Age Management AMMG Medicine Group

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THE USE OF NUTRACEUTICALS IN CHRONIC LIVER DISEASE: MYTHS, FACTS AND DANGERS

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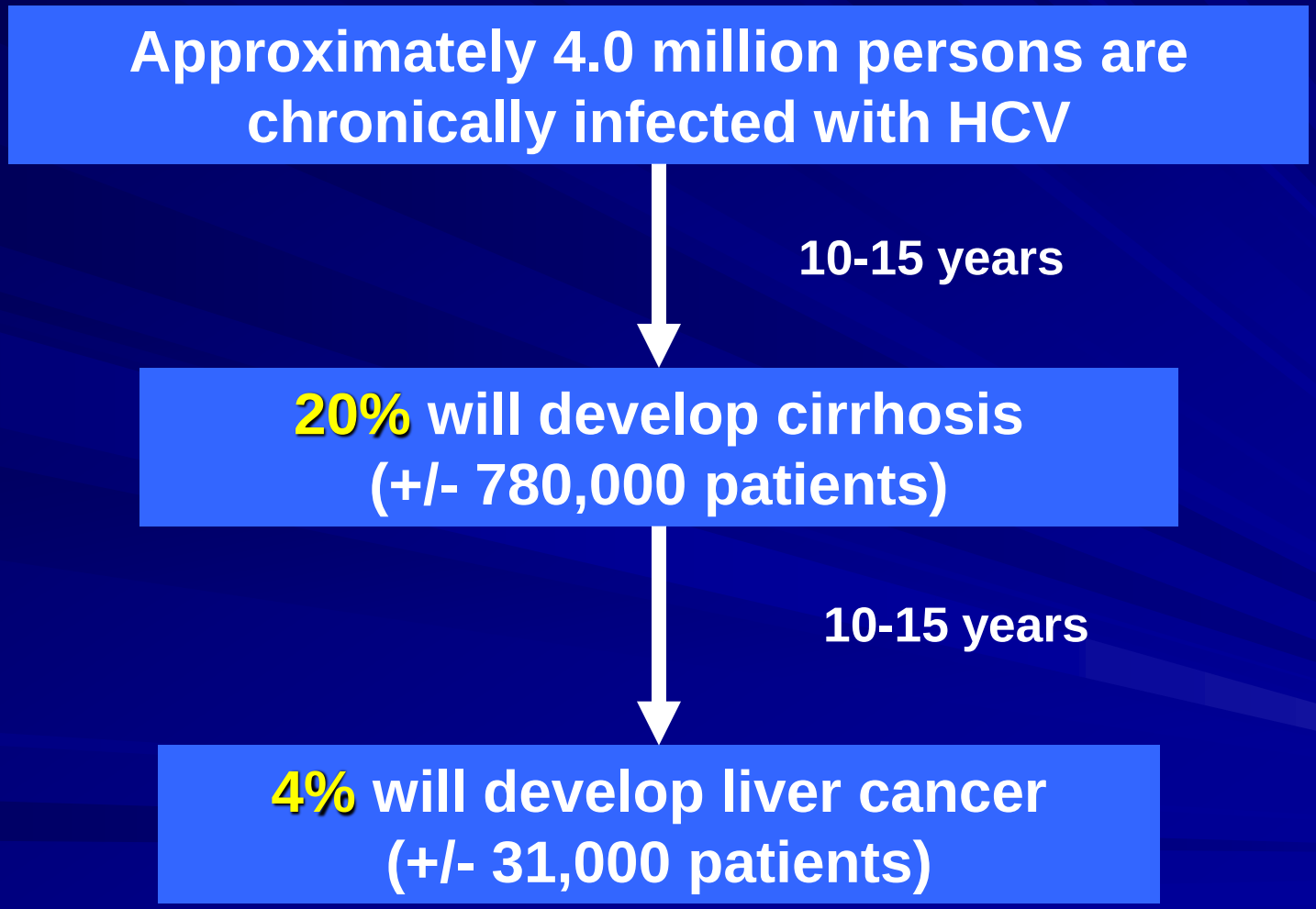
Impact of HCV Infection in the US



Di Bisceglie, Hepatology, 2000

Chronic hepatitis C is a major health care problem

Projected prevalence of cirrhosis and its complications in the US over the next 20 years

Cirrhosis / Complication	Year		Change (%)
	2000	2020	
HCV infection	2,940,678	2,681,556	-9.7
Cirrhosis	472,103	858,788	45.0
Decompensated cirrhosis	65,294	134,743	51.5
Hepatocellular carcinoma	7,271	13,390	44.9
Liver-related death	13,000	36,483	64.4
Patients listed for transplant	10,893	~30,000	—
Transplants performed	4893	unknown	—
Transplants performed for HCV	1920	unknown	—

Organ Procurement and Transplantation Network (OPTN) Database
Davis GL et al., Liver Transplant 2003

The prognosis of HCV-induced cirrhosis is poor

Annual incidence of complications (%)	
Clinical decompensation	3.6 – 6.0
• Hepatocellular carcinoma	1.4 – 2.6
• Ascites	2.2
• Variceal bleeding	0.5
• Hepatic encephalopathy	0.3
5-year survival	
Compensated cirrhosis	91%
After 1st major complication	50%

Fattovich G et al., Gastroenterology 1997
Benvegnú L et al., Gut 2004

Why should we treat HCV patients?

Short term endpoints

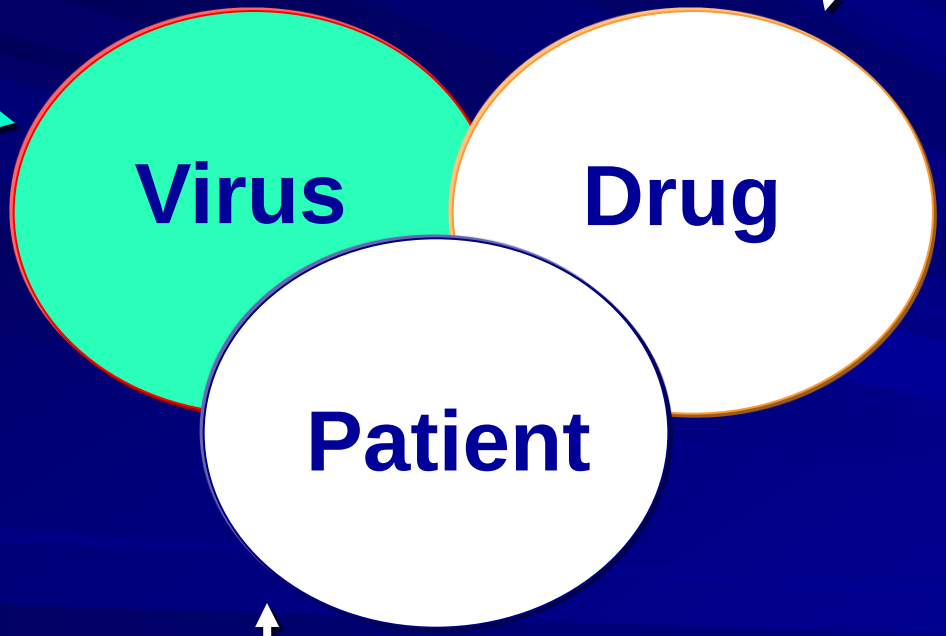
- Eradicate HCV
- Reduce/Stop necroinflammation
- Reduce/stop fibrosis progression

Ultimate aims

- Prevent/delay cirrhosis
- Prevent/delay liver decompensation
- Reduce the risk of HCC

Limits to successful antiviral therapy

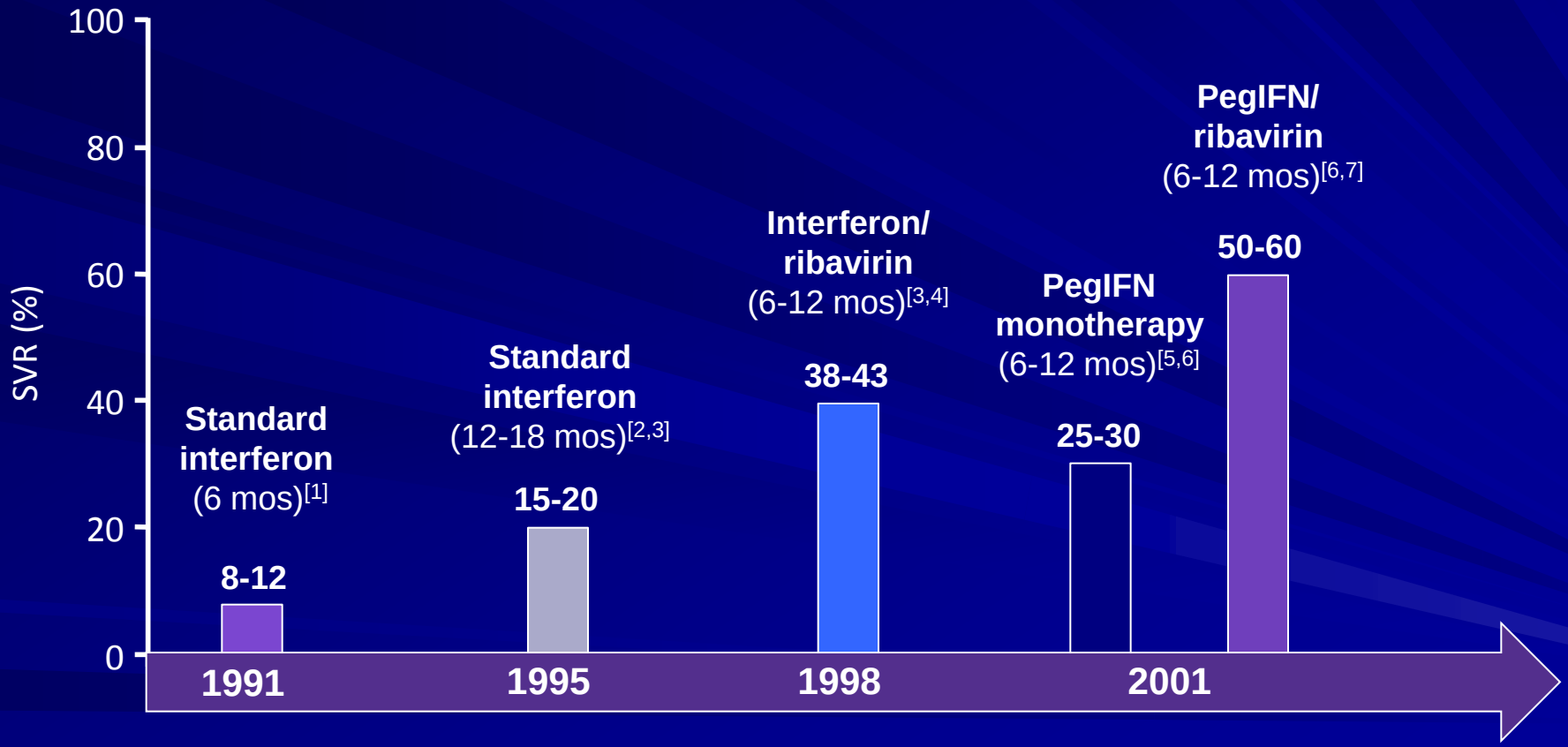
- High viral replication rate
- High mutation frequency
- Selection of resistance



- Low potency
- Short term efficacy
- Poor pharmaco-kinetic & -dynamic
- Poor tolerability
- Difficult to take
- Selection of resistance

- Adherence <100%
- Intolerance

Treatment of Chronic Hepatitis C



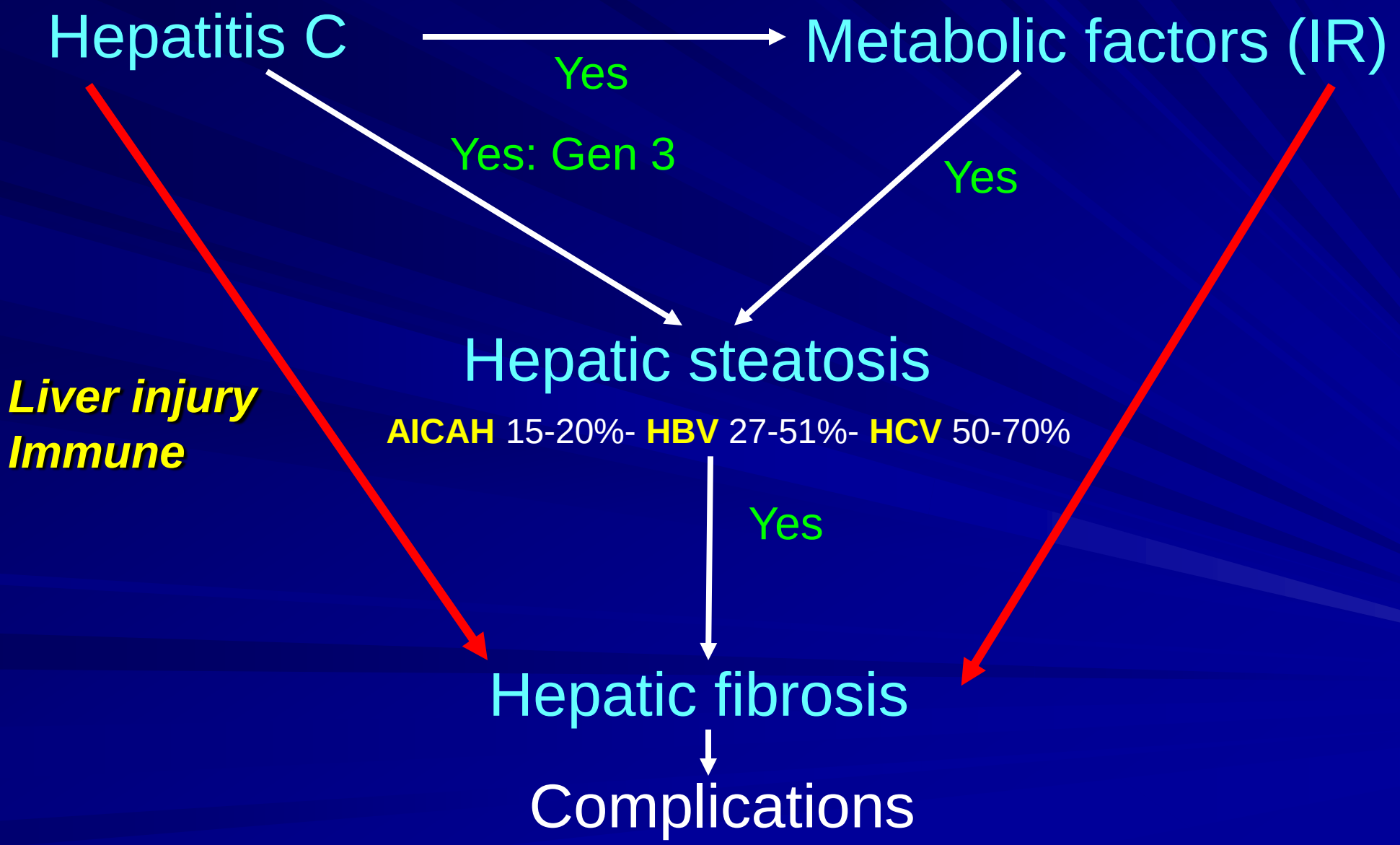
1. Carithers RL Jr., et al. Hepatology. 1997;26(3 suppl 1):83S-88S. 2. Zeuzem S, et al. N Engl J Med. 2000;343:1666-1672. 3. Poynard T, et al. Lancet. 1998;352:1426-1432. 4. McHutchison JG, et al. N Engl J Med. 1998;339:1485-1492.
5. Lindsay KL, et al. Hepatology. 2001;34:395-403. 6. Fried MW, et al. N Engl J Med. 2002;347:975-982. 7. Manns MP, et al. Lancet. 2001;358:958-965.

Evaluating Factors Associated With Poor Response to HCV Therapy

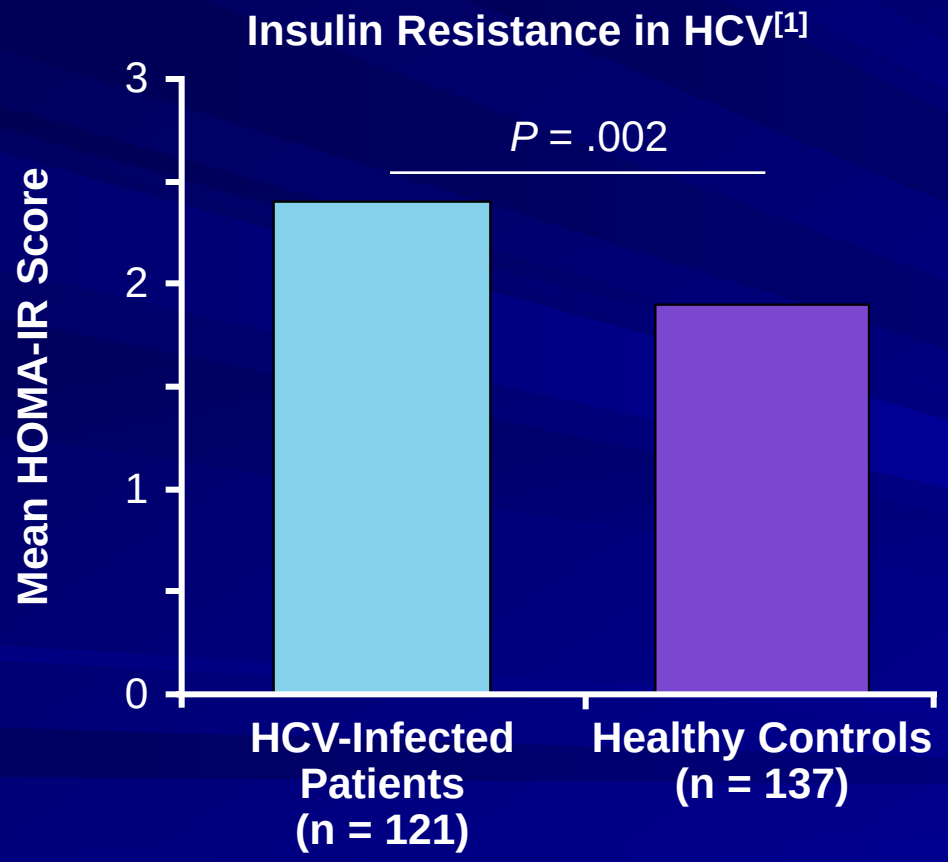
Factors Associated With Poor Response to HCV Therapy	
Fixed Factors	**Correctable Factors**
■ HCV genotype ■ Race ■ Patient age ■ Serum HCV RNA level ■ Cirrhosis ■ Morbid obesity	■ Pretreatment – Prescription of optimal course of therapy – Substance abuse – Fatty liver disease – Obesity/metabolic syndrome – Psychiatric comorbidities – Other comorbidities ■ On treatment – Noncompliance with treatment – Management of adverse effects

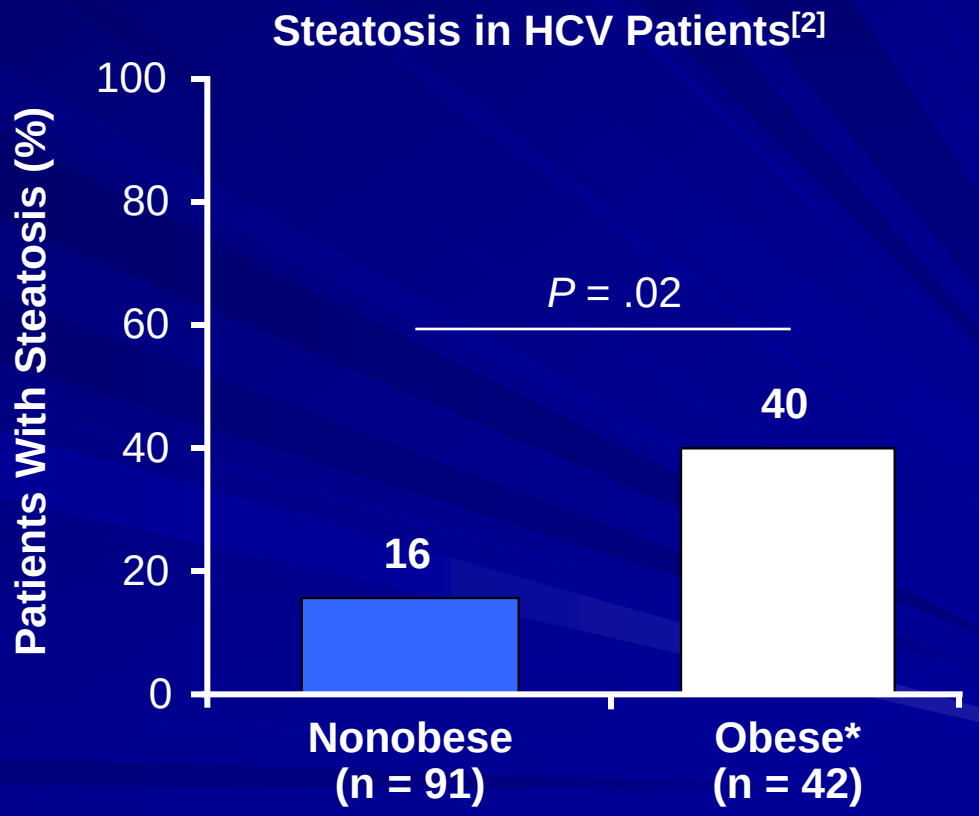
Shiffman ML, et al. Gastroenterology. 2004
Krawitt EL, et al. J Hepatol. 2005

Hepatic steatosis, IR and chronic hepatitis C infection



Evidence: Metabolic Syndrome, Steatosis, and HCV





*BMI ≥ 30.

1. Hui JM et al. Gastroenterology 2003;125:1695-1704.
2. Cesario K, et al. J Hepatol. 2005;42(suppl 2):201-202.

more ***FIBROSIS***.....Yano M, et al. Hepatology. 1996;23:1334-1340

Fibrosis Score	Description of Fibrosis	Patients Progressing to Cirrhosis by Year 10, %
≤ 1.9 (n = 27)	None; too mild to alter portal tract size	29.6
2.0-2.9 (n = 28)	Portal/periportal ± portal-portal bridging	42.9
3.0-3.45 (n = 15)	Septal + regions of partial nodular regeneration	100

more ***INFLAMMATION***..... Ghany MG, et al. Gastroenterol. 2003;124:97-104.

