Nuclear anomalies in exfoliated buccal cells in healthy and diabetic individuals and the impact of a dietary intervention

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This study aimed to compare the frequencies of nuclear anomalies in buccal cells between diabetic and non-diabetic individuals and to assess the impact of a ‘healthy diet’—a cornerstone in the treatment of diabetes. Seventy-six diabetic and 21 non-diabetic individuals participated in this parallel, randomised, intervention trial. All participants received information about the importance of a healthy diet, while participants randomly assigned to the intervention group received additionally 300 g of vegetables and 25 ml of plant oil rich in polyunsaturated fatty acids (PUFA) per day for 8 weeks. Cytogenetic damage in buccal cells was assessed at baseline and after 8 weeks using the buccal micronucleus cytome assay. Micronucleus (MN) frequency at baseline was significantly higher in participants with diabetes (0.58 ± 0.30‰) compared with non-diabetic individuals (0.28 ± 0.29‰). Further analysis of baseline data revealed significantly higher MN levels in participants of the highest tertile of waist circumference (+40%), fasting plasma glucose (+55%), glycated haemoglobin (+41%) and cardiovascular disease risk (+39%) relative to participants of the lowest tertile. The dietary intervention had no effect on MN frequencies. Glycated haemoglobin and biomarkers reflecting cytokinetic defect or acute cell death were reduced in both the intervention and ‘information only’ groups. The results of this study suggest a strong impact of abdominal obesity and glucose metabolism on genomic stability. Similar effects on nuclear anomalies were observed in the ‘information only’ group and the intervention group receiving vegetables and PUFA-rich plant oil.

Introduction

Type 2 diabetes is one of the most common chronic diseases and a growing health care problem worldwide. It is well recognised that it is linked to increased risk of micro- and macrovascular complications (1). Recent reviews and meta-analysis suggest associations between Type 2 diabetes and risk of liver (2), pancreatic (3), colorectal (4), bladder (5), kidney cancers (6) and non-Hodgkin’s Lymphoma (7).

Several factors, like obesity, quality of metabolic control, medical treatment and lifestyle have a major impact on the onset of diabetes-associated complications. Especially for cancer, it is not clear yet, if the association is direct or mediated via common predisposing risk factors such as aging, obesity, physical inactivity or poor diet quality (8). Hypothesised mechanisms for increased cancer risk in patients with type 2 diabetes include hyperglycaemia, insulin resistance and hyperinsulinaemia (9).

Genomic instability and damage is a central event in the development of many diseases, including cancer (10). A biomarker reflecting genomic damage is the micronucleus (MN) frequency, representing the loss of a whole chromosome or fragments (11). It was postulated that the assessment of micronuclei (MNI) in exfoliated buccal cells, a non-invasive method, might be of high relevance in future as up to 90% of all cancers are of epithelial origin (12).

Zúñiga-González et al. (13) found significantly increased MN levels in exfoliated buccal cells in diabetic patients (type 1 and type 2) compared with healthy individuals and showed a reduction in MN frequency after high dose of folic acid supplementation (15 mg/day). The effect of natural foods on genomic stability in diabetic individuals has not been investigated yet, although beneficial effects of a healthy diet are supported by inverse association between fruit consumption and buccal MN frequency (14), and intake of fruits, vegetables and cruciferous vegetables and cancer risk (15).

In order to compare DNA integrity between diabetic and non-diabetic individuals and to assess the impact of a healthy diet, realised through the replacement of saturated fatty acids (SFA) by polyunsaturated fatty acids (PUFA) and intake of 300 g of vegetables per day, a human intervention study was conducted. Apart of MN levels, frequencies of broken eggs (BE), indicative for gene amplification and binucleated cells (BNC), reflecting cytokinetic defects, were monitored. Furthermore, karyolytic (KL), pycnotic (PC) and karyorrhectic (KR) cells and cells with condensed chromatin (CC) that reflect acute cell death (11) were scored.

Material and methods

Study population

Patients with type 2 diabetes (36 treated with insulin, 40 treated with oral anti-diabetic drugs) were recruited from a local diabetes clinic (Diabetes Outpatient Clinic, Health Centre South, Vienna, Austria). Non-diabetic individuals (n = 21) were partners of the diabetic subjects.

