronic inflammation and DNA damage [127]. It was emphasised that the long-term consequences of identifying elevated γ-H2AX foci remains an open avenue.

There is consistent evidence to suggest γ-H2AX foci are elevated with adiposity (Table 6).

Villaret and colleagues reported premature senescence of visceral adipose tissue cells in obese subjects, and found the endothelial cells within to be marked with a state of inflammation and up-regulated hypoxia genes in coexistence with increased γ-H2AX foci [128]. Interestingly, this phenomenon was not documented in sub-cutaneous adipose tissue to the same extent. In addition, for the first time, γ-H2AX foci were documented as 8x fold higher in cultured PBLs of obese children when compared to healthy weight controls in a study led by Scarpato and collaborators [87]. They also assessed chromosomal aberrations, via the micronucleus assay, in a large cohort of obese children, alongside markers of chronic inflammation and attributed an increased rate of DSBs to overweightness. The confounding, observed increase in TNF-a, CRP and IL-6 manifests a causative link between chronic low-grade inflammation and DNA damage in obese children. In 2016, the same investigators deepened their investigations into the repair efficiency of DSBs between obese and normal weight adolescents by measuring the response of PBLs to mitomycin C [129]. An almost 2 fold increase was found in mitomycin C induced DSBs in the obese cohort when compared to

healthy controls, measured by y-H2AX foci. Intriguingly, the level of DNA repair in the obese cohort was repaired faster and more efficiently than in the healthy weight counterparts. This research indicates 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 warrants further investigations.

Furthermore, the documented evidence for increased DSB repair initiation linked with childhood obesity has also been implied in obese Zucker rats - a model of genetically predisposed obesity [130]. Obese Zucker rats presented a greater expression of γ-H2AX foci in lung, pancreatic, oesophageal, kidney and gut tissues when compared to lean counterparts. It was also noted that older rats (9-13 weeks) had a more prominent increase in the expression of γ-H2AX foci than younger rats (3-4), further implying that DSB events may correlate with age. Nonetheless, it is important to comprehend that the induction of γ-H2AX in tissue is a well-regulated proper response to reversal of DNA Double strand breaks, although, the relationship between the number of foci present and number of DSBs repaired by γ-H2AX foci is questionable.

6.4 Comet Tails

Comet tails are sensitive biomarkers of deteriorating chromosomal integrity as they enable inference of chromosomal breakages. The comet assay (single cell gel electrophoresis) used to detect these tails is a well-established laboratory assay that was first pioneered by Ostling & Johanson in 1984 [131]. Single cells are embedded in agarose and treated to remove nuclear membranes and histone material prior to 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 a taken as a directly proportional measure of DNA damage [132]. The comet assay has been extensively applied to measure genotoxicity, and responsiveness to

chemotherapy, and is now evolving into a diagnostic marker of cancer [133]. Several cases of malignancy have been assessed via the comet assay and found to display significant DNA damage (Table 7.). Furthermore, Katarkar and colleagues identified two remarkable observations via a case-control study using 260 participants. The first is the applicability of the comet assay in PBLs as a biomarker for pre-malignant lesions of the buccal mucosa [134]. Secondly, they uncovered that DNA damage in PBLs correlates with DNA damage in cells extracted from the buccal mucosa, as measured by the presence of micronuclei. This evidence leads to the conclusion that the comet assay in PBLs may possibly act as a cancer susceptibility biomarker for non-haematological malignancies. This avenue requires further exploration and warrants studies to investigate the relevance of non-invasive assays; such as the buccal micronucleus assay, in susceptibility to systemic DNA damage and carcinogenesis.

The comet assay has also been applied to evaluate DNA damage in ageing [135,136]. Research has suggested the comet assay may be a functional biomarker for pre-mature ageing, as increased DNA damage was observed in cells from patients with Hutchinson-Gilford progeria syndrome (HGPS) when compared to normally ageing human fibroblasts [136]. These investigations intensify the link between DNA damage and ageing, when explored using the comet assay.

