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Impact of dietary and lifestyle interventions in elderly or people diagnosed with diabetes, metabolic disorders, cardiovascular disease, cancer and micronutrient deficiency on micronuclei frequency – A systematic review and meta-analysis

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ABSTRACT

Chronic diseases such as cardiovascular diseases, type 2 diabetes or cancer are the global leading cause of mortality. Lifestyle interventions are most effective in reducing metabolic risk factors, disease progression or even side effects of a disease. They are also contributing to decelerate the aging process. Genome instability is very often associated with aging or the above-mentioned diseases, and triggered by inflammation and oxidative stress. An established method to measure chromosomal damage is the cytokinesis block micronucleus (CBMN) cytome assay. The aim of this review and meta-analysis is to collect and analyse the current literature regarding the effects of a lifestyle based (dietary) intervention on changes of micronuclei (MNi), nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs) in elderly subjects or people diagnosed with diabetes, metabolic disorders, cardiovascular disease, cancer or micronutrient deficiency.

Although the main important diseases were considered as well as the large topic of aging, the number and methodological quality in terms of samples size, duration and rationale of the intervention or an inclusion of a control group of available intervention studies with these backgrounds was low. Most of the studies used antioxidant vitamins or folate, few investigated the whole diet. Only one study showed a physical activity intervention approach. The interventions did not lead to decreased genomic marker despite a few cancer related studies, where particularly MN frequency in mucosa lesions and leukoplakia was reduced by green tea and antioxidants. The performed meta-analysis of the available RCTs did not show a significant reduction of MNi, NBUDs or NPBs of most of the interventions performed, except for green tea.

Data show in general a lack of an appropriate number of sound lifestyle based intervention studies linking cytogenetic damage and chronic diseases.

Contents

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1. Introduction

Non-communicable diseases (NCDs), such as heart disease, stroke, cancer, chronic respiratory diseases and diabetes, are the leading cause of mortality in the world. This invisible epidemic is an under-appreciated cause of poverty and hinders the economic development of many countries. Forty out of 56 million global deaths in 2015 were due to NCDs and interestingly almost 50 % of NCDs' mortality in low- and middle income countries occurred below the age of 70, whereas this was less than 15 % in high income countries [1]. In the latter countries, NCDs are predominantly a matter of the aging society, which is also a global phenomenon. By 2050, the world's population aged 60 years and older is expected to total 2 billion, up from 900 million in 2015. Today, around 125 million people are aged 80 years or older. By 2050, there will be 434 million people in this age group worldwide and 80 % of all older people will live in low- and middle-income countries, since these countries are most population dense with a total population of approx. 6.5 billion people at present.

NCDs share the same main public health risk factors – tobacco use, poor diet and insufficient physical activity, which leads to increased metabolic risk factors such as hypertension, dyslipidemia, impaired glucose metabolism, insulin resistance or obesity [2]. Other unifying topics amongst the variety of chronic diseases are inflammation or oxidative stress, since most above-mentioned metabolic risk factors induce both conditions [3,4].

Therefore, efforts to increase the application of public health and clinical interventions of known efficacy to reduce the prevalence of the major risk factors for chronic diseases and to increase the utilization of screening tests for their early detection could substantially reduce the human and economic costs of these diseases.

Genome damage including DNA strand breakage, chromosome rearrangement, aneuploidy or alterations in methylation patterns and subsequent alterations in gene dosage and gene expression have also been identified as being fundamental to the development of human diseases [5,6].

Biomarkers of chromosome damage need to be sensitive enough to reflect changes within the genome as a result of exposure to exogenous and endogenous agents or to reflect lifestyle interventions.

The cytokinesis-block micronucleus cytome assay (CBMN assay) is the preferred method in human biomonitoring studies to detect cytogenetic effects. In this assay mainly micronuclei (MNi), nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs) are scored in binucleated cells (BNC) after blocking cytokinesis with cytochalasin B (Cyt-B).

Since various lifestyle based interventions in different patient groups have been performed over the last decades using the CBMN cytome assay as intermediate marker, the purpose of this review and meta-analysis is to investigate the current literature of the effects of lifestyle based interventions on changes of MNi, NBUDs and NPBs in people diagnosed with diabetes, metabolic disorders, cardiovascular disease, cancer or micronutrient deficiency. Further, the applicability of MNi, NPBs and NBUDs as possible markers mirroring lifestyle based interventions will be presented and discussed, specifically in the context of their clinical biomarker potential.

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 20th 2020 with no restriction of language and calendar date using a pre-defined search strategy (see next chapter 2.2.). The reference lists from eligible studies were screened to identify additional relevant research. Screening and study selection (Fig. 1) were conducted by three authors independently (KHW, BF, AD). Following search terms were adopted for PubMed and Scopus: ("cytokinesis block micronucleus cytome assay" OR "CBMN cytome assay" or "Buccal Micronucleus Cytome assay" "BMcyt assay" OR "micronucleus" OR "nucleoplasmic bridges" OR "nuclear buds") AND ("cancer" OR "cardiovascular diseases" OR "metabolic diseases" OR "diabetes mellitus type 2" OR "elderly" OR "micronutrient deficiency" OR "chronic disease") AND ("human intervention" OR "nutrition" OR "exercise" OR "physical activity" OR "diet" OR "supplements" OR "RCT" OR "clinical trial")

2.2. Selection of studies

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

- Randomized controlled trials (RCTs);
- Human intervention studies;
- Study subject had to undergo a lifestyle based intervention (nutrition and/or physical activity based) linked to age (average age of 60 years or older), cardiovascular diseases, cancer,

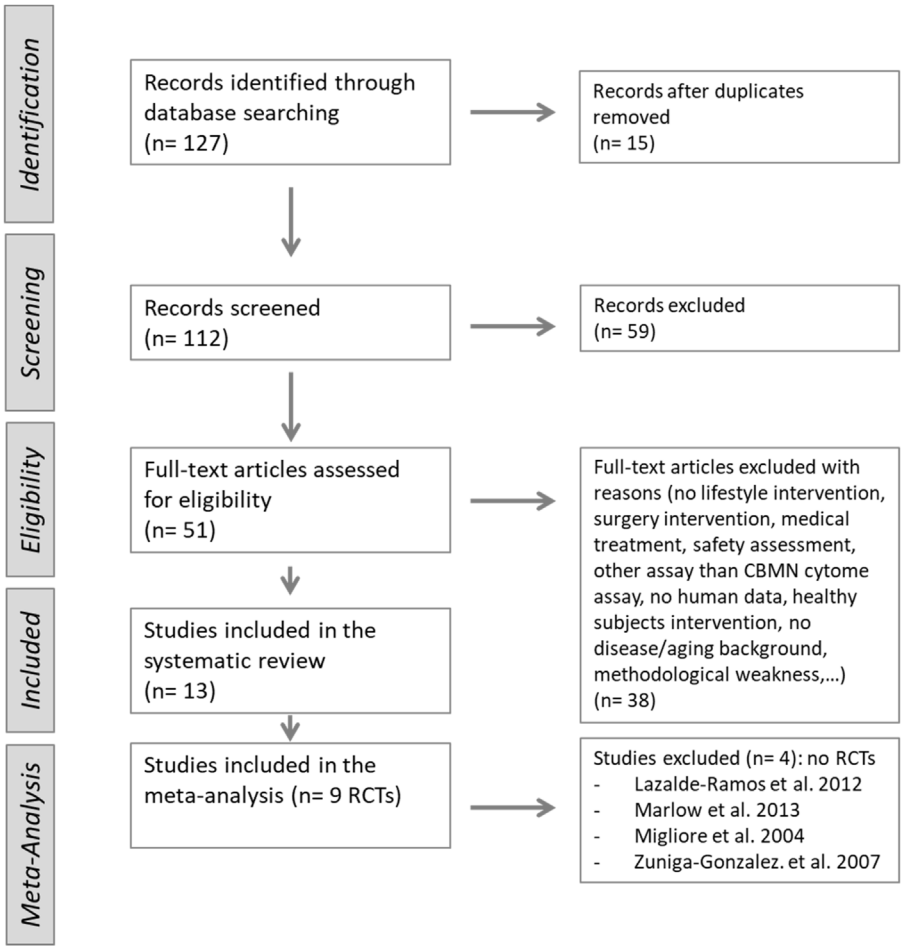


Fig. 1. Study selection flow chart.