Individuals with type 2 diabetes had to have stable metabolic control (constant medication regarding glucose, lipid and uric acid metabolism), glycated haemoglobin (HbA1c) concentration <9.5% (80 mmol/mol), serum total cholesterol (TC) <300 mg/dl (<7.76 mmol/l), serum triglycerides <500 mg/dl (<5.7 mmol/l) and serum creatinine <2.5 mg/dl (<221 μmol/l). Non-diabetic individuals had to meet the above criteria and were not allowed to take glucose-lowering drugs. Otherwise, the same inclusion criteria were applied.

All subjects had stable body weights, constant dietary habits and physical activity levels for at least 4 weeks before entry to the study. Subjects who intended to change dietary habits, frequency of physical activity or body weight within the study period were excluded from the participation. Further exclusion criteria were smoking, intake of supplements containing fish oil or other fatty acids. The medical therapies of patients were not changed during the study.

The study was performed according to the Declaration of Helsinki, approved by the Ethical Committee of the City of Vienna (EK09-218-VK_NZ).
and the trial was registered on Current Controlled Trials (ISRCTN53451803). The inclusion criteria were fulfilled by 151 subjects. Of them, 120 gave their informed, written consent. Out of these, 21 withdrew because of health problems unrelated to the study, digestive discomfort or scheduling conflicts. Two subjects, not reported not having diabetes, were excluded because of increased fasting glucose levels.

Study design and dietary intervention

All participants (diabetic and non-diabetic individuals) received information about the beneficial effects of a healthy diet with special focus on the importance of fat quality and the role of vegetables in a balanced diet. Participants were randomly assigned to the ‘intervention’ or ‘information only’ group. Subjects of the ‘information only’ group received the above-mentioned information, while subjects of the ‘intervention’ group consumed additionally 25 ml of PUFA-rich walnut oil (SFA:MUFA:PUFA: 9:3:17:4:73.3) per day and 300 g vegetables. A variety of frozen vegetables [spinach, green beans, broccoli, Brussels sprouts, soybeans, peas, carrots, romaine lettuce with peas, different vegetable mixes with broccoli, cauliflower, carrots, kohlrabi, leek, peas, corn, zucchini or pole beans; for details on dietary composition see Müllner et al. (16) and Müllner et al. (17)] were given to the participants. The participants were instructed to use the plant oil as replacement for SFA. A reference ‘cup’ and a booklet with recipes, instructions for replacement of SFA and usage of the plant oil (oil was not allowed to be heated up, but added to warm foods) were provided to the participants. A dietary diary had to be completed, and fatty acid profile and various vitamin concentrations were measured to monitor compliance.

The intervention period lasted 8 weeks, followed by a period of 8 weeks in which no intervention foods were provided. Blood samples were taken before the intervention, after 4, 8 (end of intervention period) and 16 weeks. The buccal micronucleus cytome (BMCyt) assay was performed at baseline and Week 8.

Anthropometric and blood pressure measurements and Framingham general cardiovascular risk

For all anthropometric measurements, participants were lightly dressed, without wearing shoes. Body height (stadiometer: Seca, Modell 214, Hamburg, Germany), body weight (digital scale: Seca, Bella 840, Hamburg, Germany) and waist circumference were measured. Body mass index (BMI) was calculated as kg/m².

At each study visit, three blood pressure measurements (BpTRU Medical Devices, Coquitlam, BC, Canada) with 2-min intervals in between (the mean of the last two measurements was used) were obtained after at least 5-min rest with the subject in a seated position. The Framingham general cardiovascular risk (18) was estimated, using following variables: age, sex, tobacco use, treated/unintreated systolic blood pressure, diabetes and TC, HDL cholesterol.

Blood sampling and laboratory analysis

Venous blood samples were obtained after an overnight fast using heparin or serum tubes (Becton Dickinson, Schwarz, Austria). After centrifugation, serum and plasma were aliquoted, used fresh or frozen at −80°C for further analysis. Erythrocytes were washed three times with isotonic phosphate buffer, aliquoted and stored at −80°C.

