Metalloproteinases and their inhibitors gene expression profiles in leukocytes of primary hypertension (PH), non-alcoholic fatty liver disease (NAFLD), and obese children.

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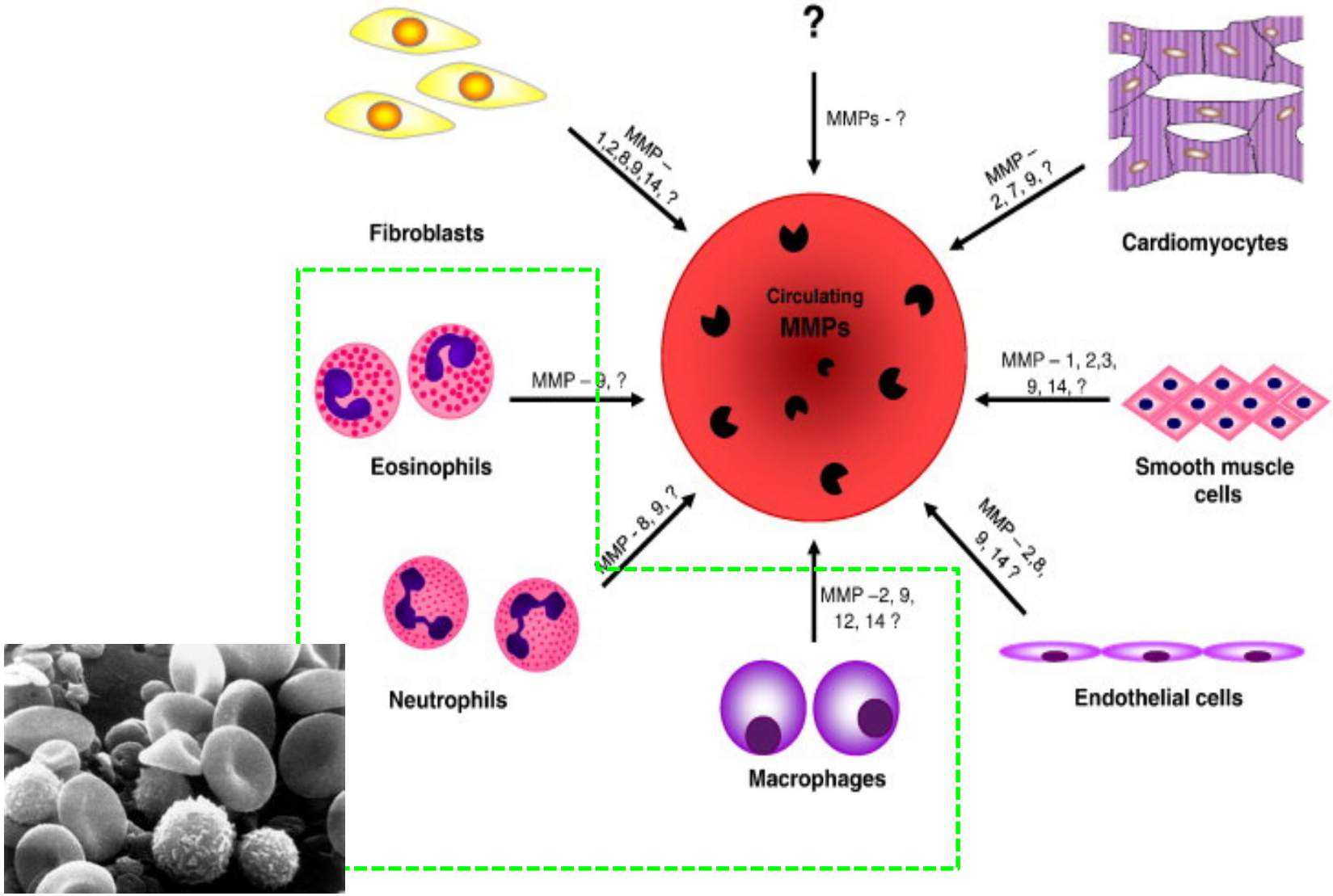
Matrix metalloproteinase - MMPs

- also known matrixins
- zinc endoproteinase
- 25 distinct MMPs identified in vertebrates, 23 in human (encoded for 24 genes)
- degrade (cleave) protein components of the extracellular matrix (ECM) collagens, elastin, fibronectin, gelatin and aggrecan, as well as non-ECM molecules – transforming growth factor (TGF)-β, pro-IL-1β, pro-IL-8, Fas ligand, and pro-TNF
- cause renewal and reconstruction of ECM (ECM turnover)
- maintain the correct structure of the ECM and basement membrane
- major players in many physiological and pathological processess

Which cells synthetise/express MMPs ?



LEUKOCYTES

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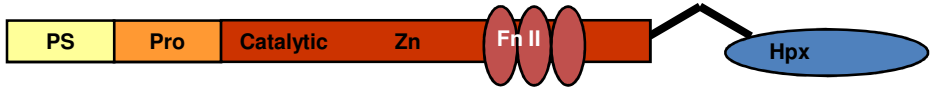
Domain structure

MMP-7, -26

MMP-1, -3, -8, -10, -12, -13, -18, -19, -20, -22, -27

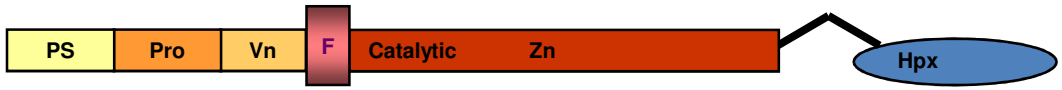


MMP-2, -9

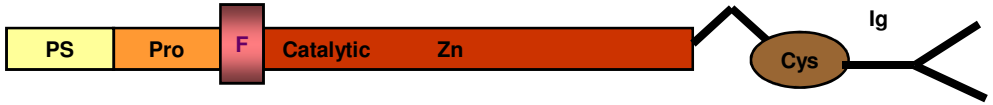
MMP-11, -28

MT-MMP 1, 2, 3, 5 (MMP-14, -15, -16, -24)

MT-MMP 4, 6 (MMP-17, -25)