metabolic or inflammatory diseases, type 2 diabetes or a proven micronutrient deficiency;
- Chromosomal damage was measured by the CBMN cytome or the Buccal Micronucleus Cytome (BMcyt) assay and 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:

- Other methods than the CBMN cytome assay were used to detect genome damage;
- Severe methodological weakness (e.g. inadequate number of counted cells);
- Short term dose response intervention trials, surgery intervention trials, trials where the CBMN assay was used as a safety marker e.g. to assess the safety of plant extract without a link to improve disease conditions

2.3. Data extraction

For included studies, three reviewers independently (AD, BF, KHW) extracted the following characteristics: name of first author, year of publication, study origin (country), study design, number of participants, disease/aging status, mean age, outcome data, and conflict of interest. The preferred outcome data (Frequency of MN, NPB, NBUD) were values with corresponding standard deviations, standard errors or 95 % CI.

2.4. Data synthesis

2.4.1. Statistical analysis

The dietary intervention group vs. control/placebo group values were pooled as mean differences (MDs) using a random effects model for each continuous outcome separately. For all outcomes standardized mean differences (SMDs) were calculated (due to different measurement methods used across included studies). The magnitude of the SMD was interpreted as follows [7]: 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=((Q−df))/Q × 100 %, where Q is the χ^2 statistic and df is its degrees of freedom [8]. An I^2-value of greater than 50 % was considered to represent considerable heterogeneity [9]. Meta-analyses were conducted using Review Manager (RevMan) Version 5.3 [10].

The investigated outcomes in the meta-analyses are presented by considering only RCTs: dietary interventions on MNi, dietary interventions on MNi in human peripheral blood lymphocytes (PBL), dietary interventions on MNi in buccal cells, dietary interventions on NPBs and NBUDs in PBL.

2.4.2. Dissemination bias

To evaluate dissemination bias, a funnel plot was created for each pairwise comparison if at least 10 individual RCTs were available for a given outcome.

3. Results

3.1. Literature search outcomes (including study selection flow chart) and description of intervention studies

After screening the available literature on MNi, NPBs and NBUDs and their assessment in human lifestyle intervention studies in subjects related to aging, diabetes, metabolic disorders, cardiovascular disease, cancer or micronutrient deficiency, the studies were evaluated according to our inclusion and exclusion criteria, as described above. A total of 13 (aging: n = 3; CVD: n = 1; diabetes type 2: n = 3; cancer: n = 4; metabolic diseases: n = 2; micronutrient deficiency (only without link to another metabolic/disease condition): n = 0) studies were identified, which were suitable for further analysis (Fig. 2, Tables 1–3). The association of micronutrient deficiency and CBMN cytome assay was mainly investigated in cross sectional studies or studies with a healthy population. Eight studies performed the MNi assay in PBL, five in buccal/mucosa cells or mucosa lesions, three of them in PBL and buccal cells.

In order to correct for intralab variation we normalized the mean (sd) data of the intervention groups against the mean (sd) data of the respective control groups (were available) and show them also in Tables 1–3.

3.2. Results for aging

Fenech et al. (2007a) performed a study to determine the prevalence of folate deficiency, vitamin B12 deficiency and hyperhomocysteinemia in 64 healthy men (mean age 61.8 ± 1.5 years in the control group and 60.6 ± 1.0 years in the folate intervention group), and evaluated the relationship of these micronutrient levels in the blood with the micronucleus frequency in peripheral blood lymphocytes. They further performed a placebo-controlled, double-blind intervention study to determine whether supplementation of the diet with a daily dose of 0.7 mg (as a supplement in cereals) or 2.0 mg (in a tablet) of folic acid over a period of 4 months resulted in alterations in the micronucleus index. Although 56 % of the investigated men showed an increase in plasma folate, and at the same time a decrease in plasma homocysteine due to the intervention, the MN frequency did not change significantly [11]. In a placebo-controlled double-blind intervention trial, the same group investigated, with 60 male volunteers aged between 50 and 70 years, whether vitamin E as d-alpha-tocopherol (VITE) above the recommended dietary intake (RDI) level has an impact on MN frequency. The intervention consisted of two eight-weeks-long phases: during the initial phase the VITE supplement dosage was 5xRDI (provided in cereal) and during the second phase the VITE supplement was 30xRDI (provided in capsules). There was no correlation between baseline genetic damage frequency and VITE status. However, a 32 % ($P < 0.007$) decrease in the MN frequency was observed in both the control and VITE-supplemented groups during the course of the study. [12].

In a more recent study, Franzke et al. (2015) investigated the effect of a six months progressive resistance training (RT), with or without protein and vitamin supplementation (RTS) or cognitive training (CT) only, on chromosomal damage in PBL in 97 Austrian institutionalized women and men (65–98 years). The mean age of the population was 83 years. All three intervention groups demonstrated a tendency of a reduced frequency of cells with MNi (−15 %) as well as for the total number of MNi (−20 %), however, no significant time-effect was observed. The six months change of B12 was negatively correlated with the six months change of the MN frequency in the RTS group ($r = -0.584$, $p = 0.009$), which was also mirrored by a significant increase in plasma

