Final Conference of the NutriAging project

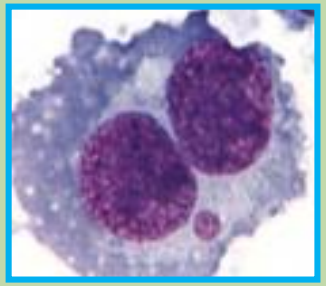
Insights into chromosmal instabilty and vitamin D supplementation in older adults

Presentation by Agnes Draxler

Wagner Working group

Department of Nutritional Sciences, Vienna

20/21 September 2022, in Bratislava



What to be expected in this talk…

Introduction into aging and its relevance

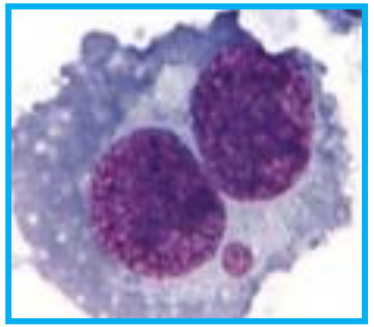
The meaning of chromosomal stability

Micronuclei frequency as a potential biomarker for…

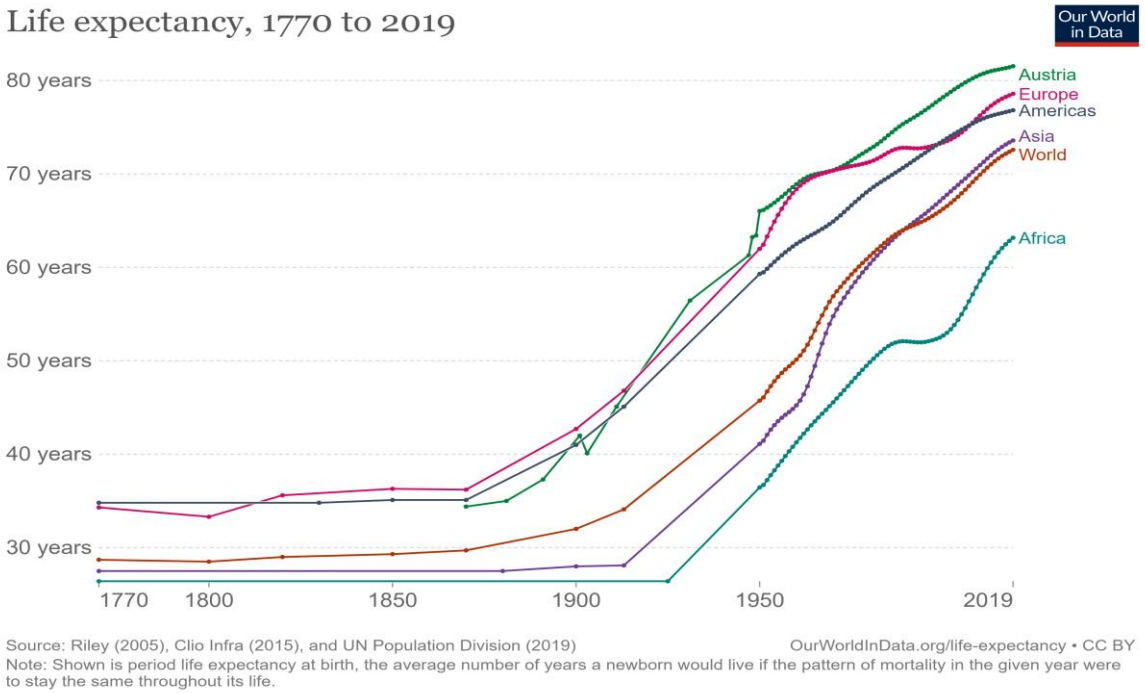
Methodology – The CBMN assay

Results

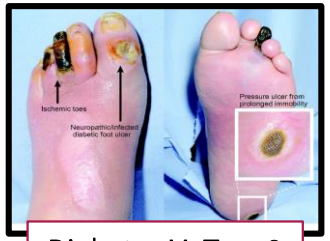
Summary and Conclusions about findings



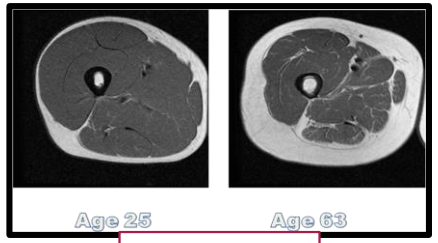
Introduction



Noncommunicable diseases

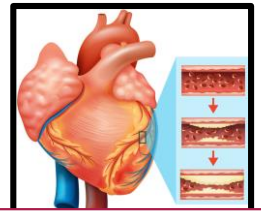


Diabetes M. Type2



Sacropenia

Cancers



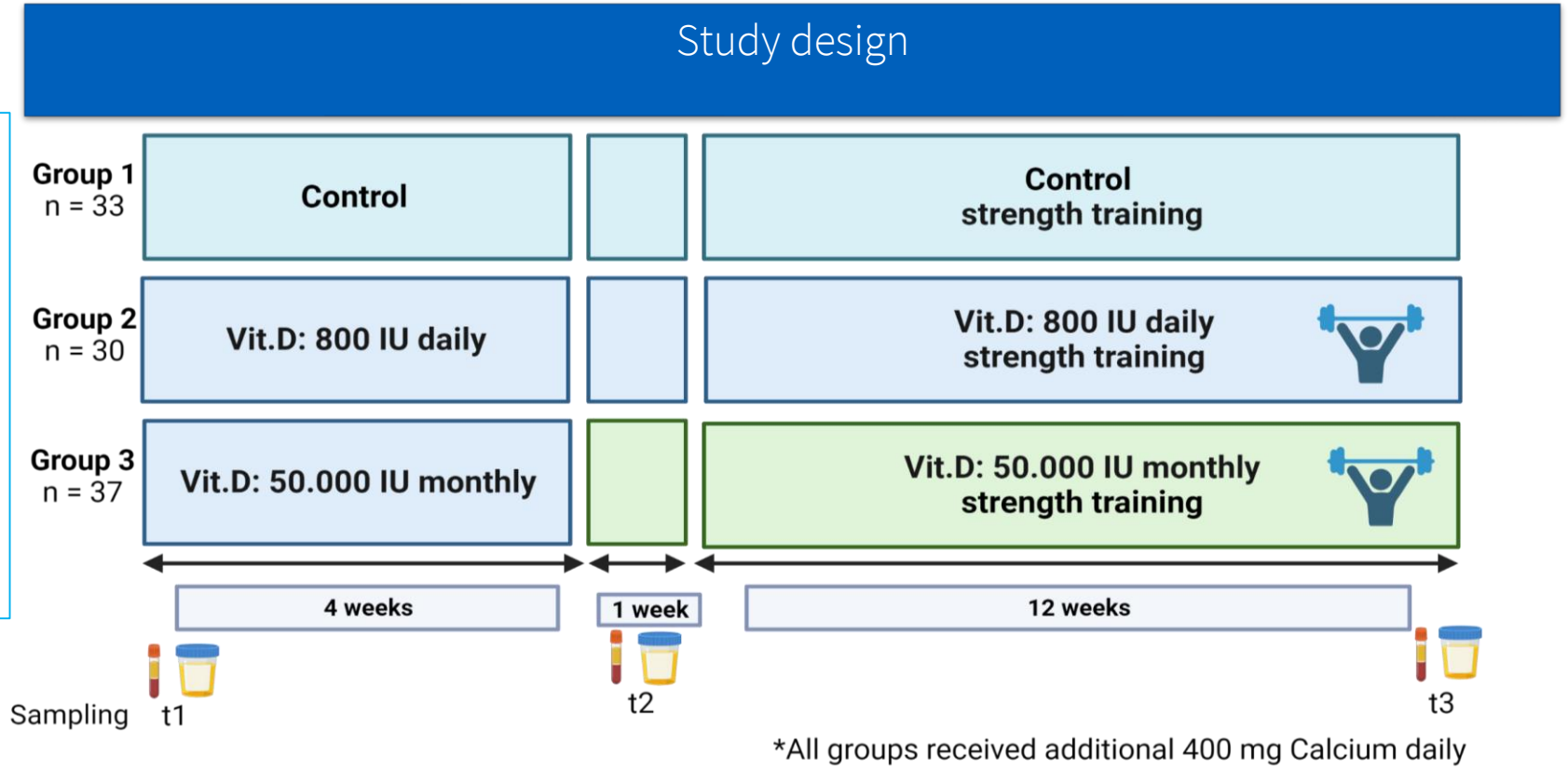
Cardiovascular diseases

Additional years in old age are **not always** years in good health
NCD are the leading cause for additional years in disability in older adults