Change in Fibrosis Score According to Necrosis Score at Baseline			
	Piecemeal Necrosis Score at Baseline		
	0-1	2-3	> 4
Patients, n	30	66	27
Mean change in fibrosis score per yr	0.05	0.19	0.37

Evidence: Steatosis and Fibrosis Progression in HCV-Infected Patients

- Younossi, et al (N = 122)
  - Predictors of advanced fibrosis: higher BMI, superimposed NASH
- Fartoux, et al (N = 135 paired liver biopsies, untreated patients)
  - After 6 yrs follow-up, steatosis was only independent predictor of progressive fibrosis
- Hui et al (N = 117)
  - Fibrosis progression predicted by HOMA-IR, serum cholesterol
- Conjeevaram et al (N = 399 GT 1 patients)
  - Bridging fibrosis or cirrhosis in patients with vs without steatosis (45% vs 23%, respectively; P < .0001)

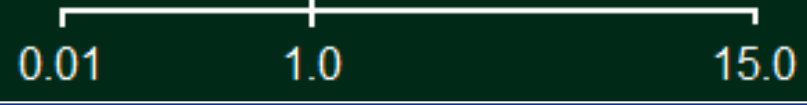
Younossi ZM, et al. J Clin Gastroenterol. 2004;38:705-709. Fartoux L, et al. Hepatology. 2005;41:82-87. Hui JM, et al. Gastroenterology. 2003;125:1695-1704. Conjeevaram H, et al. Hepatology. 2007;45:80-87.

Factors Associated With Advanced Fibrosis

Hu S, et al. J Clin Gastro. 2009

- Retrospective study of 460 pts with chronic hepatitis C (41% F3-4)
- Multivariate analysis of factors associated with F3-4

Risk Factor	Adjusted Odds Ratio (95% CI)	*P* Value
Age at entry (≥ 60 years)	3.444	.0334
Duration of infection (≥ 25 years)	1.750	.0378
BMI (≥ 30)	1.917	.0173
History of diabetes	2.251	.0304
AST (≥ 80 U/L)	4.032	.0087
AFP (≥ 15 µg/L)	3.875	.0383
Grades 2 and 3 steatosis	2.790	.0378



Steatosis, fibrosis and necroinflammation in chronic hepatitis C: a Meta-Analysis of Individual patient Data (The HCV MAID Study)

Leandro G et al

Independent predictors of fibrosis stage

	P value
HOMA-IR	<0.001
Age	<0.001
Alcohol: past	<0.001
Portal inflammation	<0.001
ALT	0.04
Platelets (negative association)	<0.001
Cholesterol (negative association)	0.001

Future Therapy of Hepatitis C

Tomorrow — **Treatment Strategies to Enhance Response to Current Therapies**

Years — **New strategies: molecular based therapy**

Therapeutic Strategies

- *To Reduce Liver Injury*
- *To Reduce Progression of Fibrosis*
- *To Decrease Hepatocytes Proliferation*

Treatment of hepatitis C
Unsolved issues

- Clinical heterogeneity of hepatitis C
- Over-treatment
- Tailoring of dose/duration
- Role of PEG-IFNs monotherapy
- Non-responders to IFN and to IFN-ribavirin
- Drug toxicity
- Co-morbidities
- Special patient populations
- The financial issue

Contraindications to therapy

Absolute

- Pregnancy
- Decompensated cirrhosis
- End stage kidney disease
- Severe or uncontrolled psychiatric disease
- Cardiopulmonary disease
- Severe Autoimmune disease
- Severe anemia
- Noncompliance

Relative

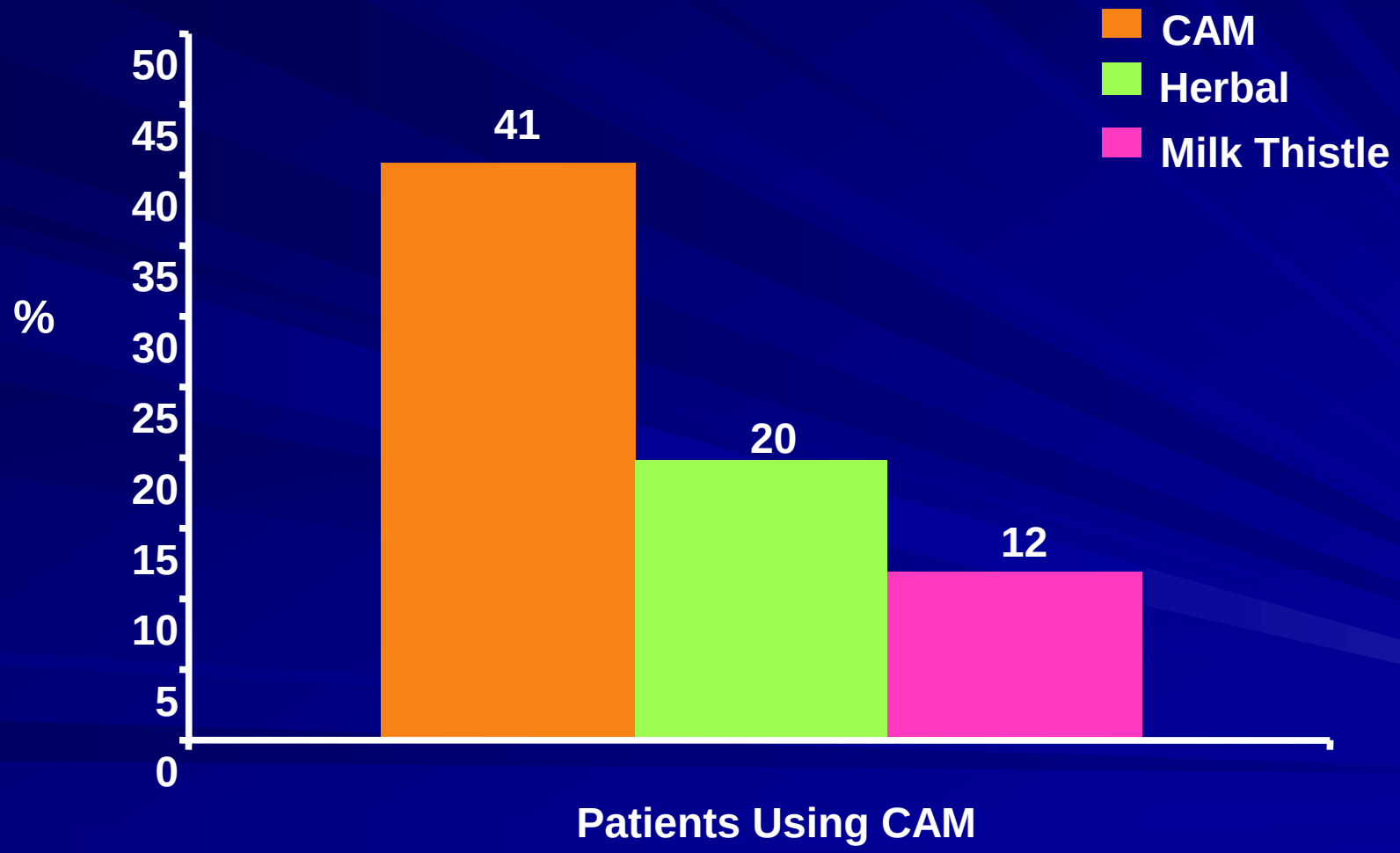
- Cirrhosis, compensated
- Controlled psychiatric disease
- Mild anemia/leukopenia
- Renal insufficiency
- Mild autoimmune disease

Epidemiology of CAM

- Prevalence of the use of complementary and alternative medicine (CAM) in US adults
  - 1990 2.5%
  - 1997 12.1%
  - 2002 18.9%
  - 2012 > %
- 2009 Estimated sales >$4 billion in the US
- Worldwide, underdeveloped countries
- Europe
  - Regulate herbs as prescription or nonprescription medicines available only through a pharmacist
  - German physicians receive medical school training in medicinal herbs (and must pass a test to become licensed)

Percent of Patients Using CAM

Liver Clinics



Seeff et al. Hepatol. 2001

Appeal of CAM Among Patients With HCV Infection

- A chronic illness with limited treatment success
- Frustration with uncertainty of prognosis
  - Limited information available from providers
  - Absence of signs and symptoms
- Lack of symptoms vs side effects of conventional treatment
- Desire for a "holistic" approach to therapy

Non-alcoholic Fatty Liver Disease

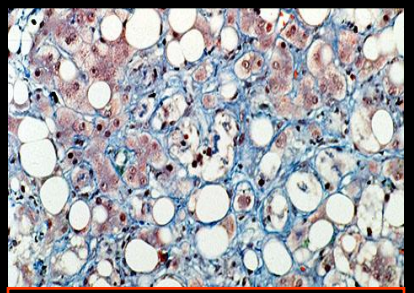
- Evidence to support important interactions between NAFLD and Metabolic Syndrome
  - Evidence #1: NAFLD and Metabolic Syndrome co-exist
  - Evidence #2: Metabolic Syndrome affects progression of NAFLD
  - Evidence #3: Treating Metabolic Syndrome influences the outcome of NAFLD

From the spectrum of NAFLD, only those patients with NASH have convincingly been shown to progress

Teli 1995
Matteoni 1999

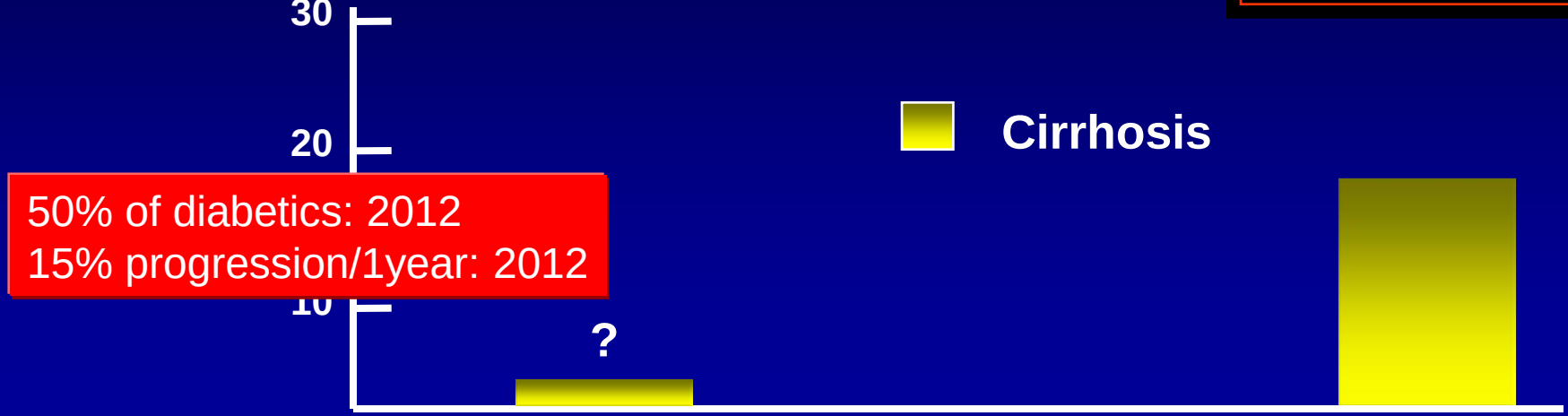
Steatosis alone

NAFLD Spectrum



NASH

Ludwig 1980
Diehl 1988
Powell 1990
Bacon 1994
Caldwell 1999
Angulo 1999
Matteoni 1999
Younossi 2000
Ong 2001
Ratziu V 2002
Harrisson 2003



50% of diabetics: 2012
15% progression/1year: 2012

Natural antiox/anti-inflammatory compounds in NASH: any relevant role?

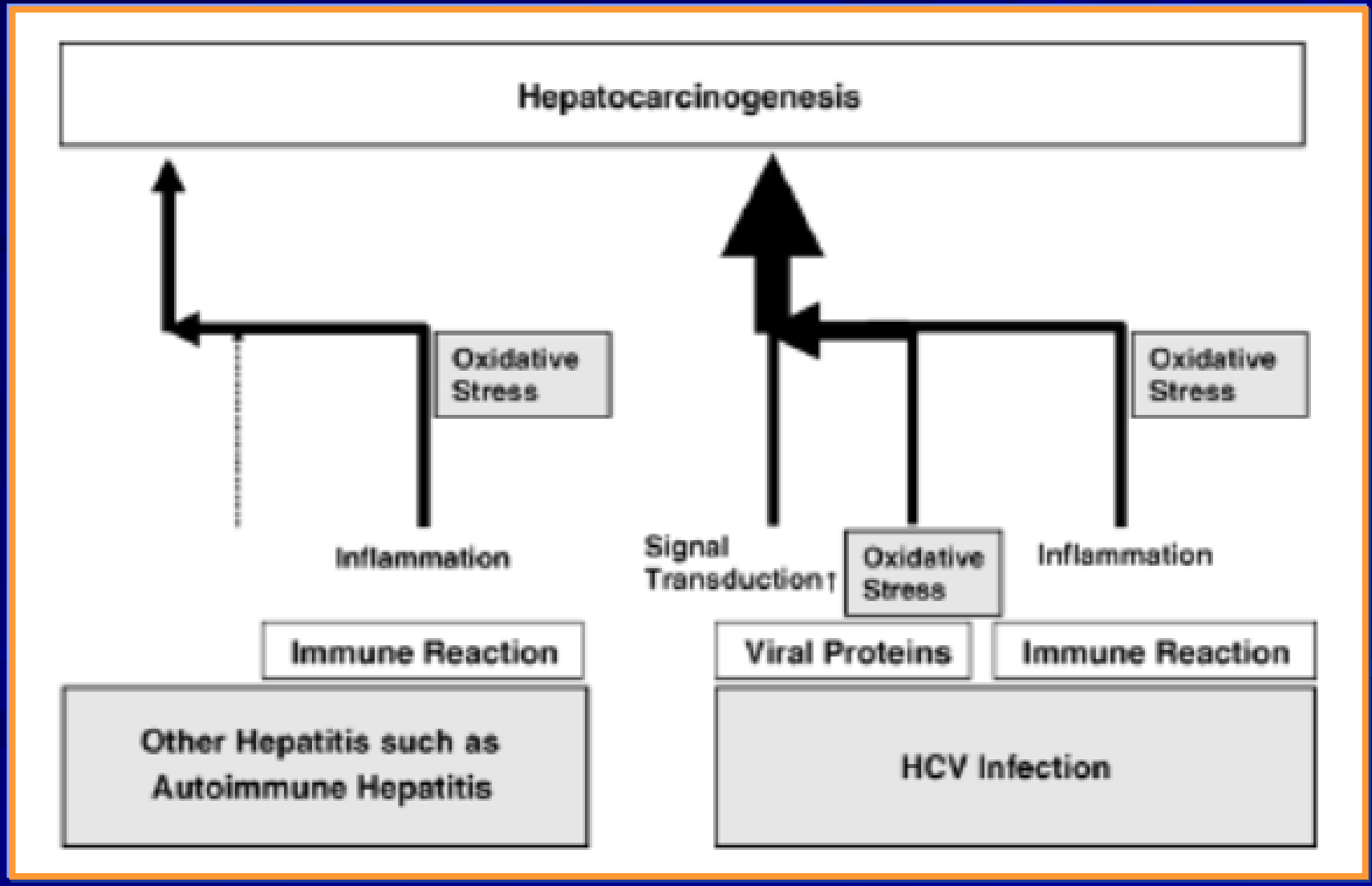
HCV: **a metabolic disease? Common pathways with NASH**. Koike et al. JSH (Japan Society Hepatology) meeting, October, 2004

There is a growing body of evidences suggesting the role of free radical generation and oxidant injury in the pathogenesis of liver fibrosis, NASH and NAFLD.

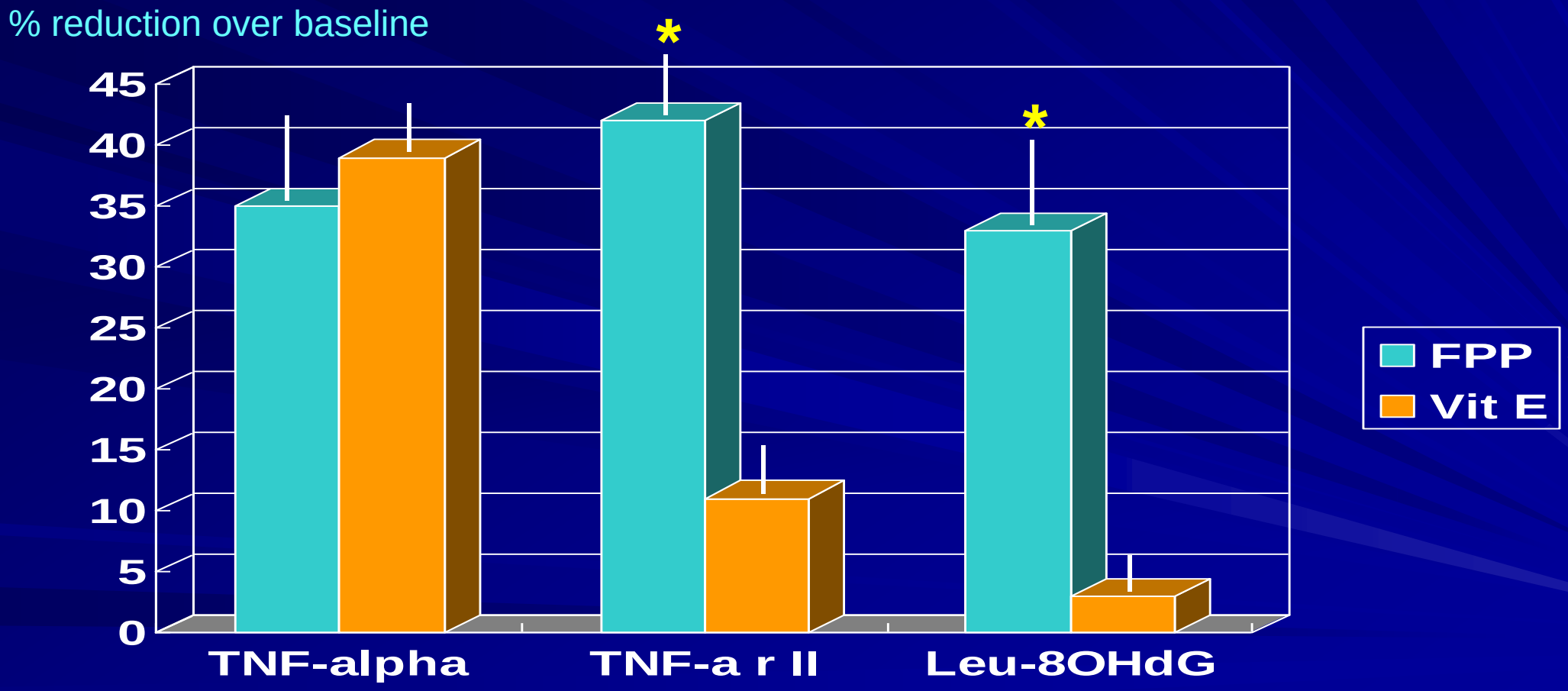
Oxidative stress and hepatitis C viral infection

Kazuhiko Koike*, Hideyuki Miyoshi

Hepatology Research 34 (2006)



Modulating leukocyte DNA damage and cytokines by nutraceuticals in HCV-CLD: a fermented papaya preparation vs vitamin E



Marotta et al. J Gastroenterol Hepatol 2006

Hepatoprotective effects of antioxidants in chronic hepatitis C

R Moreno-Otero, M Trapero-Marugán

World J Gastroenterol 2010

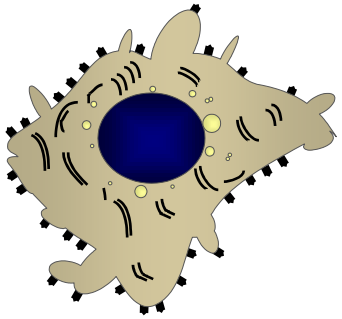
…….abundant evidence suggests that antioxidants can *effectively attenuate the oxidative and nitrosative stress in liver injury*, ultimately improving inflammation and fibrosis progression.

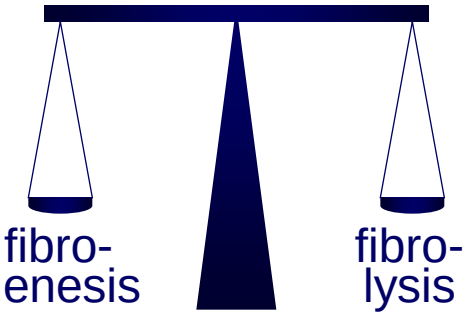
It is worth testing these drugs in future clinical trials including CHC patients, mainly those who present negative predictive factors of sustained virological response to standard antiviral regiments

But not any antioxidant naively !

Nakamura M et al.. An antioxidant resveratrol significantly *enhanced replication* of hepatitis C virus. *World J Gastroenterol* 2010

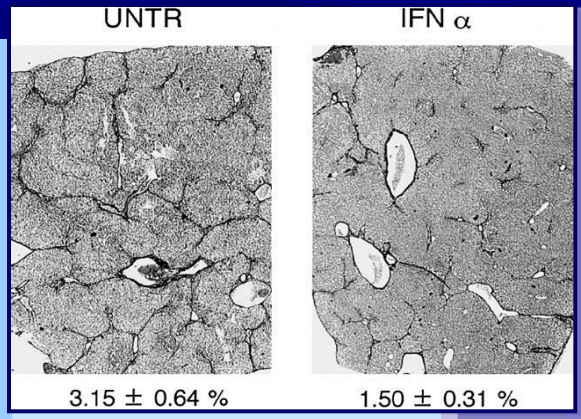
Regression of fibrosis and cirrhosis





- IFN alpha is a potent inhibitor of experi-mental fibrosis

  Inaki Y et al., Hepatology 2003



- IFN reverses fibrosis in clinical studies

  Poynard T et al., Gastroenterology 2002

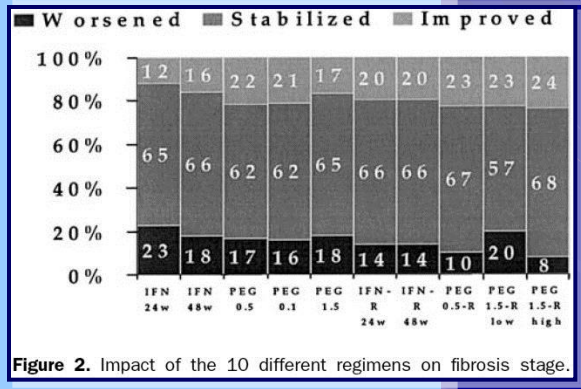


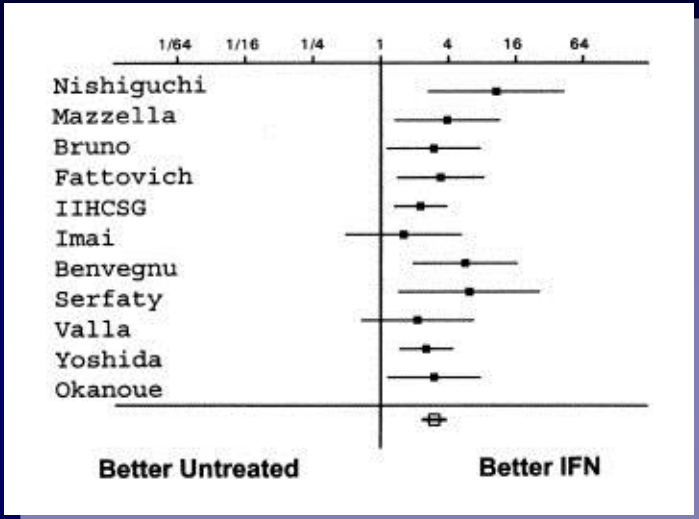
Figure 2. Impact of the 10 different regimens on fibrosis stage.

