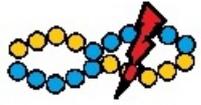


# DNA damage and arterial hypertension.



## Systematic review and meta-analysis



**Radka Hazuková, et al.**  
*FN a LF UP Olomouc*



**XXXIII. Sjezd ČKS, Brno**



# ÚVOD – ZLOMY DNA



**Intaktní DNA: zdraví, život X Neopravená: nenoc, smrt**

**Poškození DNA různá, nejhorší: 8OHdG < SSB < DSB**

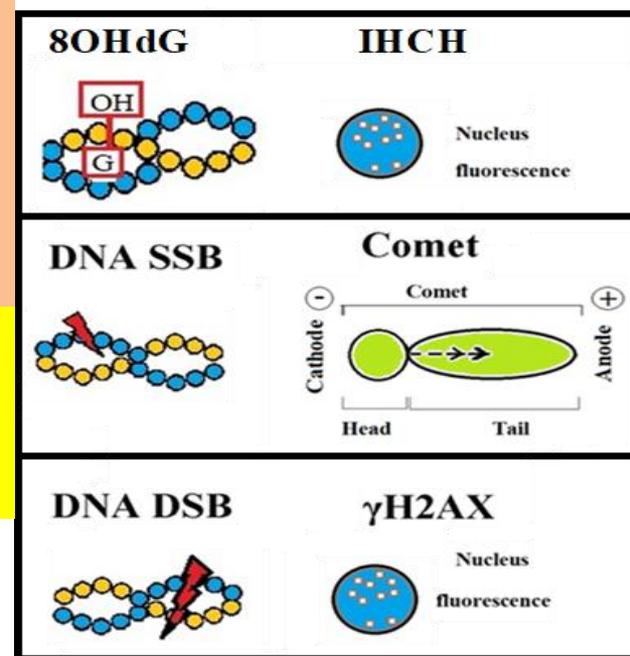
**DNA poškození = z expozice exogenním a endogenním faktorům**

**Oxidativní stres (ROS, RNS):**

- Oxidativní DNA poškození (8OHdG, SSB, DSB)
- Kardio-vaskulární onemocnění (CVD), Rakovina...

**Civilizační nem:** - CVD, Rakovina,....

- Sdílené rizikové faktory (stres,..)
- Subcel základ ? = DNA poškození ?



**Přibývá důkazů - DNA poškození souvisí s CVD**

- Subcelul cesta geneze od DNA poškození k atero/CVD

**Prokázáno - Poškození DNA ( labilita genomu) - onkogeneze**

**Neuspokojivá epidemiologie CVD = 1. místo CVD/onko Dokážeme „stabilizovat“**

**BDĚLOST K MOŽNÝM VÝZVÁM; VÝZVY SERIOZNĚ ROZPRACOVAT**

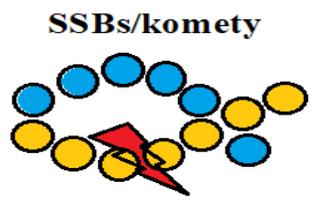


# ÚVOD – ZLOMY – VÝZVA pro CVD



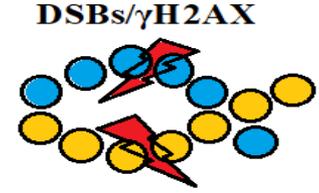
- DNA zlomy - Nová výzva pro CVD** (dostupnost vzorků, dostupnost analýz)
- žádoucí je problematiku **seriozně rozpracovat**
  - **zvýšené hladiny DNA zlomů u CVD**
  - subcelulární **cesta geneze** od DNA poškození k atero/atero-CVD...  
.....*imunitní, zánětlivé kaskády* .....**non-atheroCVD** ????
  - Dobře pospané jsou **cesty oprav DNA** poškození (DNA damage repair DDR)
  - **Substance DDR – nové cíle terapie** – inhibitory PARP jsou v klinice

