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Chromosomal damage measured by the cytokinesis block micronucleus cytome assay in diabetes and obesity - A systematic review and meta-analysis

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ABSTRACT

The percentage of people affected by overweight, obesity and/or diabetes drastically increased within the last decades. This development is still ongoing, which puts a large part of our society at increased risk for diseases, such as cancer, cardiovascular diseases and cognitive impairment. Especially the development of type 2 diabetes and overweight/obesity could theoretically be prevented. The loss of DNA and genome stability is associated with the above-mentioned metabolic diseases. Insulin resistance, high blood glucose levels or increased body fat are linked to a chronically elevated inflammatory state. This amplifies oxidative stress, might lead to oxidative DNA damage, impairs the cellular proliferation process and results in mutations; all of which increase the possibility for the development of dysfunctional cells, tissue and organs. An established method to measure chromosomal damage is the cytokinesis block micronucleus (CBMN) cytome assay. The aim of this systematic review and meta-analysis is to collect and analyse the current literature of diabetic, obese and overweight patients and their link to cellular mutations measured by the CBMN assay. A clear trend towards increased genome damage in these metabolic diseases was observed. Significantly increased frequencies of chromosomal aberrations were seen in type 2 diabetic subjects (micronuclei frequency: SMD: 1.18, 95% CI: 0.76, 1.60; I^2 = 84%). In both, type 1 and type 2 diabetics, disease progression as well as medical quality and quantity were linked to further elevated genome instability. In type 1 diabetic and overweight/obese subjects the number of studies is small and for valid and reliable results more data are needed. Besides the traditionally used material for this method, PBMCs, we extended our analysis to buccal cells in order to qualitatively compare the two cell types. Finally, we discuss knowledge as well as technical/methodical gaps of the CBMN cytome assay and its usability for clinical practice in these metabolic diseases.

Contents

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1. Introduction

The percentage of people with overweight and obesity drastically increased in the last decades. Worldwide obesity has nearly tripled between 1975 and 2016. Currently, approximately 40% of adults over the age of 18 are overweight and 13% are obese [1]. Recent data from the most affected countries, UK and USA, report even higher prevalence rates for overweight (between 65–75%) and obesity (between 25 and 35%) in these countries. Projections suggest that in 2030 there will be 65 million more obese people in the USA, as compared to 2010; in the UK within the same time-period the number of obese people will increase by 11 million [2]. An increased Body Mass Index (BMI), which is predominantly caused by an over-accumulation of fat-tissue, is a major risk factor for the development of non-communicable diseases, such as cardiovascular diseases (globally the leading cause of mortality), diabetes (mainly type 2), musculoskeletal disorders (accelerated degeneration of the joints) and several kinds of cancers (including endometrial, breast, ovarian, prostate, liver, gallbladder, kidney, and colon) [1].

Increased bodyweight, predominantly caused by excess energy consumption and the lack of physical activity, is commonly linked to a disturbed metabolic profile [3,4], although not every overweight or obese individual shows increased metabolic risk markers [5]. Specifically, the development of type 2 diabetes represents a threat to overall health and quality of life [6]. Parallel to the fast increase of overweight and obesity, the global number of people with diabetes rose from 108 million in 1980 to 422 million in 2014. According to the world health organization, 1.6 million deaths are directly caused by diabetes, and about 50 percent of all deaths, which were associated to high blood glucose, occurred before the age of 70 years [7]. Furthermore, diabetes is strongly associated with the development of cancer or cardiovascular diseases and one of the leading causes of kidney failure [6].

The metabolic syndrome is a clinical diagnosis tool, which combines the most dangerous heart attack risk factors: diabetes and increased fasting plasma glucose, abdominal obesity, high cholesterol and high blood pressure [8,9]. Consequently, overweight/obesity as well as diabetes (mainly type 2) are the main drivers for the diagnosis of the metabolic syndrome, which is linked to increased medication, reduced quality of life, dependency on others with advanced age and a reduction of years lived without disability [8].

Obesity, poor blood glucose control, insulin resistance and an impaired blood lipid profile are all associated with a chronically increased inflammatory state [10–12]. Chronic inflammation amplifies oxidative stress (by an accumulation of reactive oxygen and nitrogen species (RONS) and reduced antioxidant defense), which can generate oxidative DNA damage and further leads to increased genome instability (Fig. 1). Damaged chromosomes can negatively influence the cellular proliferation process, induce mutations and lead to cellular, tissue and organ malfunction, all of which are linked to accelerated disease development and premature aging [13,14]. Noteworthy, both conditions, type 2 diabetes and obesity, could, at least partly, be reversed by targeted lifestyle improvements, including physical activity and nutrition [15,16], leading to favorable metabolic changes as well as enhanced

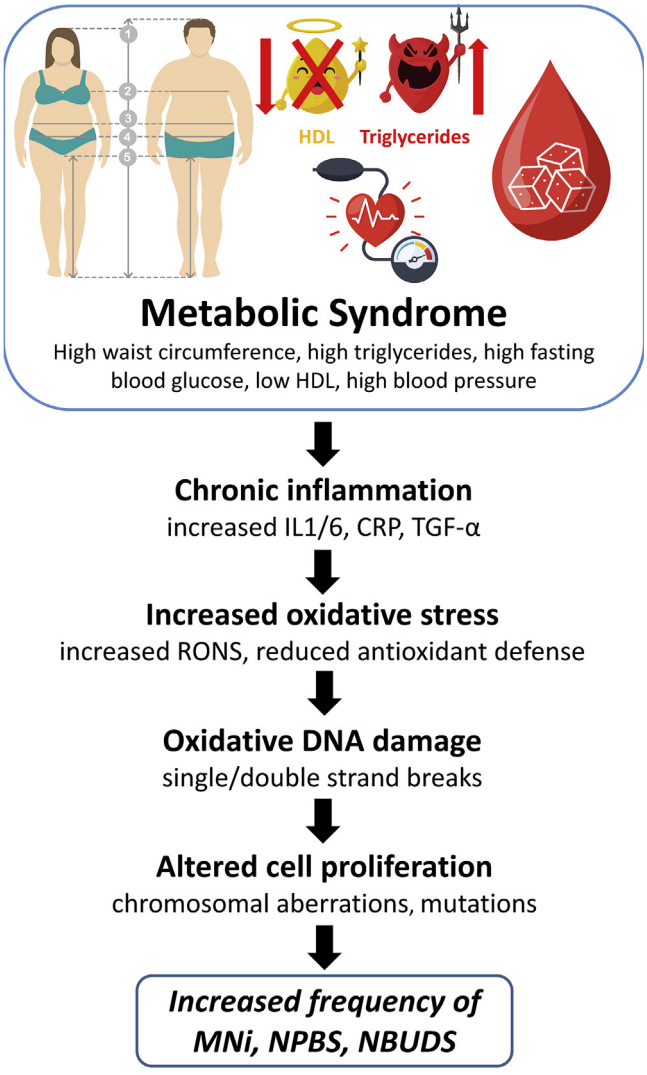

Fig. 1. Link between metabolic syndrome and markers of the CBMN cytome assay.

genomic stability, which in turn is associated with a lower prevalence of chronic diseases [11].