- Whether structural changes of cirrhosis are reversibel is still unclear

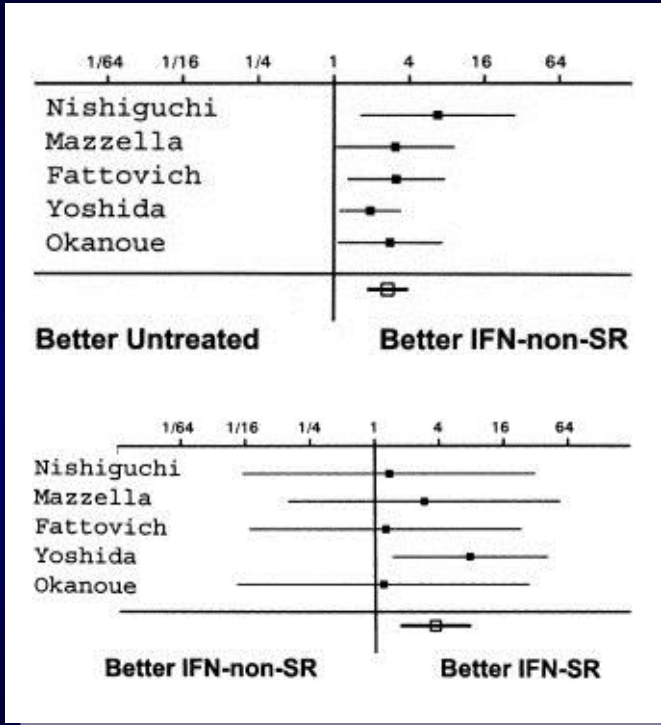
  Desmet VJ et al., J Hepatol 2004

Interferon alpha decreases development of HCC in patients with hepatitis C

Meta-analysis,11 studies, 2178 patients



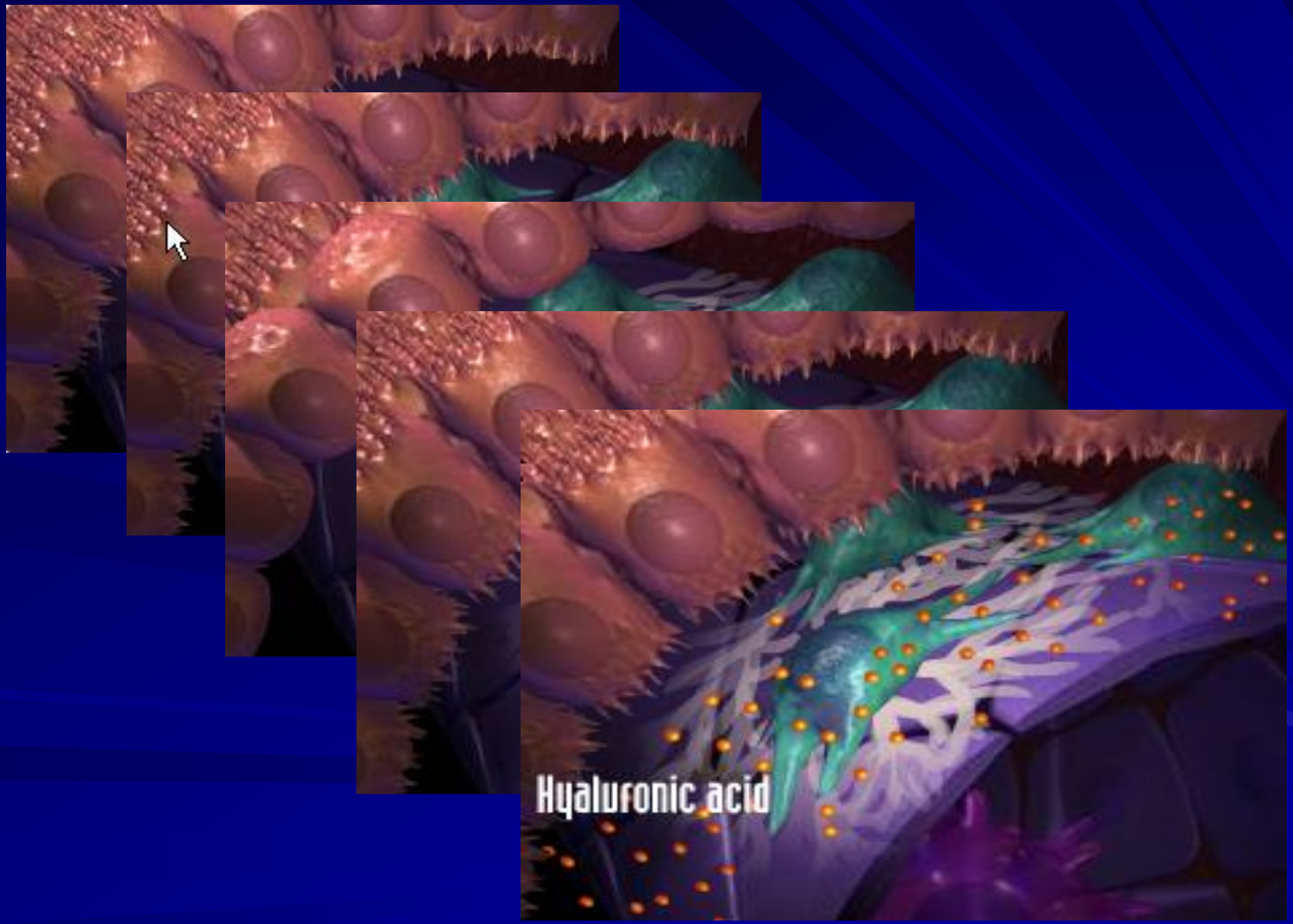
HCC: 21% → 8%
Odds ratio 3.0



HCC: 22% → 9%
Odds ratio 2.7

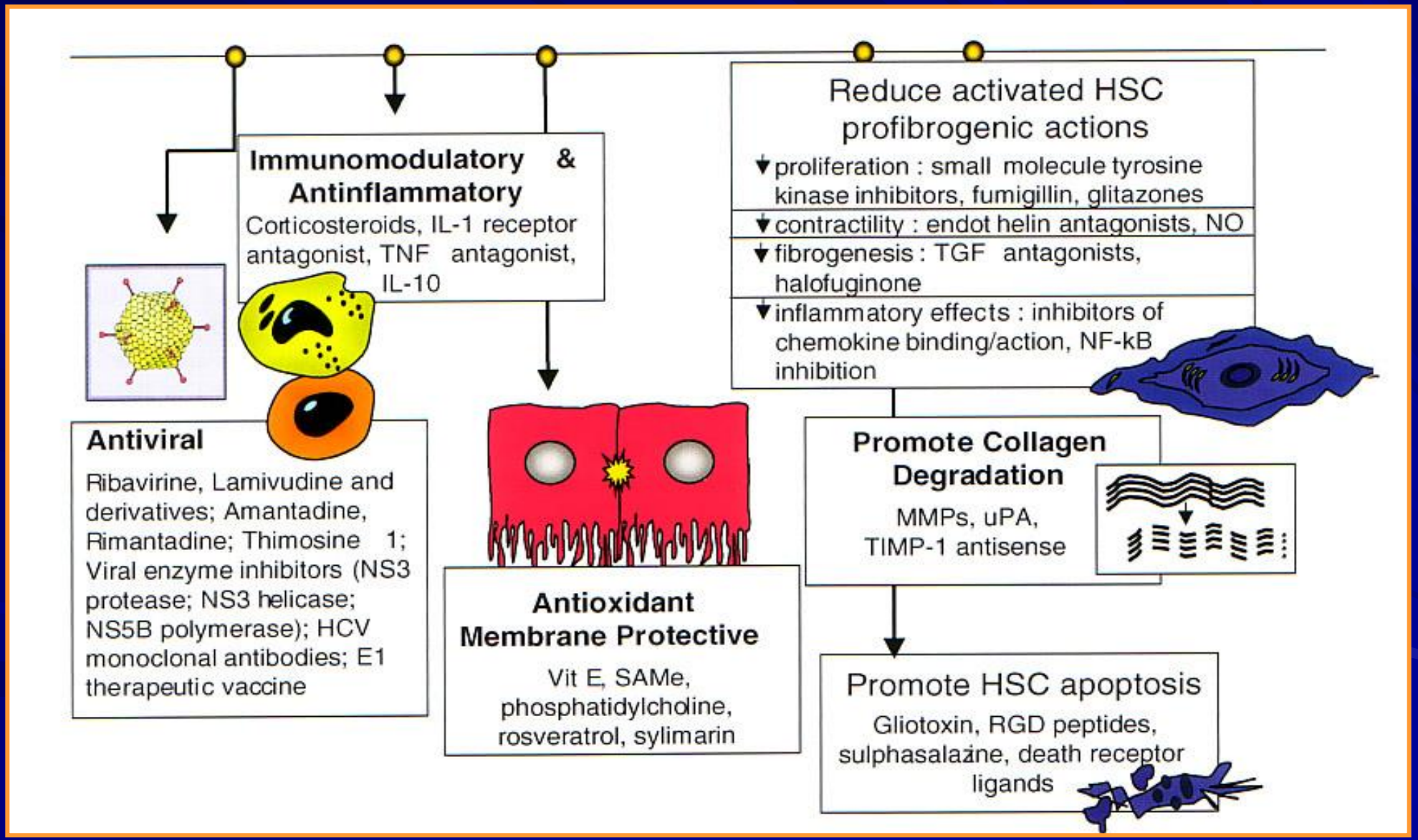
HCC: 9% → 1%
Odds ratio 3.7

Papatheodoridis GV et al., Aliment Pharmacol Ther 2001


Hyaluronic acid

Therapies for hepatic fibrosis: real hope or just academic exercise?

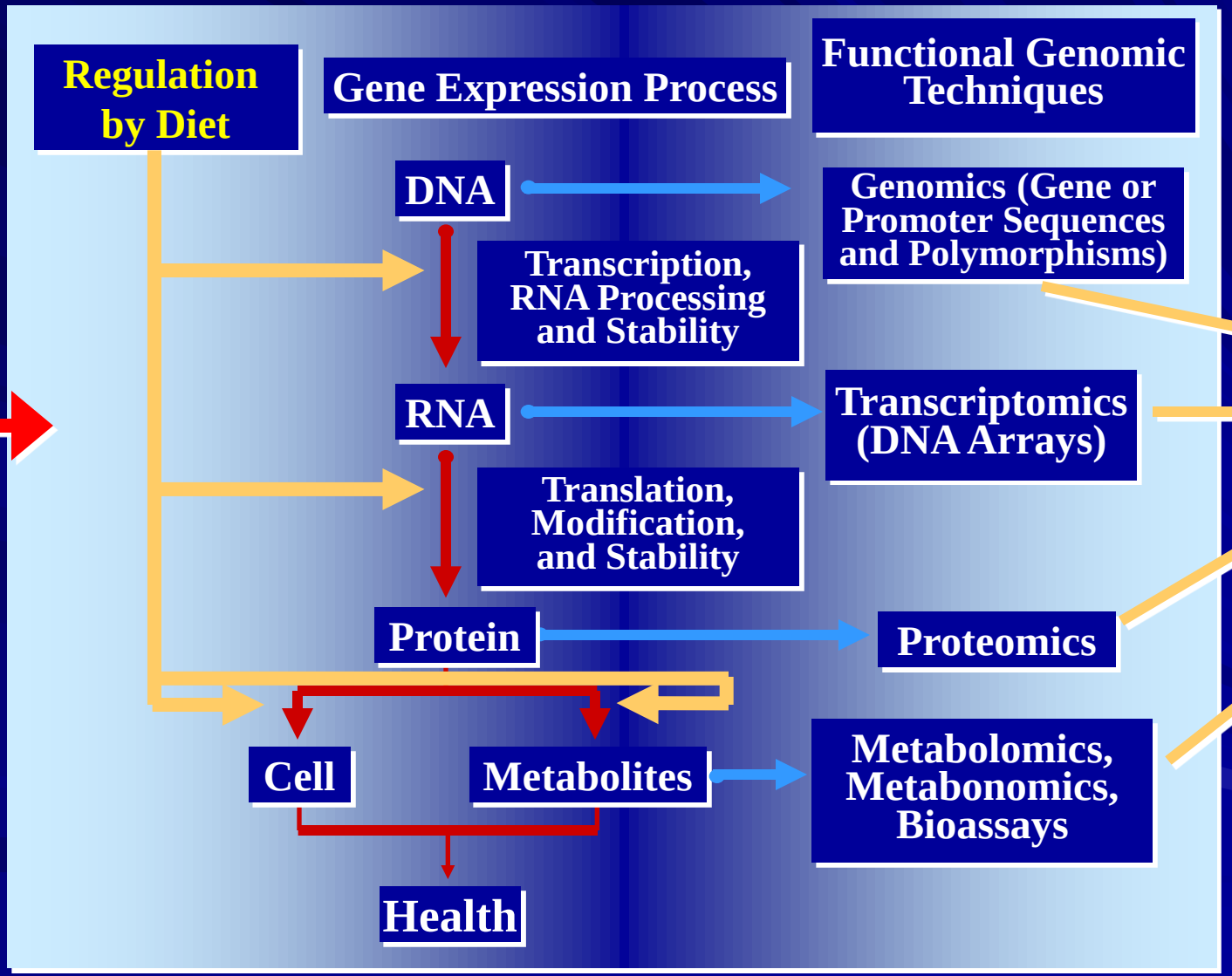
(Pinzani 2004)



Nutritional Genomics And Biomarker Discovery

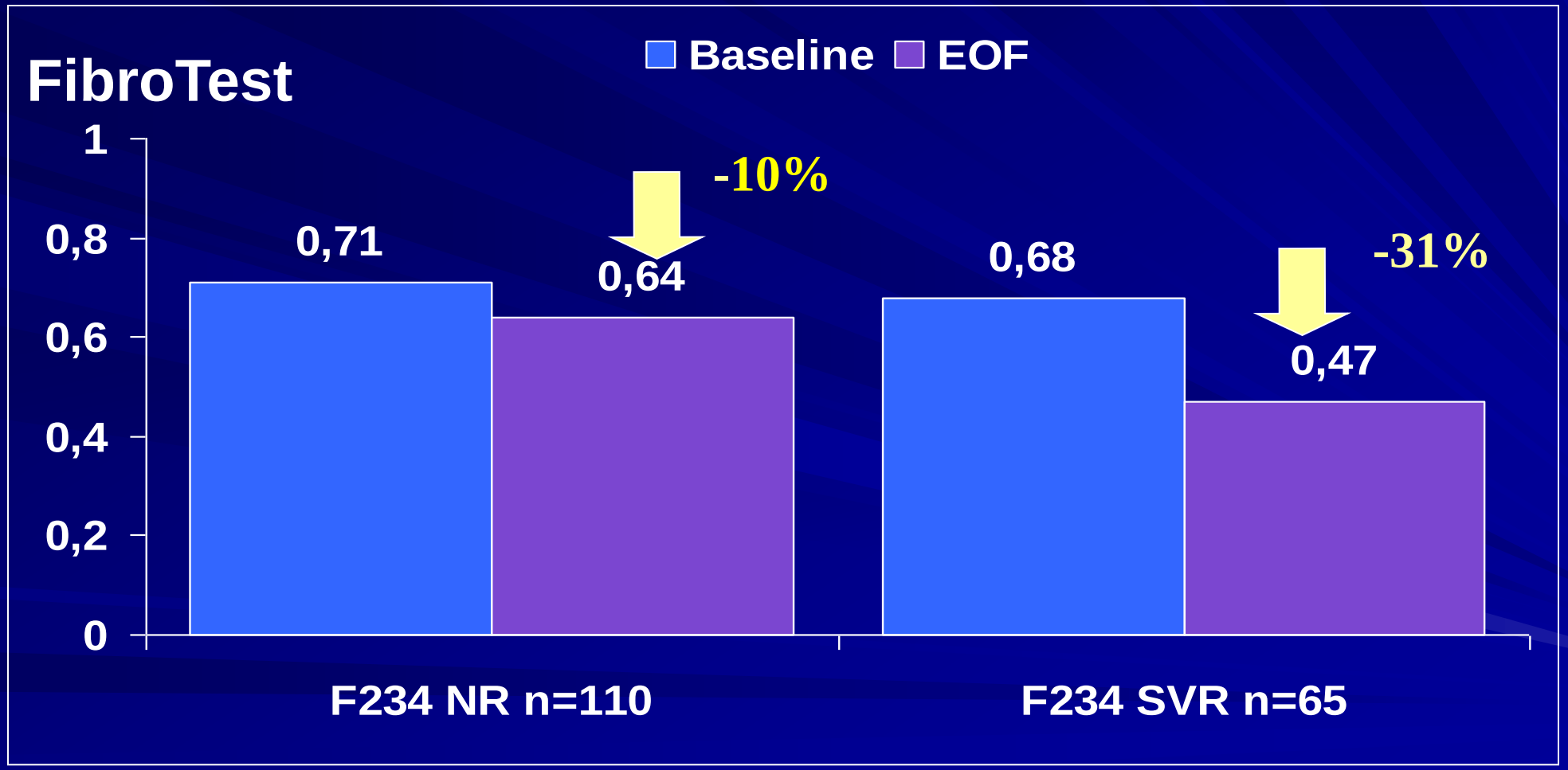
Nutrients to Modify Disease Risk

- Vitamins A
- Vitamin D
- Vitamin E
- Vitamin C
- Folic Acid
- Calcium
- Selenium
- Lycopene
- Resveratrol
- Tea Polyphenols
- Curcumin
- Genestein
- Sulforaphane
- Macronutrients
  - Carbohydrates
  - Fat
  - Protein
- Fiber & Water



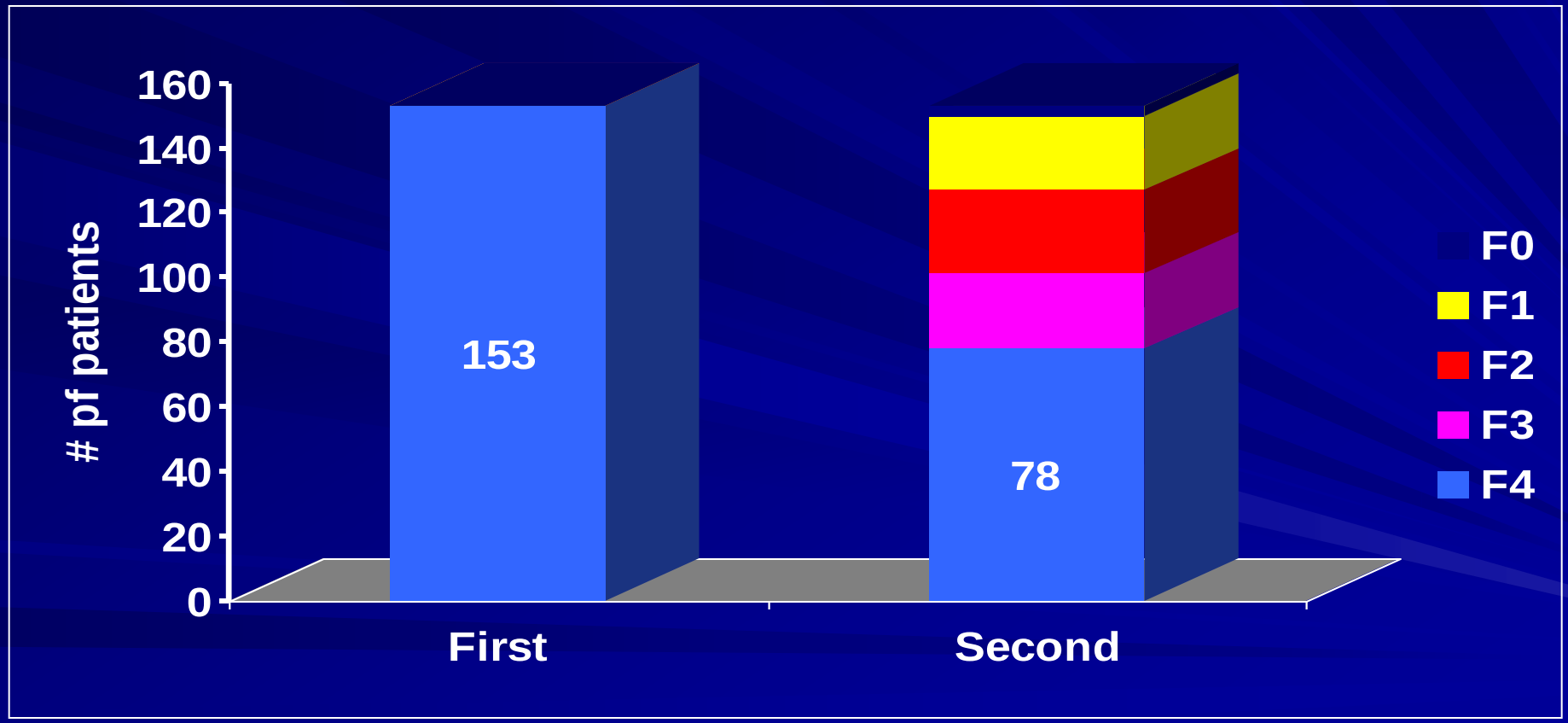
BMJ 2002; 324:1438.