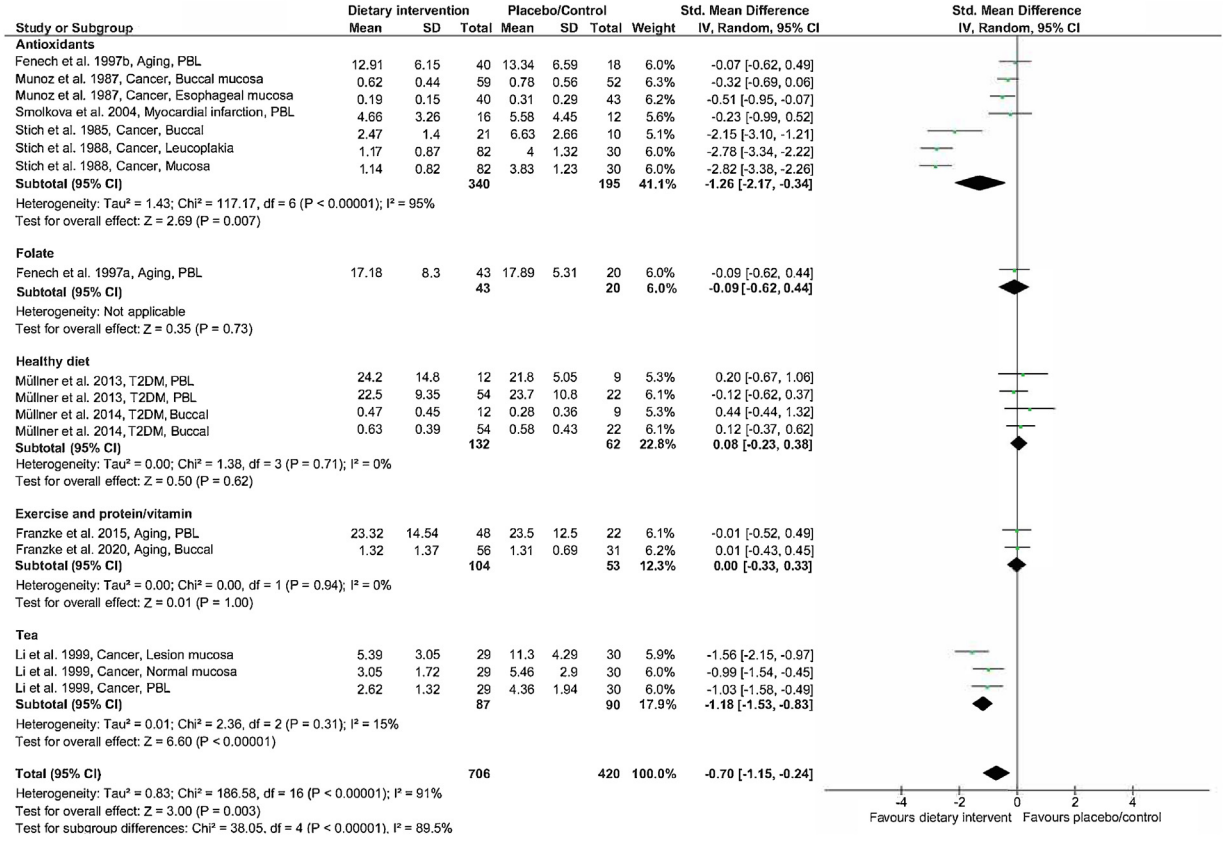


Fig. 2. Forest plot showing the effects of dietary interventions on MN frequency related to aging, type 2 diabetes, cancer, CVD and metabolic diseases in RCTs.

Table 1
Human intervention studies observing MN, NPB and NBUD frequencies related to aging.

Study ID number	Intervention/Duration	MNi per 1000 cells in PBL				Normalized values**
		Intervention group		Control group		
		N	Mean (SD)	N	Mean (SD)	Mean (SD)
Fenech et al. 1997a [11]	Folate administration vs. placebo; 16 weeks	43	Before = 18.60 (9.34) After = 17.18 (8.30)	20	Before = 17.49 (4.96) After = 17.89 (5.31)	Before = 1.06 (1.89) After = 0.96 (1.56)
Fenech et al. 1997b [12]	Vitamin E administration vs. placebo; 8 weeks	40	Before = 18.4 (9.23) After = 12.91 (6.15) *	20	Before = 20.58 (10.63) After = 13.34 (6.59) *	Before = 0.89 (0.86) After = 0.97 (0.93)
Franzke et al. 2015 [13]	Lifestyle intervention: - Nutritional supplement + resistance training (RTS) - Resistance training (RT) - Cognitive training = Control (C); 6 months	RTS = 35 RT = 29	RT: Before = 27.6 (13.9) After = 24.1 (14.9) RTS: Before: 25.0 (10.8) After = 24.1 (14.9)	33	Before = 26.0 (10.9) After = 23.5 (12.5)	RT: Before = 1.06 (1.28) After = 1.03 (1.19) RTS: Before = 0.96 (0.99) After = 0.95 (1.15)

Study ID number	Intervention/Duration	NPBs per 1000 cells in PBL				Normalized values**
		Intervention group		Control group		
		N	Mean (SD)	N	Mean (SD)	Mean (SD)
Franzke et al. 2015 [13]	Lifestyle intervention: - Nutritional supplement + resistance training (RTS) - Resistance training (RT) - Cognitive training = Control (C); 6 months	RTS = 35 RT = 29	RT: Before = 1.13 (1.14) After = 0.50 (0.49) RTS: Before = 0.88 (0.89) After = 0.59 (1.16)	33	Before = 1.23 (1.05) After = 0.64 (0.85) *	RT: Before = 0.92 (1.09) After = 0.78 (0.58) RTS: Before = 0.72 (0.85) After = 0.92 (1.37)

Study ID number	Intervention/Duration	NBUDs per 1000 cells in PBL				Normalized values**
		Intervention group		Control group		
		N	Mean (SD)	N	Mean (SD)	Mean (SD)
Franzke et al. 2015 [13]	Lifestyle intervention: - Nutritional supplement + resistance training (RTS) - Resistance training (RT) - Cognitive training = Control (C); 6 months	RTS = 35 RT = 29	RT: Before = 3.56 (2.75) After = 3.93 (3.96) RTS: Before = 3.34 (2.62) After = 2.98 (2.35)	33	Before = 4.23 (4.57) After = 3.03 (3.08)	RT: Before = 0.84 (0.60) After = 1.30 (1.29) RTS: Before = 0.79 (0.57) After = 0.98 (0.56)

Study ID number	Intervention/Duration	MNi per 1000 cells in buccal cells				Normalized values**
		Intervention group		Control group		
		N	Mean (SD)	N	Mean (SD)	Mean (SD)
Franzke et al. 2020 [14]	Lifestyle intervention: - Nutritional supplement + resistance training (RTS) - Resistance training (RT) - Cognitive training = Control (C); 6 months	RTS = 35 RT = 29	RT: Before = 1.35 (0.46) After = 1.55 (0.63) RTS: Before = 1.41 (0.5) After = 1.09 (1.45)	33	Before = 1.18 (0.39) After = 1.31 (0.69)	RT: Before = 0.84 (0.60) After = 1.30 (1.29) RTS: Before = 0.79 (0.57) After = 0.98 (0.56)

* Significant difference after intervention compared to baseline.
** Mean value (SD) of the intervention group normalized against the mean value (SD) of the respective control group.

B12 and red blood cell folate status. There was also a significant time effect towards a reduction of NPBs ($p = 0.025$), but no group effect. No changes were observed for NBUDs [13].

In the same study, genotoxicity and cytotoxicity parameters were also investigated with the BMcyt assay. In contrast to the data in PBL no changes due to the six months intervention were seen in any of the parameter of the BMcyt assay [14].

3.3. Results for type 2 diabetes

Zuniga-Gonzales et al. (2007) investigated the effect of folic acid supplementation (5 mg three times a day for 30 days) in 30 patients with type 2 diabetes on buccal mucosa samples. The mean age of 35.2 ± 15.3 years in this study was only given for the number of 78 patients (n = 48 were used for a cross sectional investigation; n = 30 for the intervention part). The folic acid intervention reduced the frequency of MNi significantly [15]. The same group repeated the study design some years later, but this time they included 30 control subjects (only for cross sectional reason – without intervention). After the first 15 days of intervention they took additional samples. Similar to the previous study, the frequency of MNi in the buccal mucosa cells was significantly reduced and even after 15 days the observed improvement was already significant [16].