MMP-21



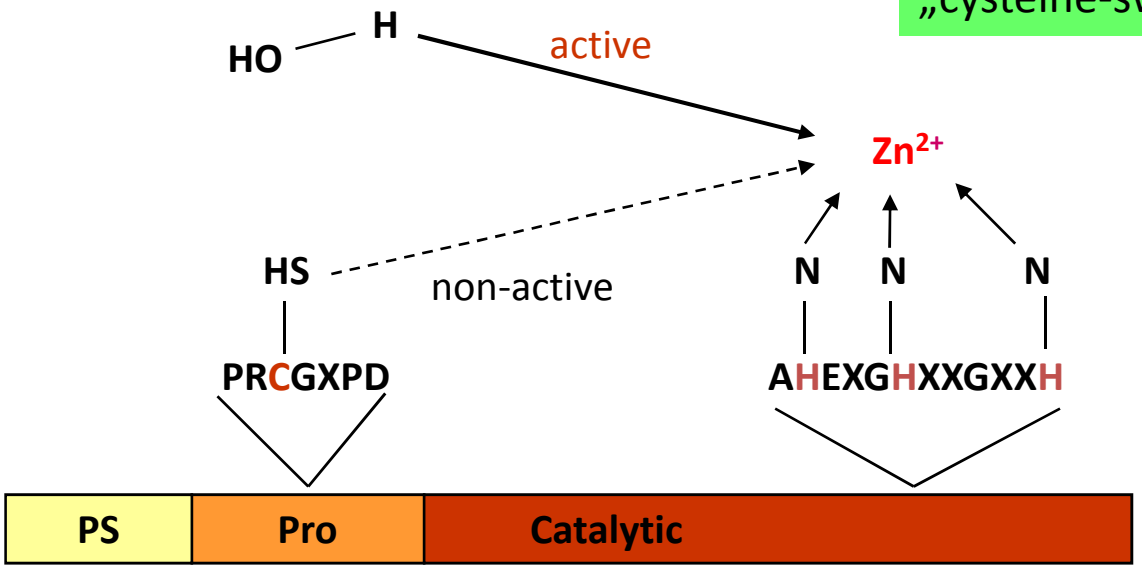
MMP-23

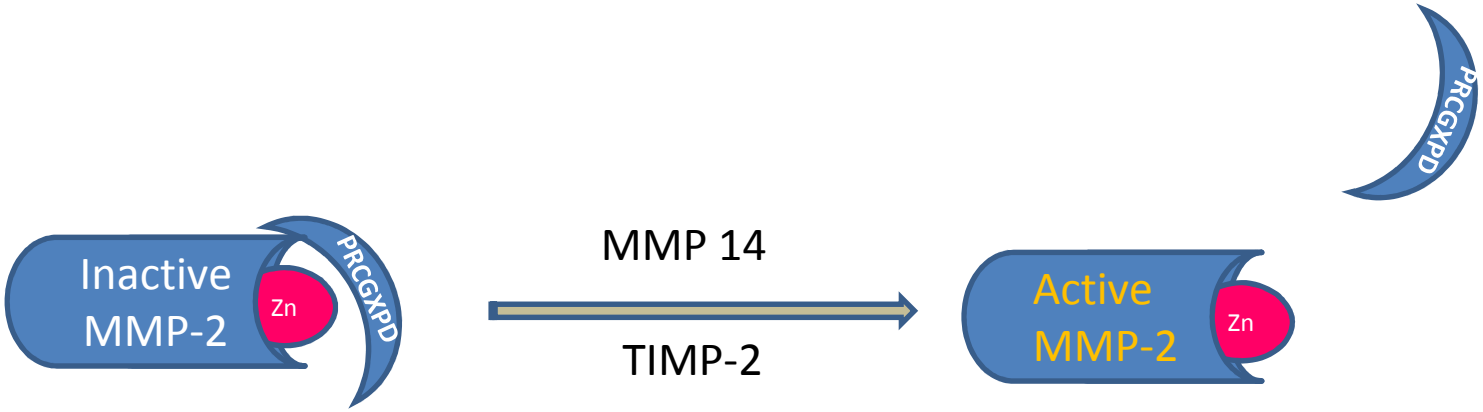
MMPs classification

Class	Common name	No MMP	Substrat - collagen	Other substrates
Collagenases	Collagenase-1	MMP-1	I, II, III, VII, VIII, X	gelatin, MMP-2, -9, proteoglycans, fibronectyn, laminin, pro TNFα
	Collagenase-2	MMP-8	I, II, III, V, VII, VIII, X	gelatin, fibronectyn, proteoglycans, ADAMTS-1, proMMP-8
	Collagenase-3	MMP-13	I, II, III, **IV**, V, VII, IX, X	gelatin, laminin, proteoglycans, fibrinogen, proMMP-9, -13
Gelatinases	**GelatinaseA**	**MMP-2**	**I, II, III, IV, V,VII,X,XI**	**gelatin, fibronectin, laminin, elastyn, proMMP-9, -13, IGFBPs, IL-1β, TGF-β, α1–antyproteinase**
	Gelatinase B	**MMP-9**	**III, IV V, VII, X,XI**	**gelatin, elastyn, laminin, fibronectin, vitronectin, CXCL5, IL-1β, TGF-β, plasminogen**
Stromelysins/ Matrilysins	Stromelysin 1	MMP-3	III**, IV**, V, VII, IX, X, XI	gelatin, fibronectin, laminin proMMP-1, -7, -8, -9, -13, proTNFα, E-cadheryn, L-selectyn
	Stromelysin 2	MMP-10	I, III. **IV**, V, IX, X	gelatin, laminin, casein, MMP-1, -8, fibronectin, proteoglycans
	Stromelysin 3	MMP-11	**IV**	gelatin, fibronectin, laminin
	Matrilysin 1	MMP-7	I, **IV**	gelatin, laminin, elastin, fibronectin, proteoglycans, proMMPs, proTNFα, E-cadheryn
	Matrilysin 2	MMP-26	I, **IV**	gelatin, laminin, elastin, fibronectin, proteoglycans, proMMPs, proTNFα, E-cadheryn
Transmembrane Type II	MT1-MMP	MMP-14	I, II, III	gelatin, fibronectin, laminin, vitronectrin, proteoglycans, proMMP-2 i proMMP-13
	MT2-MMP	MMP-15		proMMP-2
	MT3-MMP	MMP-16		proMMP-2
	MT4-MMP	MMP-17		proMMP-2
	MT5-MMP	MMP-24		proMMP-2
	MT6-MMP	MMP-25		gelatin
Other MMPs	Macrophage metalloelastase	MMP-12	**IV**	elastyn, fibronectin, gelatin, proteoglycans, plasminogen
	Collagenase 4 Xenopus	MMP-18	I	gelatin
	RASI-1	MMP-19	**IV**	elementy błon podstawnych
	Epilizyn	MMP-21, -27		gelatin
		MMP-28		gelatin, casein autocatalise
				proTNF-β

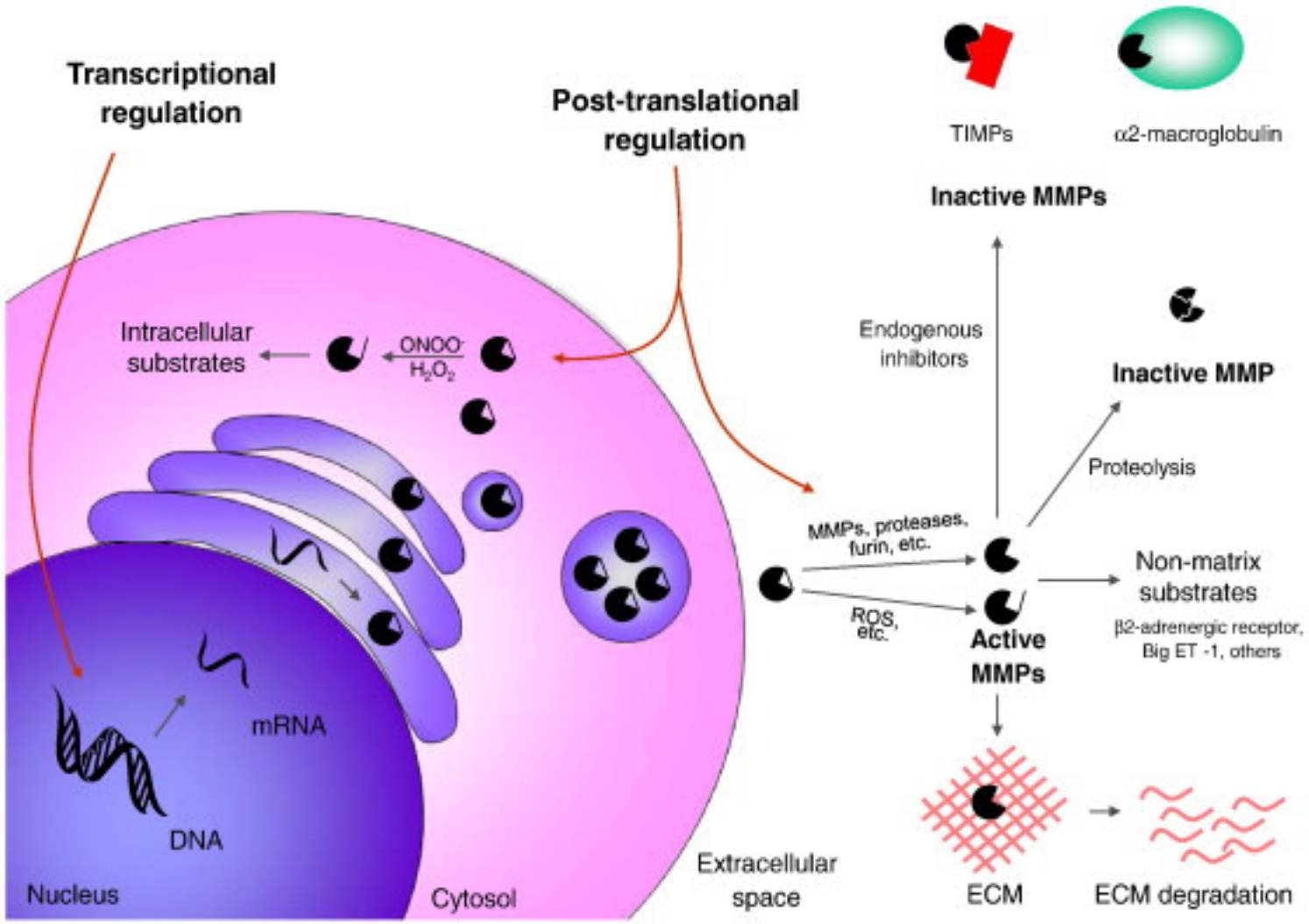
Activation

„cysteine-switch" mechanism





Regulation of MMP expression and activity



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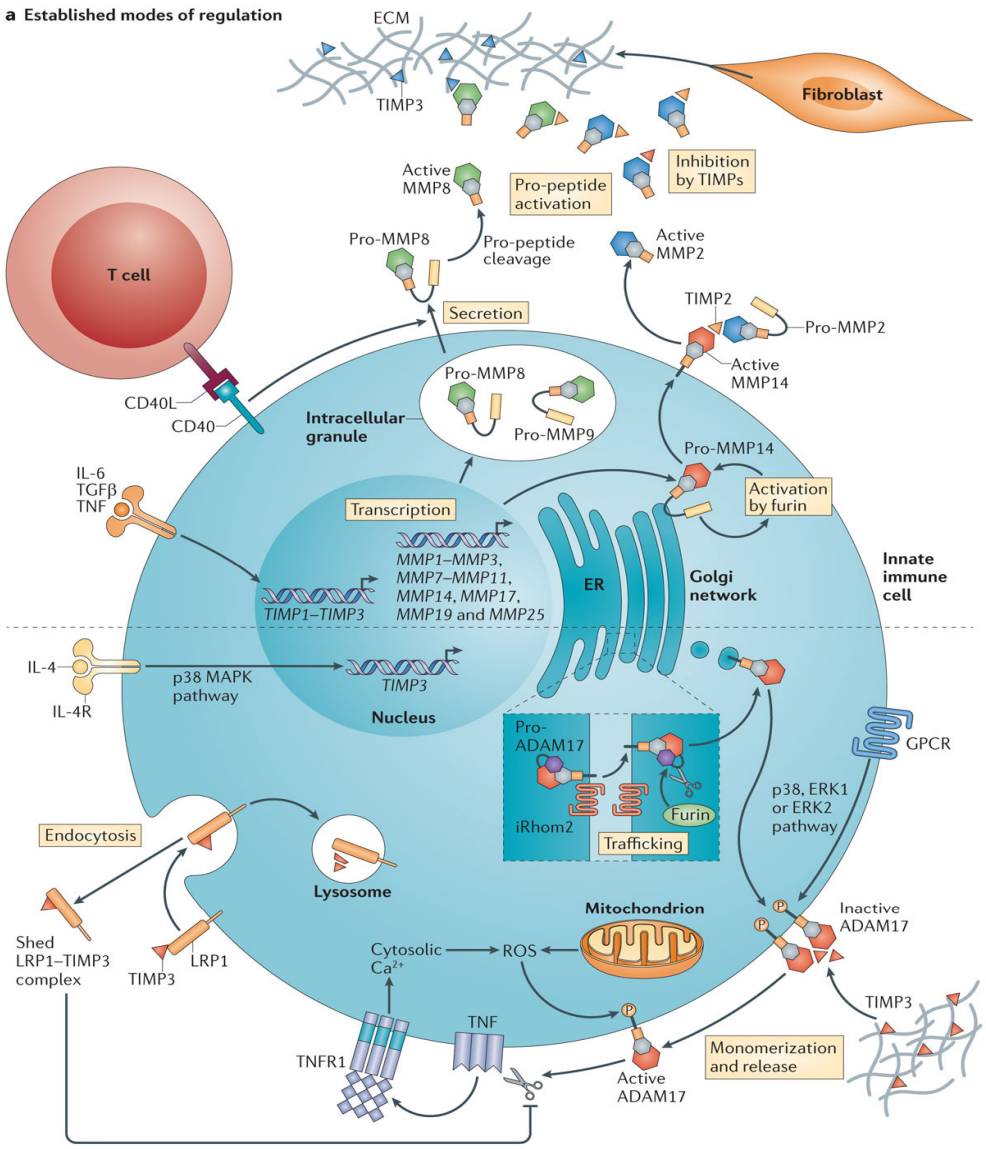
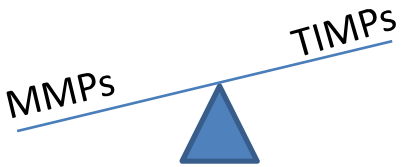
Immunological context of view…..