Fasting plasma glucose, HbA1c, high-density lipoprotein (HDL), TC and insulin were measured immediately by the laboratory of the Health Centre South, Vienna. HbA1c was analysed in whole blood by high-performance liquid chromatography (HPLC; automated Glycohaemoglobin Analyzer HLC-723G8; Tosoh, Tokyo, Japan). Fasting plasma glucose was determined enzymatically by the hexokinase method (Aerose, Abbott Diagnostics, IL, USA); plasma insulin concentrations were measured on an Immulite 2000 immunochemistry system (Siemens Medical Solutions Diagnostics, Flanders, USA). Homeostasis model assessment (HOMA) of insulin resistance was calculated as the product of fasting plasma glucose (mmol/l) and insulin (µU/ml) concentrations, divided by 22.5. Serum TC and HDL cholesterol levels were measured enzymatically by an automated method (Aerose, Abbott Laboratories, North Chicago, IL, USA) using commercial kits (Abbott).

The fatty acid profile in plasma was determined by a gas chromatograph equipped with a flame ionisation detector (19). Identification of fatty acids was based on the comparison of the samples’ retention times to those of a 37 Component FAME Mix standard (Supelco, Bellefonte, USA). TotalChrom Workstation 6.3.0, PE Nelson, Perkin Elmer was used for peak integration.

Plasma concentrations of vitamin K, α- and γ-tocopherol, lutein and β-carotene were determined by reverse phase HPLC (20). Each vitamin was quantitated on the basis of peak area using a calibration curve generated from standard solutions.

Plasma concentrations of vitamin B12 and folic acid were measured according to routine diagnostic tests on Siemens Immulite 2000 analyzer (Siemens Healthcare Diagnostics, Tarrytown, USA) at the laboratory of the Health Centre South, Vienna. Folic acid in erythrocytes was measured using radioimmunoassay (MP Biomedical, Germany).

Plasma homocysteine was measured by reverse phase HPLC with a fluorescence detector (emission wavelength: 515 nm; excitation wavelength: 385 nm) on a LiChrospher column (5 µm, 125 × 4 mm; Merck, Hitachi, LaChrom, Austria). Potassium hydrogen phosphate buffer with 4% acetonitrile was used as mobile phase (21).

Adiponectin was measured with a magnetic bead–based assay (Bio-Plex Pro diabetes assay, Bio-Rad Laboratories, Inc.) and a Bio-Plex array reader using Bio-Plex Manager™ Software 4.1.1.

Buccal micronucleus cytome assay

Cells were collected with toothbrushes from both cheeks after subjects rinsed their mouths with tap water. Subsequently, slides were prepared, stained and scored according to the method of Thomas et al. (11). Cells suspensions were diluted to a concentration of >80 000 cells/ml. One hundred and twenty microlitres of cell suspension were transferred to slides by cytocentrifugation (Shandon Cytocentrifuge Cytospine 4) and fixed with cold methanol (80%) for 30 min, and ≥2000 cells/sample were evaluated.

For Feulgen staining, cells were placed in beakers with 5.0 M HCl at room temperature for 15 min, rinsed with distilled water (15 min) and subsequently stained with Schiff’s reagent (90 min). Cells were scored under bright field with 400-fold magnification using oil immersion with Eclipse E 600 microscope (Nikon, Tokyo, Japan) and then confirmed as positive under fluorescence. MNI were scored in a combination of both basal and differentiated cells according to the criteria defined by Thomas et al. (11). The analyses of the slides were carried out at the Institute of Cancer Research (Vienna, Austria) by two experienced scorers and recorded after consensus.

As biomarkers for genome damage, MN cells, total MNI, BNC and BE and for cell death, KL, KR + CC, and P were scored. Since KR and CC cells are difficult to discriminate, they were scored together.

Statistical analyses

Statistical analyses were performed using SPSS 20.0 for Windows (SPSS, Chicago, IL, USA). Normal distribution within the data set was tested by histograms and Kolmogorov-Smirnov test. To assess differences between two groups, independent samples t-test (for parametric data) or Mann–Whitney U-test (for non-parametric data) was conducted.