## DNA DAMAGE REPAIR (DDR) cesty



**BER/SSBs**

**PARP 1**  
 PARP  
 Ogg1, APE 1, XRCC1  
 PNKP, APTX, Mag 1  
 POLβ  
 LIG 3



**HR/DSBs závislá na templátu**

MRN  
 ADC1, ATM  
 RAD 51, PALB 2  
 BRCA 1, BRCA 2, XRCC 2  
 ChK1, ChK2  
 p53, p21, cdc 25A, CDK2  
 MDM 2, RPA, ATR

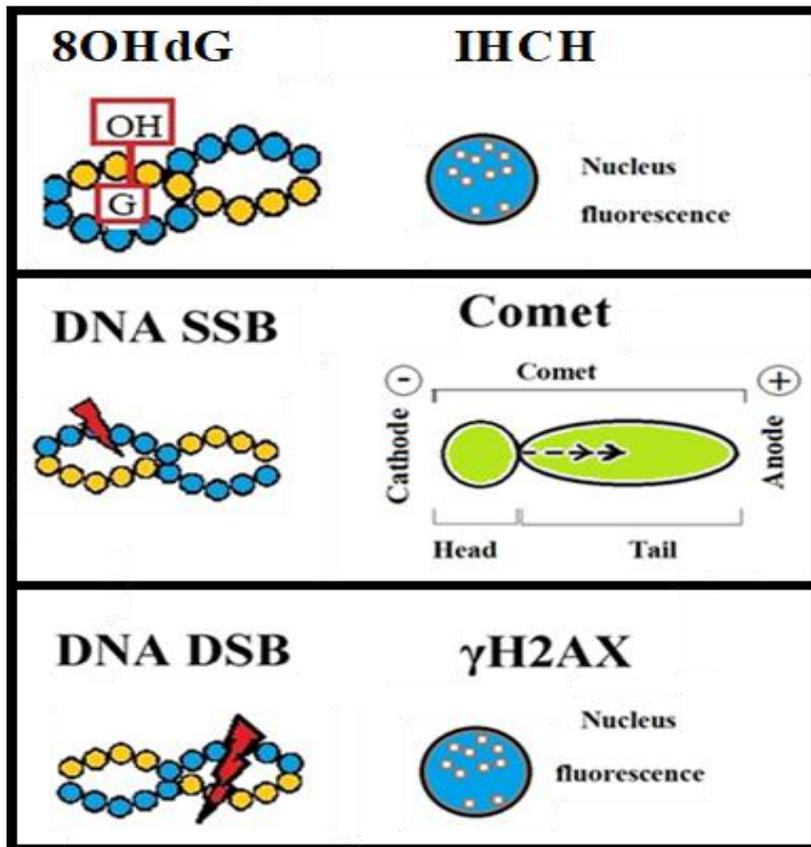
**NHEJ/DSBs nezávislá na templátu**

Ku 70/Ku 80  
 DNA-PK  
 ARTEMIS  
 XLF  
 XRCC 4  
 LIG 4

**Alt NHEJ/ DSBs nezávislá na templátu**

MRN  
 CtIP  
**PARP 1**  
 POLQ  
 XRCC 1  
 LIG 1/3

- Popsat nejzávažnější formy oxidativního poškození DNA (**8OHdG**, **SSBs**, **DSBs**) u arteriální hypertenze.





# METODIKA



## ANALÝZA MNOHOČETNÉ DATABÁZE

Do 30. 6. 2022

Klíčová slova: arteriální hypertenze, DNA poškození, SSBs, DSBs, SBs, Comet assay, 8OHdG,  $\gamma$ H2AX

### INCLUSION CRITERIA

- 8OHdG, SSBs, DSBs
- Arteriální hypertenze
- Studie - humánní
  - animální
  - buněčné kultury

### EXCLUSION CRITERIA

- Failure of key words
- Apoptosis of relevant analysis (TUNEL assay)
- Telomerase changes
- Epigenetic DNA modifications
- Cell free DNA
- Children
- Extra cardio-vascular (CV) issues (neoplasma, ..)
- Other types of DNA damage markers
- No relevant controls