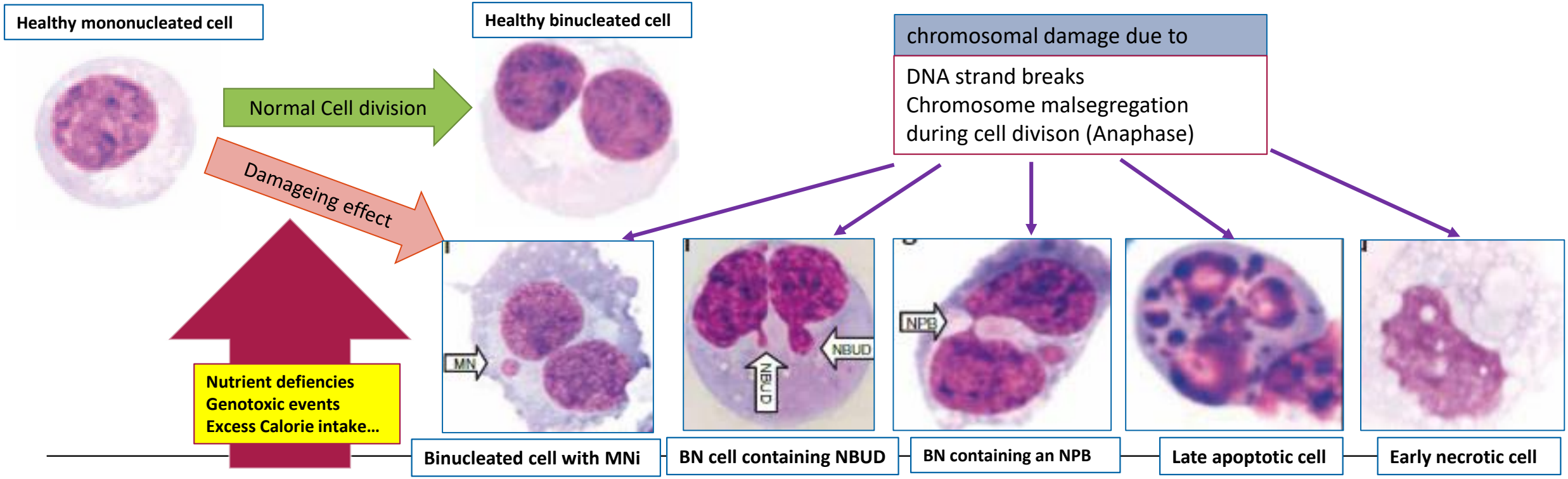
- 100 participants

aged between 65 and 85 years

- completely healthy community-dwelling pensioners
- physically inactive (no sports prior to the intervention)
- divided into 3 groups



Parameters for chromosomal stability



Main methodology

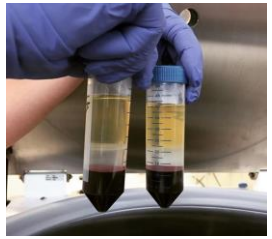
Cytokinesis-block micronucleus (CBMN) assay

M. Fenech

1

Blood drawing

2



Peripheral blood mononuclear cells (PBMCs)

3 Addition of PHA

4 Incubation for 44 hours, 37°C, 5% CO_2

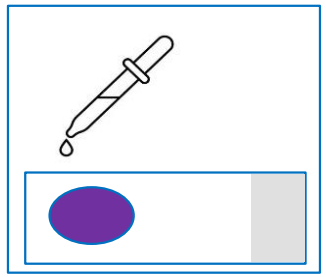
5 Addition of Cytochalasin B

6 Incubation for 28 hours, 37°C, 5% CO_2

7

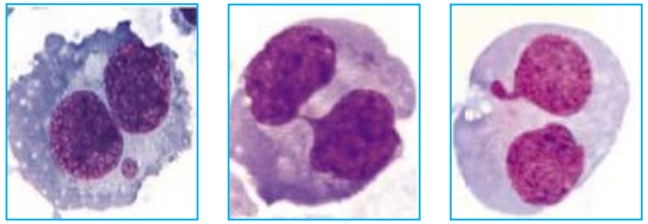
Concentrating PBMCs on microcscope slides using the Cytospin centrifuge