The cytokinesis block micronucleus (CBMN) cytome assay is an established method to analyze chromosomal aberrations, genome mutations, cytostatic effects or cellular cytotoxicity which occur during cell division. The most discussed markers of this assay are micronuclei (MNi), nucleoplasmic bridges (NPBS) and nuclear buds (NBUDS) [17,18]. For this review and meta-analysis we only used data from human studies with in vivo application of the micronucleus assay.

Therefore, the purpose of this systematic review and meta-analysis is to investigate the current literature on type 1 and type 2 diabetes, overweight and obesity and their associations with MNi, NBUDs and NPBs. Our primary focus hereby was on observational studies; randomized controlled trials (RCTs) with adequate baseline data were also included, to identify knowledge gaps and potential ways for future research as well as possible ways for the application of the CBMN cytome assay within these diseases. The utility of MNi, NPBS and NBUDS as possible markers for disease severity and/or early disease risk estimation will be discussed, specifically in the context of clinical usability.

2. Materials and methods

2.1. Literature search strategy, eligibility criteria and study selection methods

The literature search was performed in the electronic database PubMed until April 15th 2020 with no restriction of language and calendar date using a pre-defined search strategy. The reference lists from eligible studies were screened to identify additional relevant research. Screening and study selection (Fig. 2) were conducted by two authors independently (BF, KHW).

2.2. Selection of studies

Studies were included in the systematic review if they met the following criteria:

1. Randomized controlled trials with adequate baseline data (RCTs; with a parallel or cross-over design);
2. Cross sectional studies;
3. Case-control studies;
4. Only studies from human trials (all available age-groups) were analyzed;
5. Study subjects had to be categorized into a) overweight and/or obese, or b) diabetes type 1 or type 2, and c) healthy controls;
6. Chromosomal damage was measured by the CBMN cytome or the buccal micronucleus cytome (BMcyt) assay;
7. At least data from one of the main parameters (MNi, NBUDS and/or NPBS) of the CBMN cytome or the BMcyt assay must be available.

The following studies were excluded:

i) Other methods than the CBMN cytome assay were used to detect genome damage;
ii) Severe methodological weakness (e.g. inadequate number of cells was counted);
iii) Studies, which did not investigate human subjects;
iv) RCTs with inadequate baseline analysis.

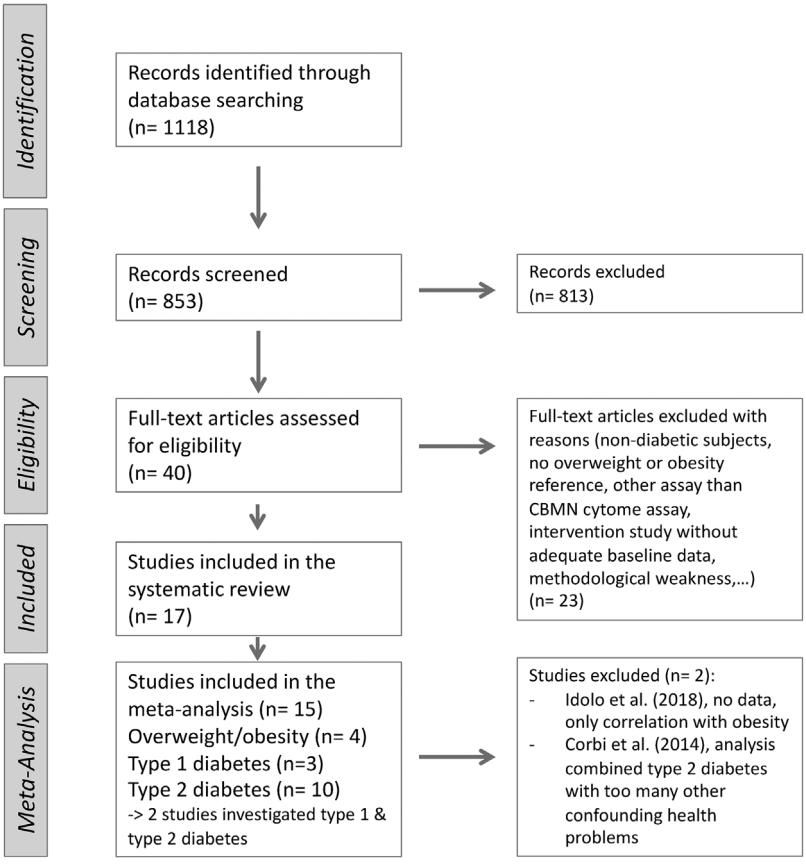

Fig. 2. Study selection flow chart.

2.3. Data extraction

For included studies, two reviewers independently (BF, KHW) extracted the following characteristics: name of first author, year of publication, study origin (country), study design (RCTs; cross-sectional, case-control), number of participants, disease status (i.e. healthy, type 1 or 2 diabetes, obesity), mean age, mean Body Mass Index (BMI), % type 2 diabetics, % female, outcome data, and conflict of interest. The preferred outcome data (Frequency of MNi, NPBS, NBUDS) were values with corresponding standard deviations, standard errors or 95% CI.

2.4. Data synthesis

2.4.1. Statistical analysis

The disease group (diabetes; overweight/obese) vs. control values were pooled as standardized mean differences (SMDs) using a random effects model for each continuous outcome separately. For all outcomes SMDs were calculated, (due to different measurement methods used across included studies). The magnitude of the SMD was interpreted as follows [19]: small/minor SMD: 0.2 or less; medium SMD: 0.2 to 0.8; large SMD: 0.8 or greater.

Heterogeneity in meta-analyses was tested with a standard χ^2 test. The I^2 parameter was used to quantify any inconsistency: $I^2 = \left(\frac{Q-df}{Q}\right) \times 100\%$, where Q is the χ^2 statistic and df is its degrees of freedom [20]. An I^2-value of greater than 50% was considered to represent considerable heterogeneity [21]. Meta-analyses were conducted using Review Manager (RevMan) Version 5.3 [22].

The investigated outcomes were presented as forest plots by considering: disease status (overweight and/or patients with obesity vs. normal weight), diabetic status (type 1 or 2 diabetes vs. control), cell/tissue types (peripheral blood mononuclear cells (PBMCs) vs. buccal cells).