### STATISTIKA

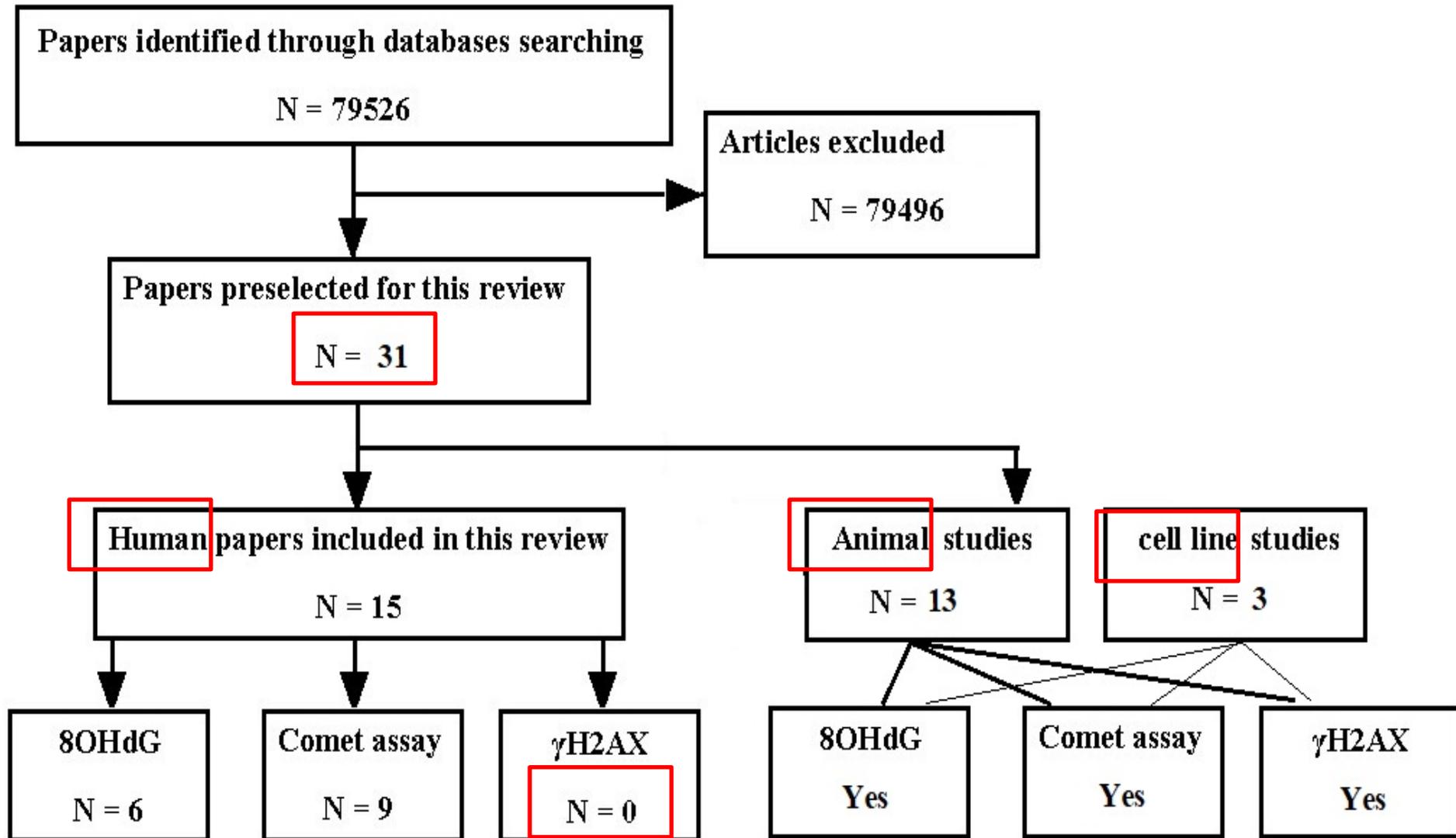
Pearson korelační koeficient ( $r$ ); Meta-analýza průměrů (mean differences, 95% Confidence limit, Forest plot,  $x \pm SD$ ,  $P < 0.05$ )