8



Cell staining

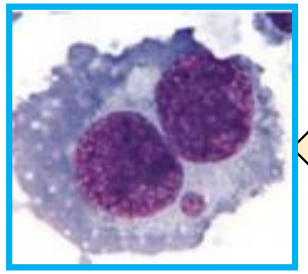
9



Counting dividing cells displaying micronuclei, bridges, buds but also nectrotic and apoptotic cells microscopically

Vitamin D deficiency and low physical inactivity ….

considered as driving factors for **genomic instability**, expressed as the **frequency of micronuclei**



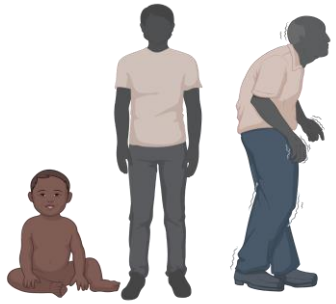
…No studies so far…

Vitamin D supplements

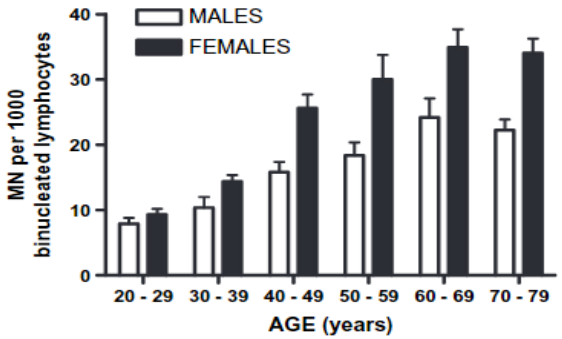
Low plasma vitamin D levels

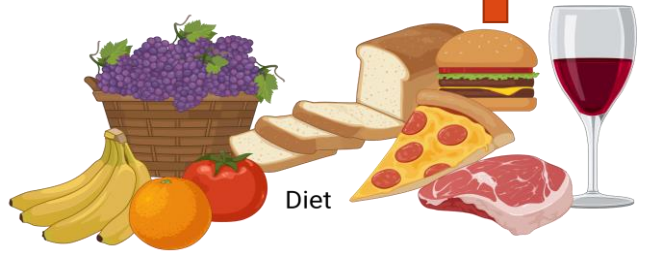
- other factors that influence Micronuclei frequency:

Physical activity



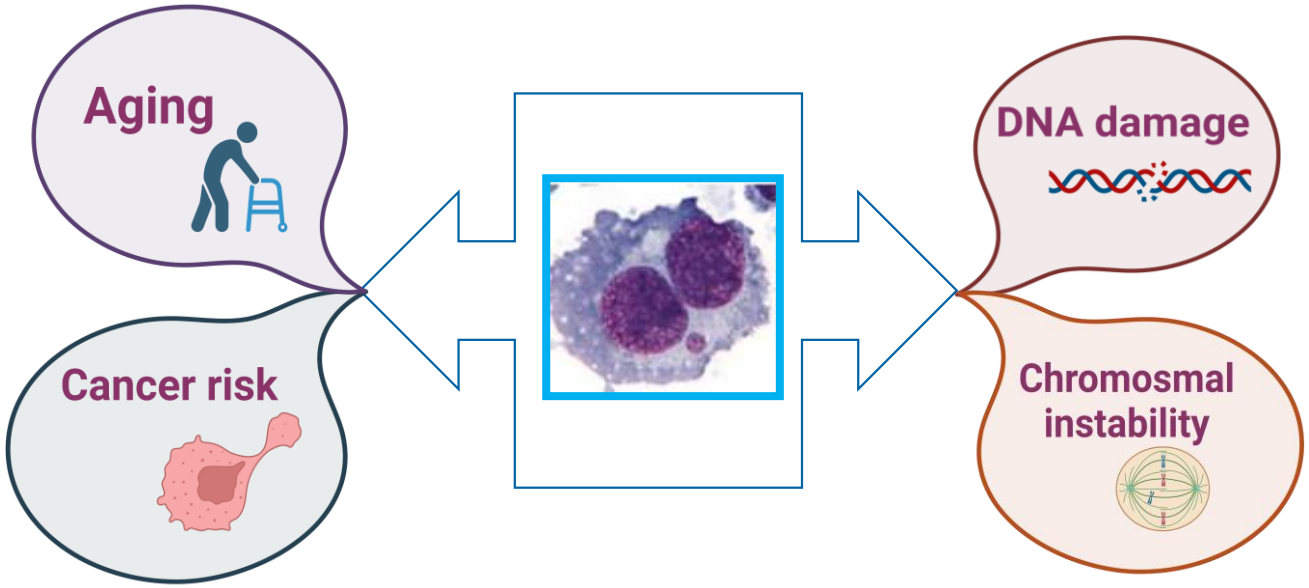
Age



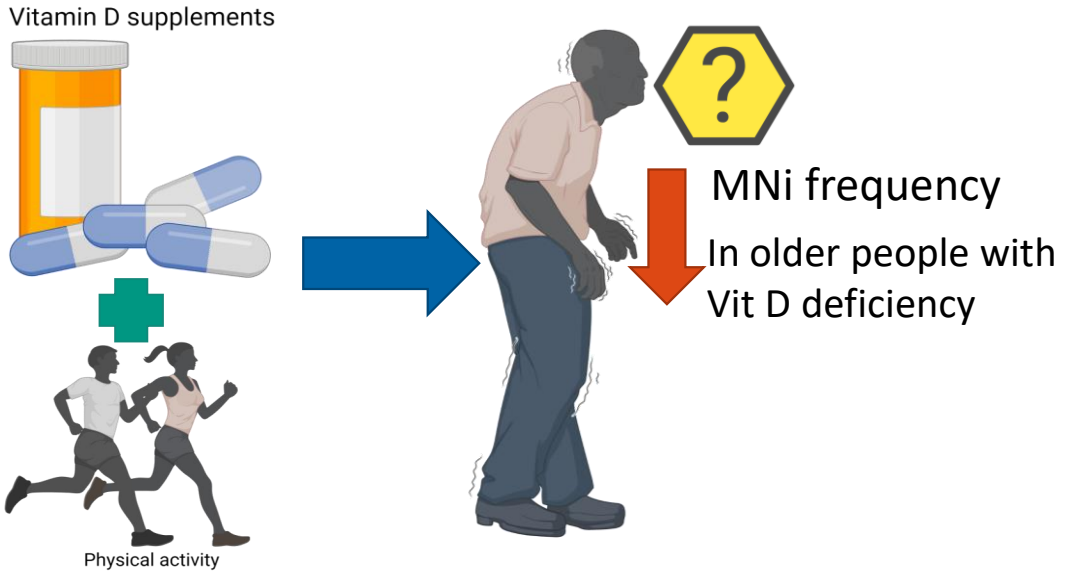


Diet

Micronuclei frequency is a potential biomarker for



Hypothesis



Results: baseline correlations

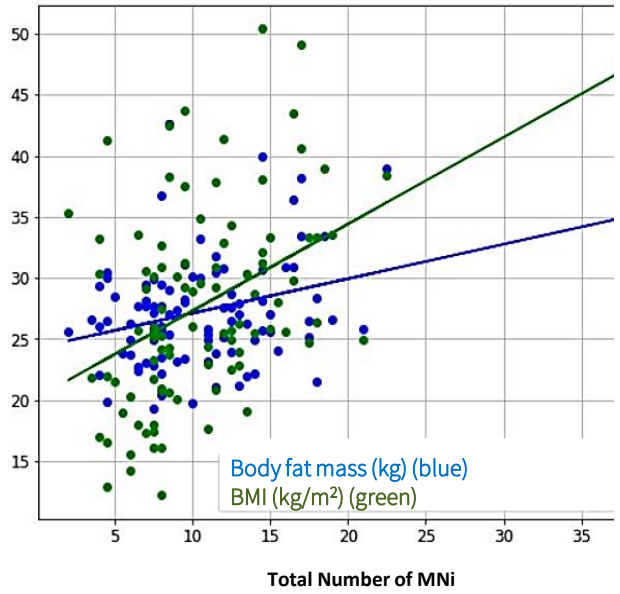
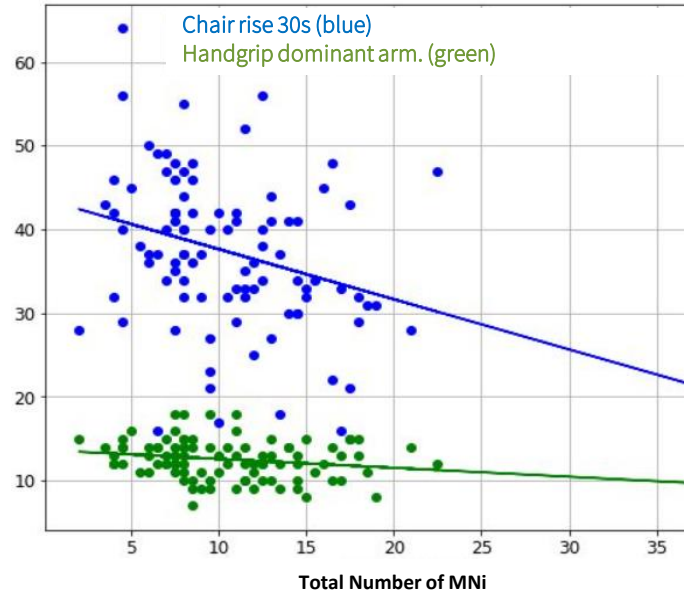