Baseline data were categorised into tertiles, and MN frequencies were compared between them (Mann–Whitney U-test and correction for multiple comparisons). Chi-square test was used for comparisons of binary variables.

The effect of the intervention was assessed by comparing baseline values with values after 8 weeks (Wilcoxon test or paired t-test) and by comparing the changes after 8 weeks between the two treatment groups (Mann–Whitney U-test or independent samples t-test). Pearson correlation and Spearman rank correlation were used to evaluate associations between variables. Results were considered significant at P < 0.05.

Results

Baseline data

Baseline characteristics of diabetic and non-diabetic individuals are presented in Table I. Diabetic individuals had significantly higher BMI and waist circumference and higher levels of fasting blood glucose, HbA1c and HOMA-insulin resistance. MN frequency and total number of MNi were approximately 2-fold higher in diabetic patients compared with non-diabetic participants (Table I).

All participants were non-smokers and no significant differences between the two groups regarding smoking behaviour in the past were observed. Levels of genomic damage were not significantly different between ex-smokers (n = 49; MN cells: 0.57 ± 0.37‰) and never-smokers (n = 48; MN cells: 0.46 ± 0.25‰). However, smoking duration (r = 0.305, P = 0.033) and number of cigarettes smoked per day (r = 0.354, P = 0.013) were significantly correlated with the MN frequency among ex-smokers. Sex had no impact on genome damage rates (male: 0.55 ± 0.27‰; MN cells; female: 0.50 ± 0.35‰; MN cells).
Analysis of the relationships between anthropometric measurements, clinical chemistry parameters and MN frequency revealed significant associations (Figure 1). Participants in the highest tertile of waist circumference, fasting plasma glucose, HbA1c and cardiovascular disease (CVD) risk had significantly higher MN frequencies (+40%, +55%, +41%, +39%, respectively) compared with participants in the lowest tertile. A similar trend (P < 0.1) was observed for insulin and HOMA-insulin resistance.

In contrast, a trend towards a protective effect of adiponectin was observed (first tertile (≤3.58 µg/ml): 0.56 ± 0.30‰ MN cells; third tertile (≥5.89 µg/ml): 0.48 ± 0.33‰ MN cells; P = 0.102). No associations between vitamin B12, plasma folate, red blood cell folate, homocysteine and MN frequency were observed (data not shown).

At baseline, there were no significant differences between information and intervention groups, apart from levels of pycnotic cells, which were significantly higher in participants of the information group (Table II).

**Effect of the intervention**

Dietary compliance was monitored via dietary diaries, showing an average consumption of 294 ± 28.5 g vegetables per day. Furthermore, oil bottles were collected and weighed and showed an intake of 23.4 ± 4.01 ml plant oil per day (density of oil 0.85 g/cm³).

Plasma and red blood cell analyses confirmed good dietary compliance and showed significant increases in lutein (+58%; P < 0.001), α-carotene (+92%; P < 0.001), β-carotene (+53%; P < 0.001), vitamin K (+154%; P < 0.001), plasma folate (+13%; P < 0.05), red blood cell folate (+10%; P < 0.05), γ-tocopherol...
(+37%; \( P < 0.001 \)), linoleic acid (+13%; \( P < 0.001 \)) and linolenic acid (+60%; \( P < 0.001 \)) after 8 weeks of intervention with vegetables and plant oil. None of the above-mentioned parameters, apart from \( \beta \)-carotene, were significantly increased in participants of the intervention group. Changes in concentrations of the above-mentioned vitamins and antioxidants were significantly higher in participants of the intervention group compared with the information group [for details see Müllner et al. (16) and Müllner et al. (17)].

Body weight, BMI and waist circumference were not altered during the study, neither in the intervention nor in the information group (data not shown). Glycemic control improved significantly during the course of the study: HbA1c decreased in diabetic individuals after 8 weeks from 7.58 ± 0.93% to 7.36 ± 0.80% in the intervention group and from 7.48 ± 0.80% to 7.25 ± 0.89% in the information group. Therefore changes in HbA1c were not significantly different between the two treatment groups. No changes in HbA1c levels were observed in non-diabetic individuals (information group, baseline: 5.71 ± 0.28%, after 8 weeks: 5.73 ± 0.30%; intervention group, baseline: 5.83 ± 0.24, after 8 weeks: 5.79 ± 0.47).