# METODIKA



## FLOW DIAGRAM





# VÝSLEDKY



Table 3 – Oxidative DNA damage – hypertensive humans<sup>7</sup>

Authors, year	Hypertensives (HT) <i>N</i> (age; years)	Healthy controls (C) <i>N</i> (matched)	DNA damage type/method <i>source, units</i>	Comparison using DNA damage level, <i>p</i> < 0.05
			<b>8OHdG/ELISA</b>	
Lee J, 2005	38 (54.0 ± 12.0)	22	Plasma, ng.ml <sup>-1</sup>	C < HT
Toljic M, 2017	21 (32.0; 23.0–38.0)	28	Plasma, nM	C = HT
Zhao Y, 2020	63 (72.1 ± 7.9)	84	Serum, ng.ml <sup>-1</sup>	C < HT
Negishi H, 2000	38 (52.0 ± 0.5)	22	Urine, ng.mg <sup>-1</sup> creatinine <sup>-1</sup>	C < HT
Negishi H, 2001	8 (52.0 ± 0.7)	22	Urine, ng.mg <sup>-1</sup> creatinine <sup>-1</sup>	C < HT
Subash P, 2010	105 (45.0 ± 9.0)	75	Urine, ng.mg <sup>-1</sup> creatinine <sup>-1</sup>	C < HT
Kotani K, 2014	45 (62.0 ± 9.0)	31	Urine, ng.mg <sup>-1</sup> creatinine <sup>-1</sup>	C = HT
Yavuzer S, 2016	30 (72.6 ± 8.7)	30	Urine, ng.mg <sup>-1</sup> creatinine <sup>-1</sup>	C < HT
Yavuzer S, 2016	30 (43.6 ± 3.9)	30	Urine, ng.mg <sup>-1</sup> creatinine <sup>-1</sup>	C < HT
Yıldırım E, 2017	40 (44.4 ± 3.9)	40	Urine, ng.mg <sup>-1</sup> creatinine <sup>-1</sup>	C < HT
			<b>SSBs/COMET ASSAY</b>	
Gur M, 2007	84 (50.0 ± 6.0)	24	PBCs/Ly/, A.U.	C < HT
Yildiz A, 2008	21 (45.0 ± 7.0)	19	PBCs/Ly/, A.U.	C < HT
Subash P, 2010	80 (50.0 ± 9.0)	50	PBCs/Ly/, A.U.	C < HT
Gür M, 2013	64 (47.9±7.0)	20	PBCs/Ly/, A.U.	C < HT
Saiedullah M, 2013	46 (41.0±5.0)	40	PBCs/Ly/, A.U.	C < HT
Subash P, 2016	100 (45.0 ± 12.0)	50	PBCs/Ly/, A.U.	C < HT
			<b>DSBs/γH2AX</b>	
NA	NA	NA	NA	NA



# VÝSLEDKY



Table 2 – Oxidative DNA damage – hypertonic animals

Authors, year <sup>Ref.</sup>	Hypertonic (HT) <i>N</i> (age; weeks)	Healthy controls (C) <i>N</i> (matched)	DNA damage type/method source, units	Comparison using DNA damage level, <i>p</i> < 0.05
			<b>8OHdG/ELISA</b>	
Negishi H, 1999 <sup>11</sup>	15 (14–18)	9	Urine, ng.kg <sup>-1</sup> per day	C < HT
Negishi H, 2000 <sup>12</sup>	5 (14)	5	Urine, ng.mg <sup>-1</sup> .creatinine <sup>-1</sup>	C < HT
Queisser N, 2013 <sup>13</sup>	8 (8)	5	Kidney, positive cells (%)	C < HT
			<b>8OHdG/MS</b>	
Schupp N, 2011 <sup>14</sup>	7 (8)	6	Kidney, LC-MS/MS, 106 dG	C < HT
Brand S, 2013 <sup>15</sup>	6 (17)	7	Urine, ng.20h <sup>-1</sup>	C < HT
			<b>SSBs/COMET ASSAY</b>	
Schupp N, 2011 <sup>14</sup>	7 (8)	6	Kidney, DNA in tail (%)	C < HT
Queisser N, 2013 <sup>13</sup>	8 (13)	5	Kidney, mean tail DNA (%)	C < HT
Dias AT, 2014 <sup>16</sup>	10 (14)	8	Kidney, DNA in tail (%), tail moment (A.U.)	C < HT
Zimnol A, 2017 <sup>17</sup>	6 (19)	8	Kidney, DNA in tail (%)	C < HT
Saheera S, 2017 <sup>18</sup>	3 (48–72)	3	Heart, tail moment	C < HT
Zimnol A, 2020 <sup>19</sup>	6 (18–22)	5	Kidney, DNA in tail (%)	C < HT
Bruic M, 2021 <sup>20</sup>	8 (24)	8	Blood, liver, heart, kidney, A.U.	C < HT
			<b>DSBs/γH2AX/FLOW CYTOMETRY</b>	
Baban B, 2013 <sup>20,21</sup>	5 (NA)	4	Heart, positive cells	C < HT
			<b>DSBs/γH2AX/IF/IHCH</b>	
Schupp N, 2011 <sup>14</sup>	7 (8)	6	Kidney, positive cells (%)	C < HT
Brand S, 2013 <sup>15</sup>	6 (17)	8	Heart, kidney, positive cells	C < HT
Queisser N, 2013 <sup>13</sup>	8 (13)	5	Kidney, positive cells (%)	C < HT
Brand S, 2014 <sup>22</sup>	6 (17)	7	Heart, kidney, positive nuclei.mm <sup>-2</sup>	C < HT
Queisser N, 2014 <sup>23</sup>	8 (6–7)	8	Liver, positive nuclei (%)	C < HT
Zimnol A, 2017 <sup>17</sup>	6 (19)	8	Kidney, positive cells	C < HT
Zimnol A, 2020 <sup>19</sup>	5 (18–22)	5	Kidney, positive cells	C < HT