Müllner et al. (2013) investigated the frequency of chromosomal damage in PBL and buccal cells of type 2 diabetics compared to non-diabetic controls (quite often partners of the diabetic study participants). Seventy-six diabetic and 21 non-diabetic individuals were randomly assigned to either an 'intervention' or an 'information only' group. All participants received information about the beneficial effects of a healthy diet, while subjects of the intervention group received additionally 300 g of vegetables and 25 mL of walnut oil rich in polyunsaturated fatty acids per day for eight weeks. There were no changes in MN, NBUD or NPB

Table 2
Human intervention studies observing MN, NPB and NBUD frequencies related to.

Study ID	Intervention/Duration	MNi per 1000 cells in PBL				Normalized values**
		Intervention group		Control group		
		N	Mean (SD)	N	Mean (SD)	Mean (SD)
Müllner et al. 2013 [17]	300 g vegetables +25 mL plant oil daily for 8 weeks - Type 2 diabetes (T2D) subjects vs. health controls; 8 weeks	TD2 = 54 HC = 12	T2D: Before = 20.7 (7.44) After = 22.5 (9.35) Healthy: Before = 23.3 (11.3) After = 24.2 (14.8)	TD2 = 22 HC = 9	T2D: Before = 23 (8.51) After = 23.7 (10.8) Healthy: Before = 22.5 (4.68) After = 21.8 (5.05)	T2D: Before = 0.90 (0.87) After = 0.95 (0.87) Healthy: Before = 1.04 (2.62) After = 1.11 (2.92)
Study ID	Intervention/Duration	NPBs per 1000 cells in PBL				Normalized values**
		Intervention group		Control group		
		N	Mean (SD)	N	Mean (SD)	Mean (SD)
Müllner et al. 2013 [17]	300 g vegetables +25 mL plant oil daily for 8 weeks - Type 2 diabetes (T2D) subjects vs. health controls; 8 weeks	TD2 = 54 HC = 12	T2D: Before = 1.56 (1.24) After = 1.85 (1.39) Healthy: Before = 2.01 (1.36) After = 2.42 (1.41)	TD2 = 22 HC = 9	T2D: Before = 1.9 (1.48) After = 2.12 (1.64) Healthy: Before = 1.44 (0.62) After = 2.06 (0.95)	T2D: Before = 0.90 (0.87) After = 0.95 (0.87) Healthy: Before = 1.04 (2.62) After = 1.11 (2.92)
Study ID	Intervention/Duration	NBUDs per 1000 cells in PBL				Normalized values**
		Intervention group		Control group		
		N	Mean (SD)	N	Mean (SD)	Mean (SD)
Müllner et al. 2013 [17]	300 g vegetables +25 mL plant oil daily for 8 weeks - Type 2 diabetes (T2D) subjects vs. health controls; 8 weeks	TD2 = 54 HC = 12	T2D: Before = 6.38 (5.02) After = 5.47 (3.85) Healthy: Before = 3.92 (2.03) After = 5.01 (2.66)	TD2 = 22 HC = 9	T2D: Before = 5.08 (2.97) After = 4.73 (2.33) Healthy: Before = 2.69 (1.81) After = 3.22 (1.95)	T2D: Before = 0.77 (0.68) After = 1.06 (1.15) Healthy: Before = 2.37 (2.77) After = 1.70 (1.97)
Study ID	Intervention/Duration	MNi per 1000 cells in buccal cells				Normalized values**
		Intervention group		Control group		
		N	Mean (SD)	N	Mean (SD)	Mean (SD)
Zuniga-Gonzalez. et al. 2007 [15]	Folic acid administration only to T2D; 30 days	N = 30	Before = 1.24 (0.72) After = 0.34 (0.28) *		No control group	
Lazalde-Ramos et al. 2012 [16]	Folic acid administration only to T2D; 30 days	N = 30	Before = 0.97 (0.84) After = 0.21 (0.32) *		No control group	
Müllner et al. 2014 [18]	300 g vegetables +25 mL plant oil daily for 8 weeks - Type 2 diabetes (T2D) subjects vs. health controls; 8 weeks	TD2 = 54 HC = 12	T2D: Before = 0.76 (0.39) After = 0.63 (0.39) Healthy: Before = 0.36 (0.36) After = 0.47 (0.45)	TD2 = 22 HC = 9	T2D: Before = 0.60 (0.34) After = 0.58 (0.43) Healthy: Before = 0.27 (0.35) After = 0.28 (0.36)	T2D: Before = 1.27 (1.14) After = 1.09 (0.91) Healthy: Before = 1.33 (1.03) After = 1.68 (1.25)

* Significant difference after intervention compared to baseline.
** Mean value (SD) of the intervention group normalized against the mean value (SD) of the respective control group.

frequency in the information or in the intervention group observed. However, there was a significant treatment × health interaction (P = 0.015) with respect to the changes in NBUDs. The intervention with vegetables and plant oil led to a significant increase in apoptosis, but there was no effect on necrosis and the nuclear division index (NDI) [17]. In the buccal cells however, the numbers of BNC and karyorrhectic cells and cells with condensed chromatin were decreased in both the information and intervention groups. Pycnotic cells were only reduced in participants of the intervention group, and changes after eight weeks were significantly greater in participants of the intervention group compared to the information group [18].

3.4. Results for cancer, CVD and metabolic/inflammatory diseases

For the diseases described in this chapter we identified seven studies. Similar to type 2 diabetes, we excluded all studies in humans which considered drug interventions (e.g. metformin), surgery interventions (e.g. bariatric surgery) or when the CBMN cytome assay was used for safety assessment studies (e.g. Bonassi et al. [19]).

Smolkova et al. (2004) investigated the effect of modest supplementation with α-tocopherol (100 mg/day), β-carotene (6 mg/day), vitamin C (100 mg/day) and selenium (50 mg/day) on chromosomal damage in mid-aged men differing in cardiovascular risk. Forty-six survivors of myocardial infarction (MI) before the age of 50 and 60 healthy controls were randomly divided into equal groups to receive either antioxidants or placebo for 12 weeks. MNi in PBL did neither change significantly in MI patients nor in the control group after antioxidant supplementation. Interestingly the placebo supplement increased the MN frequency in the placebo group but not in the MI patients. MN frequency showed the strongest decrease in MI patients with normal folate levels (p = 0.015). In subjects with low folate levels, a high correlation was found between MNi and homocysteine, both before (r = 0.979, p = 0.002) and after antioxidants supplementation (r = 0.922, P = 0.009) [20].

Migliore et al. (2004) investigated the effects of a two week ubidecarenone (a coenzyme Q10 analogue) intervention of 100

Table 3
Human intervention studies observing MN, NPB and NBUD frequencies related to.