Body fat can be proposed as a pathological factor for chromosomal breaks following investigations with the comet assay in rat models of obesity and in humans (Table 8.) A correlation study first indicated a positive association between BMI in females and oxidative DNA damage via the comet assay [108]. Later, A positive correlation between increased chromosomal damage and BMI (>30) was demonstrated by the comet assay in pregnant women of a Pakistani population [137] and was subsequently confirmed in an Indian population too [138]. Remarkably, DNA damage was also identified via the comet assay in women with a normal BMI but elevated fat mass [139]. 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 warrant for investigations of DNA damage

in obesity to consider measures of adiposity wider than BMI, as fat mass may have a stronger correlation with genomic instability. DNA damage has also been confirmed via the comet assay in liver cells of obese female Zucker rats [140]. This research identified age to be an important factor when interpreting results from the comet assay, since Zucker rats younger than 15 months showed no evidence of comet tails. Comet tails are indicative of DNA damage in adult and animal models of obesity. However, there is a lack of studies investigating the expression of comet tails in adolescent or childhood obesity.

More recently, a nutritional intervention study outlined a decrease in DNA single strand breaks with weight reduction in 50 participants, even though the obese/overweight participants did not have high levels of DNA damage at baseline [141]. Intriguingly, DNA damage was associated with increased BMI and breast cancer risk in a case-control study [142]. However, in the cancer-free control cohort, BMI was inversely associated with DNA damage [142]. It has been suggested that this discrepancy could be an outcome of increased metabolic rate in lean individuals and a possible generation of more ROS. In the breast cancer cohort, the positive association between BMI and DNA damage could be a consequence of defective DNA repair and marked chromosomal instabilities that are consistently found in cancer. Furthermore, this study analysed BMI data for only 45 cancer free patients, indicating that these findings may lack statistical power. Nonetheless, the role of the comet assay as a biomarker for genomic instability in increasing adiposity is propitious.

6.5 Telomere Length

Telomeres play a fundamental role in maintaining chromosomal integrity and can substantially underpin the genomic instabilities associated with carcinogenesis [143]. Telomeres are vital segments of non-coding DNA repeat sequences (TTAGGG) that are located at the ends of chromosomes and lose their DNA bases at every round of the cell cycle, consequently shortening in length. Telomere length can be assessed in multiple tissue types via a variety of techniques that include quantitative Polymerase Chain Reaction (qPCR), fluorescence probes, Southern blot and the

gold standard - Terminal Restriction Fragmentation (TRF). Telomere shortening to a critical length should ultimately result in replicative senescence, hence the reason for telomeres long being linked to the phenomenon of cellular ageing [144,145]

Telomere shortening has been discussed as a biomarker for detecting the onset of multiple age related disorders including cardiovascular disease and diabetes [146], Alzheimer's [147], Parkinson's [148] and arthritis [149]. Telomere length has also been assessed in disease models of accelerated ageing including Werner's syndrome [150] and Hutchinson-Gilford Progeria (HGPS) [151]. 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 [152]. To conclude, telomere shortening has become a well-known, undisputed biomarker for ageing, but its role as a biomarker for detecting early pathological changes in age-related disorders such as cancer, requires clarification.

Case-control studies in cancer patients have strengthened the associations between telomere attrition, genomic instability and possible increased risk of cancer (Table 9.). Interestingly, this phenomenon may be identified systemically in cancer patients, as Broberg and colleagues reported significantly shorter telomeres in buccal cells harvested from patients with bladder cancer, when compared to cancer free controls [153]. A recent comprehensive review of over 23,000 cases has brought to light that the significance of telomere length as a marker for cancer is more complex [154]. This research presented a heterogeneous relationship across different cancer types, including a statistically significant correlation between decreased telomere length and increased risk of GI tract, head and neck cancers, but not skin cancers. Contrastingly, it was also reported longer telomere length was more likely to increase the risk of skin cancer, indicating that telomeres may play a more diverse, tissue-specific role [154]. Similarly, other studies have noted a non-linear relationship for cancers of the breast [155], pancreas [156] and oesophagus [157]. In the case of lung cancer, both telomere shortening [158] and lengthening [159] have been described as risk factors. The findings

from these studies indicate that telomere length is an important marker of genomic instability where excessive shortening or lengthening may both be drivers or indicators of cancer development. 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 [143]. 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. Increased telomere dysfunction has also been identified alongside chromothripsis in patients with increased susceptibility to developing cancer (sufferers of L-Fraumeni syndrome) [160] suggesting an additional role for telomere attrition in increased catastrophic DNA re-arrangements. However, there is a greater need for more case-control studies to confirm these speculations and to test the applicability of telomere length as a biomarker for tissue-specific cancer risk.