Table 1 – Oxidative DNA damage – hypertonic cell models

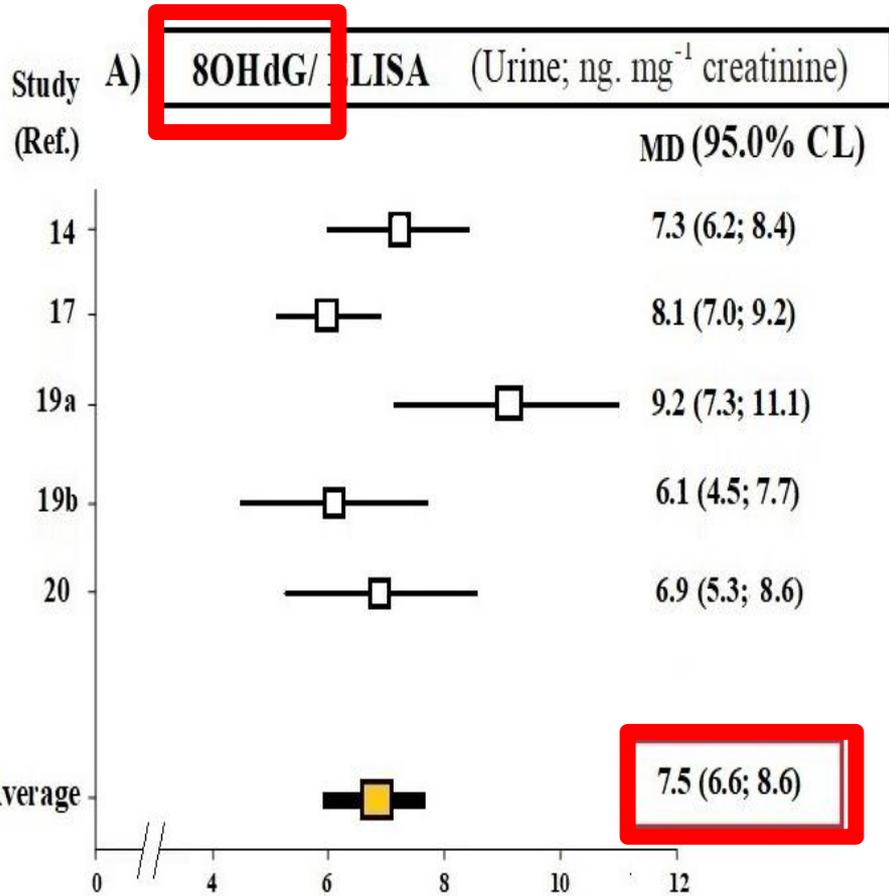
Authors, year <sup>Ref.</sup>	Hypertonic cells (HT)	Healthy cell controls (C)	DNA damage type/method source, units	Comparison using DNA damage level, $p < 0.05$
			8OHdG/IF	
Schmid U, 2008 <sup>8</sup>	Yes	Yes	Cells, DNA in tail (%)	C < HT
			SSBs/COMET ASSAY	
Schmid U, 2008 <sup>8</sup>	Yes	Yes	Cells, DNA in tail (%)	C < HT
Queisser N, 2011 <sup>9</sup>	Yes	Yes	Cells, DNA in tail (%)	C < HT
			DSBs <sub>γ</sub> /H2A <sub>X</sub> /flow cytometry	
Schmid U, 2008 <sup>8</sup>	Yes	Yes	Cells, fold	C < HT
Balhorn R, 2020 <sup>10</sup>	Yes	Yes	Cells, normalized ratio	C < HT