Study ID number	Intervention/Duration	MNi per 1000 cells in PBL				Normalized values**
		Intervention group		Control group		
		N	Mean (SD)	N	Mean (SD)	Mean (SD)
Smolkova et al. 2004 [20]	Antioxidants supplements vs placebo in survivors of myocardial infarction (MI) vs healthy controls (HC); 12 weeks	MI = 16 HC = 31	MI: Before = 7.59 (4.13) After = 4.66 (3.26) HC: Before = 7.43 (3.54) After = 7.71 (4.96)	MI = 12 HC = 27	MI: Before = 5.54 (2.69) After = 5.58 (4.45) HC: Before = 5.06 (3.59) After = 7.28 (5.47) *	MI: Before = 1.37 (1.54) After = 0.84 (0.73) HC: Before = 1.47 (0.99) After = 1.06 (0.91)
Migliore et al. 2004 [21]	Ubidecarenone supplementation in mitochondrial disease patients; 2 weeks	N = 10	Before = 27.0 (10.9) After = 16.8 (9.8) *		No control group	
Marlow et al. 2013 [22]	Mediterranean-inspired anti-inflammatory diet given to Crohn's disease patients; 6-weeks	N = 8	Before = 8.85 (15.41) After = 5.65 (5.24)		No control group	
Li et al. 1999 [26]	3 g mixed tea oral administration and topical treatment vs placebo and glycerin treatment in patients with oral mucosa leukoplakia vs. placebo; 6 months	N = 29	Before = 3.89 (1.47) After = 2.62 (1.32) *	N = 30	Before = 4.0 (1.46) After = 4.36 (1.94)	Before = 0.97 (1.01) After = 0.60 (0.68)

Study ID number	Intervention/Duration	MNi per 1000 cells in buccal, mucosa, leucoplakia, mucosa lesion				Normalized values**
		Intervention group		Control group		
		N	Mean (SD)	N	Mean (SD)	Mean (SD)
Stich et al. 1985 [23]	Oral mucosa of Inuit users of smokeless tobacco in response to the administration of beta-carotene (180 mg/week) vs. placebo; 30 days	N = 21	Before = 6.23 (3.07) After = 2.47 (1.4) *	N = 10	Before = 6.67 (3.13) After = 6.62 (2.67)	Before = 0.94 (0.98) After = 0.37 (0.53)
Munoz et al. 1987 [25]	Once-a-week supplementation with retinol, riboflavin, and zinc on prevalence of precancerous lesions of the esophagus; 1 year	N = 48	Before = 0.70 (0.58) After = 0.62 (0.44)	N = 52	Before = 0.76 (0.56) After = 0.78 (0.56)	Before = 0.92 (1.04) After = 0.80 (0.79)
Stich et al. 1988 [24]	Beta-carotene (BC) or beta-carotene + vitamin A (BCA) or placebo (PC) intervention on Fishermen who had well-developed oral leukoplakias; 6 months	BC = 31 BCA = 51	Leucoplakia: BC: Before = 4.09 (1.1) After = 1.18 (0.77) * BCA: Before = 4.01 (1.05) After = 1.16 (0.94) *	N = 30	PC: Before = 3.69 (1.22) After = 4.0 (1.32)	BC: Before = 1.11 (0.90) After = 0.30 (0.58) BCA: Before = 1.09 (0.86) After = 0.29 (0.71)
Stich et al. 1988 [24]	Beta-carotene (BC) or beta-carotene + vitamin A (BCA) or placebo (PC) intervention on Fishermen who had well-developed oral leukoplakias; 6 months	BC = 31 BCA = 51	Mucosa: BC: Before = 4.11 (1.48) After = 1.01 (0.71) * BCA: Before = 4.18 (0.78) After = 1.22 (0.88) *	N = 30	PC: Before = 4.1 (1.54) After = 3.83 (1.23)	BC: Before = 1.0 (0.96) After = 0.26 (0.57) BCA: Before = 1.02 (0.59) After = 0.32 (0.72)
Li et al. 1999 [26]	3 g mixed tea oral administration and topical treatment vs placebo and glycerin treatment in patients with oral mucosa leukoplakia vs. placebo; 6 months	N = 29	Mucosa: Before = 5.2 (2.79) After = 3.05 (1.72) *	N = 30	Mucosa: Before = 5.12 (2.04) After = 5.46 (2.9)	Before = 1.01 (1.36) After = 0.56 (0.59)
Li et al. 1999 [26]	3 g mixed tea oral administration and topical treatment vs placebo and glycerin treatment in patients with oral mucosa leukoplakia vs. placebo; 6 months	N = 29	Mucosa lesion: Before = 10.5 (5.29) After = 5.39 (3.05) *	N = 30	Mucosa lesion: Before = 10.1 (4.07) After = 11.3 (4.29)	Before = 1.04 (1.30) After = 0.58 (0.72)

* Significant difference after intervention compared to baseline.
** Mean value (SD) of the intervention group normalized against the mean value (SD) of the respective control group.

mg/d in 10 subjects with mitochondrial diseases. The relatively short intervention period was enough to reduce the MN frequency significantly [21].

Marlow et al. (2013) applied a Mediterranean-inspired "anti-inflammatory" diet to eight Crohn's disease patients (6 females, two males, mean age 45.4 years). The diet was provided to the participants and based on food items including salmon, organic avocados, kumara, a variety of vegetables, gluten-free bread, extra virgin olive oil, green tea, honey and fish oil capsules. The frequency of MN in PBL was not significantly influenced by the diet, although there was a trend for a decrease [22].

The studies regarding cancer are relatively old and were conducted before the turn of the millennium. Stich et al. (1985) studied the frequency of MN in the oral mucosa of users of smokeless tobacco in response to the administration of β-carotene (180 mg/week, given twice weekly in six capsules of 30 mg each). Oral lesions in users of snuff and chewing tobacco were classified, according to their appearance, into four classes and into three categories. Exfoliated cells were taken from the site of the oral cavity where the tobacco is preferentially held and from five additional regions which do not come in close contact with the chewing mixture. Prior to the twice-weekly administration of beta-carotene, the frequency of cells with MNi was 1.87 % ± 0.92 (n = 21) in the mucosa of the lower gingival groove where the tobacco was usually kept. It decreased significantly ($P < 0.001$) to 0.74 % ± 0.42 following the 10-week oral administration of beta-carotene capsules (n = 23). The frequency of MN did not change significantly in the group receiving a placebo (n = 10) and in snuff users who received no treatment over the 10-week trial period [23].

The same group performed a short term intervention trial with fishermen from Kerala (India) who chewed tobacco-containing betel quids daily (17.2 ± 9.6 quids per day) and had well-developed oral leukoplakias with elevated frequencies of micronucleated cells. Either beta-carotene alone (180 mg/week) (Group I, n = 31), β-

carotene (180 mg/week) plus vitamin A (100,000 IU week) (Group II, n = 51) or placebo (Group III, n = 30) capsules were given twice weekly for 6 months under strict supervision. The remission of oral leukoplakias, the inhibition of new leukoplakias, and the reduction of micronucleated oral mucosal cells were recorded after 3 months of the trial period. After the intervention period the frequency of MN was significantly reduced in Group I (from 4.09 % to 1.1 % in areas of leukoplakia, and from 4.1 % to 1.0 % in the normal mucosa) and in Group II (from 4.01 % to 1.16 % in areas of leukoplakia, and from 4.18 % to 1.22 % in the normal mucosa). There was no difference in the placebo group. At this time, remission of oral leukoplakias did not differ significantly from that observed in the placebo group [24].