3. Results

3.1. Literature search outcomes

After screening the available literature on MNi, NPBS and NBUDS and their link to overweight/obesity and diabetes, the studies were evaluated according to inclusion and exclusion criteria, as described above. A total of 15 (obesity/overweight n = 4, diabetes n = 11) studies were identified, which were enclosed for meta-analysis (Fig. 2). Three studies, all of which were done in diabetics, performed the MNi assay in buccal cells; the others examined genome stability in PBMCs (Tables 2 and 3; Fig. 1 to 3). Only one study analyzed both cell types, buccal cells and PBMCs [23,24].

We did not find any study about the link between the clinical multi-factorial diagnosis of metabolic syndrome and the CBMN cytome assay.

3.2. Description of the cross-sectional studies results

Six cross-sectional studies [25–30], four about obesity and overweight and two analyzing diabetes, were identified as being suitable for these analyses (Tables 1 and 2). Studies using the CBMN cytome assay to compare between overweight and normal weight subjects are rare, which is also true for studies in diabetics.

3.2.1. Results for overweight and obesity

Three of the four studies with overweight/obese participants showed a positive association with chromosomal damage (Table 1). Donmez-Altuntas et al. [25] investigated 125 (21 normal weight, 21 overweight, 84 obese), mid-aged women and men and observed significant correlations between BMI and the three parameters, MNi, NPBS and NBUDS. Obese subjects demonstrated significantly higher frequencies of genome damage, than normal weight and overweight subjects.

Table 1
Cross-Sectional studies observing MNi, NPBS and NBUDS frequencies in overweight and obesity.

Study ID	Obesity	MNi per 1000 cells			
		Cases		Controls	
		N	Mean (SD)	N	Mean (SD)
Donmez-Altunas et al. 2014 [25]	Normal weight Overweight Obese	Overweight = 21 Obese = 83	Overweight = 8.10 (3.30) Obese = 12.40 (4.50) * +	21	7.10 (5.10)
Li et al. 2015 [26]	Normal weight Overweight Obese	Overweight = 448 Obese = 39	Overweight = 3.27 (2.47) * Obese = 3.68 (3.07)	777	3.70 (2.69)
Scarpato et al. 2010 [27]	Normal weight Overweight Obese (children)	Overweight = 20 Obese = 61	Overweight = 2.30 (1.10) * Obese = 2.44 (1.79) *	38	0.93 (0.73)
Santovito et al. 2020 [28]	Normal weight Overweight	Overweight = 39	8.77 (2.40) *	111	6.61 (2.25)
Study ID	Obesity	NPBS per 1000 cells			
		Cases		Controls	
		N	Mean (SD)	N	Mean (SD)
Donmez-Altunas et al. 2014 [25]	Normal weight Overweight Obese	Overweight = 21 Obese = 83	Overweight = 20.0 (9.2) Obese = 30.4 (17.7) * +	21	18.6 (8.9)
Santovito et al. 2020 [28]	Normal weight Overweight	Overweight = 39	2.18 (1.32) *	111	1.47 (1.57)
Study ID	Obesity	NBUDS per 1000 cells			
		Cases		Controls	
		N	Mean (SD)	N	Mean (SD)
Donmez-Altunas et al. 2014 [25]	Normal weight Overweight Obese	Overweight = 21 Obese = 83	Overweight = 9.9 (4.3) Obese = 16.4 (8.4) * +	21	7.2 (3.4)
Santovito et al. 2020 [28]	Normal weight Overweight	Overweight = 39	2.54 (1.64) *	111	1.9 (1.77)

Significant difference to controls . . . *; significant difference to overweight group . . . +.

Table 2
Cross-Sectional studies observing MNi, NPBS and NBUDS frequencies in diabetes type 1 and type 2.

Study ID	Diabetes	MNi per 1000 cells			
		Cases		Controls	
		N	Mean (SD)	N	Mean (SD)
Grindel et al. 2017 [30]	HbA1C <7.5 HbA1C >7.5 Control (BUCCAL CELLS)	HbA1c <7.5% = 74 HbA1c >7.5% = 72	HbA1c< 7.5% = 0.84 (0.40) * + HbA1c>7.5% = 1.85 (1.40) * +	15	0.20 (0.40)
Cinkilic et al. 2009 [29]	Type 1 diabetes Control	35	0.92 (0.58)	15	0.77 (0.32)

Study ID	Diabetes	NPBS per 1000 cells			
		Cases		Controls	
		N	Mean (SD)	N	Mean (SD)
Cinkilic et al. 2009 [29]	Type 1 diabetes Control	35	2.43 (1.33)	15	2.20 (0.86)

Study ID		NBUDS per 1000 cells			
		Cases		Controls	
		N	Mean (SD)	N	Mean (SD)
Grindel et al. 2017 [30]	HbA1C <7.5 HbA1C >7.5 Control (BUCCAL CELLS)	HbA1c <7.5 % = 74 HbA1c >7.5% = 72	HbA1c< 7.5 % = 2.27 (1.00) HbA1c> 7.5% = 3.08 (1.20)	15	N.A.
Cinkilic et al. 2009 [29]	Type 1 diabetes Control	35	0.49 (0.56)	15	0.33 (0.49)

Significant difference to controls ... *; significant difference to other group of cases ... +.

Table 3
Case-Control studies observing MNi, NPBS and NBUDS frequencies in diabetes type 1 and type 2.

Study ID	Type of disease investigated	MNi per 1000 cells			
		Cases		Controls	
		N	Mean (SD)	N	Mean (SD)
Müllner et al. 2013 [23]	Type 2 diabetes Control	76	21.9 (8.0)	21	22.9 (7.99)
Müllner et al. 2014 [24]	Type 2 diabetes Control (BUCCAL CELLS)	76	0.71 (0.38) *	21	0.32 (0.35)
Palazzo et al. 2012 [31]	Type 2 diabetes Control	22	10.28 (4.3) *	22	6.81 (3.3)
Prasad et al. 2015 [32]	Type 2 diabetes Control	20	5.0 (5.0) *	42	0.3 (0.5)
Salimi et al. 2016 [33]	Type 2 diabetes Control	50	8.16 (1.47) *	50	5.82 (2.17)
Shettigar et al. 2012 [34]	Type 2 diabetes Control	25	11.4 (4.3)	24	10.3 (3.3)
Martinez-Perez et al. 2007 [35]	Type 2 diabetes Control	15	6.53 (2.03) *	10	3.10 (1.79)
Binici et al. 2013 [36]	Type 2 diabetes Control	50	3.45 (1.01) *	30	1.79 (0.67)
Gómez-Meda et al. 2016 [37]	Type 1 diabetes Type 2 diabetes Control (BUCCAL CELLS)	T1D = 32 T2D = 23	T1D = 1.17 (0.66) * + T2D = 1.43 (0.84) * +	45	0.48 (0.55)
Quintero Ojeda et al. 2018 [38]	Type 1 diabetes Type 2 diabetes Control (BUCCAL CELLS)	T1D = 10 T2D = 40	T1D = 0.75 (0.31) * T2D = 0.52 (0.27) *	40	0.07 (0.06)