Initial reports of telomere shortening in obesity were presented in a study conducted on 561 female twins and linked increased adiposity with a risk of up to 8.8 years of ageing [161]. Interestingly, a subsequent study published by Nordfjäll and colleagues confirmed these findings with a variety of obesity parameters including BMI, weight, and waist and hip circumference [162]. Although, the study reported a gender bias as these findings were only statistically significant in women. In 2009, an extensive study by Kim and collaborators also found an inverse correlation between weight gain and telomere length in 647 women [163]. Shorter telomere length has also been reported in cells of subcutaneous adipose tissue extracted from obese men and women [164]. 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 (Table 8). However, discrepancies in the evidence do exist, as some studies have found little or no association between multiple measures of adiposity and telomere dysfunction in women [165]. This highlights that telomere maintenance is a highly regulated and complex process affected by other physiological factors such as menstrual status, as well as environmental factors.

A study that established a link between obesity and telomere shortening in adults, was unable to verify this phenomenon in obese children [166]. However, subsequent studies notoriously reported findings of telomere shortening in obese children. Al-Attas and collaborators presented an inverse correlation between obesity and telomere length in boys, but not girls [167]. A subsequent, large case-control study (n=793) identified a 25% reduction in telomere length in obese children when compared to non-obese healthy controls [168]. Interestingly, Zhu and colleagues did not find an association between adiposity and telomere length in 667 adolescents aged between 14 and 18 years [169]. An extensive meta-analysis by Park and colleagues raised an interesting association between obesity in childhood and increased risk of morbidities and mortality in adulthood, independent of weight loss [170]. The associations between increased adiposity in childhood and telomere shortening are compelling, and further evidence is required to conclude upon the degree and consequences of telomere dysfunction in such obesity.

It is of interest that increased markers of inflammation have been exhibited alongside telomere attrition in obese cancer patients [171]. A high BMI in individuals with obesity-associated polymorphisms correlated with increased levels of CRP and decreased telomere length [172]. Telomere shortening was also seen in conjunction with increased CRP and adiposity in a more recent investigation [173]. This research showed BMI, waist circumference, and body fat percentage to be inversely correlated with telomere length in adults. These findings strengthen the hypothesis for chronic, low-grade inflammation as a possible etiological route to DNA damage in obese subjects.

7. Genetic Determinants of Obesity and DNA damage

Genome wide association studies (GWAS) have indicated a number of single nucleotide polymorphisms (SNPs) to be associated with obesity. Of these, particular polymorphisms (such as rs9939609) in the fat mass and obesity-associated (FTO) gene, have been confirmed to increase the

risk of non-syndromic obesity [174]. Such genetic polymorphisms are likely to alter the activity of metabolic hormones, leading to reduced feelings of satiety and an increased caloric intake [175].

It has been investigated whether such genetic determinants can be responsible for the increasing evidence of DNA damage in obesity. A study evaluated the frequency of polymorphisms in genes coding for ghrelin (rs26802), ghrelin receptor (rs572169), leptin (rs7799039), leptin receptor (rs1137101) and FTO (rs9939609) in 300 morbidly obese Caucasian women alongside 300 controls [176]. These individuals were also investigated for oxidative DNA damage via the comet assay. Obesity was associated with a significantly increased frequency of the AA-FTO genotype allele A, decreased frequency of the TT-FTO genotype, and elevated DNA strand breaks. However, the degree of DNA damage was independent of genotype, indicating that these particular polymorphisms may not play an etiological role in the development of DNA damage in obese subjects, and suggests the damage may be caused by other pathological states such as low grade inflammation and oxidative stress. However, variants of the FTO have also been associated with an increased risk of breast cancer in African American [177] but not Caucasian women [178]. This suggests SNP associations and their consequences in obesity display strong ethnic variations.