Munoz et al. (1987) conducted a randomized double-blind intervention trial in Huixian, People's Republic of China, a population with a high incidence of esophageal cancer. Their aim was to determine whether a once-a-week treatment for one year with retinol (15 mg or 50,000 IU), riboflavin (200 mg), and zinc (50 mg) could result in a lower prevalence of precancerous lesions of the esophagus in the group receiving the active treatment as compared with the prevalence in the group receiving a placebo.

In a subsample of the original 610 study subjects, smears were taken from the buccal mucosa before and after treatment, and esophageal smears were obtained during endoscopy only after treatment.

No statistically significant difference in the prevalence of MNi in the buccal mucosa cells was observed before and after treatment or between the treatment and the placebo group at the final examination. The mean percentage of micronucleated cells in the vitamin-treated group was 0.31 and 0.39 % in the placebo group. However, a statistically significant reduction (P = 0.04) was observed in the prevalence of MNi in esophageal cells in the treatment group as compared to the placebo. The mean percentage of micronucleated cells in the vitamin-treated group (n = 40) was 0.19 % and in the placebo group (n = 43) it was 0.31 % [25].

Li et al. (1999) investigated the effect of a tea mixture in a double-blind intervention trial in patients with oral mucosa leukoplakia. Fifty-nine oral mucosa leukoplakia patients were randomly divided into a treatment group (3 g mixed tea oral administration and topical treatment) and a control group (placebo and glycerin treatment). After the 6-months trial, the size of oral lesion was decreased in 37.9 % of the 29 treated patients and increased in 3.4 %; whereas the oral lesion was decreased in 10.0 % of the 30 control patients and increased in 6.7 %. At the same time, the incidence of micronucleated exfoliated oral mucosa cells in the treated group (5.39 per 1000 cells) was lower ($P < 0.01$) than that in the control group (11.3 per 1000 cells); whereas it was 1.4 per 1000 cells in 20 healthy subjects. The micronuclei and chromosome aberration rate in the peripheral blood lymphocytes showed the same results [26].

4. Meta-analysis of RCTs

Meta-analyses regarding lifestyle interventions on the parameters of the CBMN cytome assay in the disease states mentioned in the chapter before were not possible due to the limited number and heterogeneity of the studies as well as the different interventions applied. Therefore, the meta-analyses were performed for the interventions used, independent of the type of the disease, and considered MN, NPB and NBUD frequency.

4.1. Meta-analysis of dietary RCTs related to aging, type 2 diabetes, cancer, CVD and metabolic/inflammatory diseases on MNi

Fig. 2 shows the results of the meta-analyses for the 9 interventions based on antioxidants, folate, a healthy diet, exercise/protein and vitamins, and tea independent of the cells where cytogenetic damage was measures to give an overall overview on available data. A more detailed differentiation into PBL and buccal/mucosa is shown in Figs. 3 and 4. Interventions with antioxidants and with green tea did significantly decrease MN frequency ($p < 0.01$ and $p < 0.0001$, respectively), which was

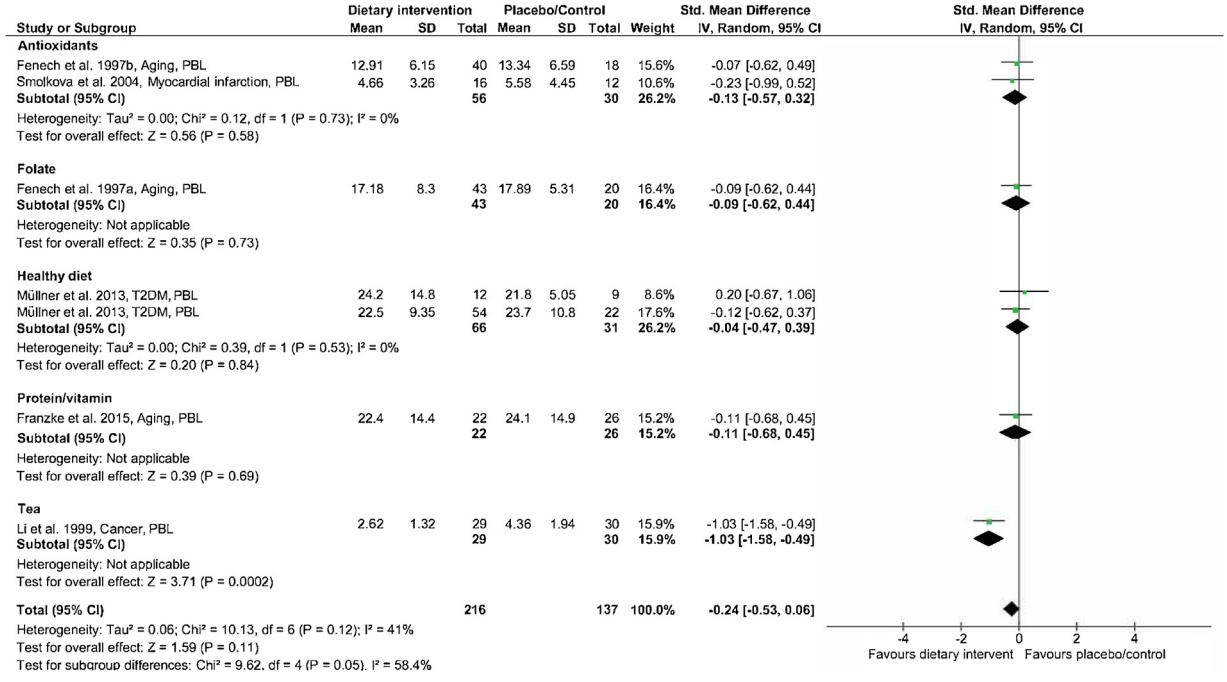


Fig. 3. Forest plot showing the effects of dietary interventions on MN frequency in PBL related to aging, type 2 diabetes, cancer, CVD and metabolic diseases in RCTs.

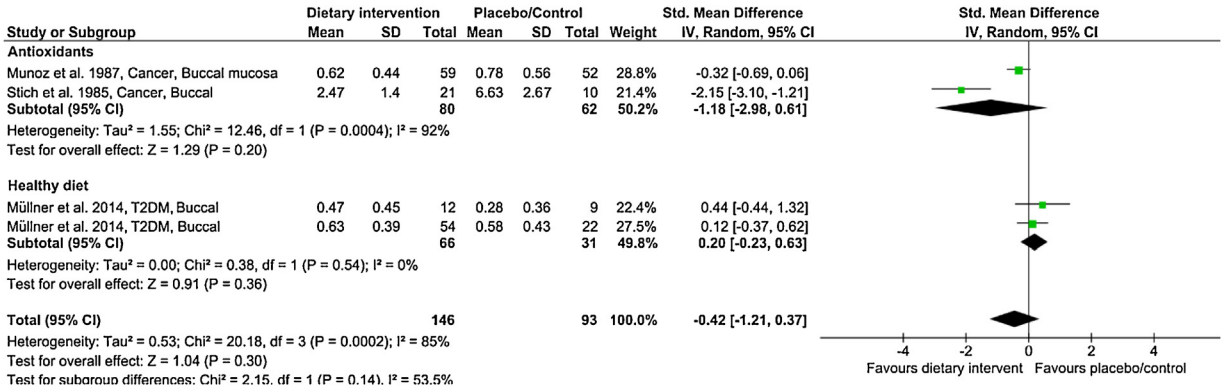
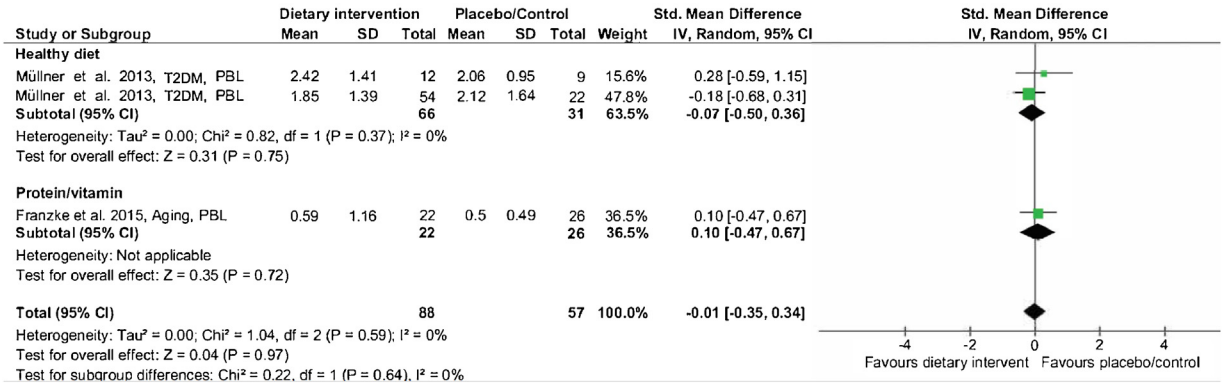