Nature Reviews | Immunology

Physiological processes	Pathological processes
• embryogenesis • angiogenesis • apoptosis • bone growth, tooth enamel • development of the nervous system • wound healing • repair of spinal cord injury • reconstruction of the endometrium • development and implantation of the embryo during pregnancy • processes associated with the development and reconstruction of connective tissue	• etiology and progress of inflammatory processes • fibrosis • cancer • dysplasia of bone • muscular dystrophy • cardio - vascular diseases • atherosclerosis • myocardial infarction aneurysms • hypertension • autoimmune diseases • degenerative rheumatoid arthritis - RA • multiple sclerosis • neurological diseases • chronic obstructive pulmonary disease COPD 1. Primary Hypertension - PH 2.Non-alcoholic fatty liver disease - NAFLD 3. Obesity

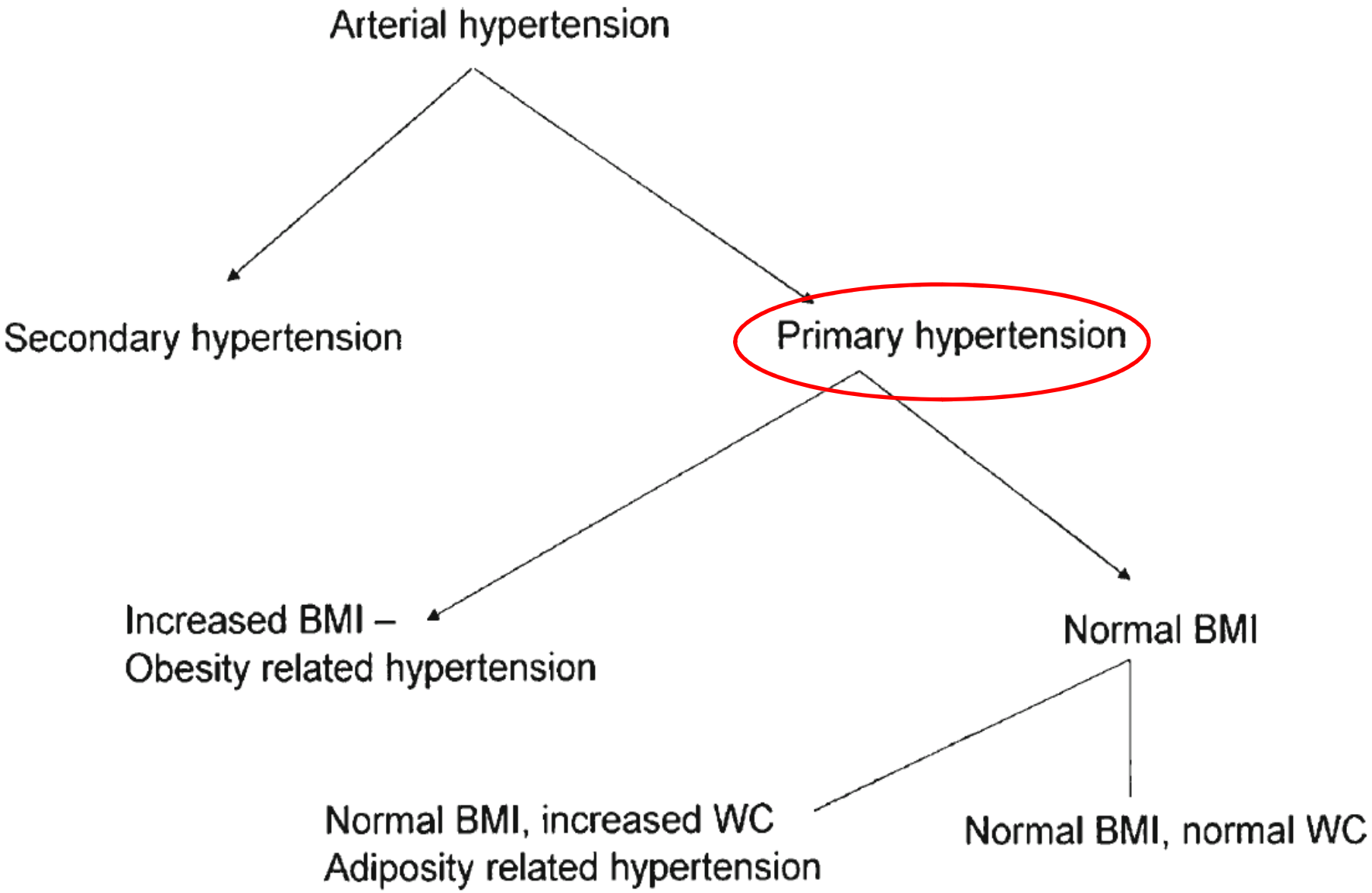
Tissue inhibitor of metalloproteinases - TIMPs



- ensure a balance of MMPs / TIMPs
- inhibit excessive degradation of ECM
- form a coordination bond stable and reversible MMP in a stoichiometric ratio of 1: 1 or 2: 2
- blocking access of substrate to the catalytic site of MMPs
- four types of the vertebrate
- involved in all processes of development and tissue remodeling
- in pathological processes disturbed balance of MMPs / TIMPs

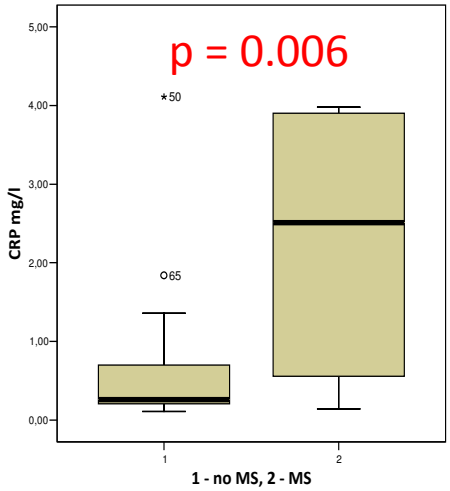
Classification of children's blood pressure level

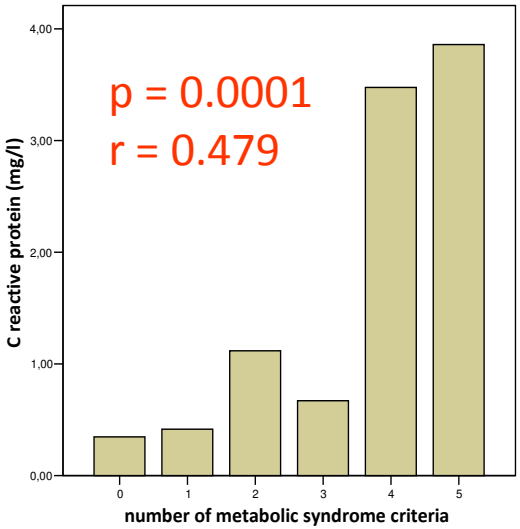
Classification (USA)	Children (percentile values of systolic and/or diastolic)	Adults [mmHg] systolic	diastolic
• Normal blood pressure	< 90 cc	< 120	< 80
•Prehypertension	> 90 cc and < 95 cc (always ≥ 120/80 mmHg even if it corresponds to <90 cc values)	120-139	80-89
• Stage I hypertension	≥ 95 cc + 5 mmHg > 99 cc	140-159	90-99
• Stage II hypertension	≥ 99 cc + 5 mmHg	> 160	>100



Litwin M et al. Curr Hypertens Rep 2013

Immune activation in children with primary hypertension – association with metabolic abnormalities and visceral obesity





Increased serum concentrations of MIP-1β and RANTES of hypertensive children in comparison with normotensive controls

The greater number of metabolic abnormalities, the greater immune activity.

The greater immune activity, the greater cIMT.

Litwin M et al. Pediatr Nephrol 2010; 25: 1711-8

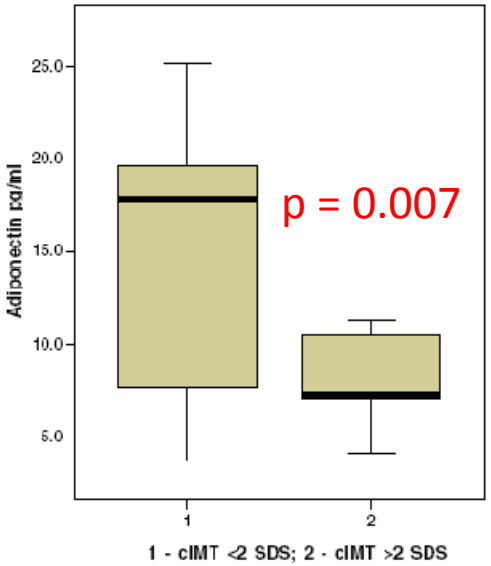


Fig. 1 Comparison of serum adiponectin concentrations in relation to carotid intima-media thickness (*cIMT*) below (group 1) and above (group 2) two standard deviation scores (*SDS*) from the median of the normal values ($p=0.006$)

Evidences for MMPs/TIMPs implications in hypertensions

- Remodeling of arterial wall structure
- Target organ damage
- Interactions with RAAS system
- Plasma/serum level
- Implication in other metabolic disease

- Fold change mRNA level in PH leukocytes !!!

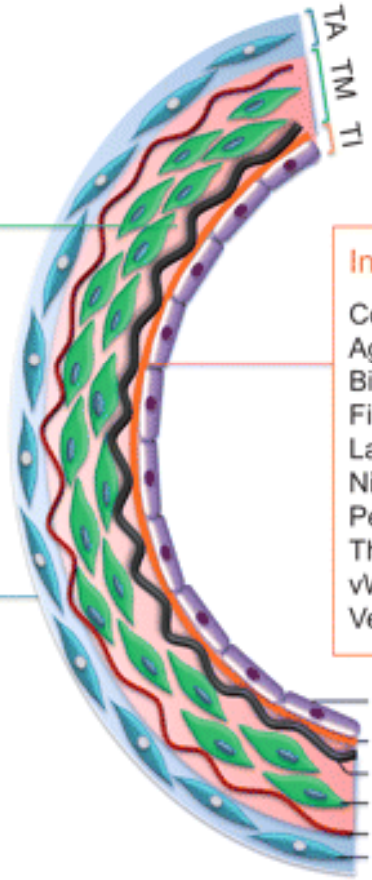
Arterial wall structure and its extracellular matrix components

Media

Collagen type I>III, V, VI
Emiln
Fibrillin-1, fibrillin-2
Fibulin
Fibronectin
Heparin
Hyaluronan
Laminin
Lumican
Microfibril
Osteopontin
Proteoglycans *e.g.* versican and biglycan
Tenascin
Vitronectin