During the course of the study, the numbers of BNC and KR + CC were decreased in both the information and intervention groups (Table III). PC were only reduced in participants of the intervention group, and changes after 8 weeks were significantly greater in participants of the intervention group compared with the information group. For all other BMCyt assay parameters, changes after 8 weeks were not significantly different between the two treatment groups.

### Discussion

The results of this study show that levels of buccal MNi in diabetic individuals are approximately 2-fold higher than in non-diabetic participants. MNi in exfoliated buccal cells are a novel and non-invasive biomarker of genomic stability, formed during mitosis in the basal cell layer of the epithelium and represent the loss of chromosome fragments or a whole chromosome that failed to be incorporated in the main nuclei (11). Genomic instability is a hallmark of tumourigenesis (10); in addition, cancer patients show increased levels of buccal MN frequency (22–25) compared with healthy individuals. Therefore, our results strongly suggest higher genomic instability in patients with diabetes mellitus compared with non-diabetic participants, which is consistent with epidemiological data indicating increased cancer risk in individuals with type 2 diabetes (26).

Alcohol intake, smoking and dietary habits are known as major modifiable variables influencing genomic stability in healthy individuals (14). Only few studies concerning the

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### Table II. Characteristics of participants at baseline

<table>
<thead>
<tr>
<th></th>
<th>Information</th>
<th>Intervention</th>
<th>( P )-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (diabetic/</td>
<td>31 (22/9)</td>
<td>66 (54/12)</td>
<td>–</td>
</tr>
<tr>
<td>non-diabetic)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>61.7 ± 6.66</td>
<td>65.9 ± 7.19</td>
<td>0.006</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.7 ± 7.03</td>
<td>32.5 ± 5.79</td>
<td>0.820</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>108.5 ± 17.6</td>
<td>109.4 ± 14.5</td>
<td>0.795</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>7.41 ± 0.27</td>
<td>8.13 ± 0.27</td>
<td>0.150</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.01 ± 1.05</td>
<td>7.26 ± 1.08</td>
<td>0.295</td>
</tr>
<tr>
<td>HOMA-insulin resistance</td>
<td>5.10 ± 3.84</td>
<td>7.09 ± 10.1</td>
<td>0.959</td>
</tr>
<tr>
<td>MN cells (%)</td>
<td>0.44 ± 0.31</td>
<td>0.56 ± 0.32</td>
<td>0.323</td>
</tr>
<tr>
<td>Total MN (%)</td>
<td>0.50 ± 0.37</td>
<td>0.69 ± 0.41</td>
<td>0.106</td>
</tr>
<tr>
<td>BNC (%)</td>
<td>21.7 ± 12.7</td>
<td>21.6 ± 13.6</td>
<td>1.000</td>
</tr>
<tr>
<td>BE (µE)</td>
<td>0.48 ± 0.55</td>
<td>0.78 ± 0.86</td>
<td>0.065</td>
</tr>
<tr>
<td>KR + CC (‰)</td>
<td>25.0 ± 19.1</td>
<td>23.5 ± 17.1</td>
<td>0.816</td>
</tr>
<tr>
<td>KL (‰)</td>
<td>60.7 ± 76.2</td>
<td>56.2 ± 67.2</td>
<td>0.997</td>
</tr>
<tr>
<td>P (‰)</td>
<td>0.67 ± 0.84</td>
<td>1.16 ± 1.32</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. BMCyt values are per 1000 differentiated cells. *Calculated by unpaired t-test (age, BMI, waist circumference, fasting plasma glucose, HbA1c, basal cells) and Mann–Whitney \( U \)-test (HOMA-insulin resistance, MN cells, total MN, BNC, BE, KR + CC, KL, P).