Fig. 4. Forest plot showing the effects of dietary interventions on MN frequency in Buccal cells to aging, type 2 diabetes, cancer, CVD and metabolic diseases in RCTs.



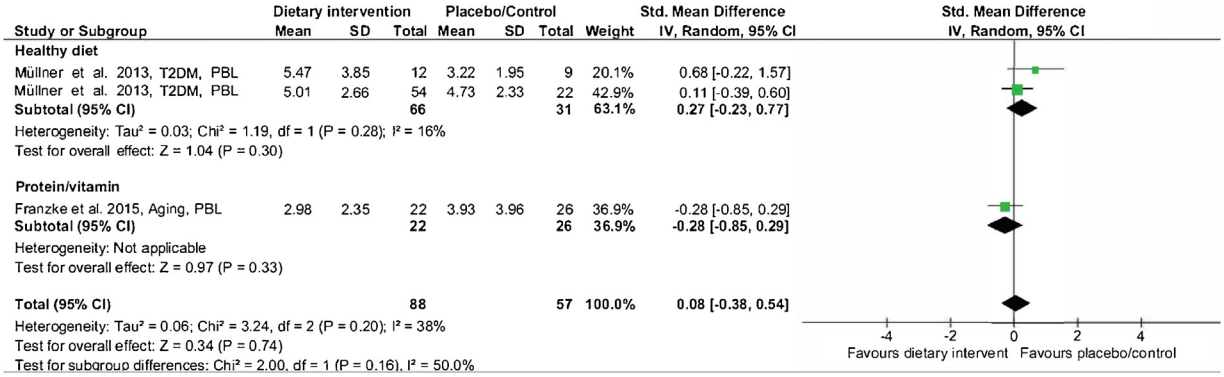


Fig. 5. Forest plot showing the effects of dietary interventions on NPBs (A) and NBUDs (B) frequency in PBL related to aging, type 2 diabetes, cancer, CVD and metabolic diseases in RCTs.

mainly based on reductions in three cancer related studies [23,24,26] in the mucosa, mucosa lesions or mucosa leuplakia. When considering MN frequency in PBL, the only reduction was observed for a tea intervention in patients with oral mucosa leukoplakia ($p < 0.001$) (Fig. 3). There was no significant impact of lifestyle interventions on MNi in buccal cells (Fig. 4).

4.2. Meta-analysis of dietary RCTs related to aging, type 2 diabetes, cancer, CVD and metabolic diseases on NPBs and NBUDs

Surprisingly, there were only very few intervention studies in this context where additionally to MNi also NPBs and NBUDs were investigated. Fig. 5 shows the results of the meta-analyses. Neither NPBs nor NBUDs were significantly influenced by the interventions in elderly or type 2 diabetes.

5. Discussion

NCDs are the world's leading causes of death and disability, are the main drivers of increasing health care costs, and undermine the economic stability of individuals, families, communities, and countries [27]. A plethora of studies support the concept that regular physical activity, maintenance of a proper bodyweight, sound nutritional practices, and avoiding tobacco products all significantly reduce the risk of chronic diseases. Therefore, the United Nations Sustainable Development Goals aims to reduce the risk of premature death among people aged 30–69 years from CVD, cancer, diabetes, and chronic lung disease by one third by 2030. Lifestyle interventions/changes are among other initiatives, such as tobacco control, the most important drivers to potentially achieve this goal.

Many of the possible consequences of increased risk factors for chronic diseases are linked to reduced quality of life, faster aging, loss of years in good health or disability and dependency on others [28–30]. Physiologically, inflammation and oxidative stress are increasing and at the same time genome stability is reduced, indicated by increased chromosomal damage and higher frequencies of genome-mutations. These DNA- and genome-based changes are strongly associated with the development of cancer and other chronic diseases and therefore potential biomarker candidates for cancer-risk-evaluation [28,31,32]. The CBMN cytome assay is a well established and widely used method to measure genotoxicity and chromosomal instability. It was the intention of this review and meta-analysis to investigate the effect of human lifestyle intervention studies in patients suffering from the above-mentioned chronic diseases, or who are in a preliminary state of these diseases, on the main markers MNi, NPBs and NBUDs [33].

The total number of intervention studies with these links was relatively low and the quality quite diverse in terms of number of subjects included (eight to almost 100), duration of the lifestyle intervention (2 weeks to 1 year) or the rationale behind (single antioxidant vs. complex dietary and physical activity intervention). Most of the studies used single or mixed supplements (beta-carotene, vitamin A, E or zinc), whereas, more rarely, food-based interventions (vegetables, plant oil, Mediterranean style diet) or physical activity approaches were realized. The available studies were primarily performed in PBL, but particularly those few referring to cancer also used buccal cells, the buccal mucosa or mucosa lesions. Only three studies investigated MNi in PBL and the buccal/mucosa. Only two studies included MNi, NPBs and NBUDs in their analyses. The available 13 studies were conducted by only eight research groups, showing the relatively low number of groups performing lifestyle based intervention studies in elderly or patients suffering from chronic diseases, which might be based on the complexity of the marker together with the challenge to run human intervention studies in patients or elderly subjects.

There are also other published studies using the CBMN cytome assay within an intervention such as medication interventions, studies where the CBMN cytome assay was used as safety assessment tool or where surgeries were used as intervention (mainly bariatric surgeries) [19,34,35], however, they were not considered in this review.