Adventitia

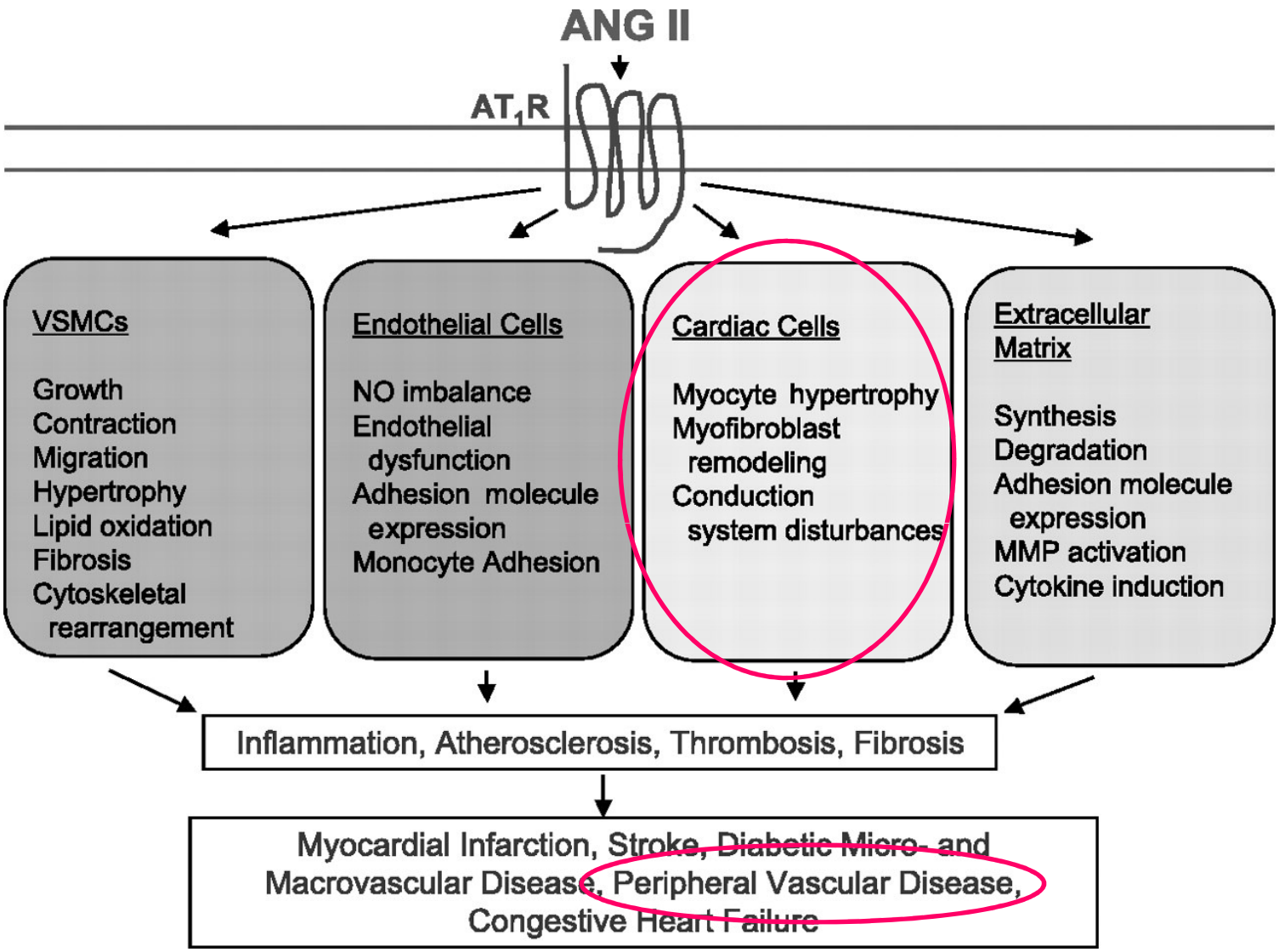
Collagen III>I, IV, V, VI
Decorin
Elastin
Fibronectin
Fibrillin
Laminin
Lumican
Proteoglycans like versican and biglycan
Vitronectin



Intima

Collagen IV, VI, VIII, XV, XVIII
Aggrecan
Biglycan, BM-40
Fibronectin, fibulin-1
Laminin
Nidogen/entactin
Perlecan (HSPG-2)
Thrombospondin-1 and -2
vWF
Versican

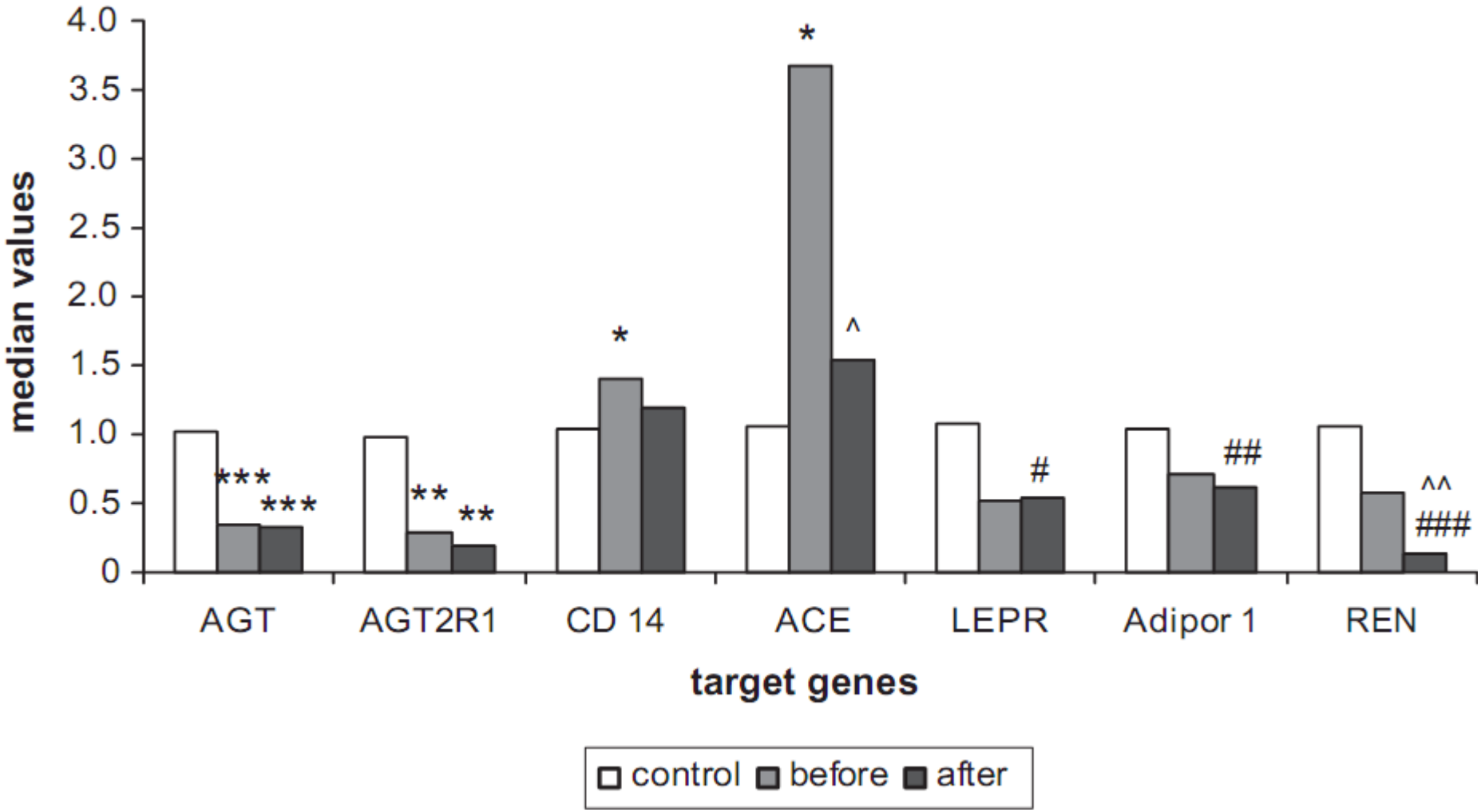
Chelladurai P. et al.Eur Respir J, 2012



Mehta PK et al. Am J Cell Physiol

Genes expression in peripheral blood leukocytes of 23 hypertensive adolescents (15 ±2.1 yrs) before and after 6 mts of non-pharmacological treatment – comparison with normotensive control group (n = 23). Normalization to constitutive genes expression in PBLs.

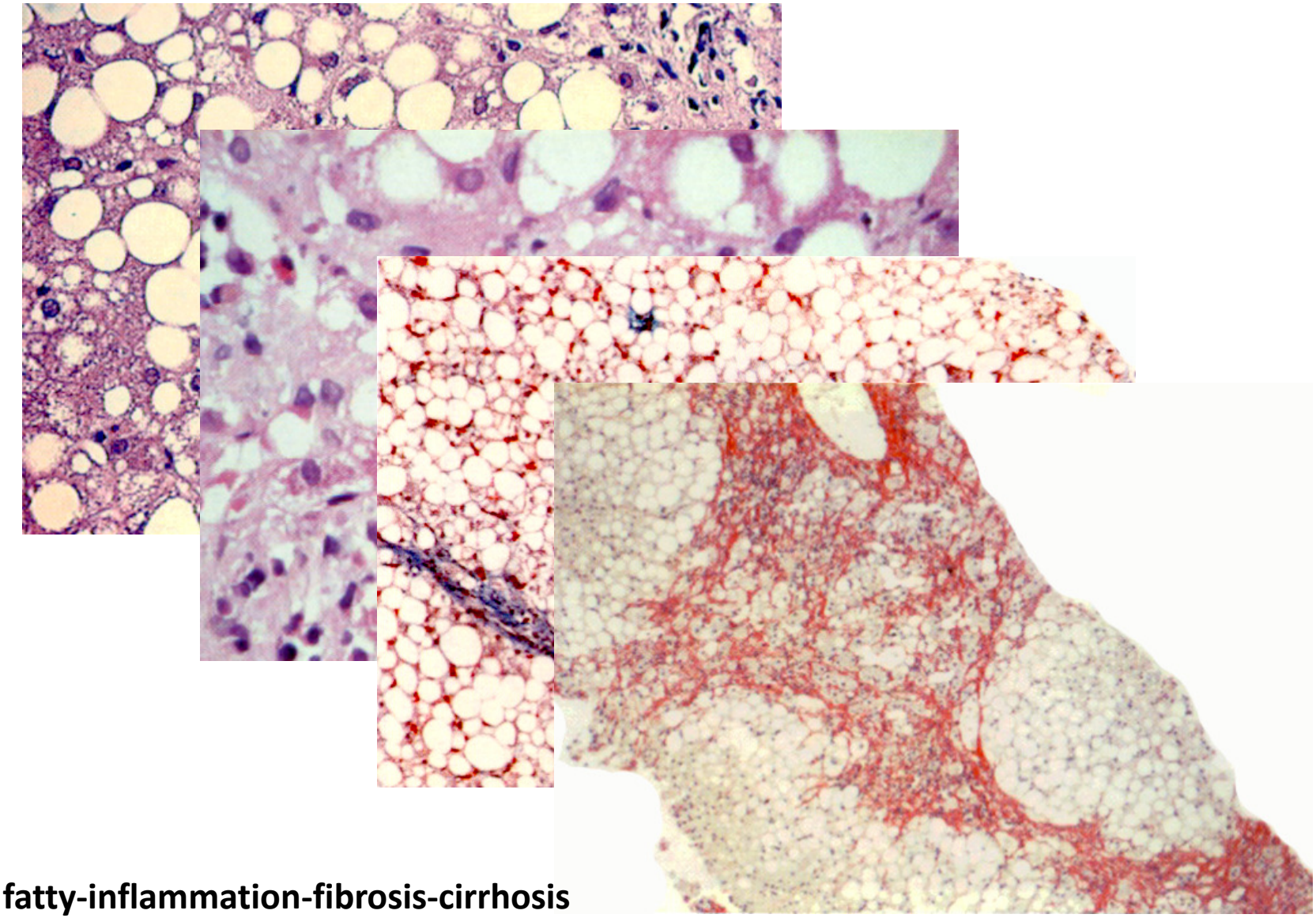
Litwin M & Michalkiewicz J, Hypertension 2013



Selected inflammatory mediators level in plasma of children with PH, NAFLD and obesity

Parameters	PH children (n=113)	NAFLD children (n=51)	OBESE children (n=31)	CONTROL (n=40)
MMP9 [ng/ml]	49 ±27*	54 ±23*	54 ± 26*	38 ± 18
TIMP1 [ng/ml]	115 ± 50*	172 ± 126*	133 ± 72	98 ± 51
IL-6 [pg/ml]	11 ± 8	8 ± 5	9 ± 8	12 ± 10,5
s CD14 [ng/ml]	861 ± 211*	1296 ± 172*	1282 ±249*	1039± 311

NAFLD – progress of the disease



fatty-inflammation-fibrosis-cirrhosis

Fibrosis- 58% NAFLD children wg *Nobili, Hepatology 2006*

NAFLD - some information

- excessive accumulation of fatty substances in hepatocytes

Reasons - depend on the patient age

1. systemic (e.g. obesity / metabolic syndrome, anorexia, diabetes mellitus type 1; polycystic ovarian syndrome; Hepatitis C)

2. metabolic (e.g.. Wilson's disease, deficiency of a 1-antitrypsin; cystic fibrosis; other congenital diseases)

3. toxic (eg. Ethanol, ecstasy, cocaine, solvents, pesticides, etc.)