FibroTest: Estimates Anti-Fibrotic Impact



Poynard et al Hepatology, 2003

Reversal of cirrhosis in 75 (49%) of patients

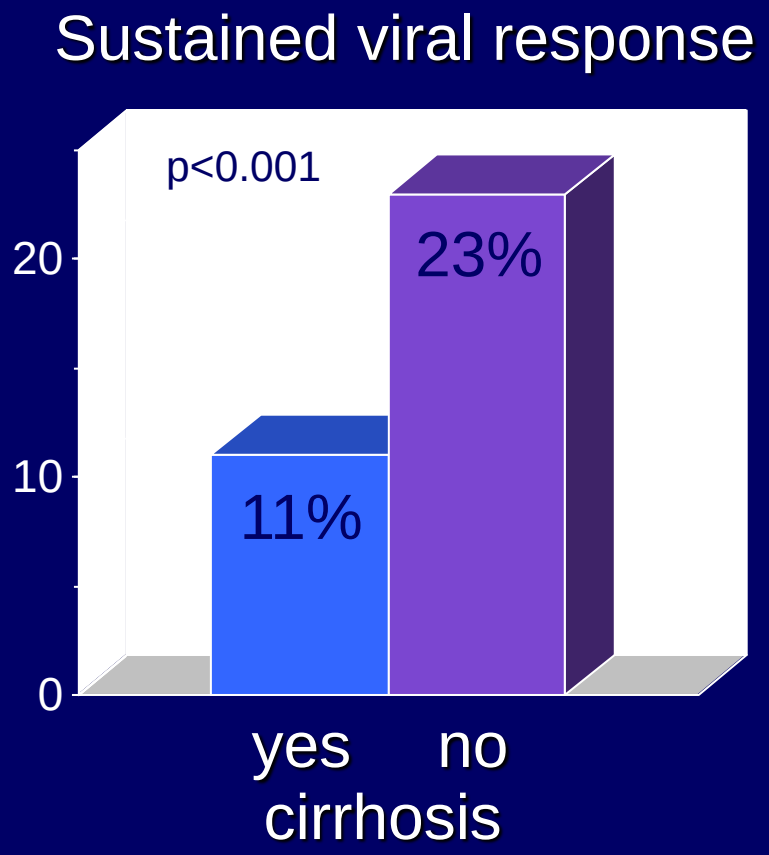


Poynard et al Gastroenterology 2002, Pol et al Hum Pathol 2004, Camma et al Hepatology 2004

Peginterferon Alfa-2a and Ribavirin in Patients With Chronic Hepatitis C Who Have Failed Prior Treatment

HALT-C trial

- Multicenter, 604 patients
- 233 cirrhosis, 371 bridging fibrosis
- Peginterferon alfa-2a + ribavirin 20 + 28 weeks
- **Cirrhosis is a negative predictor of therapy response**



Shiffman ML et al., Gastroenterology 2004

TABLE 1. Candidate Compounds With Possible Efficacy in Liver Diseases

Compound	Putative Biological Mechanism*	Targeted Liver Disease†
Silymarin (milk thistle)[17,18,24,27,28]	Biologically active compound—silibinin Acts as an antioxidant and free radical scavenger In animals, prevents glutathione depletion free radical formation in the liver May also be antifibrotic through undeterminate mechanism(s)	Cirrhosis In Europe—chronic liver disease, digestive disorders, and gallbladder disease
Glycyrrhizin[29,30,31,35]	Licorice root—multiple constituents appears to inhibit enzyme 11-beta-hydroxysteroid dehydrogenase, thus anti-inflammatory in inhibiting prostaglandin production and modifies arachodonic acid metabolism Also antioxidant properties—induces glutathione-S-transferase and catalase	Used traditionally for cough, bronchitis, gastritis, liver inflammation Fibrosis
Plantago asiatica seed[39,40]	Aucubin—active ingredient, iridoid glycoside Transient inhibition of viral replication	Hepatitis B virus
Herbal Medicine 861[40,42]	Herbal mixture, blocks stellate cell activation through inhibiting cell cycle progression	Fibrotic liver disease
TJ-9 (Sho-saiko-to)[44-48]	Herbal mixture, blocks stellate cell activation Inhibits lipid peroxidation in hepatocytes and stellate cells	Fibrotic liver disease In Japan, recommended for hepatitis B virus
TJ-41[50,51]	Herbal mixture, induces cellular apoptosis via P 53.	Hepatocellular carcinoma
TJ-108[51]	Herbal mixture with active compound gomisin A. Antiviral	Hepatitis C virus
Liv-52[52]	Herbal mixture—hepatoprotective	In India, alcohol-induced liver disease
Phyllanthus amarus[54,55]	Extract inhibits hepatitis B viral polymerase by inhibiting the virus enhancer I activity—complexes transcription factors	Hepatitis B virus

some Clinical studies

Plantago

1997 10mg/kg/day i.v. x 4-month: 10-40% ↓HBV-DNA;

Compound 861

1995 2-years, CHB: 83% subj. improv., ↓41% spleen size,
↓AST,ALT (73% to normal range), PIIINP;
1998 6-month, CHB: histological improvement (infl. & fibrosis);

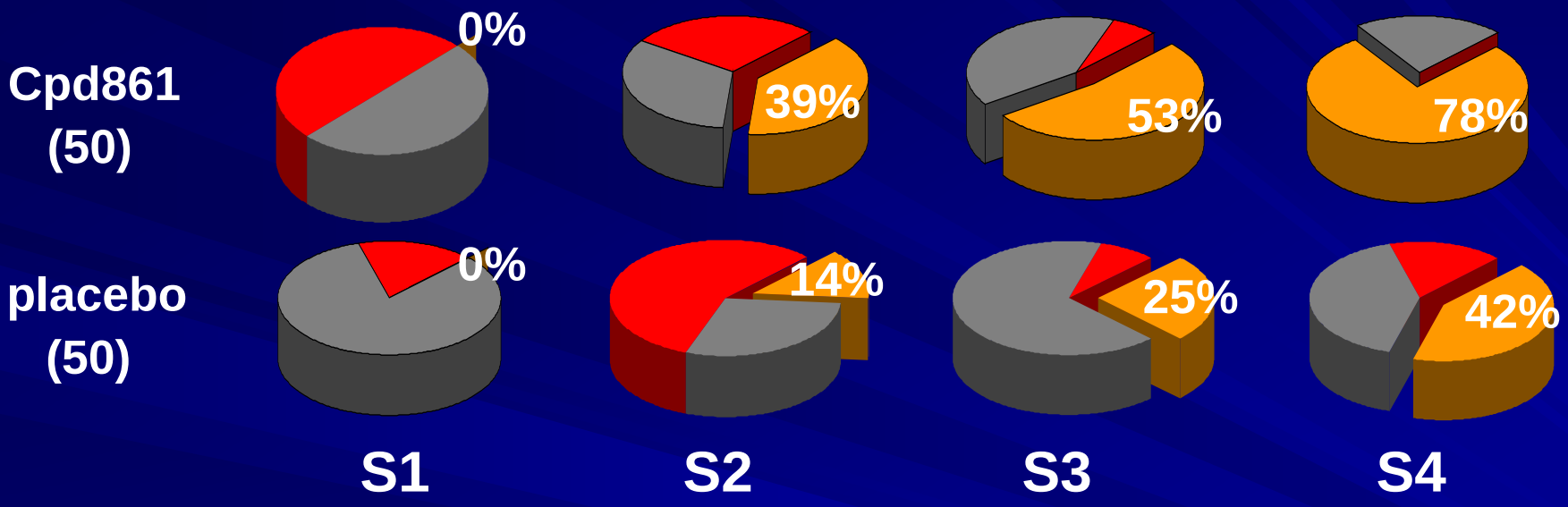
CH-100 1998 RCT - HCV pts: significant ALT reduction;

TJ-9 1995 5-year study, 260 cirrhotics, ↑survival, ↓HCC;
TJ-108 2000 ↓HCV-RNA in 21% HCV +ve patients;

YHK/K-17.22 1998-2004 HCV pts.: ↓ALT;

Compound 861 in HBV CLD

Salviae miltiorrhizae (丹参): Reversal rate



reversed: score↓ >2

worse: score↑ >2

no change: score <2

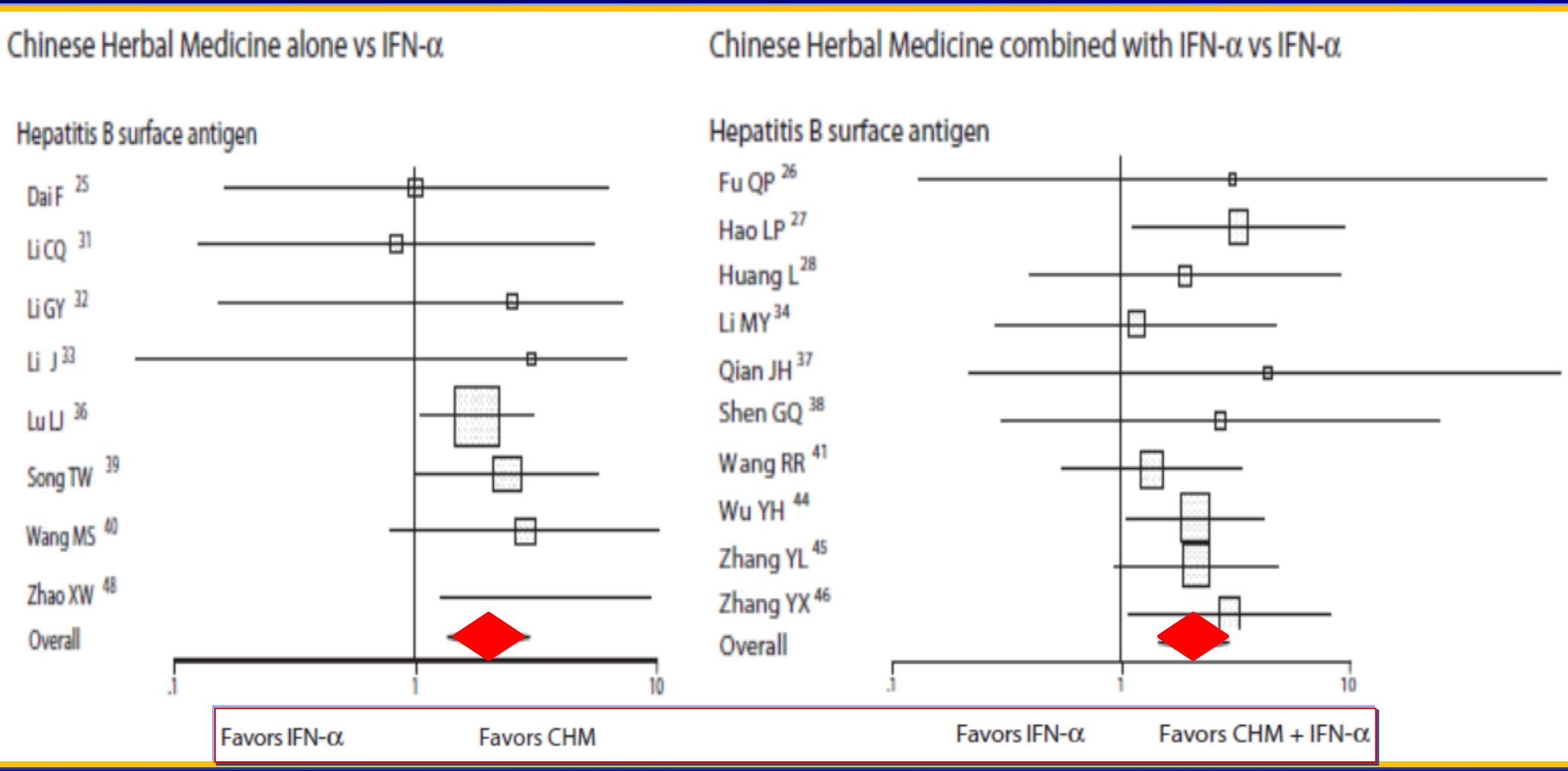
	861	placebo
	Reverse(%)	Reverse(%)
s3+s4	66*	33*
total	52	20

*p<0.05

Liu EY et al. Chin J Hepatol 1993
Shanti Wasser et al. J Hepatol,1998

Chinese Herbal Medicine and Interferon in the Treatment of Chronic Hepatitis B: A Meta-Analysis of Randomized, Controlled Trials

Am J Publ Health, 2002



Traditional Chinese medicine causing hepatotoxicity in patients with chronic hepatitis B infection: a 1-year prospective study

Aliment Pharmacol Ther, 2006

Traditional Chinese medicine-related hepatotoxicity resulted in **high mortality** in chronic hepatitis B patients.

Prospective RC trials with the same stringent criteria as western medicine clinical trials are required for Chinese medicines, to document their efficacies and safety before they can be advocated for the treatment of patients.

Funded by a grant from the Hepatology Research Fund, The University of Hong Kong

Glycyrrhizin

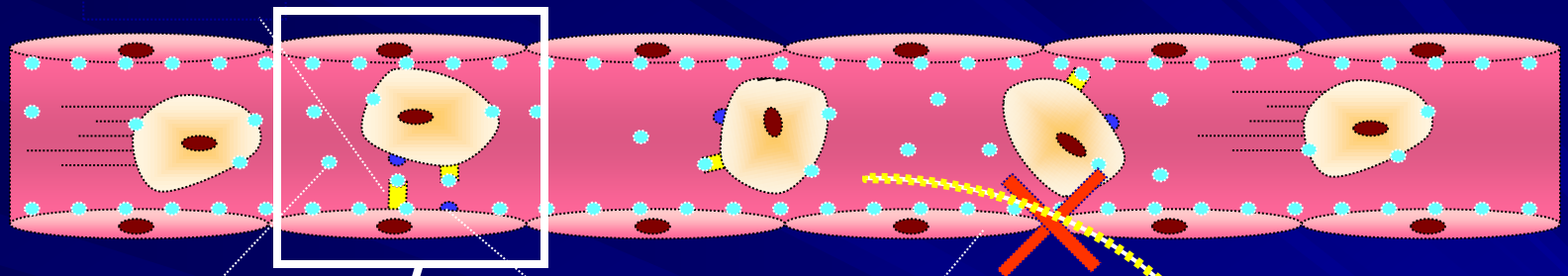
1991 4-wks Gly + 4 wks IFN: 70% loss of HbeAg after 6 months;

1994 Gly + IFN vs IFN: 33% vs 13% HCV-RNA negativization;

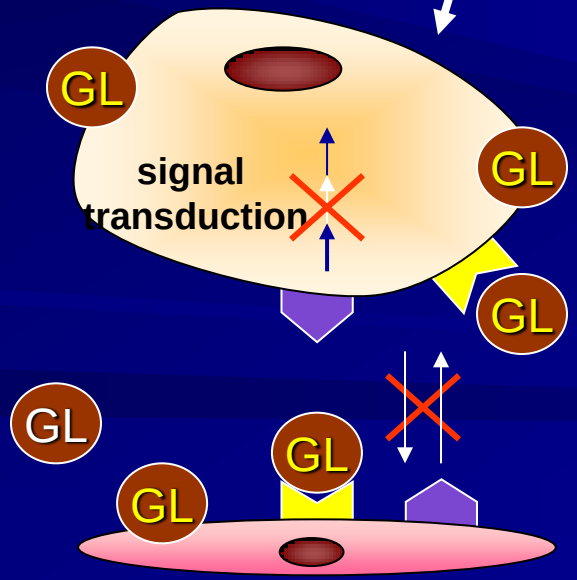
1997 80mg x 2 weeks → AST, ALT in >60% of CAH pts;

1997 2-7/weekly i.v. Gly x 10 years: 2.5-fold decrease of HCC and 1.5-fold decrease of cirrhosis;

Mechanism of Pharmacological Action of Glycyrrhizin (GL)



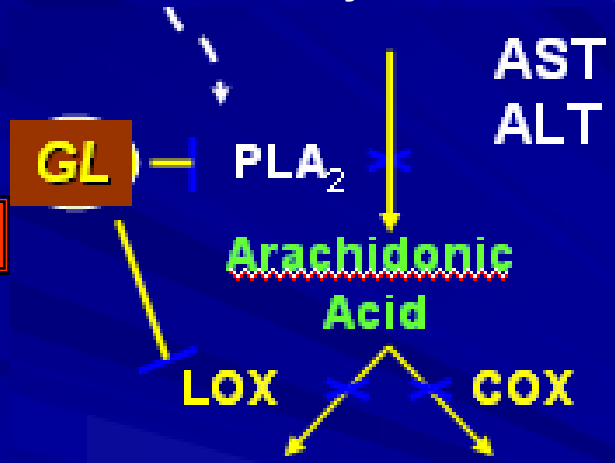
Glycyrrhizin binds to selectin



SLe^X
selectin

Inhibition of Infiltration and Chemotaxis of Leukocytes

Anti-inflammation



Rao BN. et al., JBC 269, 19663-19666, 1994

Inflammation ↓

Chronic Hepatitis C Trial

Indian Council Medical Research

Multicenter Double-Blind Randomized controlled

130pts, HCV+ve, ALT >60IU, HAI >3

IFN + Ribavirin | IFN + **SNMC**

Genotype 3 – 1 – 4: 71%, 27%, 2%

median ALT: 100.5 IU → 38.0 IU p<0.0001

Sustained Virological Response: Genotype 1:100%, Gen. 3: 70%, Gen 4: 100%

Viral Load: Genotype 1: all -ve, Gen 3: Gen 4: -ve

HCV-RNA +ve: 25% - HCV-RNA -ve: 75%

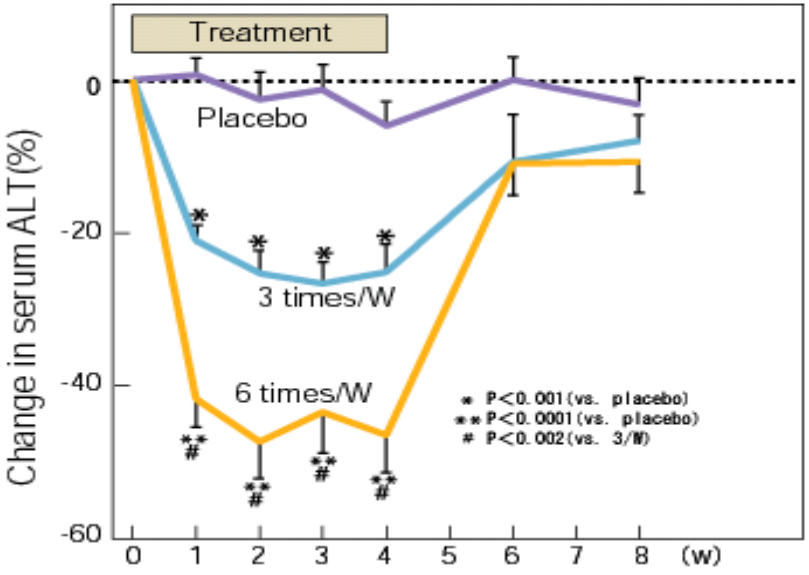
Therapeutic Effect of SNMC to IFN Non-responders in Patients with Chronic Hepatitis C

Van Rossum TGJ. et al.、Am. J. Gastroenterol.、2001

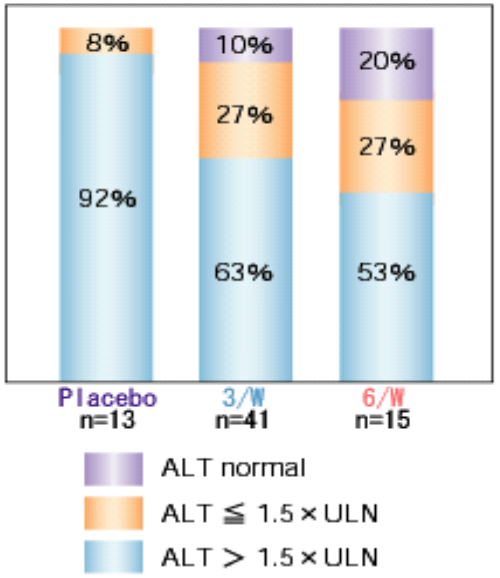
	Placebo	SNMC 3 times/W (40, 80, 120 mL)	SNMC 6 times/W (100 mL)
Number of patients	13	41	15
Male/Female	13/0	32/9	12/3
White/Other	8/5	23/18	11/4
Median age*(yr)(range)	47(37-60)	46(32-69)	49(39-70)
Noncirrhosis/Cirrhosis	7/6	24/17	7/8
Previous interferon(ribavirin)Yes/No	12/1	32/9	13/2
Median ALT ULN**(range)	3.1(1.5-6.8)	2.6(1.4-11.8)	3.0(1.6-12.5)
Median HCV-RNA Mgeneq#/mL(range)	4.5(1.4-39.2)	14.9(0.2-104)	14.1(0.7-76.3)
Genotype-1/Genotype non-1	7/6	20/21	7/8

*at start of treatment **upper limit of normal #Mega genome equivalent

The mean percentages ALT change from baseline



Distribution of ALT at the end of treatment



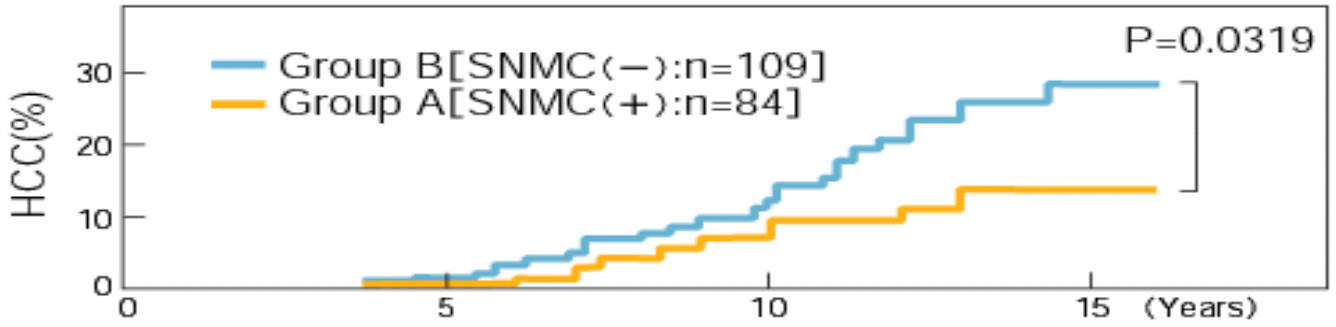
The Long-Term Efficacy of SNMC in Chronic Hepatitis C Patients