Table 3: Correlations between CBMN parameters and health related biomarker at baseline (n = 100)

Parameters	Total number of MNi (per 1000 BN cells) r	p-value
Body composition		
BMI (kg/m²)	0.311**	0.002
Waist to Hip Ratio	-.205*	0.044
Muscle mass[kg]	-0.221*	0.012
Body fat [kg]	0.372**	<0.001
Body fat [%]	0.433**	<0.001
Functional parameters		
Armcurl dominant arm 30s	-0.242*	0.012
Timed up and go [s]	0.245*	0.015
6-minute-walking test [m]	-0.262**	0.010
Chair-rise 30s	-0.256*	0.019
Handgrip arm dominant	-0.282**	0.005
Blood parameters		
Erythrocytes [T/l]	-0.264**	0.009
Haemoglobin [g/dl]	-.345**	0.001
Haematocrit [%]	-0.309**	0.002
CRP [mg/l]	0.125	0.196
hs_Troponin [ng/L]	-0.196	0.054
Creatinin [mg/dl]	-0.215*	0.034

** The correlation is signifcant at the level of 0.01 (2-sided).
* The correlation is signifcant at the level of 0.05 (2-sided).



Baseline parameters: males vs. females

Parameter	All	Men	Women	
Subjects (n)	100	67	33	
	Mean ± SD	Mean ± SD	Mean ± SD	p-value
Age (years)	70.63±4.54	70.33±4.47	71.24±4.68	0.397
Vitamin D serum level (ng/ml)	22.83±5.52	22.42±5.82	23.66±4.82	0.174
CBMN parameter				
Cells with MNi (per 1000 BN cells)	10.71±5.25	9.18±3.91	13.68±6.22	**<0.001**
Total number of MNi (per 1000 BN cells)	11.86±5.92	10.29±4.58	14.89±7.03	**<0.001**
Nuclear buds	2.62±1.67	2.6±1.71	2.67±1.62	0.713
Nucleoplasmic bridges	1.04±1.08	1.05±1.02	1±1.21	0.55
Apoptotic cells	6.06±5.52	6.88±5.94	4.47±4.23	**<0.001**
Necrotic cells	3.85±2.89	4±2.97	3.55±2.75	0.388
NDI	1.7±0.23	1.75±0.25	1.62±0.12	**0.002**

Data are presented as means±standard deviation, p-values are calculated using Mann-Whitney U test and chi-square for measuring gender differences, siginificant differences are highlighted with bold numbers.

Females displayed higher levels of Mni at baseline than males which is consistent with other studies

Baseline: Median Mni frequency

Table 2: Baseline characteristics based on the 50. percentile cut-off for the total micronucleus frequency

Parameter	All	< Median of total MNi	> Median of total MNi	
Subjects	n = 97 Mean±SD	n = 48 Mean ± SD	n = 49 Mean ± SD	p-value
CBMN parameter				
Cells with MNi (per 1000 BN cells)	10.71±5.25	6.97±1.84	14.38±4.90	**<0.001**
Total number of MNi (per 1000 BN cells)	11.86±5.92	7.60±1.84	16.02±5.56	**<0.001**
Nuclear buds	2.62±1.67	2.35±1.69	2.89±1.63	0.077
Nucleoplasmic bridges	1.04±1.08	0.94±1.03	1.13±1.13	0.279
Apoptotic cells	6.06±5.52	6.47±6.48	5.65±4.41	0.862
Necrotic cells	3.85±2.89	3.89±2.97	3.81±2.83	0.916
NDI	1.70±0.23	1.70±0.19	1.71±0.26	0.931

Data are presented as mean±standard deviation. P-values ($p < 0.05$) were calculated using the Man-Whitney U test based on the median at 10. P-value = 0.05.