Clinical symptoms:

- visceral obesity (waist circumference)
- metabolic syndrome/diabetes
- hepato-/splenomegaly, cholestasis
- other systemic disorders

OBESITY

1. The cause of fatty liver

2. More likely to have cirrhosis

3. Distribution of body fat - visceral obesity - cardiovascular risk

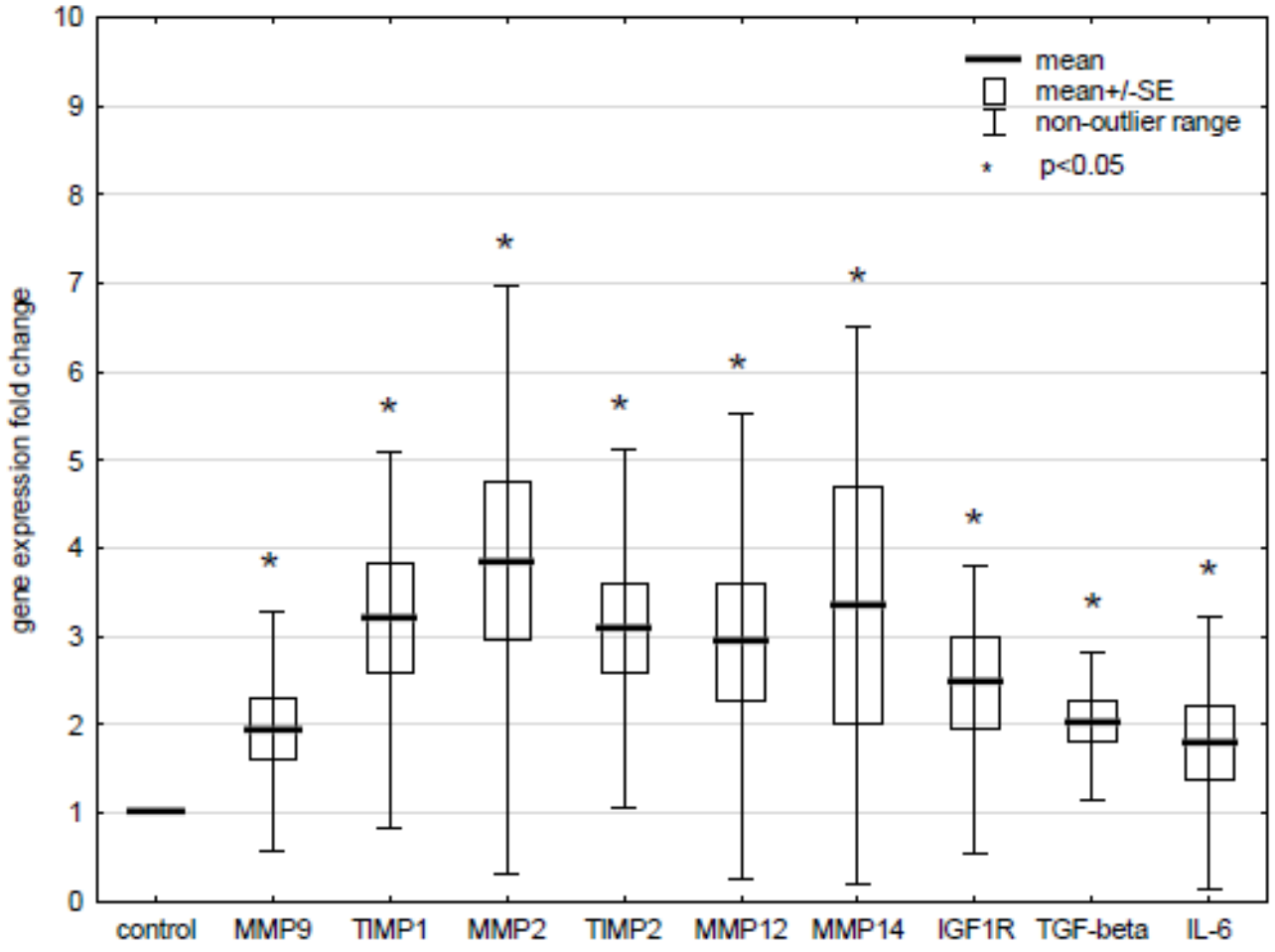
4. Lipid disorders - Insulin Resistance

CAUSES AND CONSEQUENCES

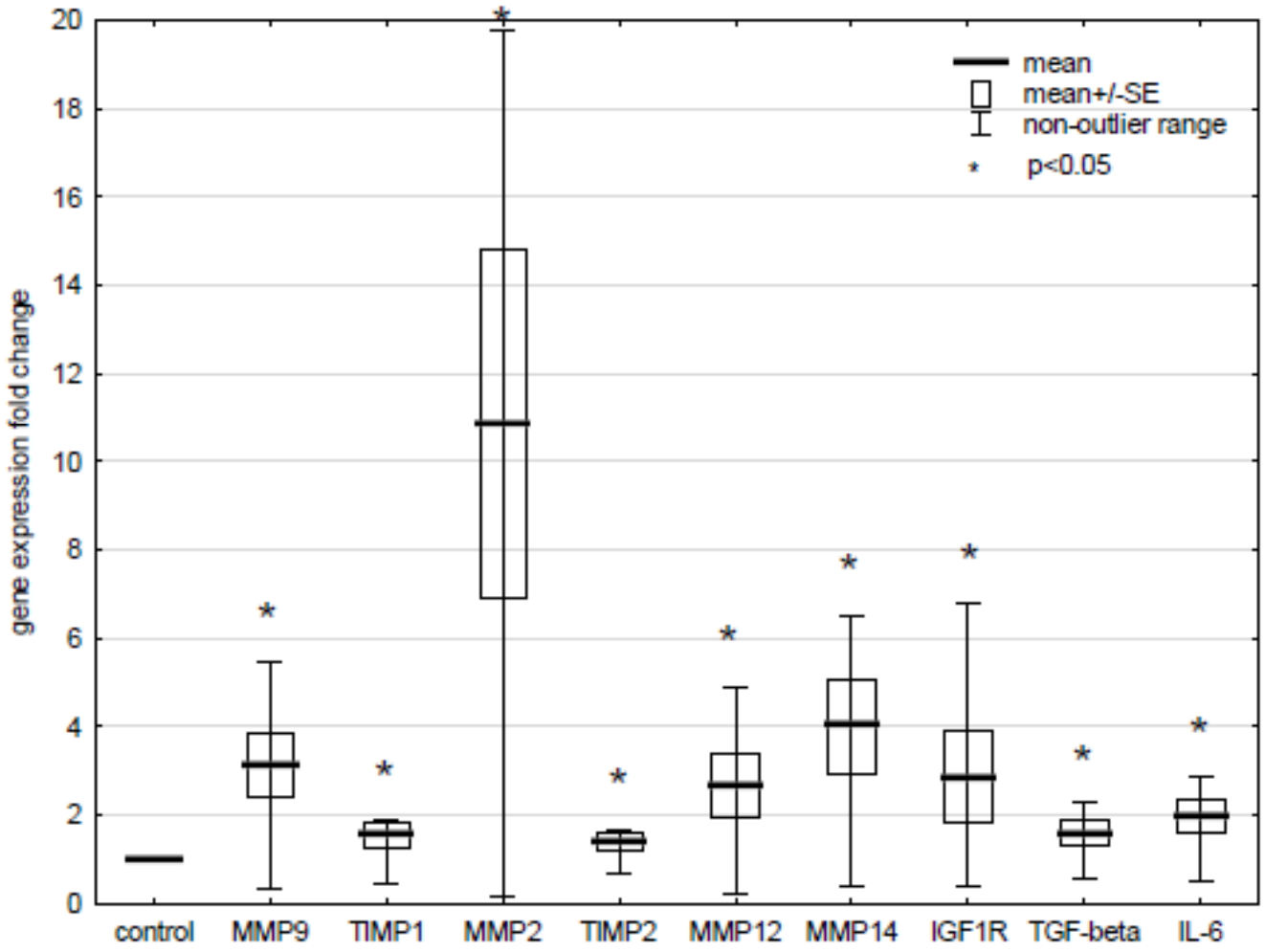
- lifestyle, lack of physical activity
- increased appetite - positive energy balance
- reducing the secretion of corticotropin
- pronounced effect of neuropeptide Y in the hypothalamus
- increased concentrations of TNF-alpha
- suppression of adiponectin secretion from adipocytes
- complications of the cardiovascular system
- the risk of coronary heart disease, hypertension, diabetes t II and hyperlipidemia

Visceral obesity=metabolic disorders
dominant intermediate phenotype
15-20% children with PH