5.1. Aging

Only three studies with lifestyle-based interventions in elderly were identified, where MNi and partly also NPBs and NBUDs were analyzed with an appropriate treatment duration between 2 and 6 months [11–14]. MNi did not decrease significantly in either of the studies after the intervention. Only in one study MN frequency was reduced, however in both, the intervention and the control group [12]. NPBs and NBUDs, determined only in one study, decreased significantly only in the control group of elderly, which received no training or nutritional intervention but performed cognitive training [13]. The number of subjects in the studies was appropriate with groups between 20 and 43 participants. Despite the challenges of performing intervention studies with elderly subjects, more studies are definitely needed to obtain a better understanding of how chromosomal damage could be influenced by lifestyle interventions during the aging process. Particularly elderly people are of interest regarding the CBMN cytome assay, since we recently showed that there is a leveling-off of the MN frequency at about 60–70 years of age [36], which indicates that there might be a threshold of genome instability that limits the upper rate of MNi formation. If old people are able to reach an age above life expectancy ("survivors"), they seem to be either more resistant to MNi formation or cells with MNi are more likely to undergo apoptosis [37,38].

5.2. Type 2 diabetes

Also regarding type 2 diabetes, only three lifestyle intervention studies could be included, which were performed by only two working groups. Compared to studies in the elderly, the duration of the interventions were only 30 days up to 8 weeks. Two studies were lacking a control group and considered only an intervention in patients. All three studies investigated MN frequency in buccal cells, only one additionally in PBL. Two studies had almost the same design and considered high amounts of folic acid for one month, which was enough to reduce the MN frequency [15,16]. There was no effect of a vegetable and plant oil intervention regarding MNi, NPBs and NBUDs in PBL [17].

As discussed in another chapter of this special issue, diabetics (type 2) showed significant links to markers of genome stability. Studies observed significantly higher MNi frequencies within the patient's groups when blood glucose was poorly controlled, and higher chromosomal damage was also linked to disease progression [39], disease duration [40,41] and intensity of medication [42]. The vast majority of the identified studies showed a significant difference between diabetic patients (type 1 or 2) and healthy controls [18,40–46].

Similar to what was discussed for elderly, more interventional studies/RCTs are warranted with type 2 diabetes subjects in order to have a better data base to potentially recommend the use of the CBMN cytome assay for clinical applications.

5.3. Cancer, CVD and metabolic diseases

Although considered in the same chapter it is almost impossible to compare the available studies of the large diseases regarding baseline values. This is due to the heterogeneity of the involved laboratories, the large time range where the studies have been performed with changed techniques within this time range (years: 1985–2020), the different cells considered (PBL, buccal cells, leukoplakia or mucosa) or the average age of the subjects involved (53–83 years). Only seven studies were identified, four of them with a cancer background. The non-cancer studies lasted 2–12 weeks with a low number of participants in the intervention groups (n = 8–16 subjects) and two studies without a control group [21,22]. The MN frequency, which was only assessed in PBL, was not influenced in two studies [20,22] and significantly decreased in one study, with coenzyme Q10 supplementation of only 2 weeks [21].

The cancer related studies were mainly antioxidant supplementation studies [23–25] and one study investigated the impact of green tea [26]. In almost all studies antioxidant/tea supplementation reduced MNi formation in PBL, oral mucosa, mucosa lesions or leukoplakia [23,24,26]. The duration of these studies was appropriate with a duration between 6 and 12 months; only one study had a shorter intervention time.

A large international cohort study showed a significant association between MN frequency in healthy subjects and cancer risk. The study assembled data from 6718 individuals from 10 countries (62,980 person-years). Cancer incidence was significantly higher in groups with medium (RR = 1.84; 95 % confidence interval: 1.28–2.66) and high MN frequency (RR = 1.53; 95 % CI: 1.04–2.25). This study provided preliminary evidence that MN frequency in peripheral blood lymphocytes is predictive of cancer risk, suggesting that increased MNi formation is associated with early events in carcinogenesis [47].

The intervention studies considered here included mainly subjects with mucosa lesions or leukoplakia, however were

conducted 20 years ago or even earlier. In the last 2 decades no lifestyle interventions with cancer patients assessing the CBMN cytome assay were performed.

5.4. Meta-analyses of RCTs

Due to clinical and statistical heterogeneity and lack of enough appropriate studies it was impossible to run meta-analyses based on the disease status. Therefore, we decided to focus on high quality study designs and consequently only RCTs were enclosed for meta-analysis.

Despite the tea intervention study [26], which reduced MN frequency in PBL, no other RCT lead to a decrease in MNi in PBL after the respective lifestyle intervention. The same is true for buccal cells, where the antioxidant supplementation studies by Stich et al. 1985 [23] and 1988 [24] lead to a MNi reduction in buccal mucosa and buccal lesions, whereas all other RCTs did not show a change in the MN frequency. Similarly, only two studies considered data for NPBs and NBUDs in their analyses, but observed no change due to the interventions applied [13,17].

5.5. Clinical utility and knowledge gaps of the CBMN cytome assay for lifestyle interventions in elderly subjects and patients with chronic diseases

Chronic diseases are reflected by chronically increased genome damage, which is influenced by disease progression and the quality of medical control. Unhealthy lifestyle habits most likely lead to a huge disease burden. Adopting a healthy lifestyle is the most beneficial and cost-effective strategy for preventing non-communicable diseases, yet also improves the outcome of a disease. Therefore, lifestyle interventions are essential and the assessment and evaluation of the CBMN cytome assay as one biomarker of interest seems plausible.

However, based on the heterogeneity of the available studies summarized in this review and meta-analysis, much more data is needed, in order to have a better understanding of the potential of MNi, NPBs and NBUDs frequencies as biomarkers within the disease context.

The total number of intervention studies with subjects suffering from a chronic disease is low and the quality of the studies diverse. It seems that the CBMN cytome assay is more likely/often applied in cross-sectional studies with patients or in human interventions with healthy subjects, than in clinical trials investigating diseased populations. Therefore, to decide about the applicability and utility of the CBMN cytome assay and its sensitivity to respond to lifestyle interventions in the clinical environment, more research and data about the metabolic/genomic understanding of the diseases is needed.

Conclusively, the points summarized below should be improved in future lifestyle intervention studies:

- RCTs with patients undergoing lifestyle interventions with an appropriate number of participants are needed.
- Medium to long term duration of the intervention should be considered, to be able to detect changes measured with the CBMN cytome assay, since lymphocytes divide on average once every 30 days with an average lifetime of around 3–4 months. Therefore, short term interventions of few weeks are not reflected by changes in the CBMN cytome assay. For analyzing short term interventions, the BMcyt assay should be preferred, since buccal cells divide much faster.
- Better/comprehensive background data when assessing patients, such as type of medication, number of drugs taken or duration of the diagnosed disease should be collected to thoroughly interpret the data.
- Not only MNi but also other parameters of the CBMN cytome assay, at least NPBs and NBUDs, should be considered.
- A better rational for the interventions used and a better characterization of the disease states would further enhance data quality.

6. Conclusions

Our review and meta-analysis about the effect of lifestyle interventions in elderly or patients with different chronic diseases on the parameters of the CBMN cytome assay revealed a lack of a sound number of appropriate data, although some RCTs indicate a beneficial effect, of lifestyle interventions in these patient groups and/or the elderly population. The number of intervention studies must be increased for elderly, type 2 diabetes subjects or in the context of CVD and cancer in order to have a better understanding about the potential of the CBMN cytome assay to reflect intervention changes and to be routinely used in the clinical setting. So far, a reduction of cytogenetic damage can only be found after antioxidant supplementations in the context of buccal/mucosa lesions or leukoplakia, however, data are rather old and the study quality moderate.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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