A population-based study discovered variants in the DNA repair genes *MGMT* (rs12917), *MSH2* (rs3732182 and rs4583514), *MSH3* (rs1650663), *XPG* (rs17655), and *XRCC1* (rs25487) that code for BER, NER and MMR pathways to elevate the risk of postmenopausal breast cancer in obese women [179]. Simultaneously, the research found obese women to be at increased risk of oxidative stress if they possess a polymorphism in the *MPO* gene. Similar findings were outlined in a meta-analysis that concluded polymorphisms of *GPX1* and *GPX7* genes that are associated with higher body fat and insulin resistance, contribute to oxidative stress in obesity, by lowering anti-oxidant defence [180]. Therefore, there is a possibility that certain genetic variants that predispose to obesity also promote pathogenesis of oxidative stress and DNA damage. If this pathological process is coupled with a lack of sufficient DNA repair, then it may increase the risk of certain cancers.

Since genetic determinants of TL in adults do not correspond with TL in children, and genetic determinants of TL remain unconfirmed [181], there is a likelihood that telomere length shortening in children may be associated with obesity related ROS rather than genetic make-up. However, there is a greater need for further studies to identify the relationship between telomere length in obesity and the long-term risk of pathological conditions.

8. Weight loss interventions and DNA Damage

Decreasing adiposity to a certain degree may improve oxidative stress and DNA health in obesity. Bariatric surgery is an established method of weight-loss which has shown positive implications on obesity associated complications, including cardiovascular disease [182], obstructive sleep apnoea [183], non-alcoholic fatty liver disease [184] and a rapid improvement in type 2 diabetes [185]. Bariatric surgery has also been suggested to reduce the risk of cancer by restoring genome stability and integrity [186–188]. 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 [189]. The same surgical procedure has demonstrated a reduction in DNA double strand breaks with decreased levels of y-H2AX positive cells up to 7 years after surgery [190] and restoration of telomere length in obese men [191]. However, a study conducted on 107 obese subjects with and without metabolic syndrome reported no extension in telomere length after a 12 month follow up of bariatric surgery [192]. Although more recently, Bankoglu and colleagues reported a reduction in DNA breaks assessed by the comet assay in 56 blood samples 12 months after surgery [193]. Therefore, bariatric surgery appears to be propitious for improving DNA integrity and stability in obese adults. Whether such is the case in severe adolescent obesity could be explored.

Dietary and lifestyle modifications have also been associated with a reduction of DNA damage in obesity. Following a two month intervention programme, governed by improvements in diet and physical activity levels, increased telomere length in 74 obese adolescents [194]. Similarly, dietary

modifications and consequent weight loss also reduced DNA damage in obese women with PCOS [195]. From these studies it can be deduced that DNA damage in obesity may be a reversible phenomenon. Although, it is also possible that gene mutations that have been induced and accumulated during the phase of obesity may persist beyond weight-loss.

9. Conclusion

Oxidative stress in obesity poses a significant threat to DNA stability and integrity as indicated by the growing number of investigations reporting a positive correlation between markers of oxidative DNA damage and increased adiposity. These associations raise concerns that the obese state may manifest with accelerated genome ageing and promote carcinogenesis. Monitoring of acquired DNA damage may become an important part of clinical investigations in the management of obesity, alongside markers of inflammation and micro nutritional deficiencies in order to detect pre-pathological changes. Potential benefits of monitoring 'genome health' in obesity include early detection of pre-cancerous alterations to establish urgency of personalised intervention measures and assess their progress. These avenues should be explored as DNA damage in obesity appears to be a reversible phenomenon.

Declaration of Interest: none

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Table 1. Associations between micronuclei (MNi) frequency and site-specific cancers.