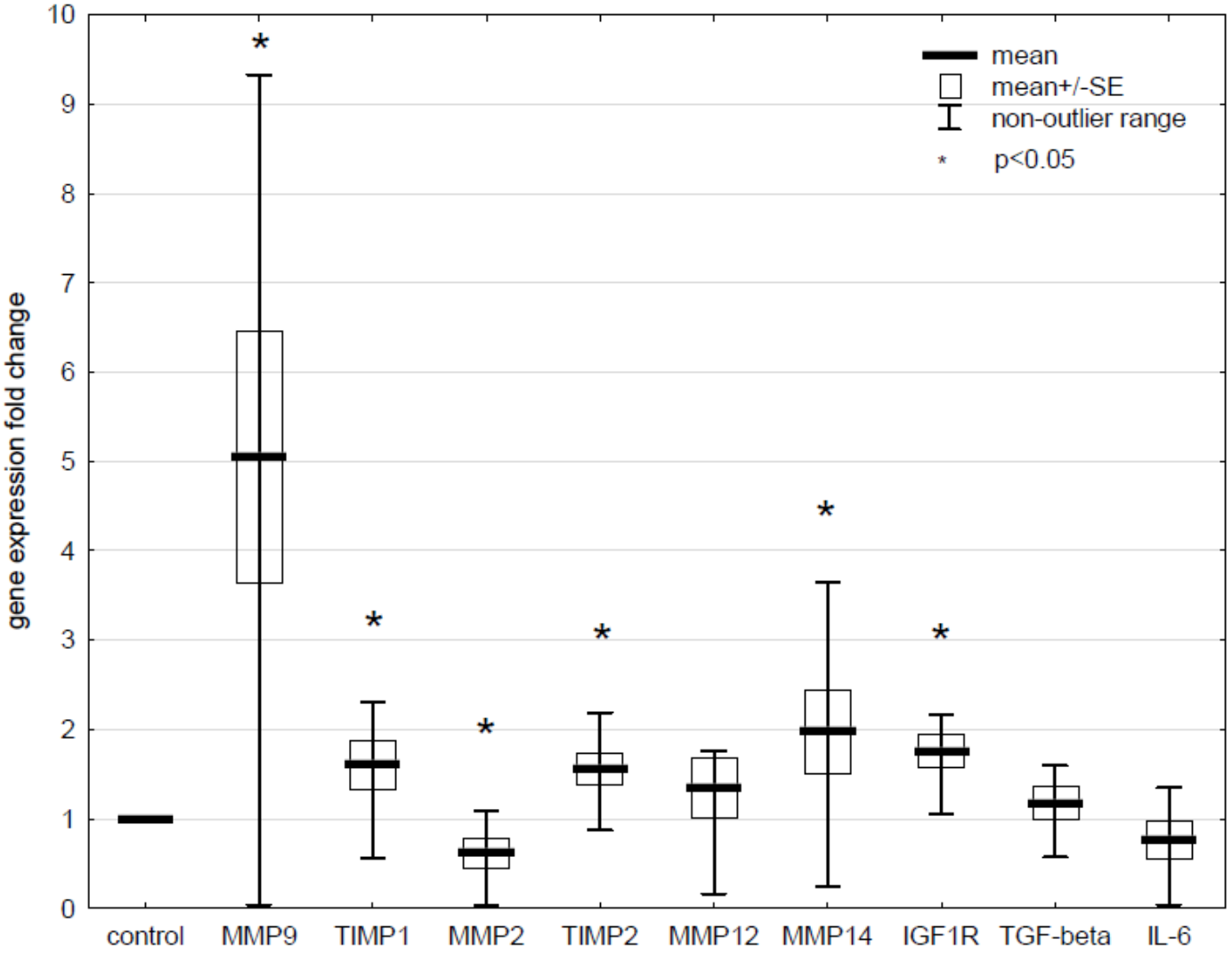
Genes expression -1 (PH nw/Contr nw)



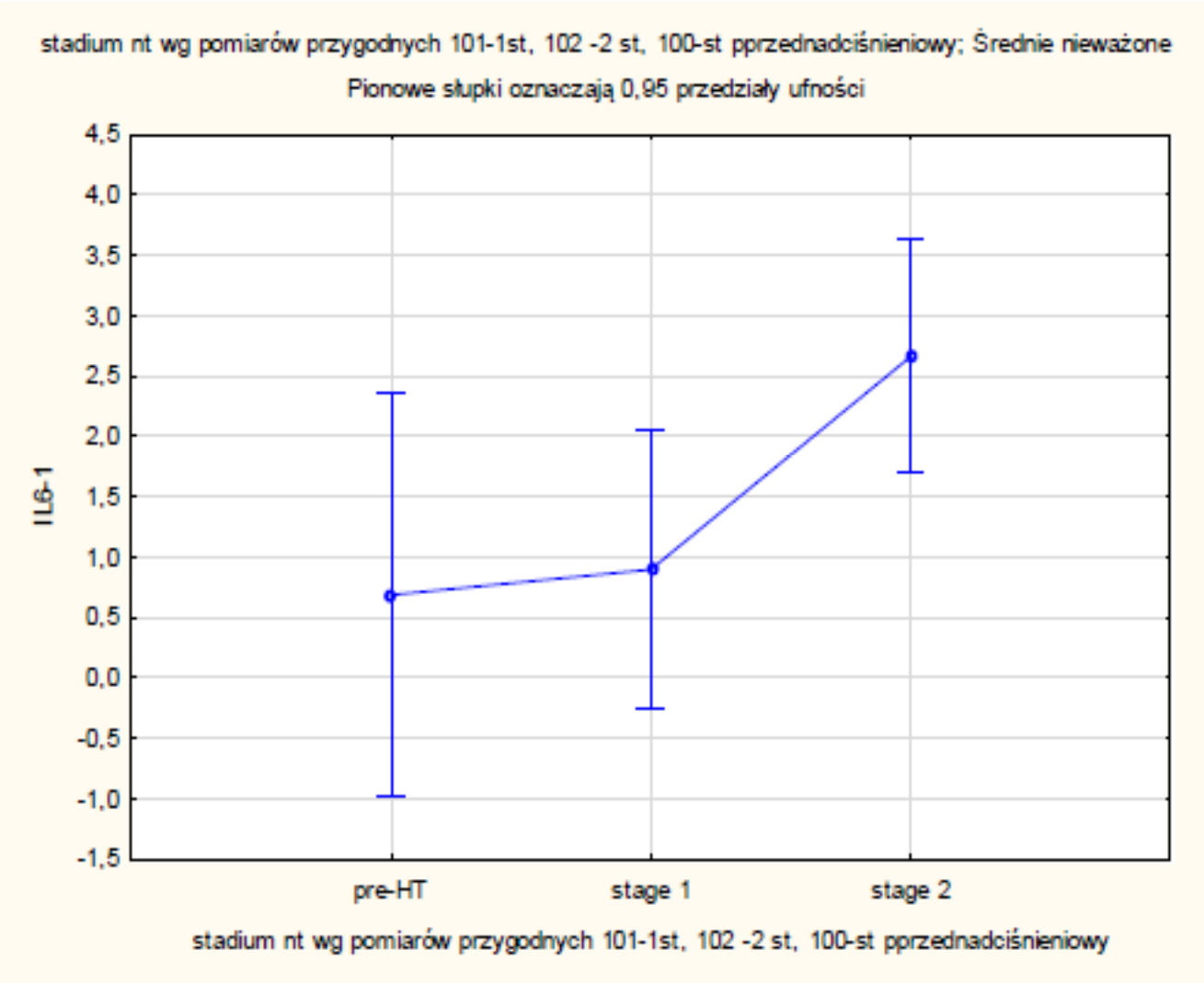
Gene expression -2 (PH Ob/Contr Ob)



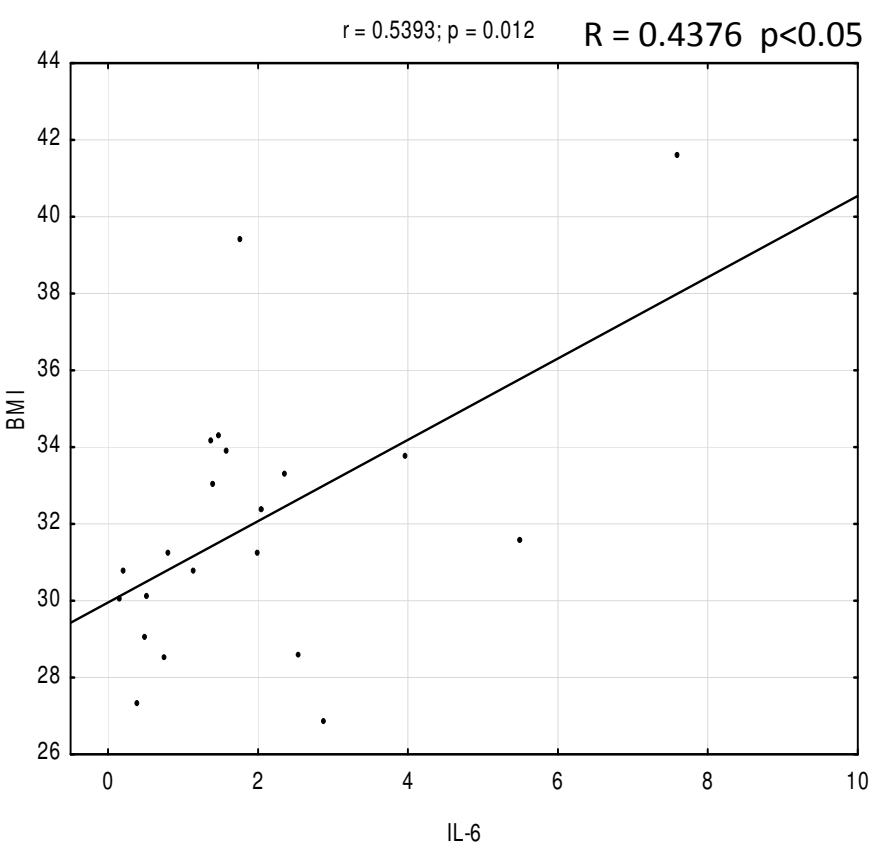
Gene expression – 3 (Contr Ob/Contr nw)