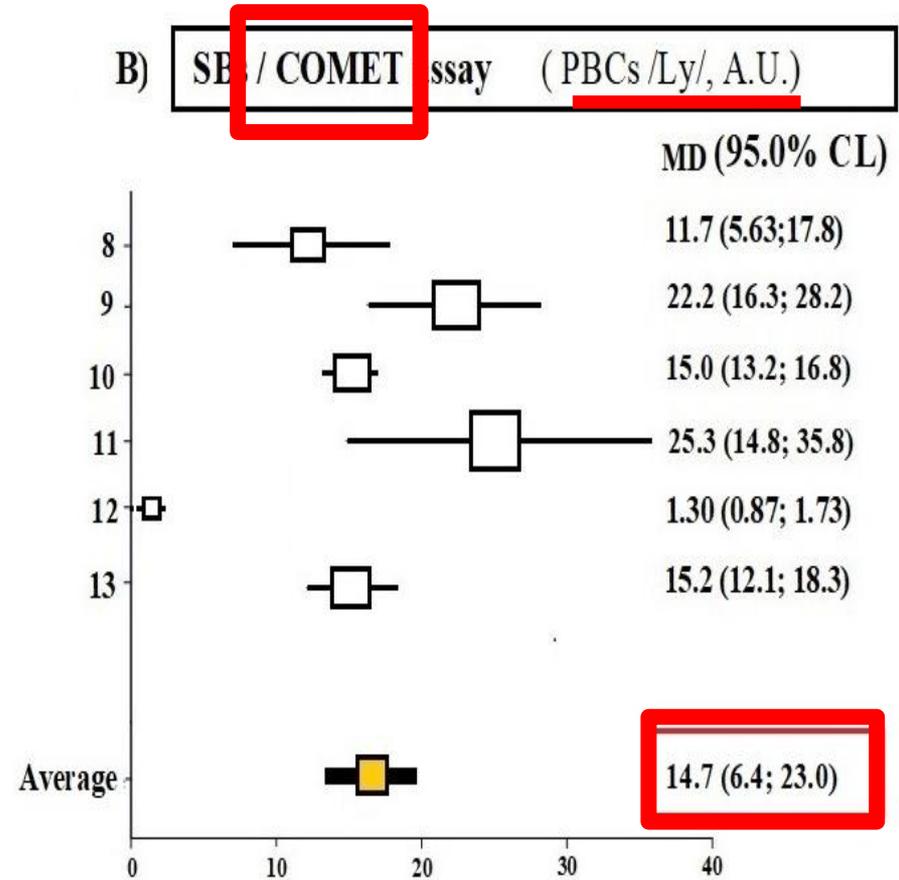
# VÝSLEDKY



## DNA DAMAGE - mean differences between hypertensive patients and controls (forest plot – related to Table1)



Mean differences in DNA damage (8OHdG) between hypertensive pts and controls (Urine; ng.mg<sup>-1</sup> creatinine)



Mean differences in DNA damage (SBs) between hypertensive pts and controls (PBCs, /Ly/ A.U.)