Cancer Site	Study Population	Cell type USED for Micronuclei (MNi) test	Key Findings	Authors, Date
Bladder	158 patients/ 158 controls (age matched)	PBLs	Increase in MN frequency associated with increased risk of cancer	Pardini *et al.*, 2017 [196]
Breast	91 patients/ 96 controls	PBLs	Higher frequency of MNi in breast cancer patients	Varga *et al.*, 2006 [197]
	45 patients/ 85 controls (age matched)	PBLs	Higher frequency of MNi in breast cancer patients	Santos *et al.*, 2010. [198]
	220 patients/ 295 controls	PBLs	No significant difference of MNi frequency between patients and controls	Bolognesi *et al.*, 2014 [199]
	Follow up of 1650 adults	PBLs	Higher frequency of MNi at baseline in cancer patients.	Murgia *et al.*, 2008 [78]
Colorectal	25 cancer patients/ 26 polyp patients/ 31 controls	PBLs	2.1x higher frequency of MNi in cancer patients than controls. Polyp patients had 1.5x higher frequency of MNi than controls.	Maffei *et al.*, 2014 [200]
Encephalon	Follow up of 1650 adults	PBLs	Higher frequency of MNi at baseline in cancer patients.	Murgia *et al.*, 2008 [78]
Endometrial Cancer	59 patients/ 59 controls	Cervical squamous epithelial cells	20% higher frequency of MNi in patients.	Aires *et al.*, 2011 [201]
	20 endometrial hyperplasia patients/ 20 cancer patients/ 20 controls	PBLs	2.9x higher frequency of MNi in women with endometrial cancer.	Kiraz *et al.*, 2016 [202]
Lung	216 small cell lung cancer patients/ 173 Non-small cell lung cancer	PBLs	Higher MNi frequency in lung cancer patients.	El-Zein *et al.*, 2006 [203]

	patients/ 204 controls			
Lymphoma	Follow up of 1650 adults	PBLs	Higher frequency of MNi at baseline in cancer patients.	Murgia et al., 2008 [78]
Oral	Follow up of 1650 adults	PBLs	Higher frequency of MNi at baseline in cancer patients.	Murgia et al., 2008 [78]
Pancreatic	346 patients/ 449 controls	PBLs	1.6x higher MNi frequency in pancreatic cancer patients.	Chang, Li and Li, 2011 [204]
Stomach	6718 adult participants across 20 sites	PBLs	Higher frequencies of MNi were associated with increased risk of stomach cancer.	Bonassi *et al.*, 2007 [205]
Urogenital	6718 adult participants across 20 sites	PBLs	Higher frequencies of MNi were associated with increased risk of urogenital cancer.	Bonassi *et al.*, 2007 [205]

Table 2. Associations between micronuclei (MNi) frequency and adiposity

Date	**Study type**	**Study population**	**Cell type for MNi test**	**Key findings**	**Authors**
2006	Case control	19 PCOS patients/19 controls	PBLs	Higher frequency of MNi in PCOS patients. Positive correlation between BMI and MNi frequency.	Yesilada *et al.*, [84]
2008	Case control	14 PCOS patients/16 controls (Age & overweight matched)	PBLs	Higher frequency of MNi in overweight PCOS patients. Insulin resistance was positively associated with aneuploidy of X chromosomes.	Moran *et al.*, [206]
2010	Case control	36 overweight PCOS patients/ 29 controls	PBLs	Higher frequency of MNi in overweight PCOS patients.	Hamurcu *et al.*, [86]
2011	Case-control	60 obese/ 20 overweight/ 38 children controls	PBLs	Higher frequency of MNi and y-H2AX foci in overweight and obese children.	Scarpato *et al.*, [87]