### Table III. Changes in genome damage rate in participants of the information and intervention group

<table>
<thead>
<tr>
<th></th>
<th>Information</th>
<th>Intervention</th>
<th>( P )-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Week 8</td>
<td></td>
</tr>
<tr>
<td>MN cells (%)</td>
<td>0.53 ± 0.29</td>
<td>0.52 ± 0.36</td>
<td>0.816</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.22 ± 0.26</td>
<td>0.27 ± 0.26</td>
<td>–</td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>0.60 ± 0.34</td>
<td>0.58 ± 0.43</td>
<td>–</td>
</tr>
<tr>
<td>Total MN (%)</td>
<td>0.27 ± 0.35</td>
<td>0.28 ± 0.36</td>
<td>–</td>
</tr>
<tr>
<td>BNC (%)</td>
<td>21.3 ± 13.90</td>
<td>15.7 ± 7.42</td>
<td>–</td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>22.8 ± 9.81</td>
<td>13.6 ± 8.45</td>
<td>–</td>
</tr>
<tr>
<td>BE (µE)</td>
<td>0.62 ± 0.56</td>
<td>0.49 ± 1.29</td>
<td>–</td>
</tr>
<tr>
<td>KR + CC (‰)</td>
<td>24.3 ± 19.4</td>
<td>15.4 ± 11.1</td>
<td>–</td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>26.7 ± 19.2</td>
<td>23.8 ± 22.0</td>
<td>–</td>
</tr>
<tr>
<td>KL (%)</td>
<td>63.3 ± 77.9</td>
<td>60.0 ± 76.9</td>
<td>–</td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>54.3 ± 76.1</td>
<td>41.5 ± 52.7</td>
<td>–</td>
</tr>
<tr>
<td>PC (‰)</td>
<td>0.74 ± 0.94</td>
<td>0.84 ± 0.88</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>Week 8</td>
<td></td>
</tr>
<tr>
<td>MN cells (%)</td>
<td>0.53 ± 0.30</td>
<td>0.51 ± 0.29</td>
<td>0.331</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.32 ± 0.31</td>
<td>0.40 ± 0.36</td>
<td>–</td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>0.76 ± 0.39</td>
<td>0.63 ± 0.39</td>
<td>–</td>
</tr>
<tr>
<td>Total MN (%)</td>
<td>0.36 ± 0.36</td>
<td>0.47 ± 0.45</td>
<td>–</td>
</tr>
<tr>
<td>BNC (%)</td>
<td>21.5 ± 12.78</td>
<td>15.0 ± 7.50</td>
<td>–</td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>22.0 ± 17.31</td>
<td>13.3 ± 6.26</td>
<td>–</td>
</tr>
<tr>
<td>BE (µE)</td>
<td>0.76 ± 0.80</td>
<td>0.71 ± 0.82</td>
<td>–</td>
</tr>
<tr>
<td>KR + CC (‰)</td>
<td>23.0 ± 15.9</td>
<td>18.6 ± 15.9</td>
<td>–</td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>24.5 ± 22.5</td>
<td>21.3 ± 15.5</td>
<td>–</td>
</tr>
<tr>
<td>KL (%)</td>
<td>56.0 ± 65.7</td>
<td>49.3 ± 54.7</td>
<td>–</td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>57.4 ± 76.7</td>
<td>43.9 ± 74.8</td>
<td>–</td>
</tr>
<tr>
<td>PC (‰)</td>
<td>1.24 ± 1.43</td>
<td>0.59 ± 0.59</td>
<td>–</td>
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</tbody>
</table>
| Data are presented as mean ± SD. BMCyt values are per 1000 differentiated cells. *\( P \)-values refer to difference between baseline and Week 8 in the information and intervention group (diabetic and non-diabetic individuals together) and were calculated with Wilcoxon test.
frequencies of buccal MN in diabetic individuals were performed so far, and they are either of limited quality (27,28) or did not address the issue of modifiable risk factors (13,29).

This study suggests associations between abdominal obesity, glucose metabolism, CVD-risk and buccal MN frequency, while no associations were observed with nutrients involved in DNA synthesis such as folic acid and vitamin B12 or homocysteine. Interestingly, we observed correlations between smoking duration, number of cigarettes smoked per day and buccal MN frequency. This finding is somehow surprising, since buccal cells have a rather fast turnover and are replaced after 7–21 days (11). Since other studies reported a trend towards higher proliferation index in ex-smokers compared with non-smokers (30), one may suggest that smoking can lead to permanent epithelial alterations. However, further studies are needed to confirm this association.