Y. Arase et al., Cancer, 1997

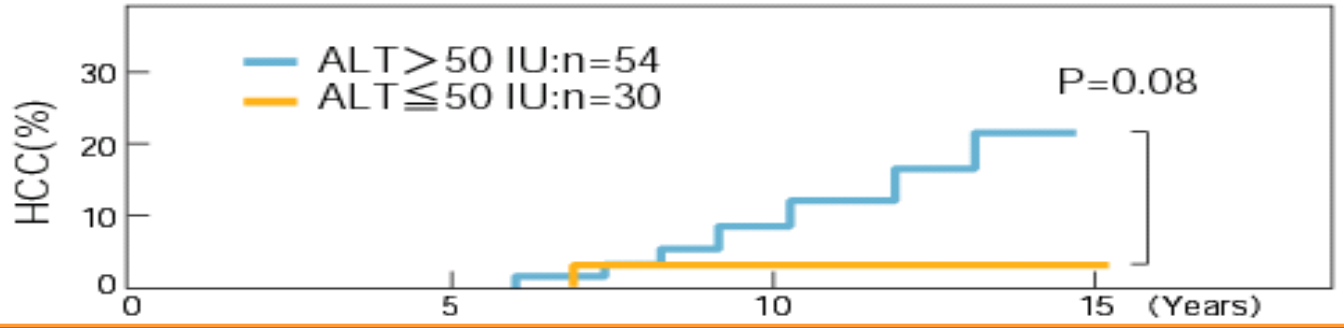
	SNMC(+)	SNMC(-)
Number	84	109
Age(years)[a]	47(31-64)	48(30-64)
Gender (male/female)	73/11	92/17
Transfusion(+/-)	39/45	48/61
Histology(F1/F2 or F3)	51/33	61/48
HCV genotype(1b2a or 2b)	60/16	62/21
ALT(IU/L)[a]	200(100-726)	186(104-698)
ICG R15(%)[a]	14(9-24)	15(8-26)

a: Data are expressed as the median value(range)

Cumulative HCC appearance rate with or without SNMC administration



Cumulative HCC appearance rate based on the average ALT after SNMC administration



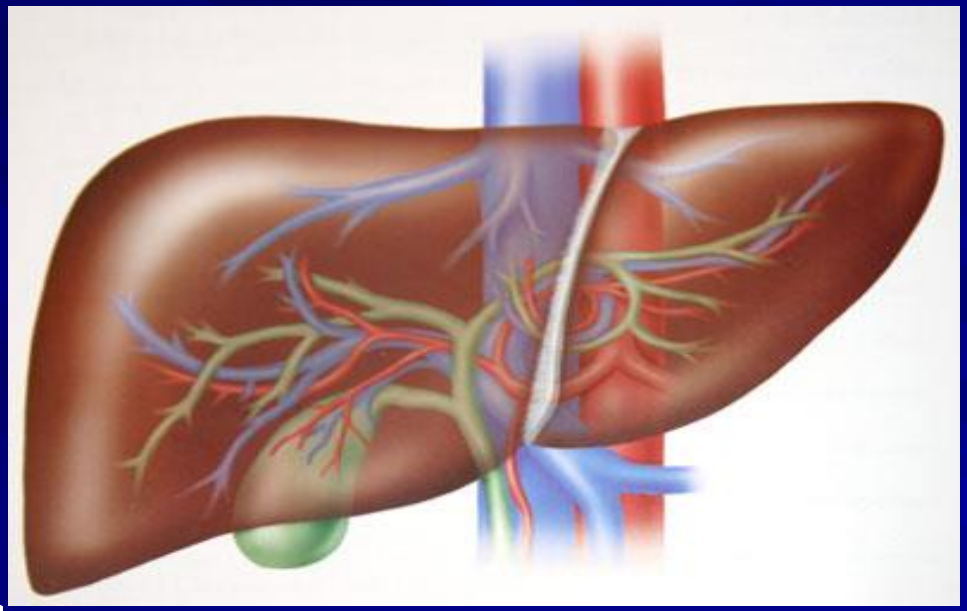
Silymarin

- Extract of crushed milk thistle seeds:
  - Milk Thistle:
    - Silymarin:
      - Extract from seeds of Milk Thistle
      - a complex of at least 7 flavonolignans and 1 flavonoid that comprise 65-80% of milk thistle extract
- Prevents liver disease in many experimental animal models
- Used widely by HCV patients as a hepatoprotectant
- Clinical studies indicate that Silymarin is very well tolerated and safe

Hepatoprotection

Antiviral

Antiinflammatory

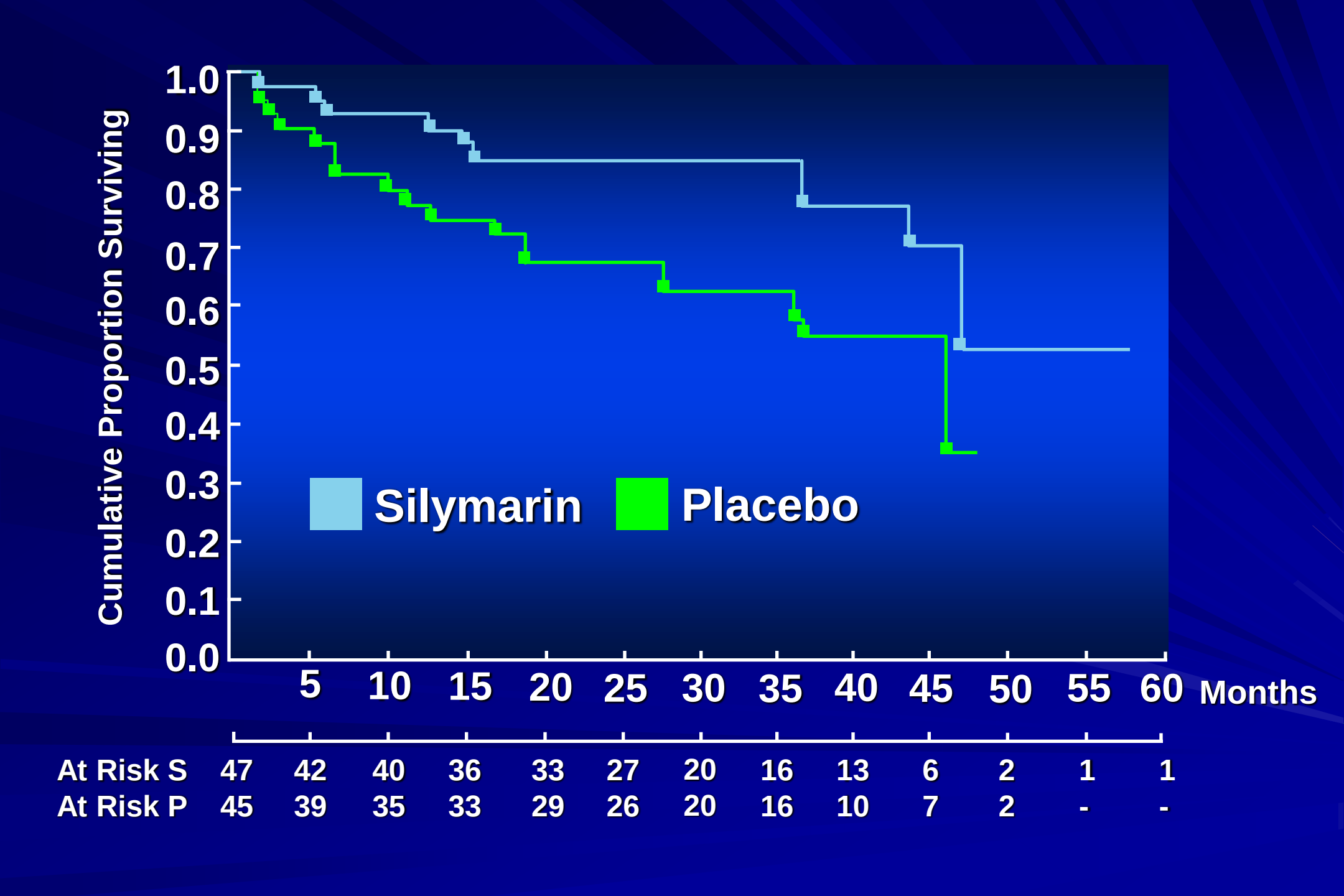


Antioxidant

Immunomodulatory

Milk Thistle (Silymarin)

- Choose a brand that has silibin and phosphotidyl choline
  - Better absorbed
- Typical dose 140-420 mg per day in divided doses of 2-3 times per day of 70-80% silymarin
- Large doses can cause loose stools


Cumulative Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Silymarin
Placebo
5
10
15
20
25
30
35
40
45
50
55
60
Months
At Risk S 47 42 40 36 33 27 20 16 13 6 2 1 1
At Risk P 45 39 35 33 29 26 20 16 10 7 2 - -

Silymarin

1978 expedites recovery after acute A or B hepatitis;
1980 expedites recovery in alcohol-related hepatitis;
1982 2-fold decrease of death rate due to Amanita intoxication;
1989 41 months follow-up: higher survival in cirrhotics;
1998 previous data not confirmed !

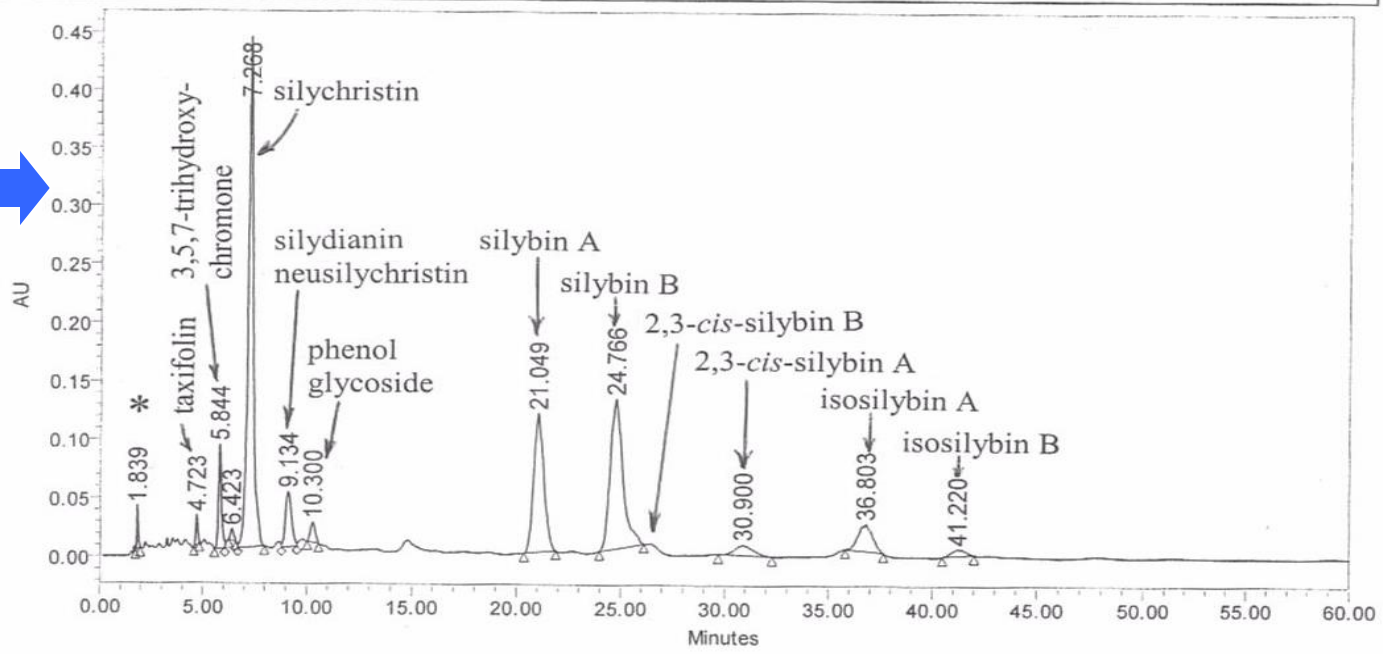
…….lack of reliable formulations, erratic pharmacokinetics

Molecular Profile of Silymarin

Silybum marianum seeds

SAMPLE INFORMATION

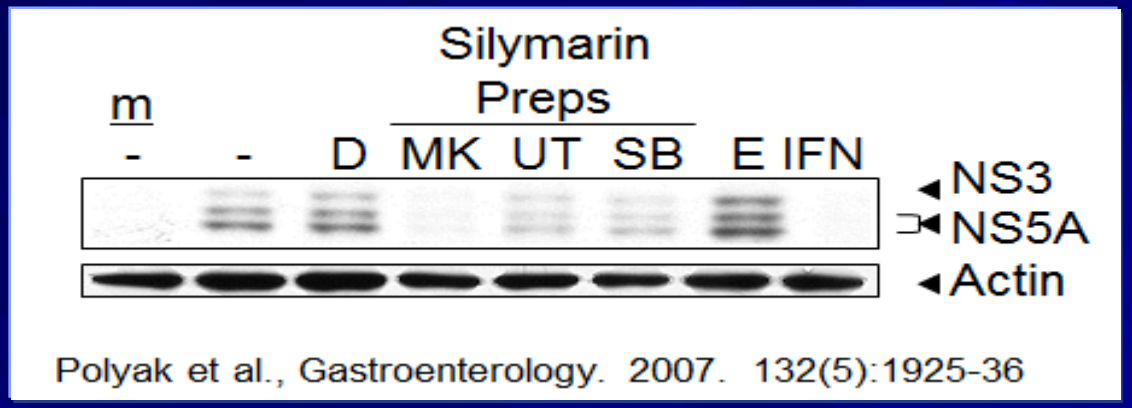
Sample Name:	**Standardized Silymarin (MK-001)**	Acquired By:	System
Sample Type:		Date Acquired:	3/11/04 11:34:10 AM
Vial:	1	Acq. Method:	Liu_Silymarin_4555
Injection #:	31	Date Processed:	3/11/04 2:37:33 PM
Injection Volume:	5.00 ul	Channel Name:	2487Channel 1
Run Time:	60.00 Minutes	Sample Set Name:	



HPLC Fingerprint of Standardized Milk Thistle Product (MK-001)

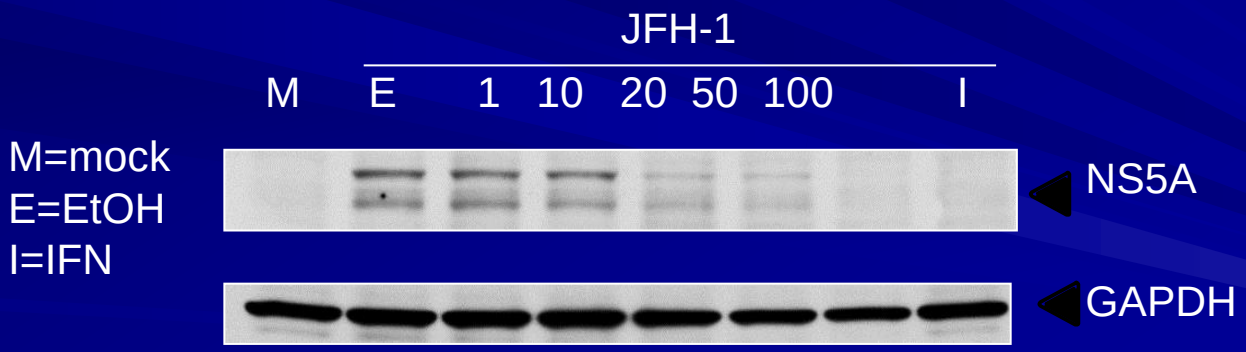
Silymarin Inhibits HCV Infection

HCVcc, (m.o.i. 0.01)



Polyak et al., Gastroenterology. 2007. 132(5):1925-36

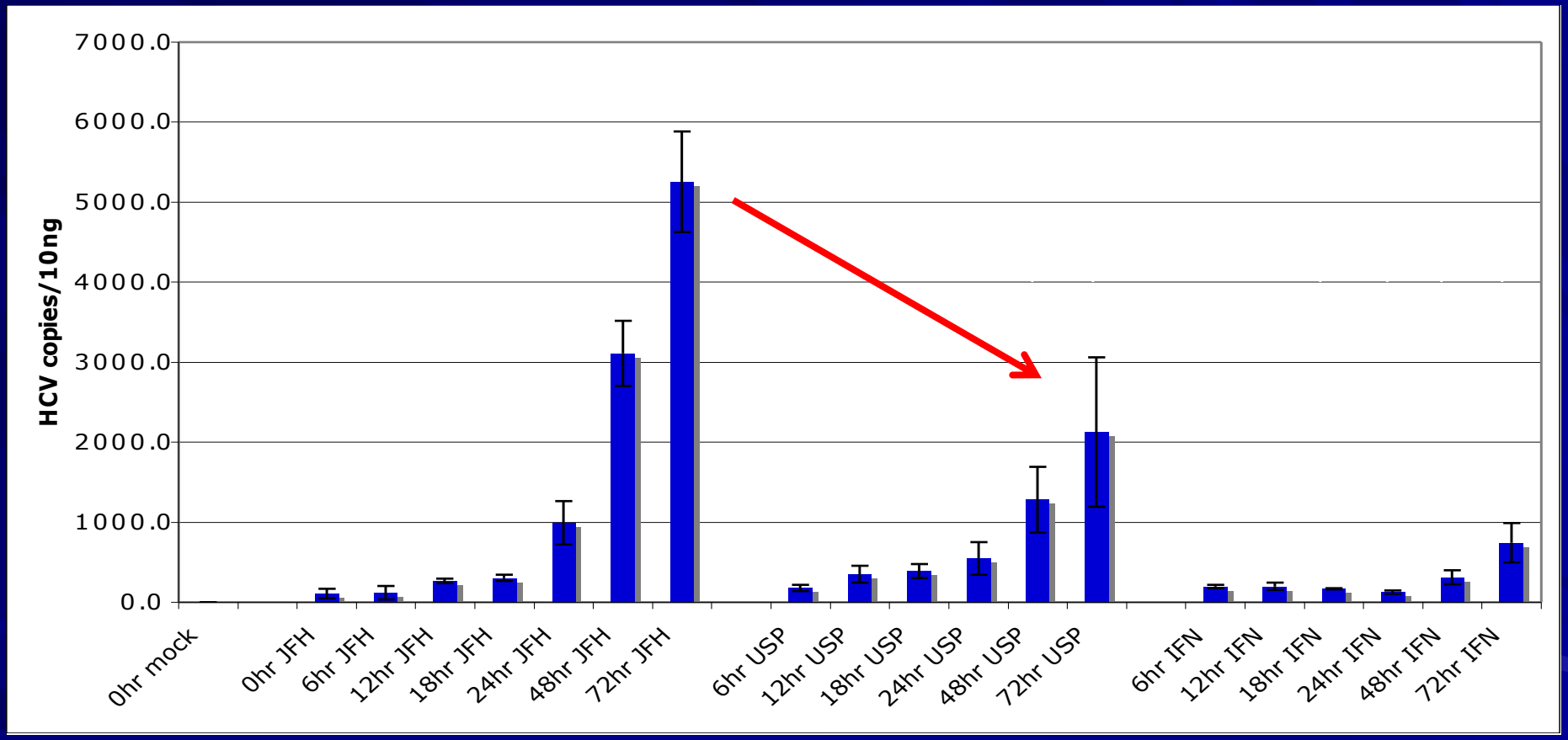
Therapeutic Design



US Pharmacopoeia Milk Thistle

HCV RNA Synthesis

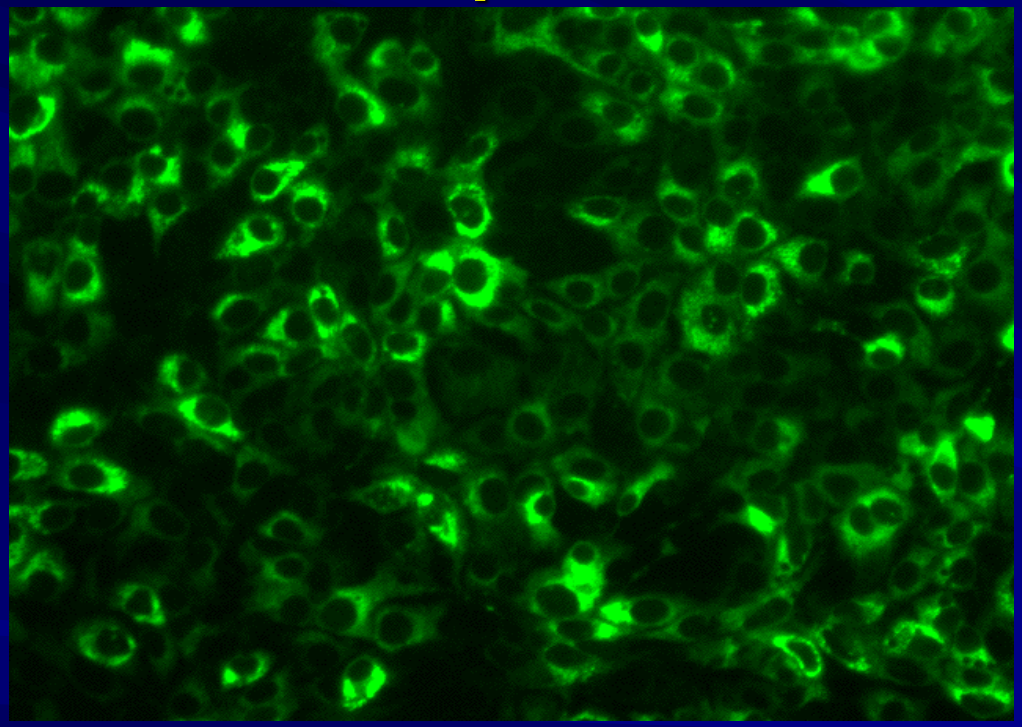
HCVcc,
(m.o.i. 0.01)