Study ID	Type of disease investigated	NPBS per 1000 cells			
		Cases		Controls	
		N	Mean (SD)	N	Mean (SD)
Müllner et al. 2013 [23]	Type 2 diabetes Control	76	1.73 (1.36)	21	1.73 (0.99)
Palazzo et al. 2012 [31]	Type 2 diabetes Control	22	0.84 (1.48)	22	0.84 (0.62)
Prasad et al. 2015 [32]	Type 2 diabetes Control	20	5.0 (4.0) *	42	0.7 (0.8)
Salimi et al. 2016 [33]	Type 2 diabetes Control	50	5.40 (2.18) *	50	1.84 (1.04)

Study ID	Type of disease investigated	NBUDS per 1000 cells			
		Cases		Controls	
		N	Mean (SD)	N	Mean (SD)
Müllner et al. 2013 [23]	Type 2 diabetes Control	76	4.50 (2.50)	21	4.54 (3.42)
Müllner et al. 2014 [24]	Type 2 diabetes Control (BUCCAL CELLS)	76	0.72 (0.74)	21	0.58 (0.94)
Palazzo et al. 2012 [31]	Type 2 diabetes Control	22	10.91 (2.97) *	22	1.78 (1.81)
Prasad et al. 2015 [32]	Type 2 diabetesm Control	20	4.0 (3.0) *	42	0.6 (0.8)
Salimi et al. 2016 [33]	Type 2 diabetes, Control	50	1.56 (0.83) *	50	1.00 (0.82)
Gómez-Meda et al. 2016 [37]	Type 1 diabetes Type 2 diabetes Control (BUCCAL CELLS)	T1D = 32 T2D = 23	T1D = 1.34 (1.32) * + T2D = 1.65 (1.72) * +	45	0.89 (1.17)
Quintero Ojeda et al. 2018 [38]	Type 1 diabetes Type 2 diabetes, Control (BUCCAL CELLS)	T1D = 10 T2D = 40	T1D = 1.24 (0.48) T2D = 2.40 (1.80) *	40	0.76 (0.42)

Significant difference to controls ... *; significant difference to other group of cases ... +.

A very recent study of Santovito et al. [28] examined 150 (111 normal weight, 39 overweight) women (n = 84) and men (n = 66) with a mean age of 30.6 years. In this study BMI correlated with MNi, NPBS and NBUDS, with overweight subjects showing significantly higher levels of chromosomal damage compared to normal weight participants.

A study in 119 (38 normal weight, 20 overweight, 61 obese) children (63 females, 56 males), with a mean age of 11.0 ± 3.0 years, showed significantly higher frequencies of MNi in obese and overweight children, as compared to the normal weight group [27]. Higher levels of genome damage were further associated with increased inflammation markers (C-reactive protein, interleukin-6, tumor necrosis factor-α).

In contrast, a large study (n = 1333, age = 42.6 ± 8.5 years) by Li et al. [26] observed a significantly lower frequency of MNi in male subjects with BMI ≥ 25 kg/m^2 compared to the normal weight group, which was also supported by a reduced risk of getting lung cancer in the overweight group (Table 1).

3.2.2. Results for type 1 and type 2 diabetes

Grindel et al. [30] investigated genome stability in buccal cells of 146 female diabetes type 2 patients (age = 76.5 ± 9.9 years) and 15 healthy controls, reporting significantly lower frequencies of MNi and NBUDS in the latter group. Furthermore, the patients were divided into two groups based on their HbA1c being higher or lower than 7.5%. Those with worst managed blood glucose (HbA1c> 7.5%) showed significantly higher frequencies of chromosomal damage than subjects under 7.5%, and both groups significantly differed from the control group (Table 2). Additional analyses indicated a link between medication intensity and MNi frequency. In diabetes type 2 patients lowest to highest rates of genome damage were observed from no medication or non-insulin monotherapy, over non-insulin combination therapy, to insulin medication.

A study on 35 (20 males, 15 females, age = 31.9 ± 10.0 years) type 1 diabetics and 15 age- and sex-matched, healthy controls found no difference in MNi frequency in-between the study groups, despite observing a higher frequency of sister chromatid exchanges in the group of diabetic subjects [29].

3.2.3. Description of the case-control studies results

We identified nine case-control studies [23,24,31–38] that fitted our criteria, all of which were performed in diabetic patients. No case-control studies in obese and/or overweight subjects were found. Müllner et al. [23,24] did their analysis in buccal cells and PBMCs.

3.2.4. Results for type 1 and type 2 diabetes

Müllner et al. [23] investigated the frequency of chromosomal damage in PBMCs of type 2 diabetics compared to non-diabetic controls (partners of the diabetic study participants). They observed no statistical difference regarding any parameter of the CBMN cytome assay between the two groups. However, they could see significantly higher fasting plasma glucose and glycated haemoglobin levels in individuals with a MNi frequency over the 50th percentile. Within the same study population, Müllner et al. [24] performed the BMcyt assay to assess genome stability in buccal cells. Contrary to PBMCs, cytogenetic damage was significantly higher in diabetic subjects than in non-diabetic individuals in buccal cells. Further, in subjects of the highest tertile of waist circumference, fasting plasma glucose, glycated haemoglobin and the Framingham general cardiovascular risk score, MNi frequency was significantly higher compared to those in the lowest tertile.

Both, Quintero Ojeda et al. [38] and Gómez-Meda et al. [37] analyzed chromosomal stability in buccal cells (but not in PBMCs) of type 1 and type 2 diabetics versus controls and observed significantly more nuclear abnormalities compared to healthy controls. Poorly controlled type 1 diabetics had significantly higher frequencies of chromosomal aberrations than well controlled patients [37].

Type 2 diabetics demonstrated in general higher frequencies of MNi compared to healthy controls [31–33,35,36]. Within type 2 diabetes patients MNi frequency also correlated with disease severity and duration [32,33], as well as with DNA damaged, measured by the comet assay [31]. Blood glucose management seems to play important role in the context of chromosomal stability. Binici et al. [36] observed a significant correlation between glycated haemoglobin and the frequency of sister chromatid exchanges, yet not with the MNi frequency. Shettigar et al. [34] observed significantly higher frequencies of MNi in poorly controlled type 2 diabetics, as compared to patients with glycated haemoglobin within a normal range. This association was not seen in healthy controls. Although fasting glucose, glycated haemoglobin and post glucose levels significantly differed between patients and controls, MNi frequency showed no difference.