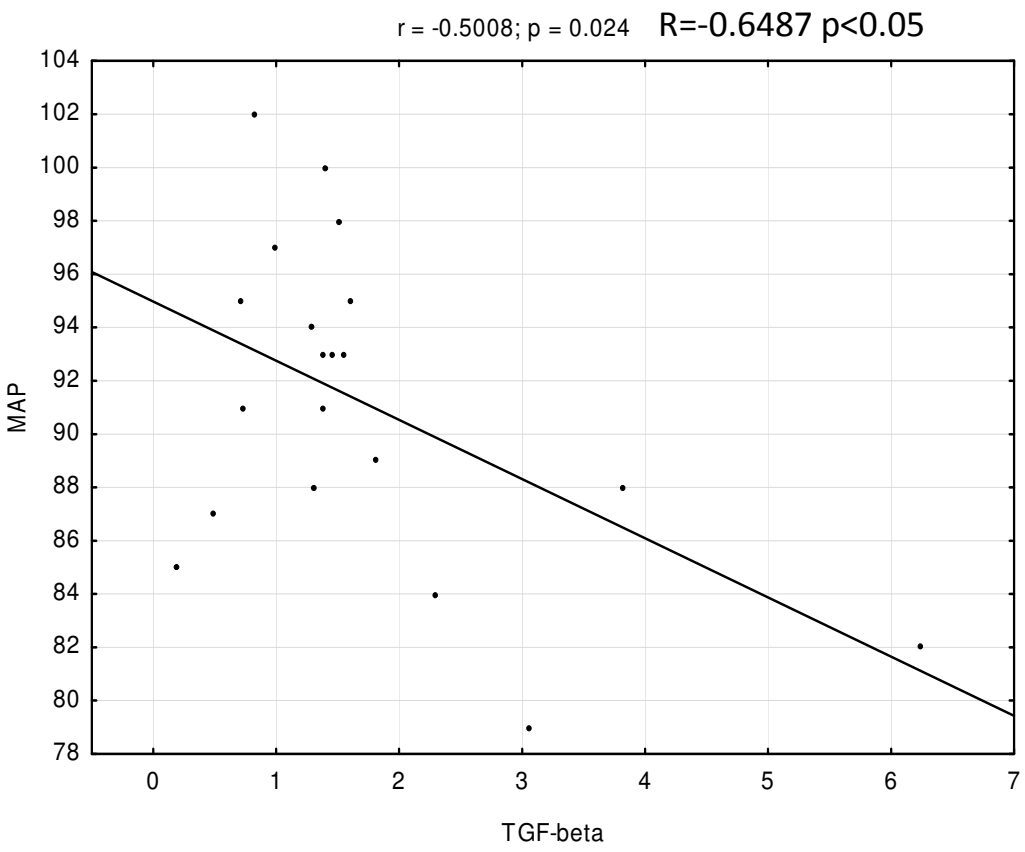


Correlations -1



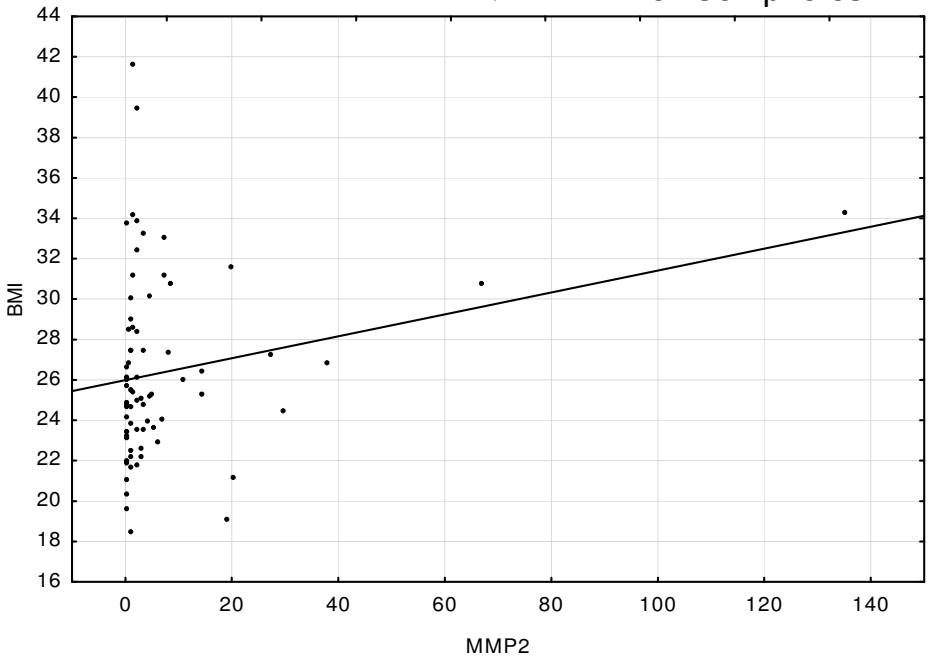
Correlations - 2 (PH ob/Contr ob)



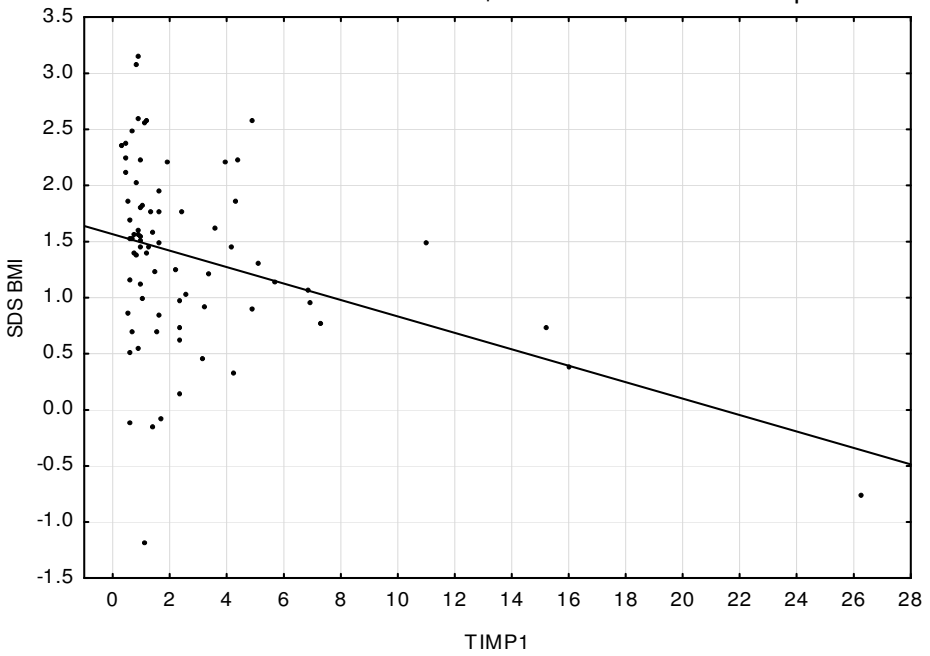


Correlations - 3 (PH nw/Contr nw)