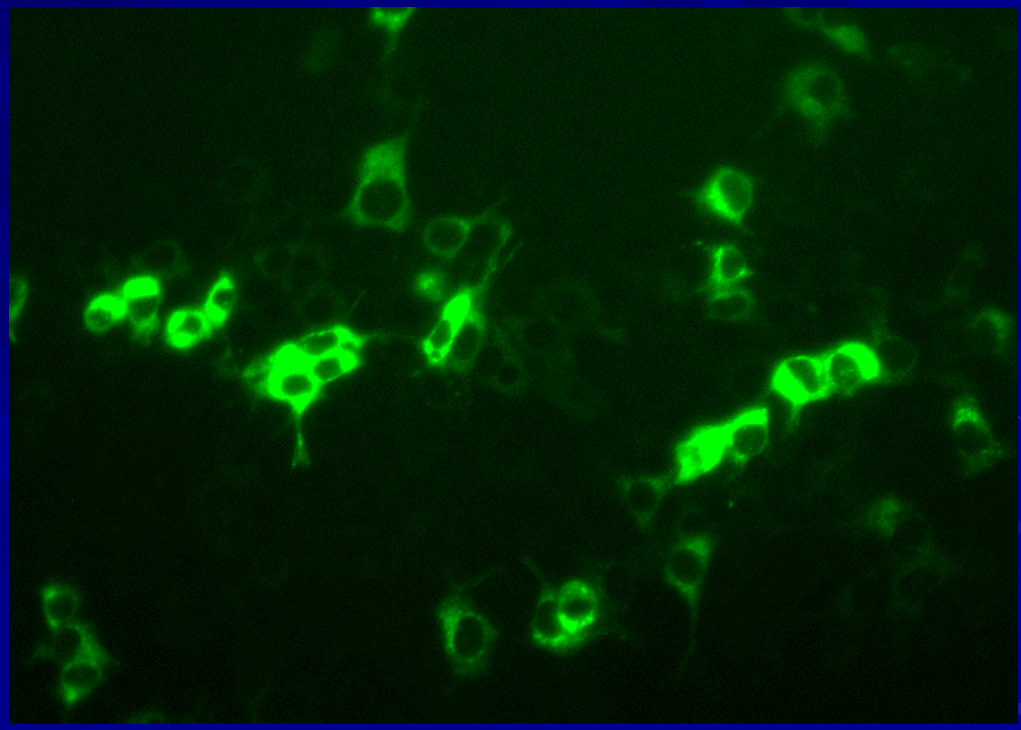
Therapeutic Design

Infectious Virus Release

Supes From 48 Hours Post-Treatment



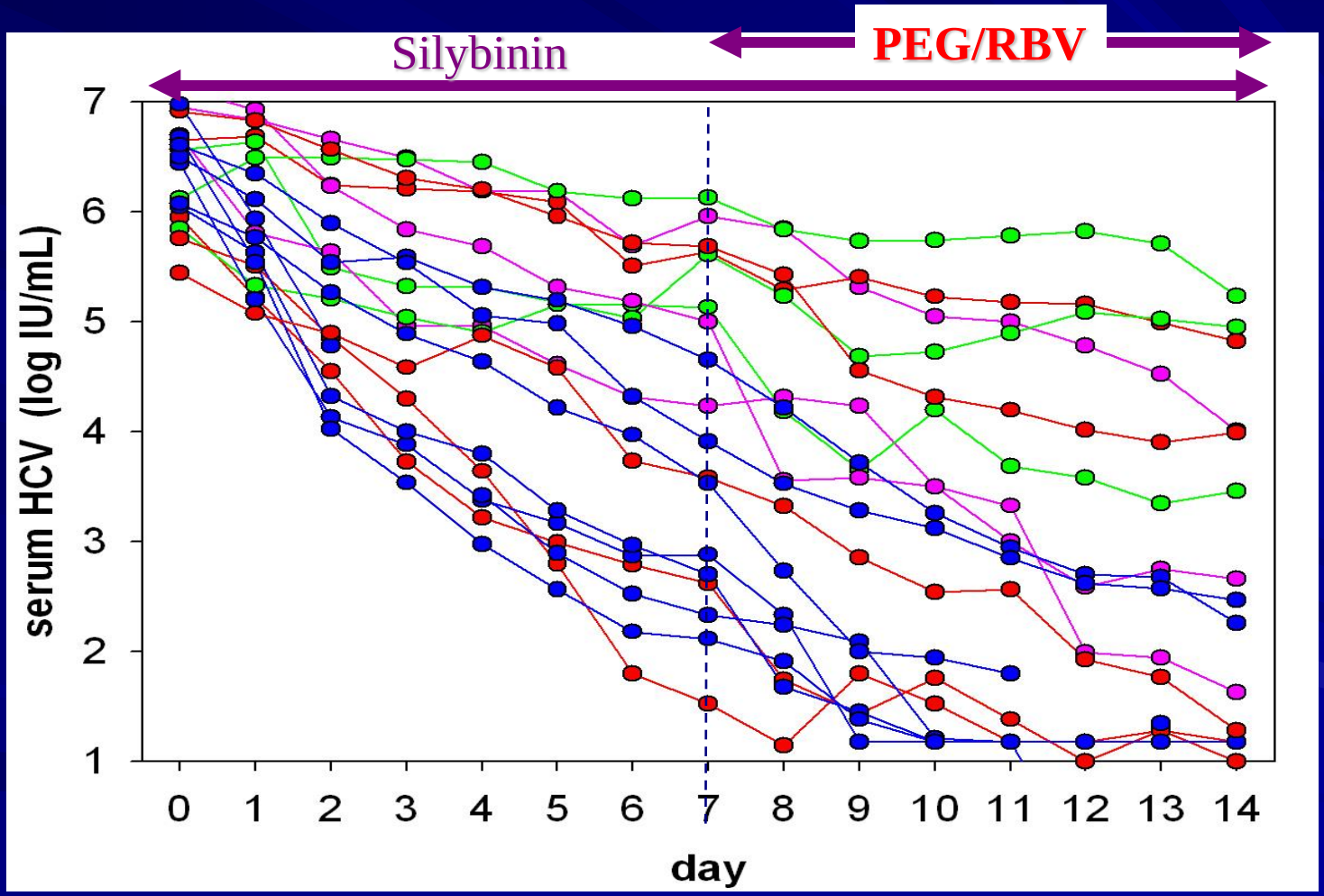
DMSO



Silymarin

Huh7.5.1 & Huh7

Intravenous Silymarin Reduces Viral Loads in IFN Nonresponders

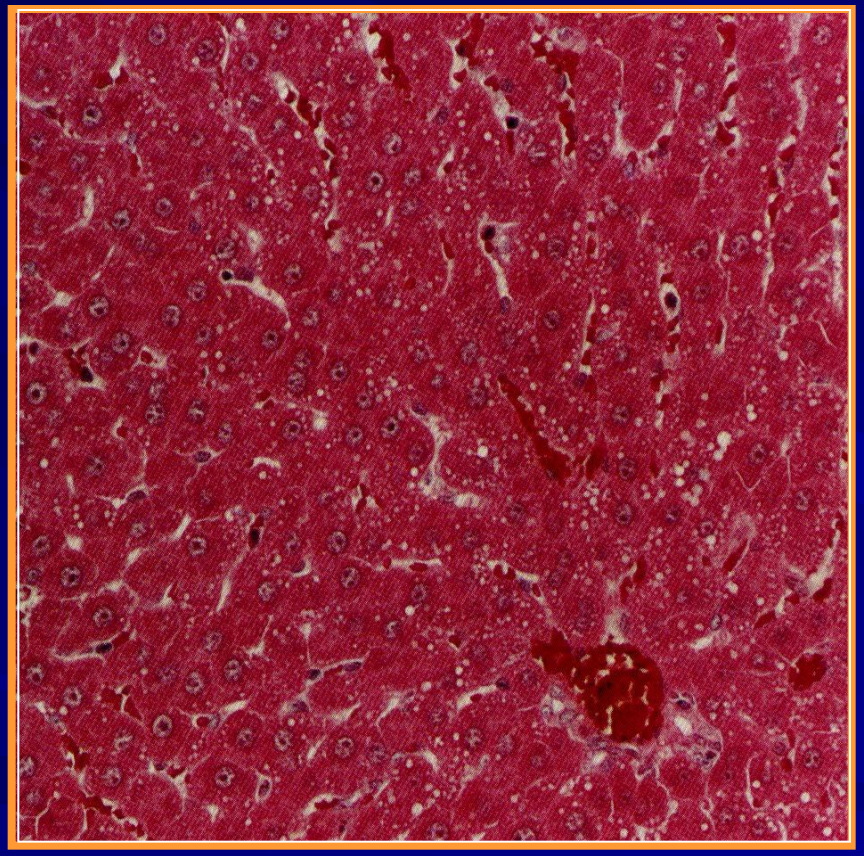


Silibinin
5 mg/kg
10 mg/kg
15 mg/kg
20 mg/kg

Ferenci et al., Gastroenterology 2008

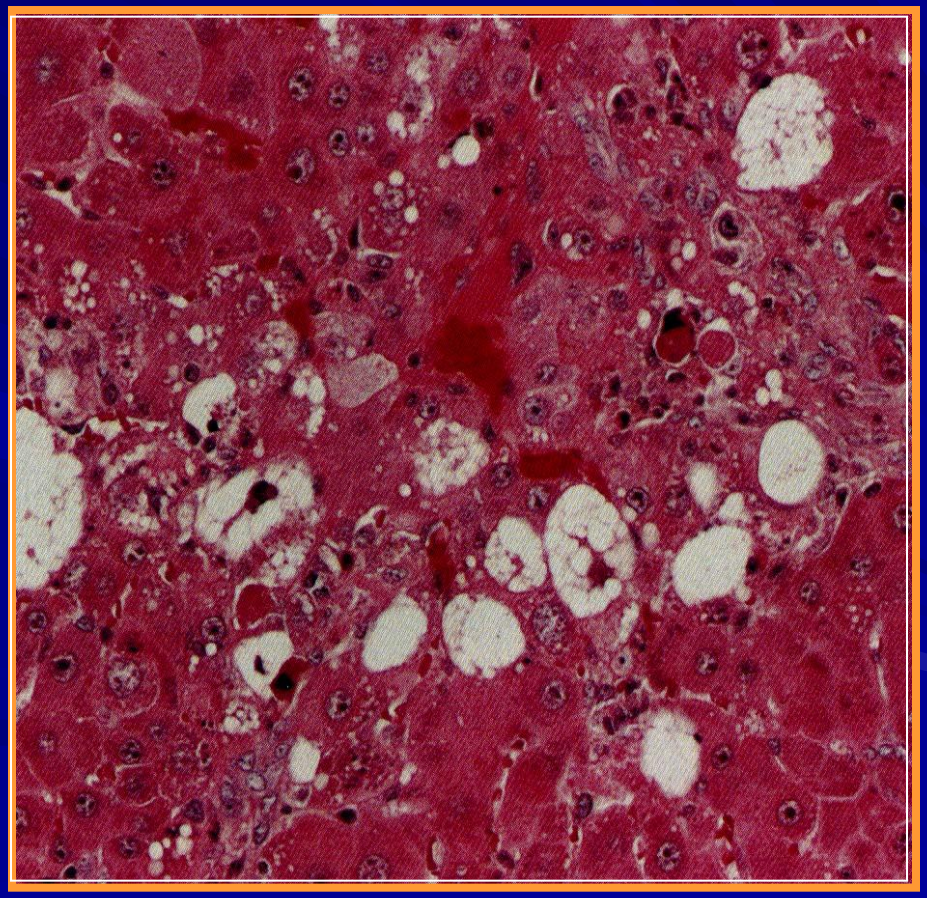
A novel ISO-controlled nutraceutical: YHK

CCL_4 Model



YHK-Treated

Untreated

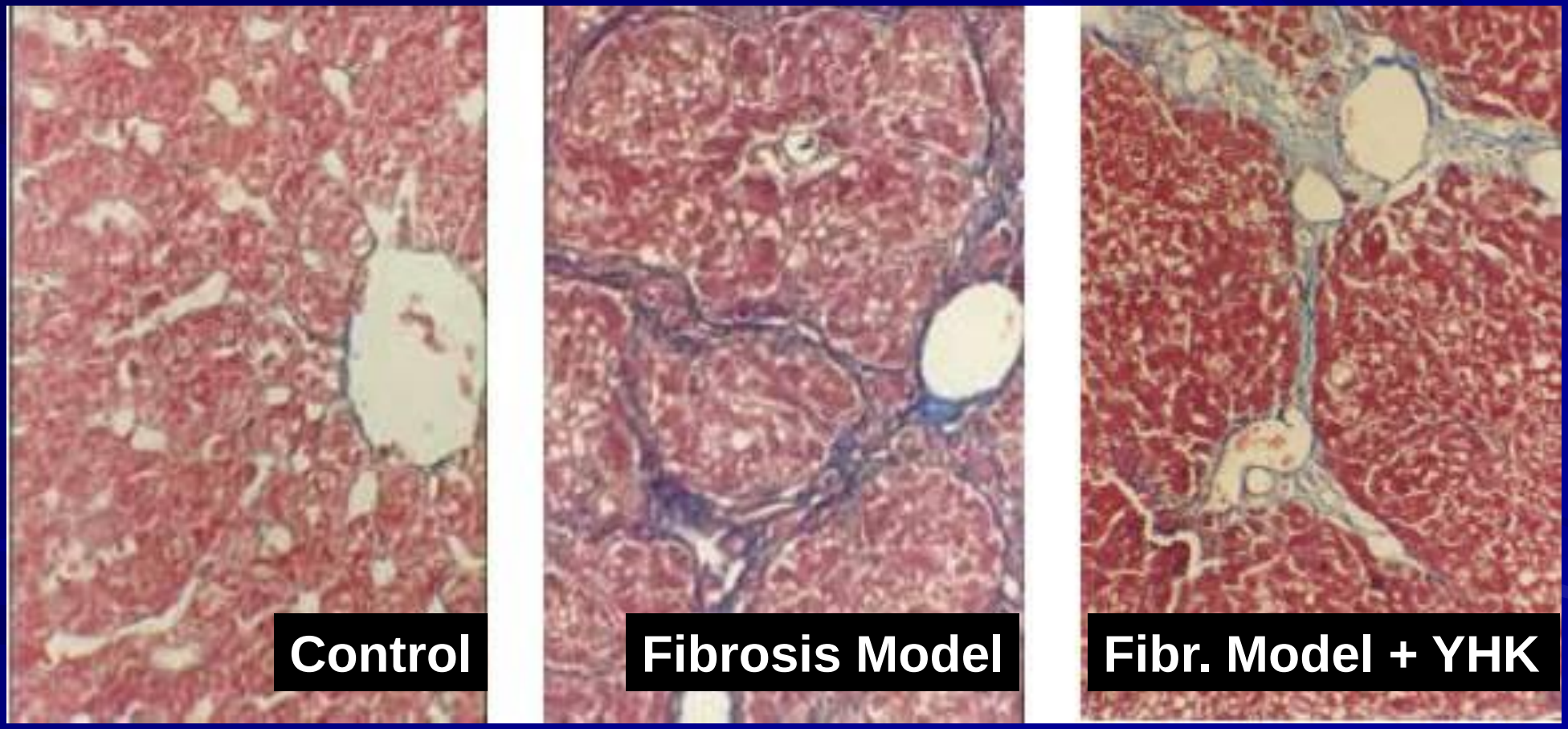


Hydroxyproline content of the liver

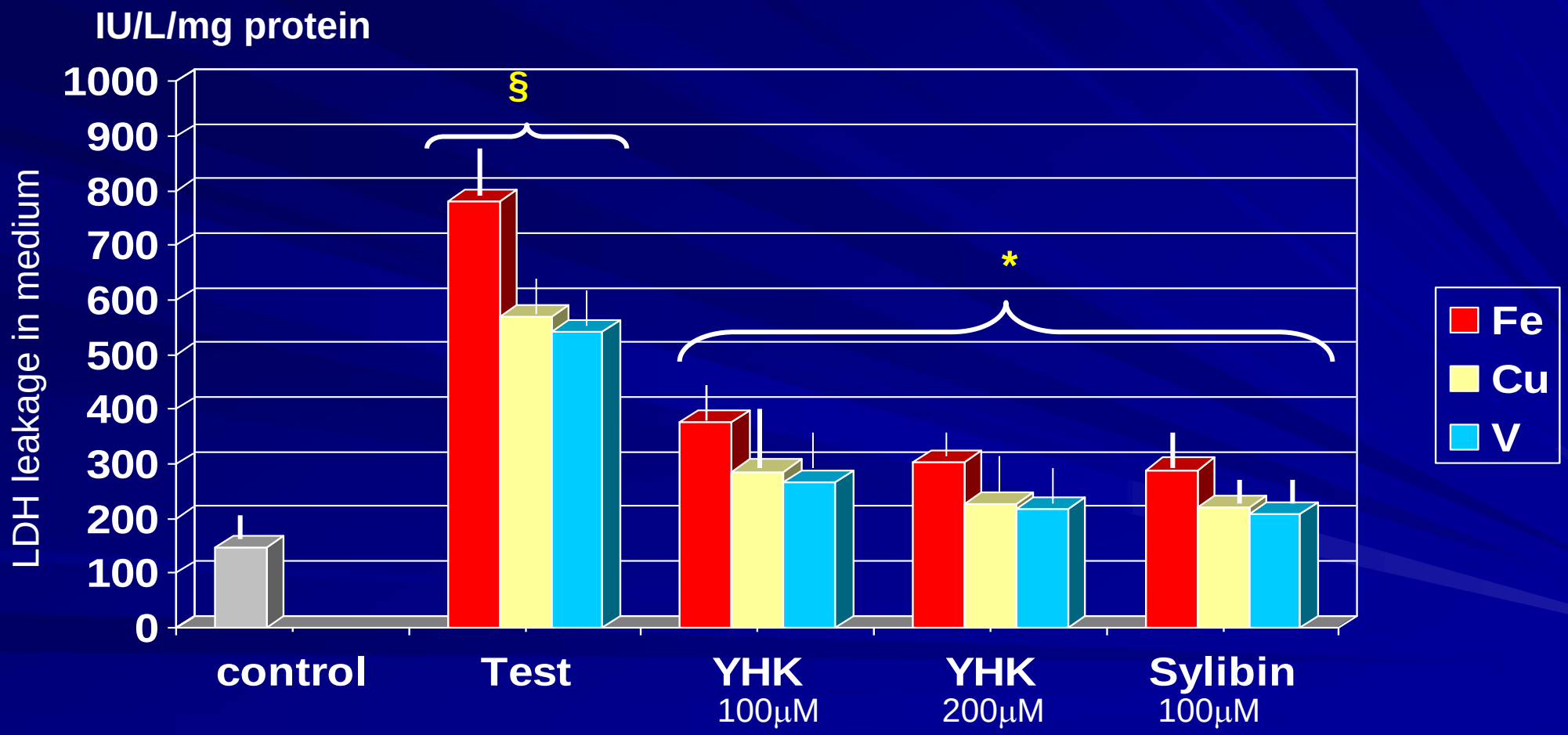
weeks	Control	CCL_4	CCL_4 + YHK
0	367 ± 75	344 ± 87	401 ± 110
10	389 ± 93	839 ± 147*	563 ± 132*§
20	343 ± 61	1190 ± 205*	718 ± 151*§

Serum markers of fibrosis

weeks	Control	CCL_4	CCL_4 + YHK
	Hyarulonic acid		
0	*8.3±4.3*	*4.6±3.7*	*6.2±4.0*
10	*6.7±3,6*	*133.8±55.6**	*67.8±24.7*§*
20	*11.3±5.4*	*224.6±77.5**	*15.5±7.2§*
	Type IV collagen 7s		
0	*4.3 ±0.2*	*4.4 ±0.2*	*4.2 ±0.3*
10	*4.2 ±0.2*	*4.2 ±0.5*	*4.1 ±0.5*
20	*4.3 ±0.6*	*4.9 ±0.4*	*4.7 ±0.1*


Control
Fibrosis Model
Fibr. Model + YHK

EFFECT OF YHK AND SYLIBIN ON LDH LEAKAGE DUE TO METAL IONS DAMAGE IN CULTURED HEPATOCYTES



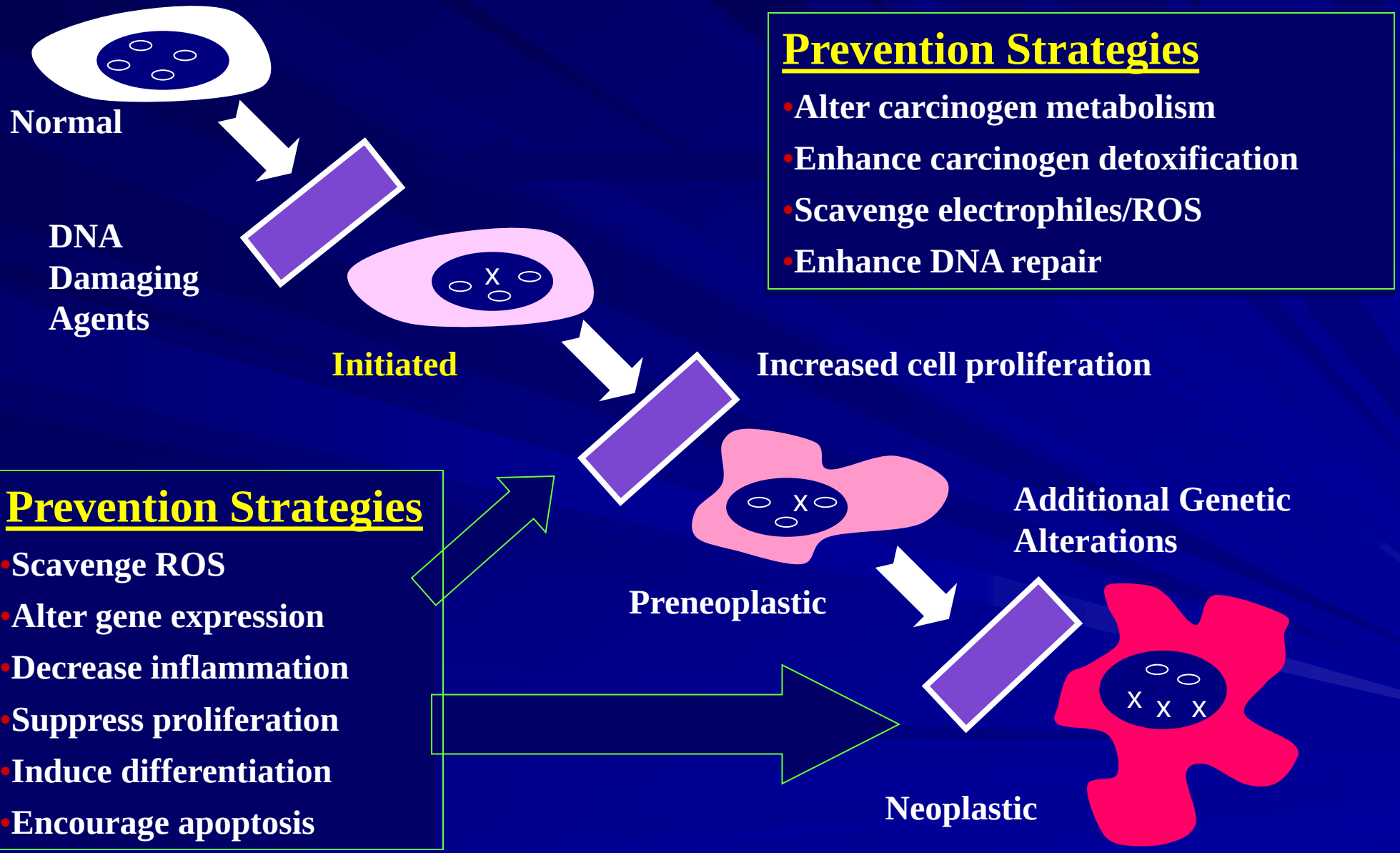
Inhibiting activity of YHK and sylibin on $FeSO_4$-, $Cu\ SO_4$- and VCl_3 -induced lipid peroxidation in normal hepatocytes *(mean ± SD)*

Metal ion	YHK		Silybin
	100μM	200μM	100μM
$FeSO_4$	15. 6 ± 4.6§	12. 2 ± 4.4§ *	18.9 ± 3.2§
$Cu\ SO_4$	7.9 ± 0.3	6.7 ± 0.7	7.3 ± 0.3
VCl_3	8.7 ± 0.99	9.4 ± 0.85	10.8 ± 1.2

Values represent the concentrations that inhibit lipid peroxidation by 50% (IC50, μM). IC50 is calculated from the concentration-activity curves.