Subjects that showed lower Mni levels than the Mni Median (= total Mni of 10) displayed also:

- lower body fat (kg)
- lower BMI
- Performed better in functional testings including
  - Arm-curl test
  - Chair-rise test
  - Hand-grip test

Intervention effects on MNi

Table 4: Development of CBMN parameter and important blood biomarker after vitamin D intervention and resistance training (n=67)

Parameter	Group	Mean±SD			p-value	p-value		
		T1	T2	T3	Friedman	T1-T2	T1 -T3	T2-T3
CBMN parameters								
Cells with MNi	CON	10.41±3.94	10.27±6.50	12.82±6.23	**<0.001**	0.383	0.027	**0.008**
(per 1000 BN cells)	Vit.D daily	11.43±8,18	9.02±6.10	11.60±4.15	**0.041**	0.11	0.313	0.039
	Vit.D monthly	10.60±3,89	9.02±3.54	14.00±6.76	**<0.001**	0.049	**0.001**	**0.001**
Total number of MNi	CON	11.36±4.33	11.25±7.4	14.45±7.22	**0.002**	0.413	0.025	**0.005**
(per 1000 BN cells)	Vit.D daily	12.48±9.16	10.02±6.76	13.33±5.28	**0.03**	0.217	0.179	0.047
	Vit.D monthly	11.77±4.51	10.27±4.75	16.06±7.81	**<0.001**	0.059	**<0.001**	**0.001**
Nuclear buds	CON	2.75±1.35	3.5±1.79	2.93±2.36	0.102	0.054	0.919	0.143
	Vit.D daily	2.81±2.09	3.76±2.25	2.67±1.93	**0.047**	0.098	0.793	0.055
	Vit.D monthly	2.46±1.44	3.92±2.76	2.58±1.32	**0.033**	0.014	1,000	0.014
Nucleoplasmic	CON	1.02±0.91	0.77±0.75	0.73±0.86	0.343	0.463	0.269	0.715
bridges	Vit.D daily	1.31±1.44	0.43±0.43	1.00±0.69	**0.013**	0.009	0.344	**0.004**
	Vit.D monthly	0.77±0.92	0.88±0.80	0.54±0.72	0.107	0.734	0.228	0.05
Apoptotic cells	CON	5.20±4.23	6.18±4.62	8.11±6.37	0.063	0.154	0.048	0.071
	Vit.D daily	5.40±4.24	9.67±8.84	7.48±5.02	0.089	0.117	0.013	0.525
	Vit.D monthly	7.38±6.97	6.98±7.17	7.98±3.97	0.314	0.943	0.338	0.223
Necrotic cells	CON	3.70±2.70	4.16±2.25	4.89±3.98	0.825	0.464	0.286	0.564
	Vit.D daily	3.64±2.94	5.26±4.51	5.71±4.05	0.088	0.172	0.01	0.588
	Vit.D monthly	5.00±3.60	3.71±2.83	3.19±2.37	0.153	0.143	0.048	0.519
Nuclear Division	CON	1.70±0.36	1.66±0.17	1.77±0.27	0.861	0.627	0.445	0.101
Index	Vit.D daily	1.72±0.10	1.70±0.38	1.78±0.34	0.827	0.848	0.375	0.339
	Vit.D monthly	1.69±0.20	1.64±0.14	1.68±0.19	0.325	0.317	0.458	0.361

Blood parameters after intervention								
Vitamin D serum level	CON	11.36±4.33	11.25±7.4	14.45±7.22	**0.002**	0.413	0.025	**0.005**
(ng/ml)	Vit.D daily	20.81±4.82	21.19±5.13	23.64±8.29	0.97	0.685	0.088	0.123
	Vit.D monthly	23.93±6.00	24.36±6.63	28.02±7.61	**0.01**	0.444	0.023	0.025

Data are presented as mean±standard deviation. P-values ($p < 0.05$) were calculated using the Man-Whitney U test based on the median at the value 10 . P-value = 0.005 (Bonferoni corrected)

Increase of MNi frequency in all study groups at the end of intervention

Summary and Conclusions

- Micronucleus frequency correlated positively with anthropometric parameters such as BMI (R = 0.311*) and body fat mass (R = 0.372*) at baseline.
- Additionally, we observed negative correlations with various functional parameters with Mni frequency, such as the 6-minute walking test (R = -0.262*).