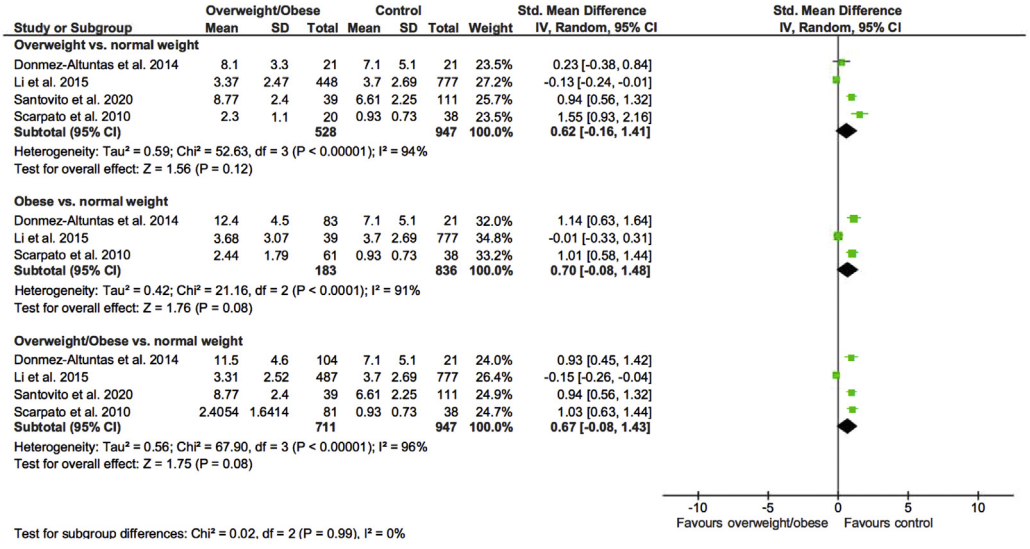

Fig. 3. Forest plot of MNi frequency in obese/overweight and controls.

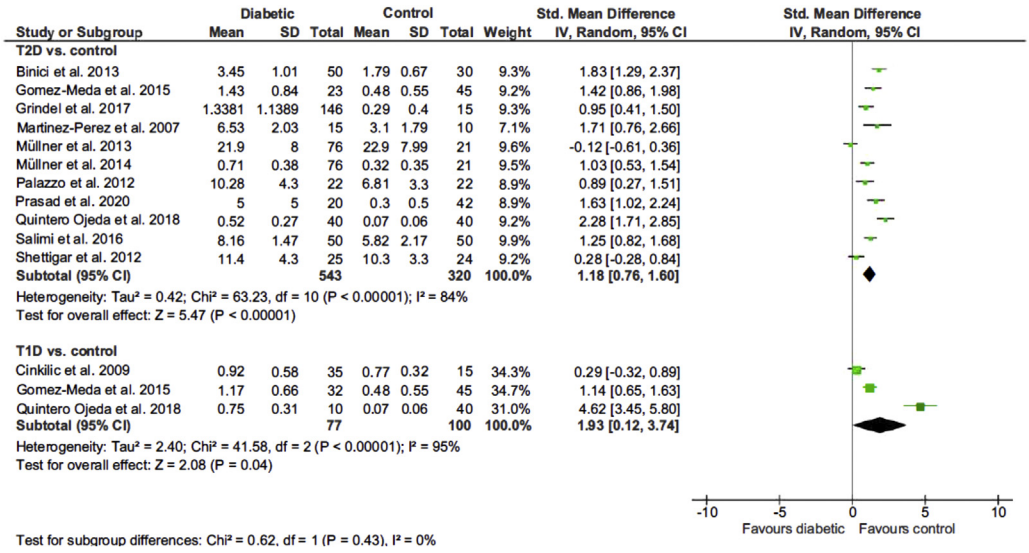

Fig. 4. Forest plot of MNi frequency in diabetics and controls.

3.3. Meta-analysis

Meta-analyses were performed for studies investigating MNi, NPBS and NBUDS frequency in overweight and obese [25–28], as well as in diabetic subjects [23,24,29–38].

3.3.1. Meta-analysis of MNi, NPBS and NBUDS frequencies in obesity and overweight

The meta-analysis only delivered useful data for MNi, but not for NPBS and NBUDS. Fig. 3 shows the results of the meta-analysis in overweight (n = 528) and obese (n = 183) compared to normal weight subjects (n = 947 and n = 836, respectively). Although a clear trend could be seen, neither MNi frequency data from overweight, from obese, nor pooled data from overweight and obese subjects were significantly different compared to normal weight subjects (p = 0.12, p = 0.08, p = 0.08, respectively).

There were not enough data available for NPBS and NBUDS to perform meaningful analyses for overweight and/or obesity.

3.3.2. Meta-analysis of MNi, NPBS and NBUDS frequency in diabetics

Type 2 diabetics (n = 543) showed significantly higher MNi frequencies than control subjects (n = 320) (SMD: 1.18, 95% CI: 0.76, 1.60; I^2 = 84%) (Fig. 4). Only three studies in type 1 diabetic patients could be included into our analysis and showed a higher MNi frequency in type 1 diabetes patients (n = 77) compared to healthy controls (n = 100) (SMD: 1.93, 95% CI: 0.12, 3.74; I^2 = 95%) (Fig. 4).

Four studies in type 2 diabetics and one in type 1 diabetics were available to analyze NPBS frequencies (Fig. 5). Studies with type 2 diabetes patients (n = 168) versus controls (n = 135) showed only a trend for lower frequencies (SMD: 0.97, 95% CI: −0.16, 2.10; I^2 = 94%). Only data from Cinkilic et al. [28] were available for type 1 diabetes and showed only a trend for higher NPBS frequencies in patients (n = 35) compared to healthy controls (n = 15).

Fig. 6 shows the results of the meta-analyses of type 1 and type 2 diabetics compared to healthy controls for NBUDS. The difference between patients and controls was significant in studies with type 2 diabetics (SMD: 1.08, 95% CI: 0.43, 1.73; I^2 = 91%), yet, three studies with type 1 diabetes patients showed significantly lower NBUDS frequencies in controls (SMD: 0.52, 95% CI: 0.08, 0.96; I^2 = 40%).

3.3.3. Meta-analysis comparing PBMCs vs Buccal Cells in diabetics vs. Controls

Figs. 7 and 8 compare chromosomal damage in buccal cells and PBMCs of diabetes patients compared to healthy controls. Both, MNi and NBUDS frequencies showed significant higher values for patients in both cell types (for MNi frequency: PBMCs: SMD: 1.04, 95% CI: 0.46, 1.62; I^2 = 86%, buccal cells: SMD: 1.41, 95% CI: 0.83, 2.00; I^2 = 78%; for NBUDS frequency: PBMCs: SMD: 1.47, 95% CI: 0.27, 2.67; I^2 = 94%, buccal cells: SMD: 0.66, 95% CI: 0.03, 1.28; I^2 = 79%).