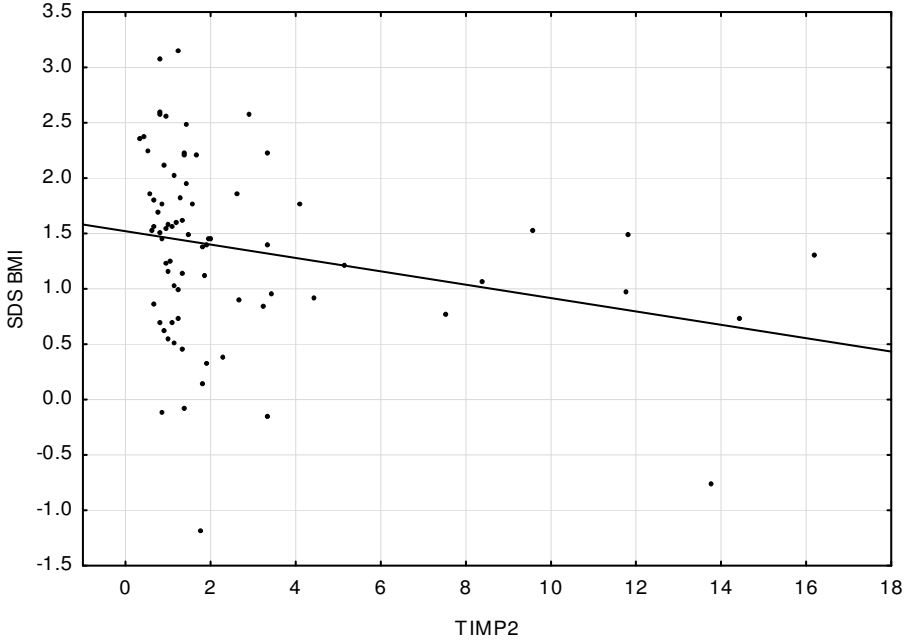
r = 0 .22171; p=0.058 R = 0.2502 p<0.05



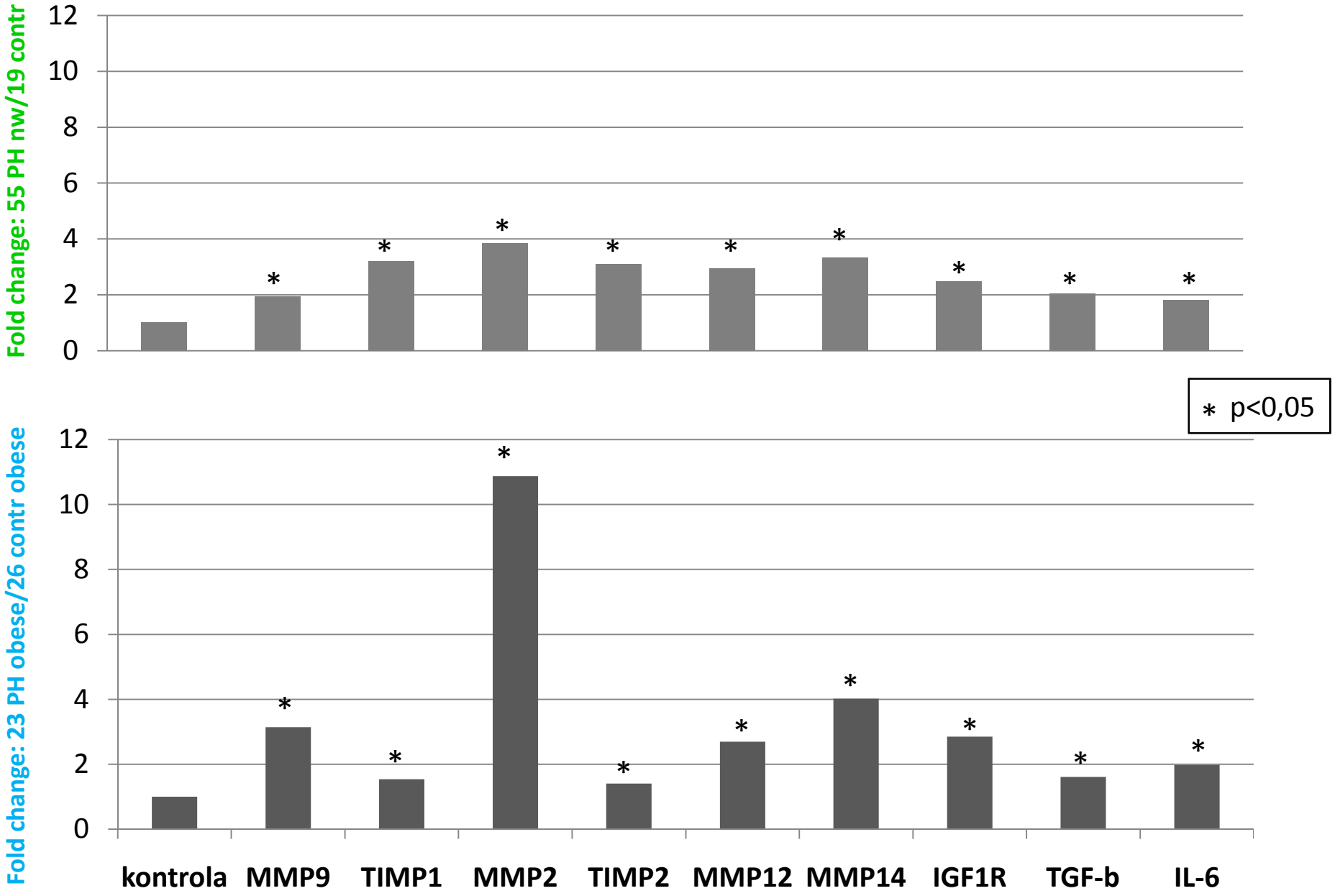
r = -0.3588; p=0.002 R=-0.3303 p<0.05



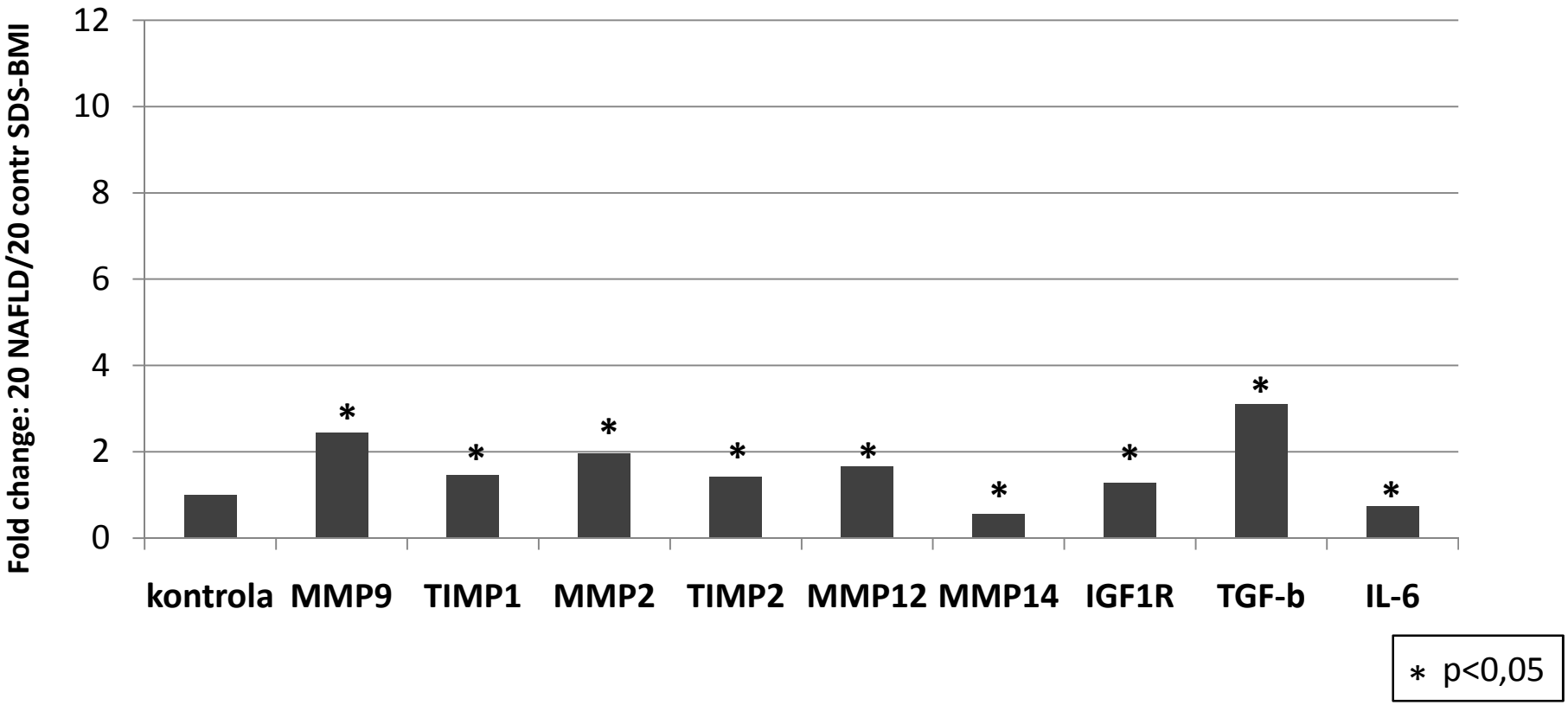
r = -0.2511; p=0.030 **R=-0.3317 p<0.05**



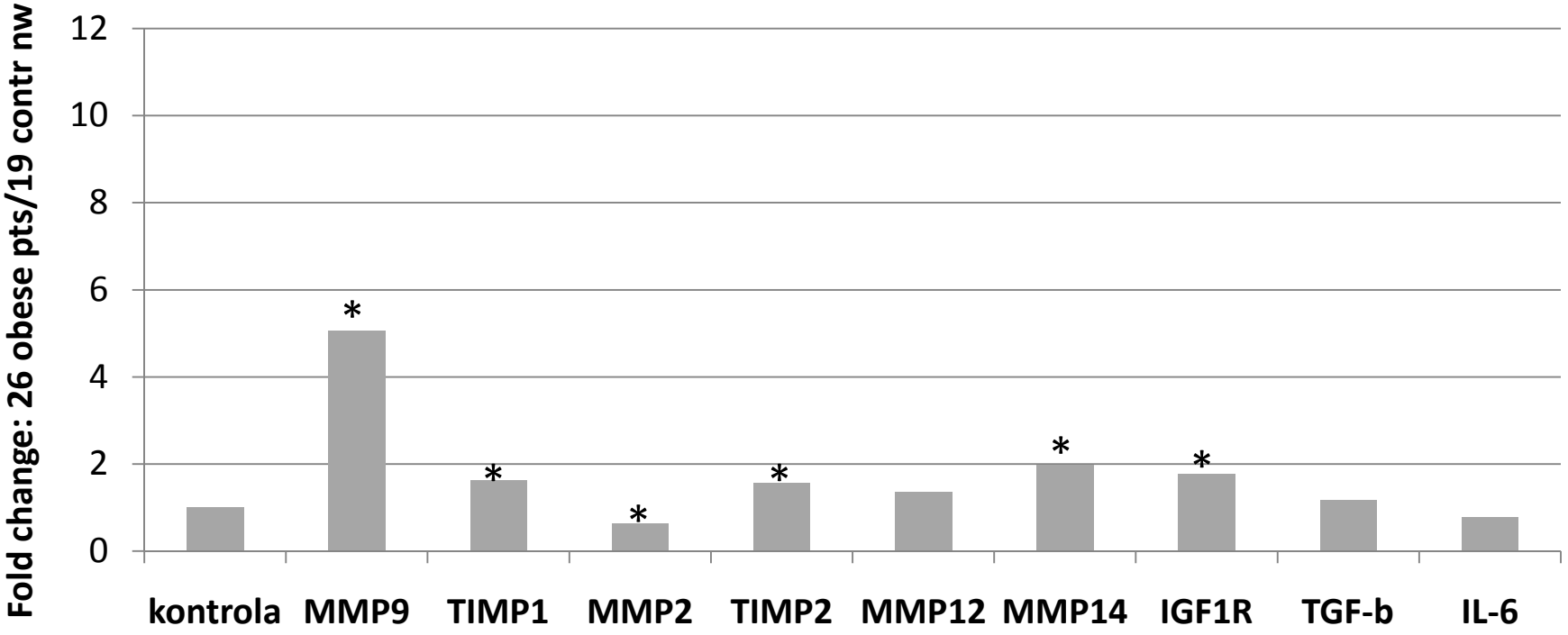
Genes expression levels in PH leukocytes



Genes expression levels in NAFLD leukocytes

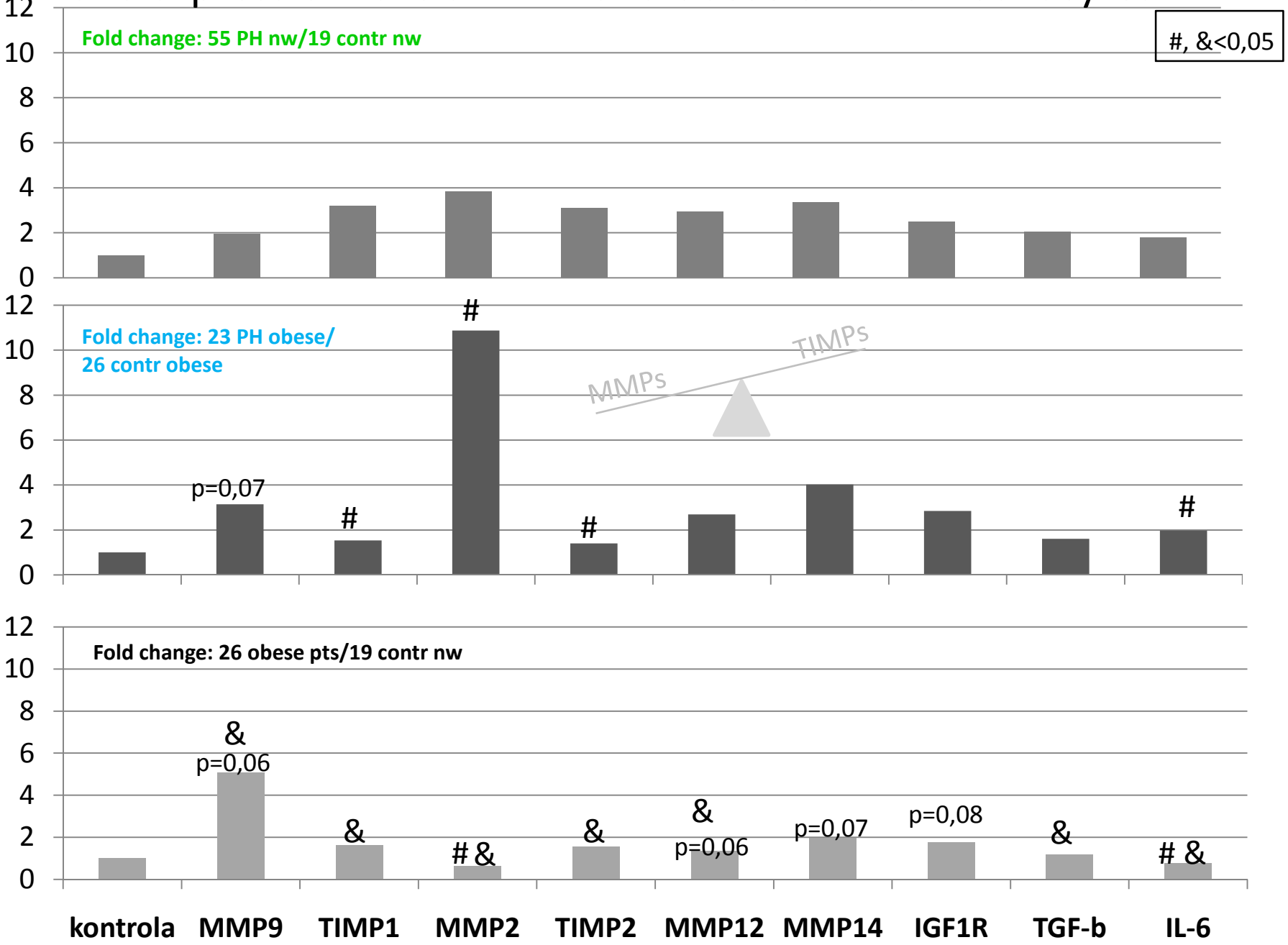


Genes expression levels in obesity leukocytes



* p<0,05

Gene expression levels in PH and obese children leukocytes



Conclusions:

1. Obesity significantly up-regulates MMP-2 expression in the PH leukocytes

2. MMP-2 over-expression in the PH leukocytes is not counter--regulated by TIMP-1 and -2

3. MMP-9 expression is highly specific for obesity but not PH

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