2014	Case-control	83 obese/ 21 overweight/ 21 healthy weight adult controls	PBLs	BMI not WHR is positively correlated with MNi frequency in all subjects.	Donmez-Altuntas *et al.*, [86]
2016	Meta-analysis	8 studies covering 47 mammalian species	Erythrocytes	Body mass is positively correlated with MNi frequency.	Croco *et al.*, [90]
2016	Case-control	18 Diet-induced obesity (DIO) mice/ 18 non-DIO control mice	Erythrocytes and reticulocytes	No difference in MNi frequency between DIO and non-DIO mice. Erythrocytes showed an increase in mutant cells (*Pig-a).*	Wickliffe *et al.*, [91]
2017	Longitudinal study	87 pregnant women and their fetal offspring	PBLs	Positive correlation of birth weight and length with MNi frequency, nucleoplasmic bridges and nuclear buds.	Singh *et al* [88]
2017	Correlation	300 Korean men and women aged 30-59 years	PBLs	Positive correlation between age and MNi but not BMI.	Cho *et al* [89]

Table 3. Associations between 8-OHdG concentration and site-specific cancers.

Cancer Site	**Study Population**	**Tissue type for 8-OHdG test**	**Key Findings**	**Authors, Date**
Buccal mucosa	30 patients/ 30 controls	Urine	8-OHdG higher in cancer patients.	Murugaiyan *et al.*, 2015 [207]
Breast	49 patients/ 49 controls	Serum	8-OHdG higher in cancer patients.	Himmetoglu *et al.*, 2009 [209]
	60 patients/ 60 controls	Urine	8-OHdG higher in cancer patients.	Kuo *et al.*, 2007 [208]
Colo-rectum	36 patients/ 40 controls	Serum	8-OHdG higher in cancer patients.	Chang *et al.*, 2008 [210]
	84 patients/ 142 controls	Urine	8-OHdG higher in cancer patients.	Guo *et al.*, 2016 [211]

Oeosophagus	18 patients/ 12 controls	Serum	8-OHdG higher in cancer patients.	Diakowska *et al.*, 2007 [212]
Ovaries	84 patients	Serum and tumour tissue	High 8-OHdG levels (>140pg/ml) are associated with poorer prognosis.	Pylvas *et al.*, 2011 [213]
Prostate	82 patients/ 82 controls	Urine	8-OHdG higher in cancer patients.	Miyake *et al.*, 2004 [214]

Table 4. Associations between 8-OHdG concentration and adiposity

Date	Study type	Study population	Tissue type for 8-ohdG test	Key findings	Authors
2006	Case control	27 non-obese hernia patients	Skeletal Muscle	Weight gain was associated with increased 8-OHdG.	*De la Maza et al.*, [107]
2006	Correlation	99 young adults	PBLs	No correlation between BMI and 8-OHdG.	Hofer, Karlsson and Möller, [108]
2007	Longitudinal	174 healthy adults	Urine	A decrease in BMI by 1.0 was associated with a 2.7% increase in 8-OHdG.	Mizoue *et al.*, [109]
2011	Correlation	103 adolescents	Serum	BMI was positively correlated with 8-OHdG.	El Wakkad *et al.*, [111]
2014	Case control	83 obese/ 21 overweight/ 21 control adults	Plasma	8-OHdG concentration was lower in the obese group.	Donmez-Altuntas *et al.*, [86]
2014	Correlation	159 healthy children	Urine	BMI was inversely correlated 8-OHdG.	Protano *et al.*, [112]
2015	Case control	24 obese/28 control children	Urine	Higher 8-OHdG in obese children.	Ramachandra et al. [113]
2016	Case control	63 obese hypertensive/40 obese-normotensive/ 27 control men	Urine	No significant difference in 8-OHdG.	Cejvanovic *et al.*, [110]

Table 5. Associations between γ-H2AX frequency and site-specific cancers.