4. Discussion

The percentage of people affected by overweight, obesity and type 2 diabetes is drastically increasing [1,6]. Worldwide, about 1.9 billion adults are overweight or obese, while 462 million are underweight. Although around 45% of deaths in children under 5 years of age are related to undernutrition and mostly occur in low- and middle-income countries, at the same time, in the same countries, the number of overweight and obese children is rising [39]. The largest part of the world's population lives in countries where overweight and obesity are responsible for higher mortality rates than underweight. The increase in the number of adults, but also children and teenagers, who are affected by type 2 diabetes follows the same pattern [7]. Both, overweight/obesity and diabetes (type 1 & 2) cause physiological and metabolic changes, all of which are strongly associated with ongoing severe disease progression. Overweight and Obesity (one of the main risk factors for the development of type 2 diabetes) can be starting points for further disease development in later life, such as cancer, kidney disease, diabetes and particularly cardiovascular disease [40,41]. Even diseases related to cognitive decline (Alzheimer's, dementia) are linked to poor blood glucose control, insulin resistance and obesity [42,43].

Many of the possible consequences of overweight and/or diabetes are linked to reduced quality of life, loss of years in good health, accelerated aging, disability and dependency on others, all of which increase socio-economic and healthcare costs [44–46]. Chronically increased inflammation and oxidative stress, both consequences of the metabolic disturbances caused by overweight, obesity and diabetes, are linked to decreased genome stability, increased chromosomal damage and higher frequencies of

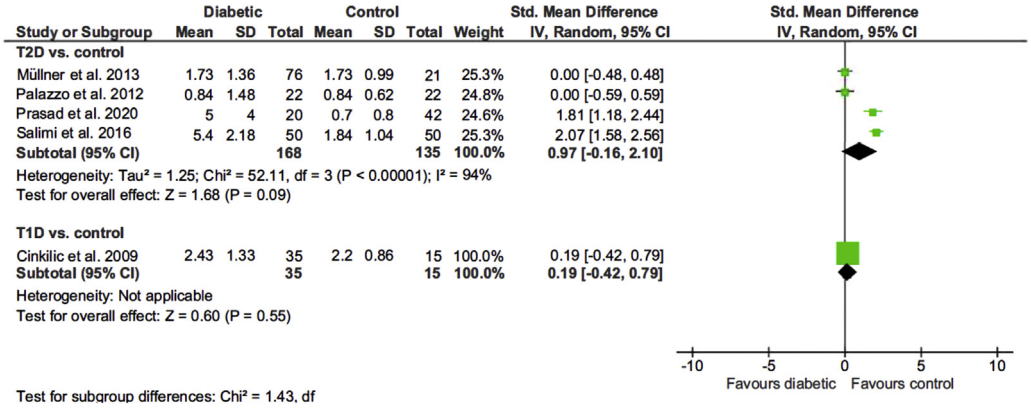

Fig. 5. Forest plot of NPBS frequency in diabetics and controls.

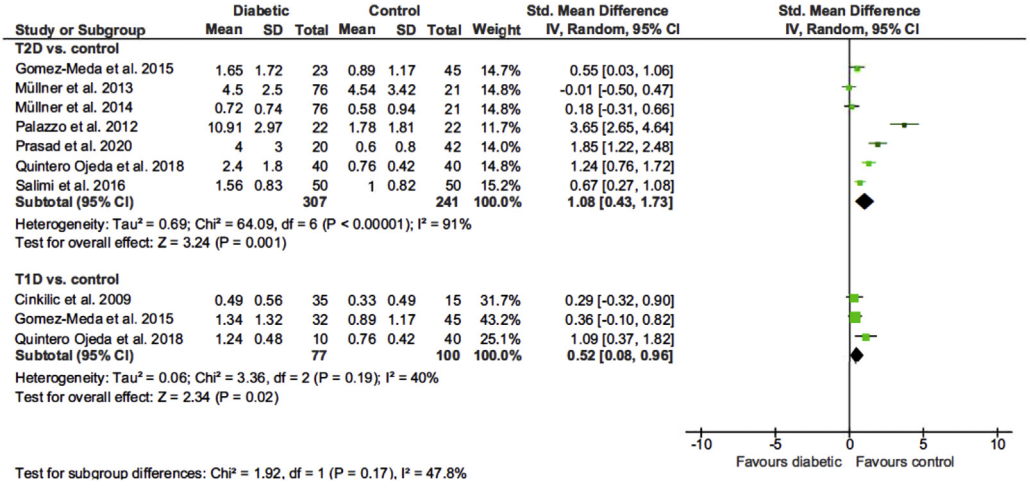

Fig. 6. Forest plot of NBUDS frequency in diabetic subjects and controls.

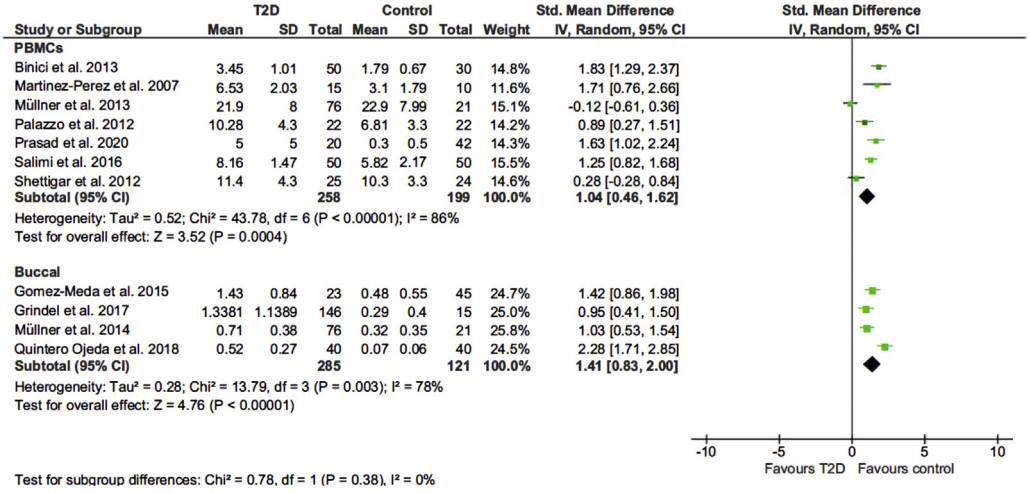

Fig. 7. Forest plot of MNi frequency in diabetics and non-diabetics; PBMCs vs. buccal cells.

genome-mutations. These DNA- and genome-based changes are strongly associated with the development of cancer and therefore potential biomarker candidates for cancer-risk-evaluation [44,47,48].