Intervention effects:

- In older adults with low plasma vitamin D levels, we could not detect significant intervention effects due to different vitamin D supplementation dosages (800 IU daily vs. 50.000 IU monthly vs. control) when it comes to chromosomal instability.
- Interestingly, strength training slightly increased the MNi frequency in all groups.

Some pictures of measurements and the training

This picture shows study participants undertaking their supervised resistance exercise twice per week.

f.l.t.r. Agnes Draxler, Patrick Zöhrer, Sandra Unterberger, Rudolf Aschauer, Bernhard Franzke, Laura Bragagna, and many more

References

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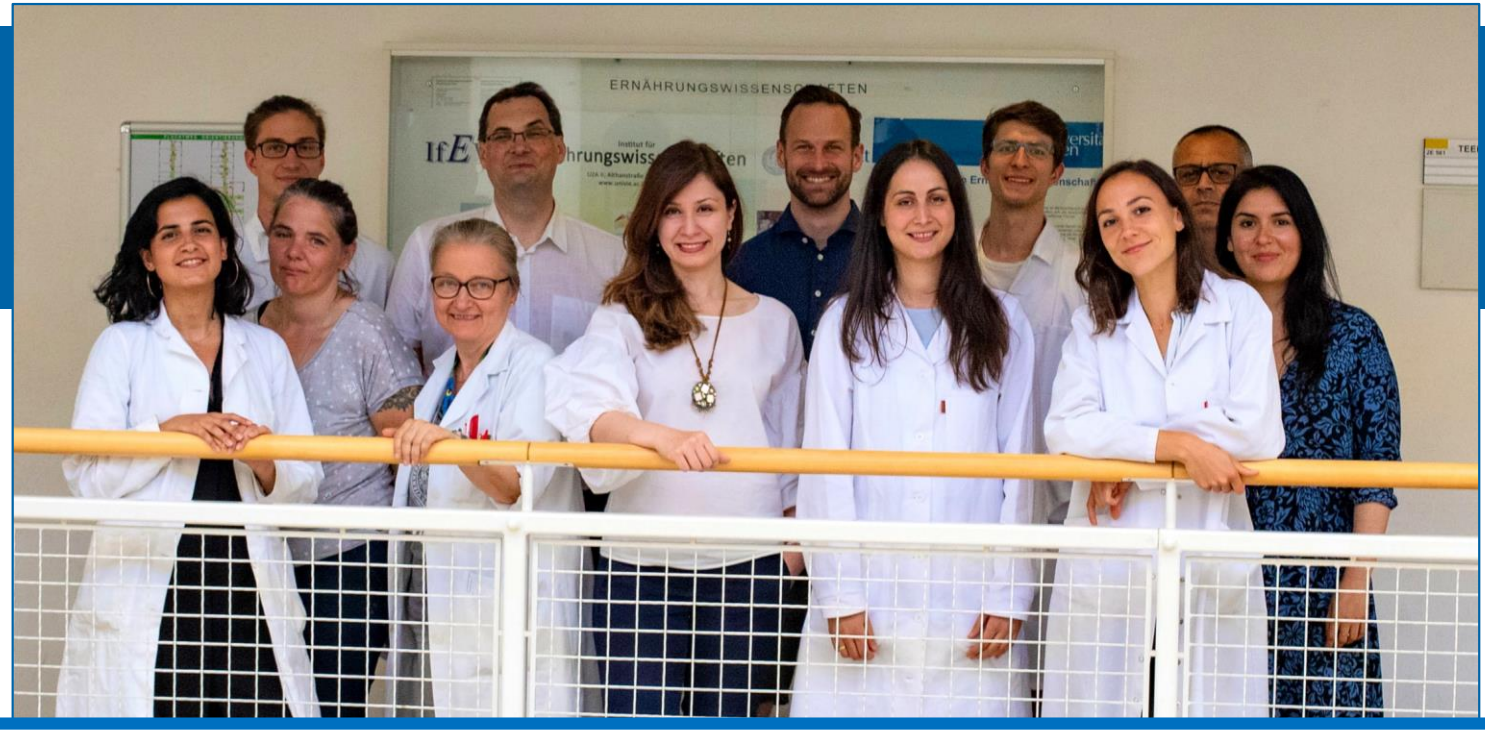

Thank you for listening!