§p<0.05 vs Cu SO4 and VCl3. *p<0.05 vs Silybin

Nutritional Modulation of Carcinogenesis



Nutritional Oncology pp. 91, 1999

American Institute for Cancer Research

Washington, 2008

Phytotherapeutic Compound YHK Exerts an Inhibitory Effect on Early Stage of Experimentally-Induced Neoplastic Liver Lesions

Marotta F et al

Hepato-Gastroenterology Dept.., S.Giuseppe Hospital, Milan, Italy
MHC Hospital, Tokyo, Japan
Hepato-GI Unit, University of Sao-Paulo, Brazil

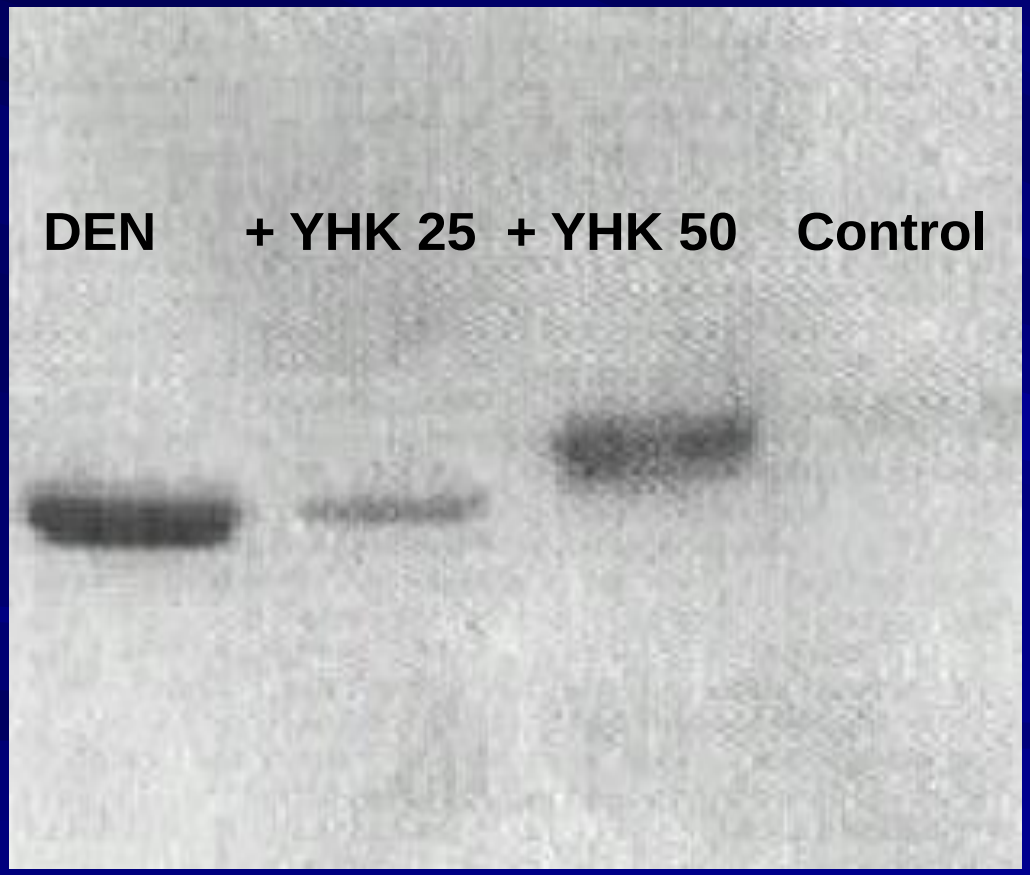
Marotta F et al. Ann Hepatol. 2006

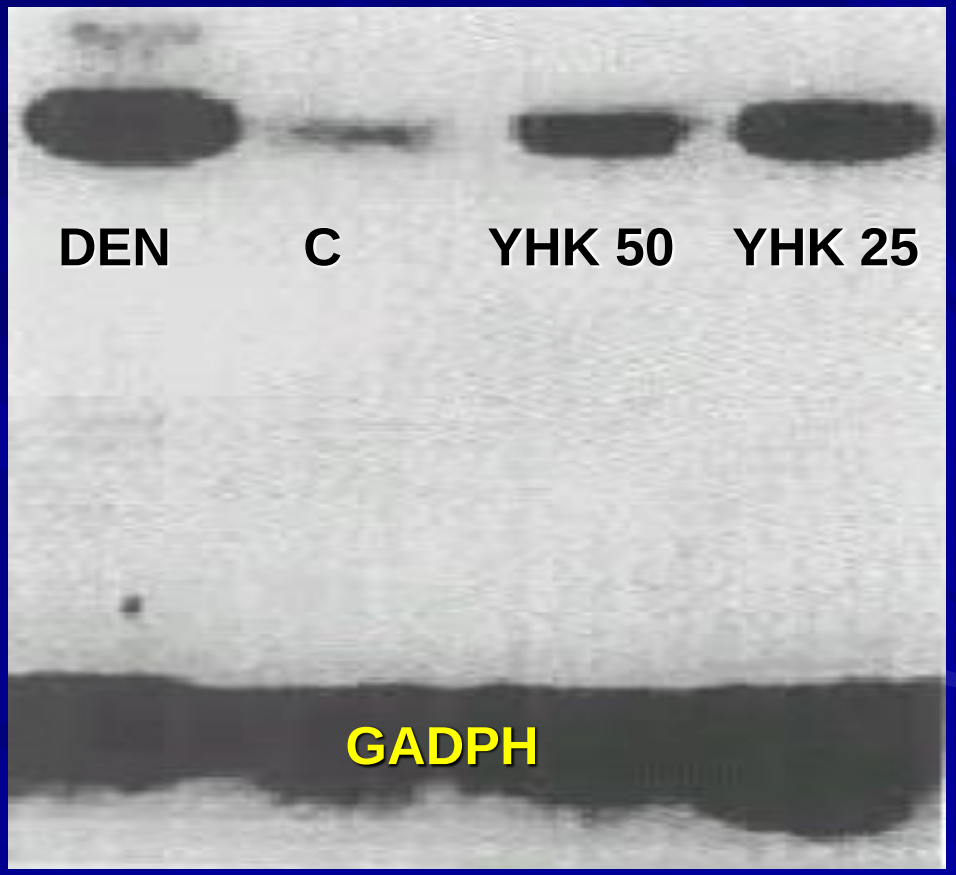
NUMBER AND SIZE OF GST-P-POSITIVE HEPATIC LESIONS IN DEN-INDUCED HEPATOCARCINOGENESIS: EFFECT OF CONCOMITANT SUPPLEMENTATION WITH YHK

Group	DEN	DEN + YHK 50mg/kg/day
No./cm^2	12 ± 4	6 ± 3 *
Mean area (mm^2)	0.32 ± 0.04	0.25 ± 0.03 *
No./cm^3	2012 ± 133	1545 ± 109 *
Mean vol. (mm^3)	0.17 ± 0.03	0.14 ± 0.02 *
Foci/tissue %	28.2 ± 2.5	21.7 ± 2.1 *

* $p<0.01$ vs DEN-only treated rats

Western blotting and Northern blot hybridization of GST-P mRNA in the liver: effect of YHK





INCIDENCE, NUMBER, SIZE AND VOLUME OF DEN-INDUCED HEPATOCELLULAR CARCINOMA: EFFECT OF CONCOMITANT SUPPLEMENTATION WITH YHK

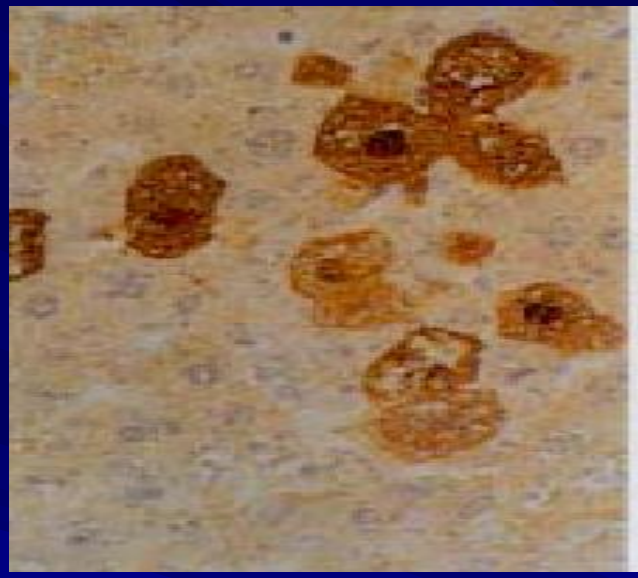
Group	DEN	DEN + YHK 50mg/kg/d
No. of rats with HCC (%)	96 ± 4	71 ± 4 *
Mean area (mm^2)	1.40 ± 0.47	0.17 ± 0.09 * *
No./cm^3	1.3 ± 0.3	0.8 ± 0.2 *
Mean volume (mm^3)	0.79 ± 0.28	0.02 ± 0.01 * *
HCC/tissue %	0.7 ± 0.2	0.2 ± 0.1 *

* $p<0.01$ vs DEN-only treated rats

DEN

DEN + YHK

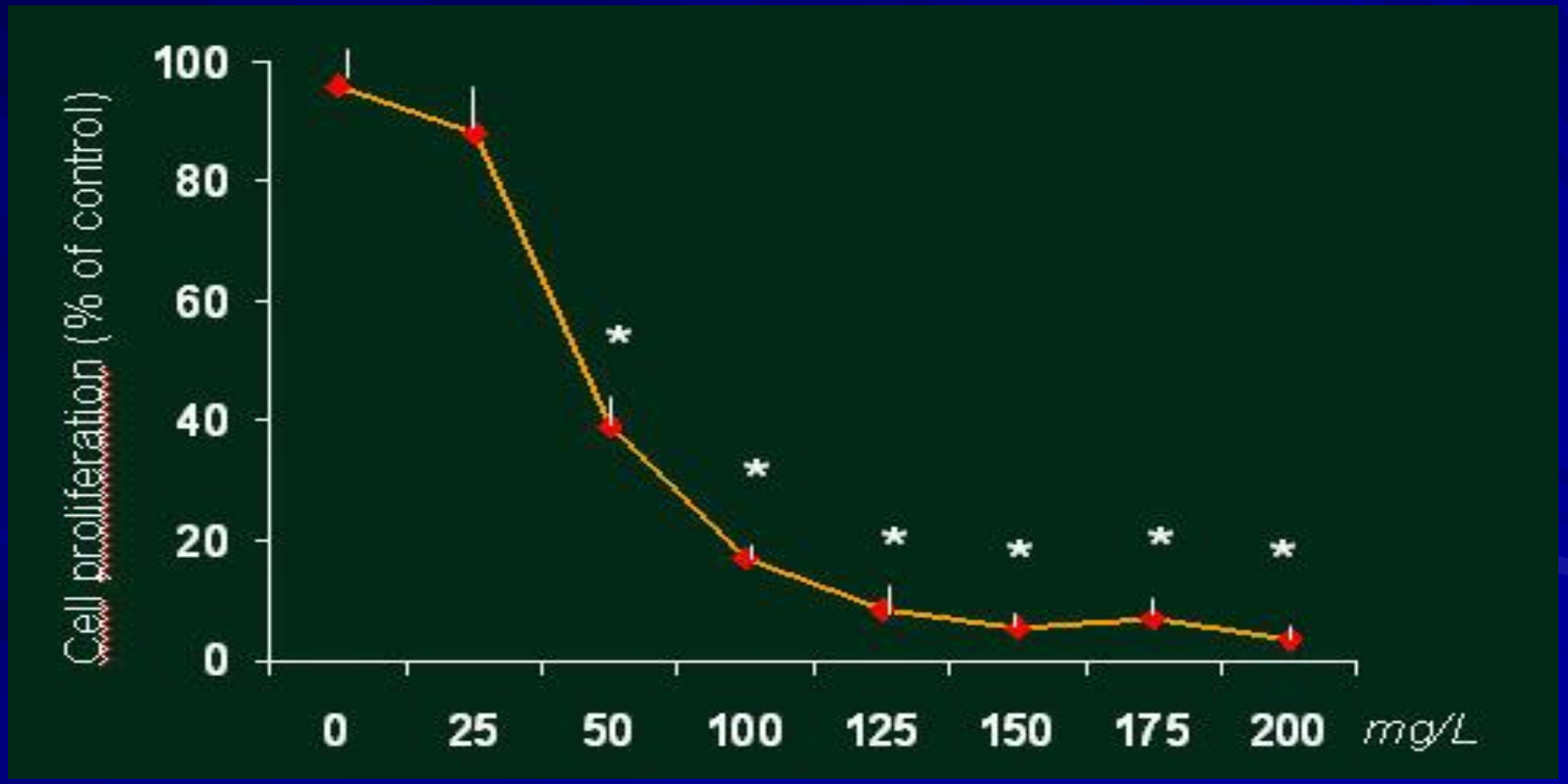
Small-multiple GST-P Foci



Large GST-P Foci

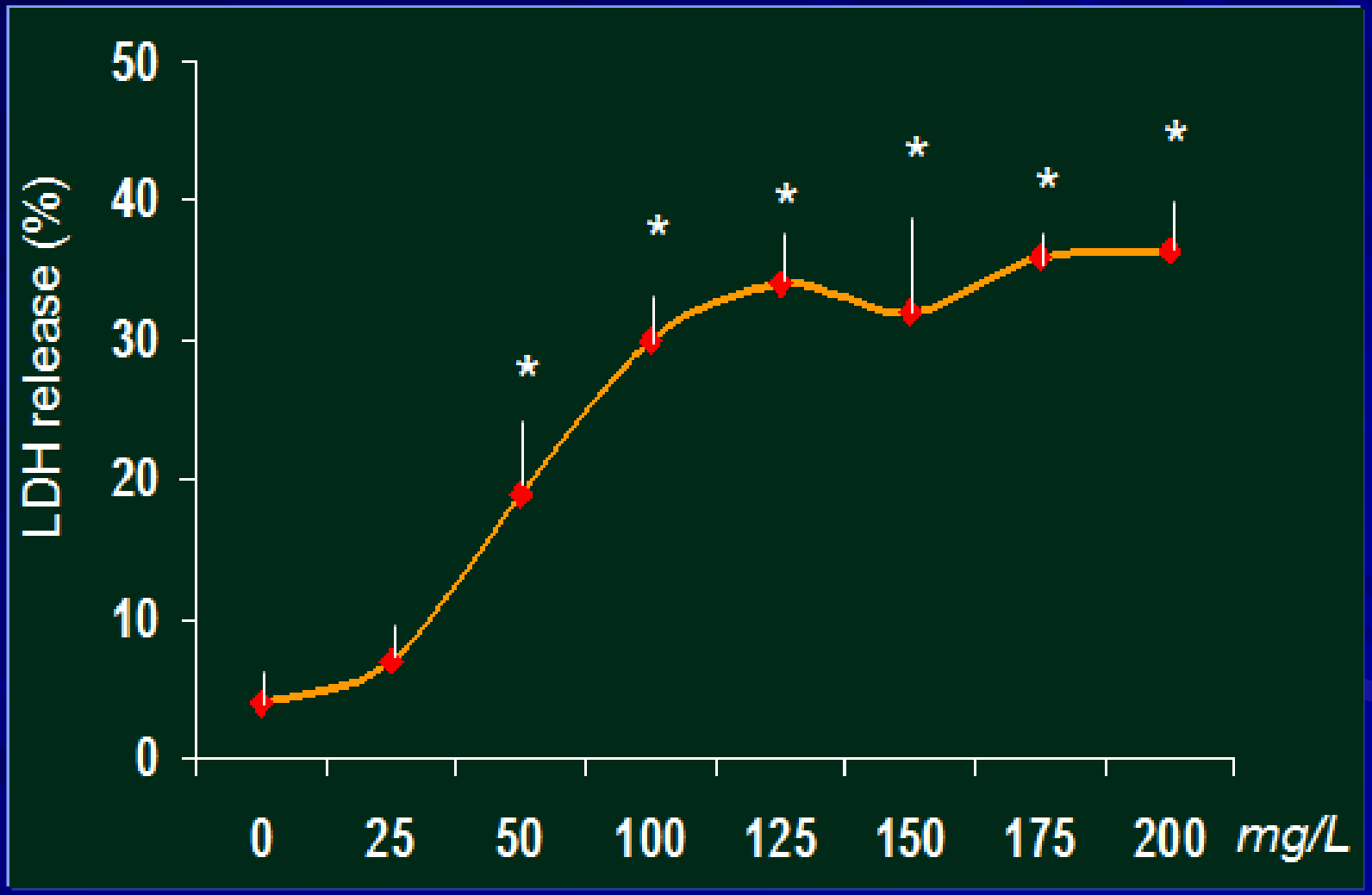
IS THERE ANY ROLE FOR SUPPORTIVE NUTRACEUTICALS IN HCC?

Effect of YHK on HepG2 cell proliferation



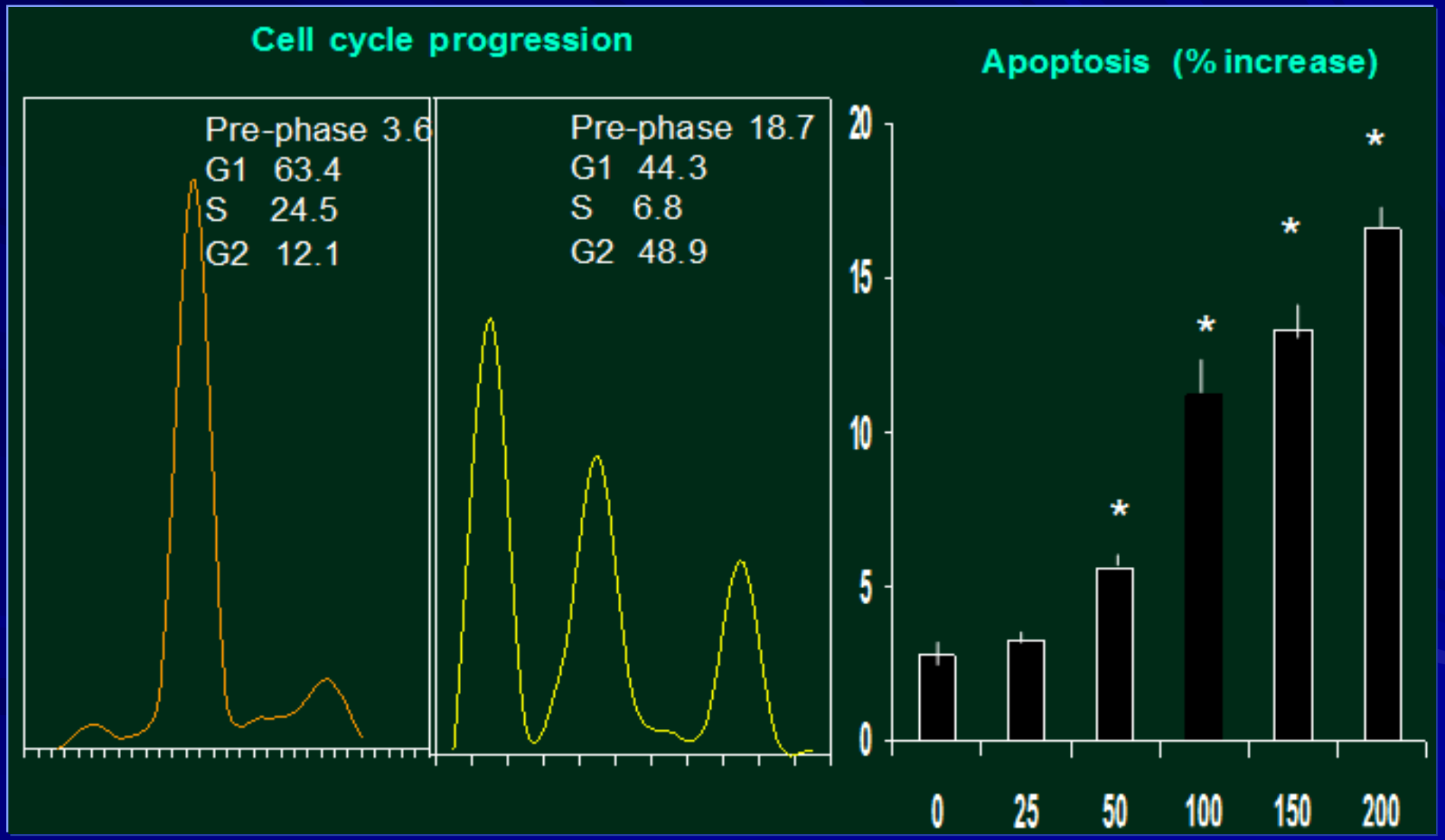
Marotta F et al. Annal Hepatol 2007

Effect of YHK on cell cytotoxicity in HepG2 cells



Marotta F et al. Annal Hepatol 2007

Effect of YHK on Cell cycle and apoptosis of HepG2 cells



Marotta F et al. Annal Hepatol 2007

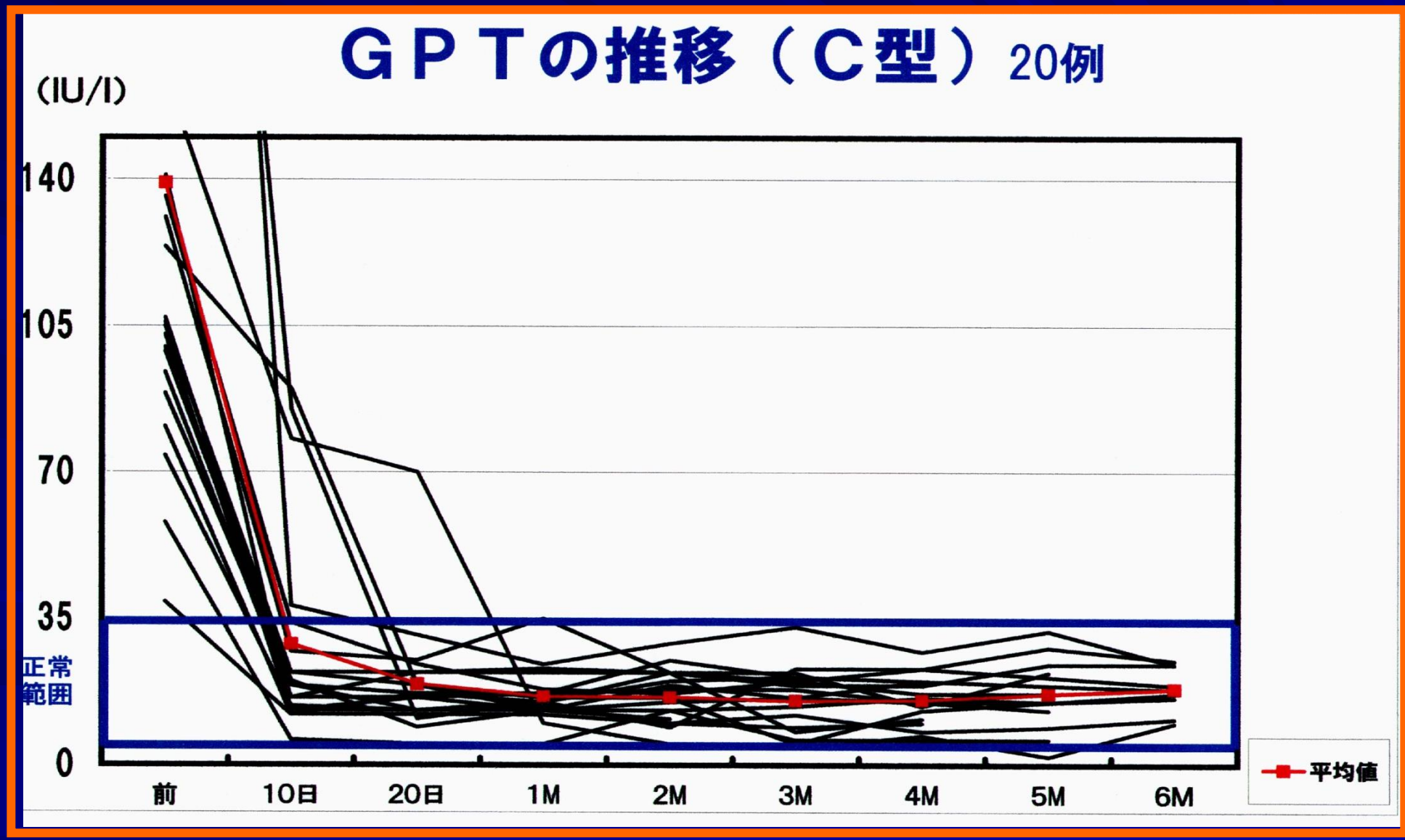
A pilot clinical study of YHK in *HCV-related CLD*

Prize-Winner
JSH 2002

BIOPSY ASSESSMENT

Fibrosis score	Necro-Inflamm. score	Outcome
$F_1 \rightarrow F_{0\text{-}1}$	$A_2 \rightarrow A_0$	*improved*
$F_2 \rightarrow F_{0\text{-}1}$	$A_3 \rightarrow A_2$	*improved*
$F_1 \rightarrow F_1$	$A_2 \rightarrow A_2$	*no change*
$F_0 \rightarrow F_0$	$A_2 \rightarrow A_{0\text{-}1}$	*improved*
$F_2 \rightarrow F_3$	$A_2 \rightarrow A_2$	*progression*
$F_{1\text{-}2} \rightarrow F_{1\text{-}1}$	$A_3 \rightarrow A_{1\text{-}2}$	*improved*

A pilot clinical study of YHK in *HCV-related CLD*



1: Liver Int. 2007 Mar;27(2):227-34.