The CBMN cytome assay is an established method to measure genotoxicity and chromosomal instability and in the center of this review and meta-analysis. It was the intention to investigate the link of obesity, overweight and diabetes to the main markers MNi, NPBS and NBUDS [49]. The available studies were primarily performed in PBMCs and only a small number of studies used buccal cells. The available data were then used to perform meta-analyses to examine genome stability in a) overweight and obese

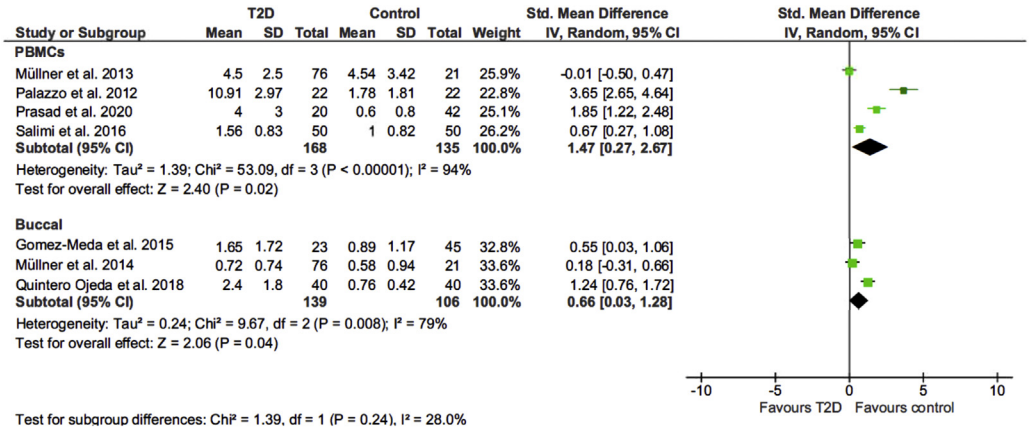

Fig. 8. Forest plot of NBUDS frequency in diabetics and non-diabetics; PBMCs vs. buccal cells.

subjects vs. healthy controls; b) type 1 and type 2 diabetes patients vs. healthy controls; c) buccal cells and PBMCs of diabetic patients.

4.1. Overweight and obesity

Three of the four available studies in overweight and/or obese subjects showed a clear favor for normal weight regarding the parameters of the CBMN cytome assay parameters [25,27,28]. These results were seen in adults [25,28] as well as in children [27,50]. Overweight and obesity are both linked to an increased inflammatory state. While adipose tissue in lean individuals preferentially secretes anti-inflammatory cytokines (adipokines), in obese people the secretions shifts to a mainly pro-inflammatory pattern [12]. A chronically increased inflammatory state is linked to increased RONS production, mainly due to altered mitochondria function [51] and reduced antioxidant defense mechanisms [52], which in sum can cause oxidative damage of the DNA and finally lead to mutations during cell proliferation. These mutations are linked to cellular and tissue disfunction, as well as to the development of cancer [53].

While the above described mechanisms support the theory of increased markers of the CBMN cytome assay in obese and overweight individuals, the study of Li et al. [26] somehow contradicts these observations. Their results indicate, that overweight subjects have lower frequencies of MNi and lower lung cancer risk than the normal-weight-group. This was seen in both of their subgroups of office- and coke-oven workers. Importantly, however, obese study participants did not show this inverse relationship between BMI and MNi frequency. We can only speculate about these unexpected results. The subjects of this study were recruited from a coke-oven plant in Wuhan, China, a region which is known to be highly affected by air-pollution [54]. A hypothetical mechanism by which overweight subjects showed lower genome instability in the presence of a permanent toxic/polluted environment, could be the theory that toxins are likely to be stored in adipose tissue, which might make the overweight more resilient by “cleaning” the circulating system and storing these toxic components in the fat tissue [55]. This might be an explanation, why also obese subjects showed similar and even lower MNi frequencies, as compared to normal weight individuals. However, this theory needs further investigation.

Results of the meta-analysis in obese and overweight individuals included data from 1658 subjects (obese: n = 183, overweight: n = 528, normal weight: n = 947). Sufficient data to perform meta-analyses were only available for MNi frequency and showed a trend in favor of normal weight subjects (Fig. 3). Our findings are supported by previous reviews describing links between oxidative stress, inflammation or DNA damage and genomic instability in obese subjects [56–58], however, meta-analyses on this topic have not been performed yet.

Concluding, overweight and/or obesity seem to negatively affect DNA-mutation and consequently increase the risk for disease development, such as cancer, diabetes, and cardiovascular disease. Yet, there is need for more research to investigate the link between CBMN cytome assay markers (and other markers for genome damage) and overweight, obesity and/or the metabolic syndrome.

4.2. Diabetes

Studies in diabetics (type 1 and 2) showed significant links to markers of genome stability, at least in some aspects (Table 3). Although Shettigar et al. [34] and Müllner et al. [23] did not observe differences between diabetes type 2 patients and controls, they observed significantly higher MNi frequencies within the patient’s groups when blood glucose was poorly controlled, which was also reported in type 1 diabetic subjects [37]. Higher chromosomal damage was also linked to disease progression [59], disease duration [32,33] and intensity of medication [30]. The vast majority of the identified studies showed a significant difference between diabetic patients (type 1 or 2) and healthy controls [24,30,32,33,35–38]. Gómez-Meda et al. [37] and Quintero Ojeda et al. [38] investigated type 1 and type 2 diabetics and found significantly higher MNi frequencies in both disease conditions compared to healthy controls.

Insulin resistance and insulin deficiency are the main drivers in the development of clinical complications of diabetes [11,60]. Type 1 diabetes (primarily characterized by insulin deficiency), as well as type 2 diabetes (initially creating insulin resistance) impair mitochondrial function, which leads to increased ROS generation, produces a state of chronically elevated oxidative stress and results in oxidative damage of the DNA and other proteins. Damaged DNA and proteins lead to cellular and tissue malfunction, which contributes to numerous diabetes-associated problems (for further mechanistic insights please refer to [61–63]). Similarly to what was discussed for overweight and obesity, diabetes can be linked to genome instability, as measured with the CBMN assay.

Data from the meta-analyses confirmed recent reviews [61–63]. We collected data from 883 subjects (543 patients, 320 healthy controls) and observed significantly lower MNi frequencies in controls compared to type 2 diabetics (Fig. 4). Despite lower numbers of subjects for meta-analyses in NPBS and NBUDS frequencies (Figs. 5 & 6), we could confirm the same outcome in

type 2 diabetics. Controls showed significantly lower frequencies of chromosomal aberrations.

Seventy-seven cases and 100 controls, from only three studies, were our data base for the analyses in type 1 diabetics (Figs. 4–6), showing increased MNi and NBUDS frequencies in patients. There is definitely a need for more data using the CBMN cytome assay to assess genome stability in type 1 diabetics.

Summarizing this part, diabetes seems to severely impact genome stability, specifically through the effect of insulin (resistance/deficiency) on mitochondrial function. As studies in type 1 diabetes patients using the CBMN assay are rare, a valid comparison with type 2 diabetic subjects is impossible and needs further investigation.