The observed link between waist circumference and decreased genomic stability is consistent with findings of epidemiological studies showing that obesity in general (31,32) and waist circumference in particular (33–36) are associated with genomic instability, manifested as increased risk of site-specific malignancies. Mechanisms linking obesity and genome instability are insulin resistance and chronic hyperinsulinaemia (37,38). Hyperinsulinaemia leads to reduced hepatic production of insulin-like growth factor–binding protein 1 and 2 and thereby to increased levels of free, bioactive insulin-like growth factor-1 (IGF-1) (39). IGF-1 induces several biological actions, like cell proliferation, differentiation and inhibition of apoptosis, which may lead to genomic instability and favour tumour growth (40). A trend ($P < 0.1$, Figure 1) for significantly different MN levels between participants in the highest and lowest tertiles of insulin or HOMA-insulin resistance was observed in this study.

Insulin resistance is among other factors a link between abdominal obesity and hyperglycaemia. High glucose levels may contribute to decreased genomic stability via increased cell proliferation, enhancing the risk of genetic errors and the possibility of cancer development (41,42). Glucose is also used as an energy substrate in tumour cells and might thereby have a direct tumour promoting effect (43). Within this study, participants in the highest tertile of fasting plasma glucose and HbA1c showed significantly higher levels of genomic damage compared with participants in the lowest tertile.

Furthermore, a positive association between genomic instability and CVD risk, assessed by the Framingham general cardiovascular risk score (18), was observed. It was postulated that genomic instability is a relevant contributor to atherosclerosis (44) and that patients at increased risk for CVD (17) or with coronary artery disease (45) have higher MN frequency in lymphocytes. Mercer et al. (44) suggested that persistent DNA damage in plaque cells changes the ratio of cell proliferation and apoptosis and thereby promotes the risk of atherosclerosis. Folate and vitamin B12 are important modulating factors of chromosomal stability due to their role in DNA biosynthesis pathways (46). The MN frequencies in lymphocytes are higher under conditions of folate and B12 deficiencies (47), and intervention studies in diabetic individuals with folate showed significant reductions of buccal MN frequencies (13,29). However, the administered dose (15 mg/day) within these trials was 37.5-fold higher than the dietary reference intake and exceeded the upper safe level for folate intake several folds, which cannot be reached via a dietary intervention with whole foods.

The study duration of the present trial (8 weeks) is supposed to be long enough to see positive effects of an intervention since the time frame for cells to migrate from the basal layer to the oral cavity is suggested to range from 7 to 21 days (11). The above-mentioned intervention trials with high dosage of folic acid could show improvements in MN frequency already after 1 month (13,29).

Within the present trial, no associations with folate or vitamin B12 were observed, and the vitamin B12, plasma and red blood cell folate status were satisfactory (17). Significantly increased plasma levels of antioxidants and vitamins and reductions in HbA1c during the intervention did not lead to a reduction in buccal MN frequency. Our findings are in contrast with high-dose supplementary trials in healthy individuals, showing reductions in MN levels after supplementation with folate (48), vitamin A, β-carotene and canthaxanthin (49). On the contrary, levels of BNC and KR + CC were significantly reduced within this study in both the intervention and information groups. Reasons for these improvements are unknown, however, might be related to the reduction in HbA1c, which was improved in the intervention and information groups.

In conclusion, the results of this study provide important novel information regarding associations between waist circumference, glucose metabolism and genomic damage. The mentioned associations with MN frequency were observed in a population of diabetic and non-diabetic individuals. The highest tertiles of waist circumference, CVD risk, fasting plasma glucose and HbA1c were comprised almost solely diabetic individuals. However, the lowest tertiles consisted of both diabetic and non-diabetic individuals. Therefore, diabetic individuals should aim on achieving waist circumference and blood glucose levels as observed in the first tertile, which is accompanied by better genomic stability.

An intervention with vegetables and walnut oil, which was accepted by the subjects and proven for everyday use, led only to moderate improvements of genomic stability; nevertheless, results of this study underline the importance of tight glycemic control and optimum body weight and show that providing participants with information reduces HbA1c as much as a nutritional intervention.

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References