# VÝSLEDKY

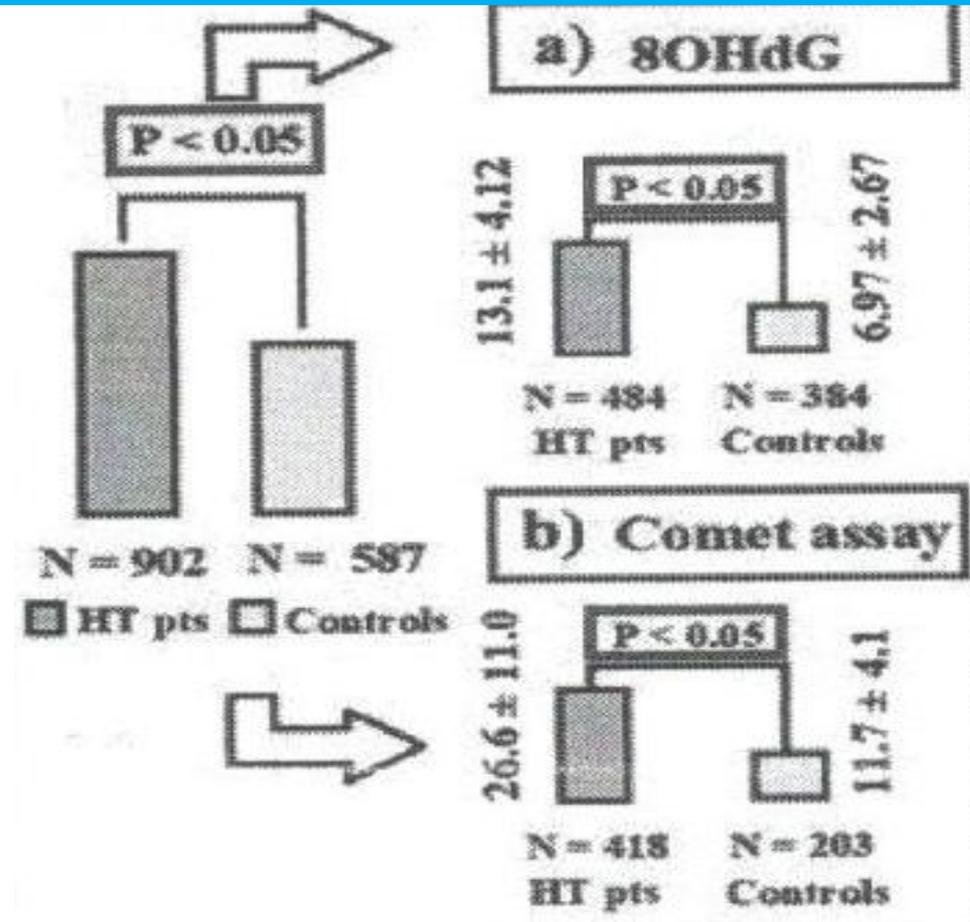
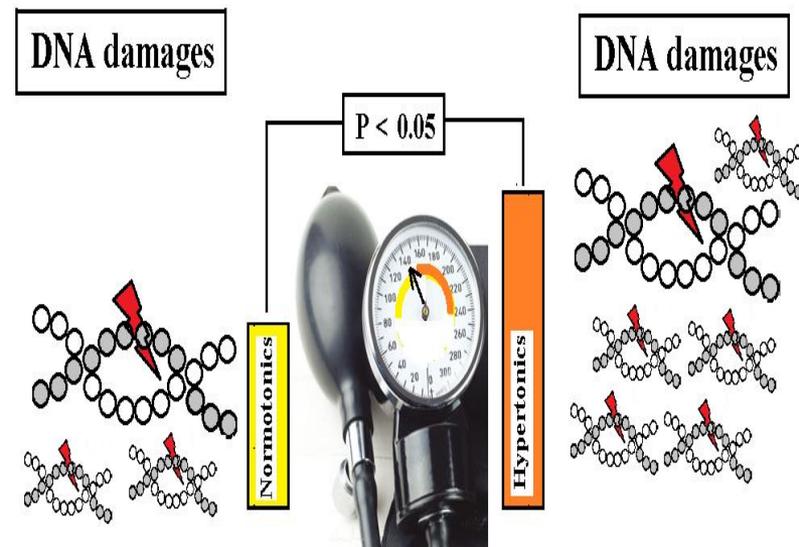