4.3. Comparison of nuclear abnormalities in buccal cells and PBMCs of diabetics

Although PBMCs are the most frequently used cell type in studies with diabetic subjects, we identified four studies with buccal cells for our comparisons between these two cell types (Figs. 7 & 8). We did not find any studies using the BMcyt assay in overweight and/or obese patients. As only one study performed the BMcyt assay in type 1 and type 2 diabetics [38], only data for type 2 diabetes were used for this cell-based comparison.

In both cell types, PBMCs (patients: n = 258, controls: n = 199) and buccal cells (patients: n = 285, controls: n = 121), there was a significant difference between type 2 diabetics and controls, showing lower MNi frequency in the latter group (Fig. 7). There was no statistical difference after subgroup analyses.

Comparably, NBUDS frequency was significantly lower in controls for both cell types, with also no statistical difference within the groups (Fig. 8).

Taken together, buccal cells seem to be a promising alternative to PBMCs to investigate genome stability in diabetic subjects. Being part of epithelial tissue, where most tumors are developing, further strengthens the use of buccal cells to measure mutation frequencies [64] and their application as a valid biomarker for cancer risk assessment [65]. The sample collection method is minimally invasive as compared to other tissues, which is of undeniable advantage. Buccal cells seem to be consistently affected by the aging process, showing an almost linear increase in the MNi frequency until very old age [65], whereas in PBMCs, the age-related increase in MNi frequency seems to level-off after the age of around 70 years [66,67].

These different, tissue specific, age-related dynamics of the MNi formation, might lead to misleading baseline values to analyze genome stability. Specifically in the context of a potential biomarker for an age-related disease such as type 2 diabetes, MNi measured in PBMCs do not seem to be a reliable marker, whereas the BMcyt assay seems to better reflect age-related genome damage [66,67]. There are still a lot of open questions such as the impact of the present metabolic condition, medical treatments, nutritional impairments or lifestyle changes. Furthermore, the presented age-related data of chromosomal damage were mainly performed in relatively healthy populations and it might easily be different in the context of metabolic instable conditions seen in diabetes and/or obesity. Therefore, much more tissue specific data in the ageing context are needed to address these questions comprehensively.

4.4. Clinical utility and knowledge gaps of the CBMN cytome assay for obesity and diabetes

As described above, obesity and specifically diabetes are reflected by chronically increased genome damage, which is influenced by disease progression and the quality of medical control. In our opinion, there are several points within the clinical context, where the CBMN cytome assay could be supportive for a more detailed diagnosis. However, clinical utility needs beforehand more research and data to be included in the metabolic understanding and the diagnosis of these diseases.

One major prerequisite for clinical utility is the automation of the assay, specifically for the cell counting step. This would improve quality, comparability, reproducibility and duration of the whole procedure. Presently, the available automated systems are not suitable and of sufficient quality for clinical practice [68–72]. The development and establishment of automated systems would enable clinicians to incorporate MNi and other genome-based parameters into their (daily) practice, which might open the window for more personalized and valid diagnosis also for the complex diseases obesity and diabetes.

Particularly for diabetes and obesity, we would suggest the following areas for the application of the CBMN cytome assay:

a) Establishment of an individual baseline when still being healthy.
b) Detection and definition of critical levels of genome damage for the disease risk assessment.
c) MNi as marker to predict/evaluate critical disease outcome.

Ad a): An individual baseline in the healthy but also in well controlled type 1 diabetics state would be necessary to better define and observe health- and disease development. This has long been established for other biomarker such as fasting blood glucose or cholesterol levels. These baseline values must not only be population-based, but could specifically respect the individual level regarding age, sex, medication, and also lifestyle related factors. This would be a necessary starting point to further evaluate variations from the baseline.

Ad b): Based on population based normative but also individual values and combined with diabetes and obesity related biomarkers (e.g. fasting blood glucose, HbA1c, insulin, BMI, waist to hip ratio, . . .), critical deviation rates to the baseline MNi values could be used for a more detailed disease risk prediction, assessment, or management.

Ad c): In the state of a disease (diabetes type 1 or 2), MNi and other markers of the CBMN cytome assay could be used to monitor disease development. Continued disease progression and/or more intense or changing medication could either increase or decrease genome damage, which could be reflected by the MNi frequency. These data could also be used for further health check-ups.

Although we are the first to perform a review including a meta-analysis about MNi, NBUDS and/or NPBS frequencies in the diseases overweight/obesity and diabetes (type 1 & 2) our study has some limitations. We performed the literature research exclusively in the database PubMed. Although researching other databases would have made our search even more complete, we decided to focus on PubMed, as all quality journals and articles in this specific field can be found there.

Further, because of the small number of studies the dissemination bias could not be evaluated.

Our review and meta-analysis on the CBMN cytome assay in obesity and diabetes revealed a lack of sufficient data for both diseases. Specifically for obesity and overweight but also for type 1 diabetes, the number of human studies is very small with only four and three, respectively, that were suitable for our analyses. The same is true for studies, which used the BMcyt assay to assess genome stability in buccal cells. Due to its non-invasive sampling method, this tissue type could be of high practicability for a clinical testing of the MNi frequency in obesity and diabetes patients. In this context, the different dynamics of various tissues regarding age- and lifestyle-related impacts on genome damage absolutely

need further evaluation in order to establish specific, individual and reliable baseline values.

Taken together, there is in our opinion definitely a potential for the clinical usability of the CBMN cytome and the BMcyt assay in the areas of obesity and diabetes [73], however, the method itself and also the application in clinical practice needs further data and, at the same time, technical development to realize the next steps.

5. Conclusions

Our review and meta-analysis about the diseases diabetes (type 1 & 2) and overweight/obesity and their association with genome damage, measured by using the CBMN cytome assay, revealed an interesting perspective. Type 2 diabetes, where most studies considering the CBMN cytome assay were published, clearly showed higher frequencies of chromosomal aberrations in patients compared to controls, with even more impaired DNA stability with further disease progression, worse blood glucose control and/or more intense medication. Type 1 diabetics showed similar data, yet, only a very small number of studies was available for our analyses.

Similarly to diabetes, obesity seems to negatively influence genome stability, however, data are insufficient to fully support this statement yet, as one of the four studies reported the opposite.

The analysis of chromosomal damage in buccal cells presents a potent and minimally invasive alternative to PBMCs in the clinical setting. However, the incorporation of either the CBMN cytome or the BMcyt assay into clinical practice for diabetics (no available studies in obese and overweight with the BMcyt assay) needs further validation and technical/methodical development in the next years (e.g. individual and population-based baseline values for health and disease, automation of the assay including counting, . . .) to be precise, valid as well as time and financially efficient.

Declaration of Competing Interest

The authors reported no declarations of interest.

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