Cancer Site	Study Population	Tissue/Cell type for γ-H2AX test	Key Findings	Authors, Date
Breast	31 patients and 89 control	Breast	γ-H2AX higher in cancer patients.	Zhou *et al.,* 2013 [120]
Bladder	94 patients and 8 controls	Bladder	γ-H2AX higher in cancer patients.	Bartkova *et al.,* 2005 [121]
GI tract	320 normal and 320 controls	Lymphocytes	γ-H2AX higher in colorectal cancer patients.	Zhao *et al.,* 2017 [215]
	92 cancer patients	Colorectal	γ-H2AX associated with poor cancer prognosis.	Lee *et al.,* 2015 [216]
	121 cancer patients	Gastric epithelial cells	γ-H2AX higher in cancerous epithelial tissue.	Kim et al., 2010 [217]
Ovaries	87 patients and 20 controls	Epithelial ovarian	γ-H2AX higher in cancer patients.	Mei *et al.,* 2015 [122]
Skin	9 benign and 108 cancer tissues	Melanocytes	γ-H2AX higher in cancer tissue.	Wasco *et al.,* 2008 [218]
Thyroid	30 benign and 153 cancer tissues	Thyroid	γ-H2AX higher in cancer tissue.	Hu *et al.,* 2015 [219]

Table 6. Associations between γ-H2AX frequency and adiposity

Date	Study type	Study population	Tissue/Cell type for γ-H2AX test	Key findings	Authors
2010	Correlation	29 males/1 female undergoing bariatric surgery	Adipocytes and endothelial cells	Endothelial cells from visceral adipose tissue had greater γ-H2AX positive foci than Subcutaneous adipose tissue.	*Villaret et al.,* [128]
2011	Case control	61 obese/ 20 overweight/ 38 control children	PBLs	Higher γ-H2AX positive foci in overweight and obese cohorts.	Scarpato *et al.,* [87]
2016	Case control	23 obese/ 15 control adolescents	PBLs	Higher γ-H2AX positive foci in obese cohort.	Azzarà *et al* [129]
2017	Case control	Obese and healthy weight 3-4 weeks old	Pancreas Lung	Higher γ-H2AX-positive foci in obese 9 to 13-week-old obese	Azzarà *et al* [130]

		rats and 9-13 weeks old rats.	Esophagus Kidney Gut	rats. No differences in the 3-4 week cohorts.	

Table 7. Associations between comet tails and and site-specific cancers

Cancer Site	Study Population	Cell type for comet assay	Key Findings	Authors, Date
Breast	70 patients and 70 controls	PBLs	Comet tails were significantly higher in cancer patients.	Smith *et al.*, 2003 [142]
	35 patients, 15 first degree relatives and 15 controls	PBLs	Comet tails were significantly higher in cancer patients and first degree relatives.	Kattimuthu et al., 2012 [220]
Bladder	114 patients and 145 controls	PBLs	Comet tails were significantly higher in cancer patients.	Schabath *et al.*, 2003 [221]
Buccal Mucosa	54 patients and 52 controls	PBLs	Comet tails were significantly higher in cancer patients.	Katarkar *et al.*, 2014 [134]
	55 patients and 55 controls	PBLs	Comet tails were significantly higher in cancer patients.	Rawat et al., 2018 [222]
Uterus	106 patients and 30 controls	PBLs	Comet tails were significantly higher in cancer patients.	Buchynska et al., 2017 [223]

Table 8. Associations between comet tails and adiposity

Date	Study type	Study population	Cell type for comet assay	Key findings	Authors
2003	Case control	70 breast cancer patients/ 70 controls	PBLs	BMI was positively correlated with comet tails in cancer patients but inversely correlated in controls.	Smith *et al.*, [142]
2006	Correlation	99 females (aged 19-31)	PBLs	BMI was positively correlated with comet tails.	Hofer *et al.*, [108]
2010	Case control	20 pregnant women/ 10 controls	Leukocytes	BMI was positively correlated with comet tails in pregnant women.	Bukhari *et al.*, [137]
2011	Case control	20 obese/ 20 normal weight	PBLs	Higher frequency of comet tails in obese and 'normal-	Tomasello *et al.*, [139]

		+ fat mass > 30%/ 20 control females		weight obese' cohorts.	
2012	Case Control	36 obese/15 control adults	Leukocytes	Higher frequency of comet tails in obese cohort.	Gandhi and Kaur [138]
2013	Case Control	Lean and obese Zucker rats at 3, 6, and 15 months old assigned to a sleep-deprived group or home-cage control (n=5 in each group)	PBLs	Higher frequency of comet tails in obese rats at 15 months old, irrespective of sleep status.	Tenorio et al., [140]
2015	Randomized, double-blind placebo controlled trial	50 adults	PBLs	Calorie restriction was associated with weight-loss and a decrease in comet tails.	Ibero-Baraibar *et al.,* [141]

Table 9. Associations between telomere length (TL) and site-specific cancers.