Author	DNA marker	r	P	Factor
Gur M	SBs/ Comet assay	0.315	0.012	hs CRP
Gur M	SBs/ Comet assay	0.479	< 0.001	Age
Yildiz A	SBs/ Comet assay	0.453	0.039	Age
Subash P	SBs/ Comet assay	0.072	NS	Age
Negishi H	8OHdG/ ELISA	-0.012	NS	Age
Subash P	8OHdG/ ELISA	0.221	NS	Age
Yavuzer S	8OHdG/ ELISA	0.39	0.002	Age (elderly)
Gur M	SBs/ Comet assay	0.382	0.002	Cholesterol
Yildiz A	SBs/ Comet assay	0.550	0.010	Cholesterol
Subash P	SBs/ Comet assay	-0.045	NS	Cholesterol
Negishi H	8OHdG/ ELISA	-0.082	NS	Cholesterol
Subash P	8OHdG/ ELISA	0.044	NS	Cholesterol
Gur M	SBs/ Comet assay	0.331	0.008	LDL
Yildiz A	SBs/ Comet assay	0.539	0.012	LDL
Subash P	SBs/ Comet assay	-0.043	NS	LDL
Subash P	8OHdG/ ELISA	0.067	0.033	LDL
Negishi H	8OHdG/ ELISA	0.059	NS	HDL
Negishi H	8OHdG/ ELISA	0.018	NS	TAG
Saiedullah M	SBs/ Comet assay	0.362	< 0.05	BP
Negishi H	8OHdG/ ELISA	0.202	NS	BP
Subash P	8OHdG/ ELISA	0.027	NS	BP
Negishi H	8OHdG/ ELISA	-0.040	NS	HR
Negishi H	8OHdG/ ELISA	-0.235	NS	BMI
Negishi H	8OHdG/ ELISA	0.670	< 0.0001	HbA1c
Kotani K	8OHdG/ ELISA	0.330	< 0.05	PWV (HT - DM)
Yavuzer S	8OHdG/ ELISA	0.400	0.002	IMA
Yildirim E	8OHdG/ ELISA	0.396	< 0.001	IMA
Yildirim E	8OHdG/ ELISA	0.372	< 0.001	PAB
Yavuzer S	8OHdG/ ELISA	0.370	0.004	PCO
Yildirim E	8OHdG/ ELISA	0.243	< 0.05	PCO
Yildirim E	8OHdG/ ELISA	0.411	< 0.001	AOPPs
Zhao Y	8OHdG/ ELISA	0.232 to 0.424	< 0.05	extent of CHD
Gur M	SBs/ Comet assay	-0.692	< 0.001	TAS
Yildiz A	SBs/ Comet assay	-0.818	< 0.001	TAS
Subash P	SBs/ Comet assay	-0.792	< 0.0001	TAS
Subash P	SBs/ Comet assay	-0.914 to -0.968	< 0.0001	TAS
Subash P	8OHdG/ ELISA	-0.933	< 0.0001	TAS
Yavuzer S	8OHdG/ ELISA	-0.290	0.025	T - SH
Yildirim E	8OHdG/ ELISA	-0.410	< 0.001	T - SH
Subash P	SBs/ Comet assay	0.204	NS	Glc
Subash P	8OHdG/ ELISA	0.170	NS	Glc

# ZÁVĚR – REKAPITULACE

## Konzistentní zvýšení DNA poškození u HT

(8OHdG, SSBs), (DSBs ???)



- Platí pro **lidi** (DSBs ?), **zvířata** i **buněčné** kultury
- **Rozdíl mezi hladinami** mezi kontrolou a hypertonikem lze čekat 14.7 jednotek (periferní lymfocyty, SSBs) a 7.5 jednotek (8OHdG, moč).



# ZÁVĚR – REKAPITULACE



**Korelace DNA poškození k TAS, glyk Hb, LDL ?**

a) 8OHdG		P < 0.05	
Age	r 0.390	PAB	r 0.372
LDL	0.067	PCO	0.243
HbA1c	<b>0.670</b>	AOPPs	0.411
PWV	0.330	extent CHD	0.232
IMA	0.400	TAS	<b>- 0.933</b>
		T-SH	- 0.410

b) Comet assay		P < 0.05	
hs CRP	r 0.315	LDL	r 0.331
Age	0.450	BP	0.362
Cholesterol	0.382	TAS	<b>- 0.692</b>

PWV brachial ankle pulse wave velocity  
IMA Ischemia modified albumin  
PAB pro antioxidant balance  
PCO protein carbonyl  
AOPP advanced oxidation protein products



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**Děkuji za  
pozornost**

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