Cancer Site	**Study Population**	**Cell type and technique for telomere length test**	**Key Findings**	**Authors, Date**
Bladder	63 patients/ 158 controls	Buccal cells via qPCR	Shorter TL in cancer patients.	Broberg *et al.*, 2005 [153]
Breast	287 patients/ 350 sister controls	PBLs via qPCR	No significant difference in TL between groups.	Shen *et al.*, 2007 [224]
	601 patients/ 695 controls	PBLs via qPCR	Shorter TL in cancer patients.	Qu *et al.*, 2013 [155]
GI tract	396 patients/ 378 controls	PBLs via qPCR	Shorter TL in cancer patients.	Liu *et al.*, 2009 [225]
Lung	243 patients/ 243 controls	PBLs via qPCR	Shorter TL in cancer patients.	Jang *et al.*, 2008 [158]

	215 patients/ 215 controls	PBLs via qPCR	Shorter TL in cancer patients.	Lan *et al.*, 2013 [159]
Ovarian	99 patients/ 100 controls (age matched)	Leukocytes via qPCR	Shorter TL in cancer patients.	Mirabello *et al.*, 2010 [226]
Pancreas	499 patients/ 963 controls	PBLs via qPCR	Shorter telomeres and extremely long telomeres both related to increased pancreatic cancer risk.	Skinner *et al.*, 2012 [156]
Prostate	6 patients with prostate adenocarcinomas	Telomere Fluorescence in Situ Hybridization of prostate cells	Cells with high-grade prostatic intraepithelial neoplasia had shorter telomeres than normal cells in same tissue.	Meeker *et al.*, 2002 [227]
Skin	218 melanoma/ 285 squamous-cell carcinoma/ 300 basal-cell carcinoma (BCC) cases/ 870 controls	PBLs via qPCR	Short TL was associated with a decreased number of moles and decreased risk of melanoma and BCC.	Han *et al.*, 2009 [228]

Table 10. Associations between telomere length (TL) and adiposity

Date	Study type	Study population	Cell type & technique for telomere length test	Key findings	Authors
2005	Case control	119 obese/ 85 healthy weight female twins	PBLs via TRF	TRF length was lowest in the obese cohort and correlated with BMI and serum leptin.	Valdes *et al.*, [161]
2008	Correlation	989 adults	Leucocytes via qPCR	Inverse correlation between BMI and TL in women.	Nordfjäll *et al.*, [162]
2008	Case control	53 children/ 23 adults	PBLs via TRF	No difference in TL in children. Obese adults had shorter TL.	Zannolli *et al.*, [166]
2009	Correlation follow-up	647 female adults	Leucocytes via qPCR	High BMI and hip circumference inversely correlated with TL. Obese	Kim *et al.*, [163]

				females had shortest TL.	
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 *et al.*, [164]
2010	Correlation	317 adults (aged 40-64 years)	Leukocytes via qPCR	No significant correlations between BMI or visceral adipose tissue and TL.	Diaz *et al.*, [165]
2010	Correlation	2284 females	Leukocytes via qPCR	Waist circumference was inversely correlated with TL.	Cassidy *et al.*, [229]
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 *et al.*, [230]
2011	Case control	793 children (aged 2-17 years)	Leukocytes via qPCR	23.9% shorter TL in obese children.	Buxton *et al.*, [168]
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 *et al.*, [231]
2013	Correlation	2,912 females (aged 40-70 years)	Leukocytes via qPCR	TL inversely correlated with BMI, waist circumference, waist-to-height ratio, weight, and hip circumference but not waist to hip ratio.	Cui *et al.*, [232]
2016	Correlation	7527 adults (aged 20-84)	Leukocytes via qPCR	Telomere length inversely correlated with BMI, waist circumference, BF% and C-reactive protein.	Rehkopf *et al.*, [173]