Yo jyo hen shi ko, a novel Chinese herbal, prevents nonalcoholic steatohepatitis in ob/ob mice fed a high fat or methionine-choline-deficient diet.

de Lima VM, de Oliveira CP, Sawada LY, Barbeiro HV, de Mello ES, Soriano FG, Alves VA, Caldwell SH, Carrilho FJ.

Department of Gastroenterology (LIM 07), University of São Paulo School of Medicine, São Paulo, Brazil.

1: Dig Dis Sci. 2006 Jul;51(7):1183-9.

Yo Jyo Hen Shi Ko (YHK) improves transaminases in nonalcoholic steatohepatitis (NASH): a randomized pilot study.

Chande N, Laidlaw M, Adams P, Marotta P.

Factors Affecting HCC Risk

- Active disease
  - **Elevated ALT**
- Persistently elevated AFP
- Low platelet count
- HBV DNA level
- Histologic changes
  - Dysplasia
  - Geographic morphologic changes
  - PCNA positive
- Use of TIPS (?)

Beasley RP, et al. Lancet. 1981. Degos F, et al. Gut. 2000. Oka H, et al. Hepatology. 1994. Zhang JY, et al. Am J Trop Med Hyg. 1998. Colombo M, et al. N Engl J Med. 1991. Ganne-Carrie N, et al. Hepatology. 1996; Lee RG, et al. Hepatology. 1997. Chen CJ, et al. JAMA. 2006.

Cirrhosis (Non-HBV) Suitable for HCC Surveillance*

- **Hepatitis C**
  - Incidence of HCC ~ 2% to 8% per year
- **Primary biliary cirrhosis**
- **Alcoholic cirrhosis**
- **Genetic hemochromatosis**
- **Nonalcoholic steatohepatitis**
- ? Alpha1-antitrypsin deficiency
- ? Autoimmune hepatitis
- ? Cryptogenic cirrhosis

***Populations with an annual HCC incidence of ≥ 1.5%.**

Takano S, et al. Hepatology. 1995. Tsukuma H, et al. N Engl J Med. 1993. Pateron D, et al. J Hepatol. 1994. Zaman SN, et al. Lancet. 1985.

Drug Development is NOT Easy

Clinical Trials – Timeline for new drug development

	Preclinical Testing	Phase I	Phase II	Phase III	FDA	Total Years	Phase IV
Years	3.5	1	2	3	2.5	12	Post-marketing
Test Population	Laboratory & animal studies	20 to 80 healthy volunteers	100 to 300 patient volunteers	1000 to 3000 patient volunteers	Review process/ Approval		
Purpose	Assess safety and biological activity	Determine safety and dosage	Evaluate effectiveness, look for side effects	Verify effectiveness, monitor adverse reactions from long-term use			

HCV ADVOCATE
www.hcvadvocate.org

HCV Drugs in Development

(as of April 21st, 2009)

- 23 drugs against HCV targets:
  - 12 targeting NS3/4a protease
  - 8 targeting NS5B polymerase
  - 2 targeting NS5A
  - 1 entry inhibitor
- 15 general drugs:
  - 6 against cellular targets: cyclophilin, miRNAs, caspases, glucosidase, phospholipids
  - 9 Immunomodulators (stimulators/inhibitors): TLR9 agonists, A3AR agonists, anti-inflammatory, anti-fibrotic
- 6 Interferons:
  - IL-29, oral IFN, albuferon, consensus IFN
- 6 vaccines
- 4 liver cancer drugs
- 42 studies cancelled

http://www.hcvadvocate.org/hepatitis/hepC/HCVDrugs.html

Risks of CAM

- Indirect risks
  - Delay/avoidance of effective treatment
- Direct health risks
  - Toxic reactions
  - Pharmacologic effects
  - Mutagenic effects
  - Drug interactions
  - Contamination
  - Substitutions or adulteration of ingredients

Hidden risks: Ginger

- Beneficial for nausea
- Be careful if you have gallstones
- Can worsen blood clotting!

Herbals supplements implicated in causing hepatotoxicity

- *Atractylis gummifera*
- Black cohosh
- *Callilepis laureola*
- Chaparral

Chinese herbal medicines

- Chaso and Onshido
- Sho (Do)-saiko-to
- Jin Bu Huan
- Ma huang
- Shou-wa-pian

- Comfrey/pyrrolizidine alkaloids
- Germander
- Greater celandine
- Kava
- Mistletoe
- Pennyroyal
- Skullcap and valerian
- Centella Asiatica
- Red yeast

Leonard B. Seeff, MD, *Clinics in Liver Disease*, August 2007

Common Chinese Herbs with potentially liver-toxic substances

- An Gong Niu Huang Wan
- Bi Tong Pian
- Bi Yan Pian
- Dendrobum Moniliforme
- Farfunoeiminkam Wan
- Gan Mao Ling
- High Strength Yin Cheng
- Huang Lien Shang Ching Pian
- Ma Hsing Zhe Ke Pian
- Marguerite Acne Pills
- Aconite or aconitum
- Acorus
- Comfrey
- Crotalaria
- Eupatorium
- Germander
- Groundsel
- Heliotropium
- Jin Bu Huan
- Mentha pulegium
- **Mistletoe**
- Pennyroyal oil
- Hedeoma pulegoides
- Sassafras
- Senicio species
- **Senna**
- Sophora
- Night Sight Pills
- Niu Huang Chiang Ya Wan
- Pe Min Kan Wan
- Da Huo Luo Wan
- Shen Ling Bai Zhu Pian
- Ta Huo Lo Tan
- Tsai Tsao Wan
- Yin Chiao Chieh Tu Pian
- Zhi Sou Ding Chuam Wan
- Zhong Gan Ling
- Amanita mushroom
- Chaparrel

In general, **combination ingredient** supplements are more likely to cause serious adverse events than single ingredient supplements!

CAM Can Be Beneficial in HCV

- 40% use in liver patients suggests benefit
- Preliminary data promising
- Need more scientific data
  - May **ameliorate side effects** of conventional therapy
  - Use in those **in whom therapy is contraindicated**
  - Use in **cirrhotics**
  - Use in **non-responders**
  - Potential **synergy** with conventional therapy
  - Bridge pending advances in conventional therapy

How Do We Counsel Patients Using Alternative Therapies?

- Consider what motivates patients to pursue alternative therapy
  - Educate patients concerning natural history of HCV infection and improving treatment options
- Obtain a thorough history of alternative treatments
- Discuss limited information on efficacy, safety, and potential risks of therapy
- Safe alternative agents are often beneficial for symptoms

Treatment Options for Hepatitis C

Western (Allopathic) Medicine Hepatitis C Specialist

Pegylated interferon/ribavirin
or
Experimental protocols

Integrated Medicine Hepatitis C Specialist

Western therapy and complementary and alternative medicine

Complementary and Alternative Medicine Hepatitis C Specialist

Combination of all/some:

- Ayurvedic medicine
- Chinese herbs and acupuncture
- Homeopathy
- Mind:body medicine
- Naturopathic treatments
- Nutrition and lifestyle

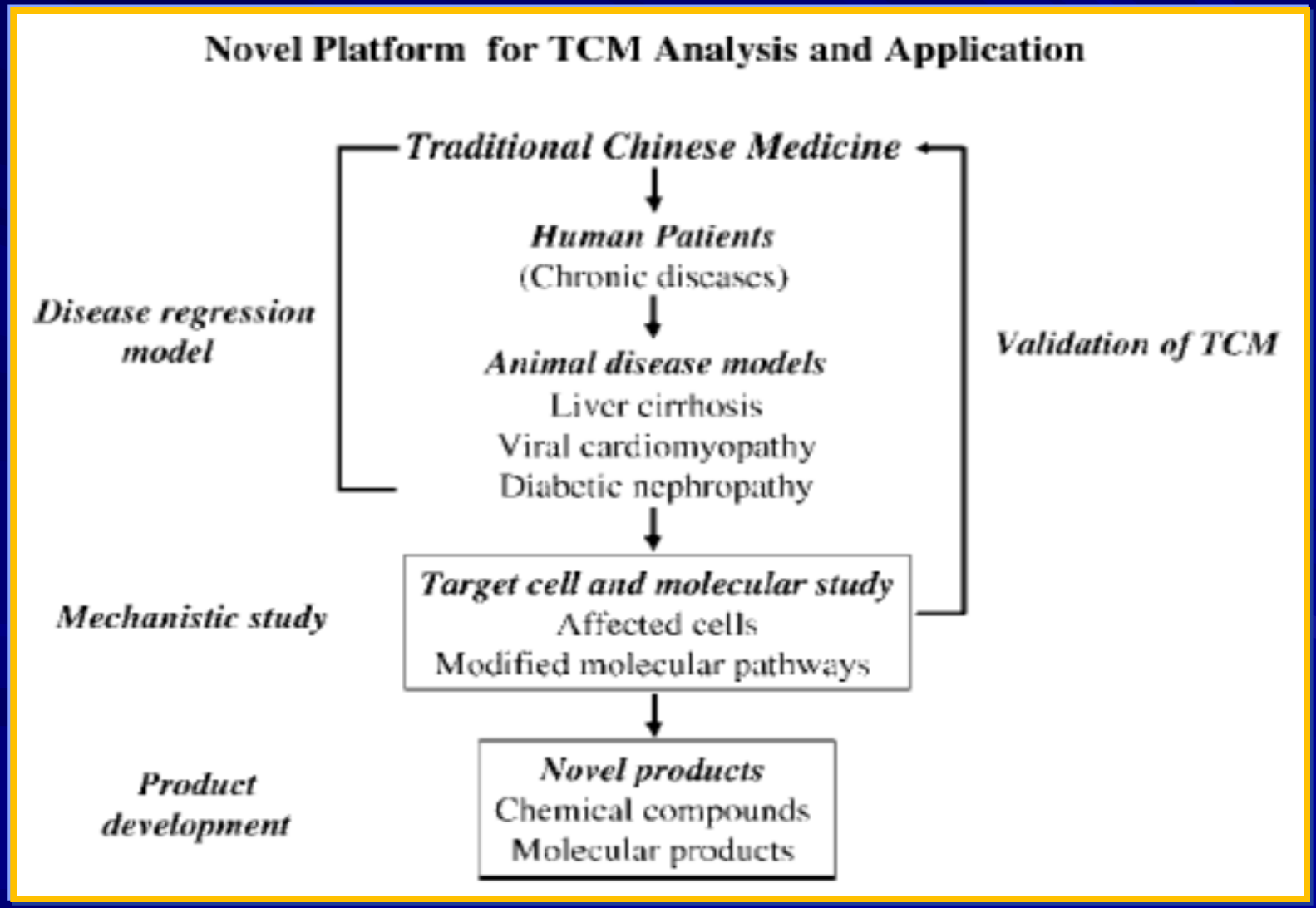
Relapse or non-responder: **Try retreatment or use supportive care while waiting for new options. Continue healthcare provider follow-up on a regular basis.**

No treatment or self-treatment

Discuss possible implications with your hepatitis C specialist/healthcare provider. Understand your risks of cirrhosis or liver cancer.

Herbogenomics: From Traditional Chinese Medicine to Novel Therapeutics

Experimental Biology and Medicine 2008



Fermented Papaya Preparation: ≈15 years of Evidence-Based studies

- **L. Packer's group -UCLA, USA** **Life Sci 2000; 67:679-94**

  FFP is a potent macrophage activator increasing NO synthesis and TNFα secretion in vitro.

  hydroxyl scavenging and iron-chelating properties of FFP prevents oxidative damage to DNA and proteins. **Anticancer Res 2000; 20:2907-14**
- **F. Marotta et al.** **Digestion 1999; 60:538-543, Hepatogastroenterol 1997, 2000**
- **FFP promotes an effective protection against ethanol-induced gastric mucosal damage and reduced ox stress and DNA damage in cirrhosis**

  FPP reduced precancerous markers of GI lesions **Ann NYAS 2004, 2006**
- **Luc Montagnier et al. Immune-stimul. in imm-NR HIV (in process)**
- **"Development of Life Living Guidance for Prevention of HIV Progression" Res. Project** **Dept. of Health Japan, 1998**

  FFP enhanced CD8+ cell count in HIV pts. No side effects.

Fermented Papaya Preparation (100g)

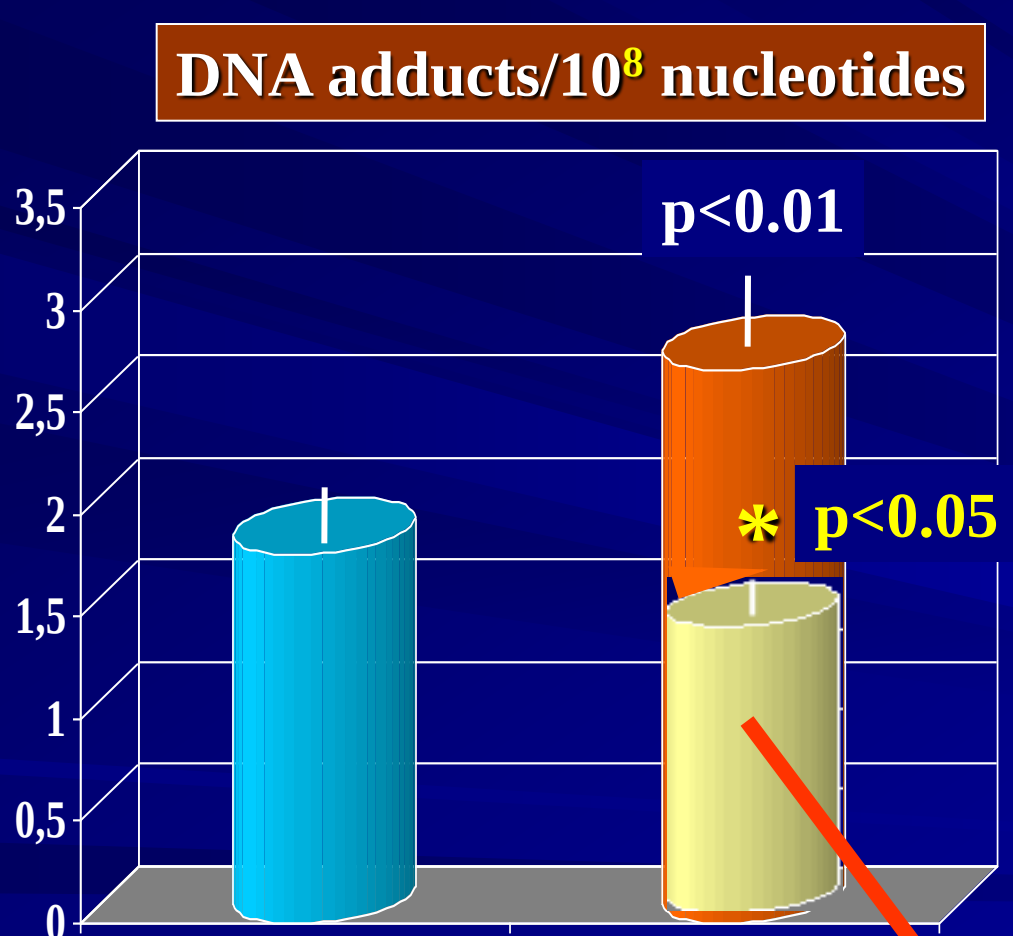
ISO 9001, ISO 14001 **Japan Food Res. Lab., report n. 397100396-007**

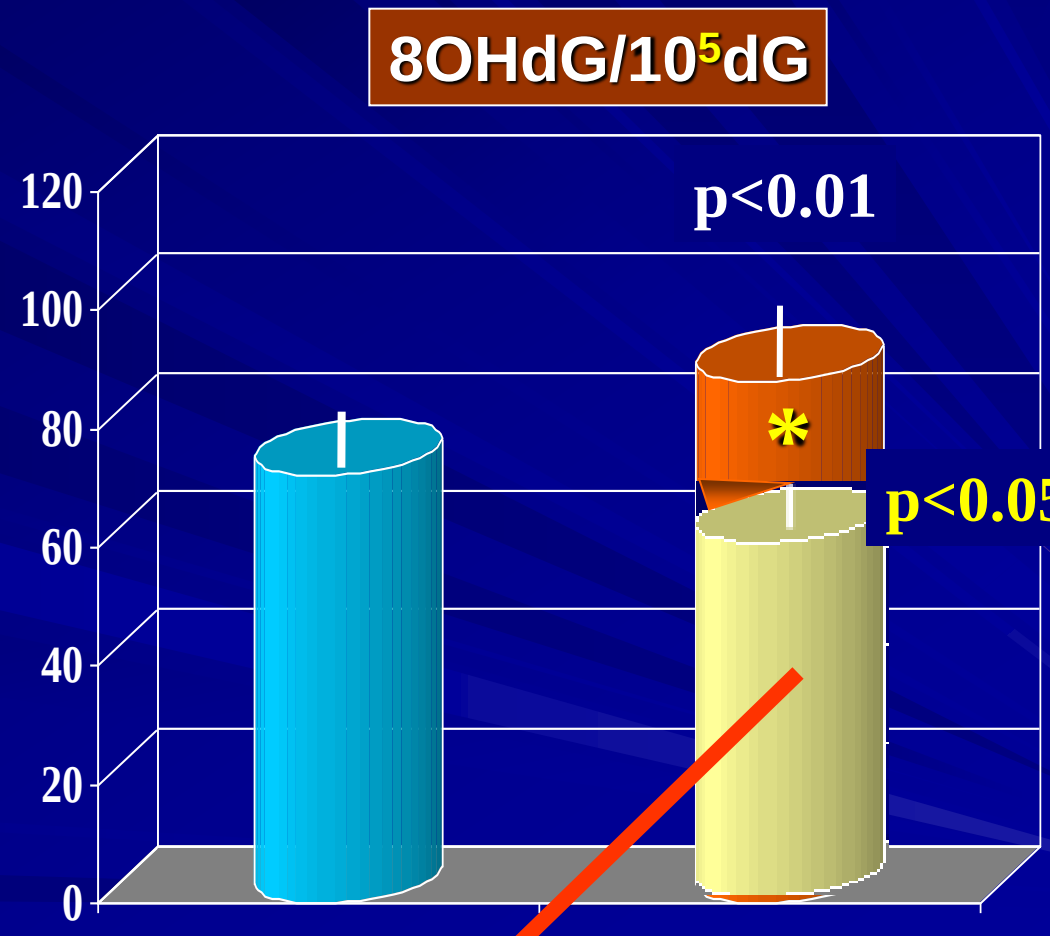
CHD	90.7g	**Phenylalanine**	11mg
Moisture	8.9g	**Tyrosine**	9mg
Protein	0.3g	**Leucine**	18mg
Fat	none	**Isoleucine**	9mg
Folic acid	2µg	**Methionine**	5mg
Niacin	0.24mg	**Valine**	13mg
Lysine	6mg	**Glycine**	11mg
Histidine	5mg	**Proline**	8mg
Aspartic ac.	27mg	**Tryptophan**	2mg
Serine	11mg	**Threonine**	8mg
Arginine	16mg	**Glutam.ac.**	37mg

SOME COMMON CONDITIONS WITH IMMUNE SYSTEM-LINKED INFLAMMATORY & OXIDATIVE STRESS PHENOMENA

- Aging per sè
- Chronic Diseases (Liver, Diabetes, etc.)
- Relates stress
- Seasonal (*flu, COPD flare up*) stress

Nutraceutical supplementation: effect of a fermented papaya preparation on redox status and DNA damage in healthy elderly individuals and relationship with GSTM1 genotype: a randomized, placebo-controlled, cross-over study.

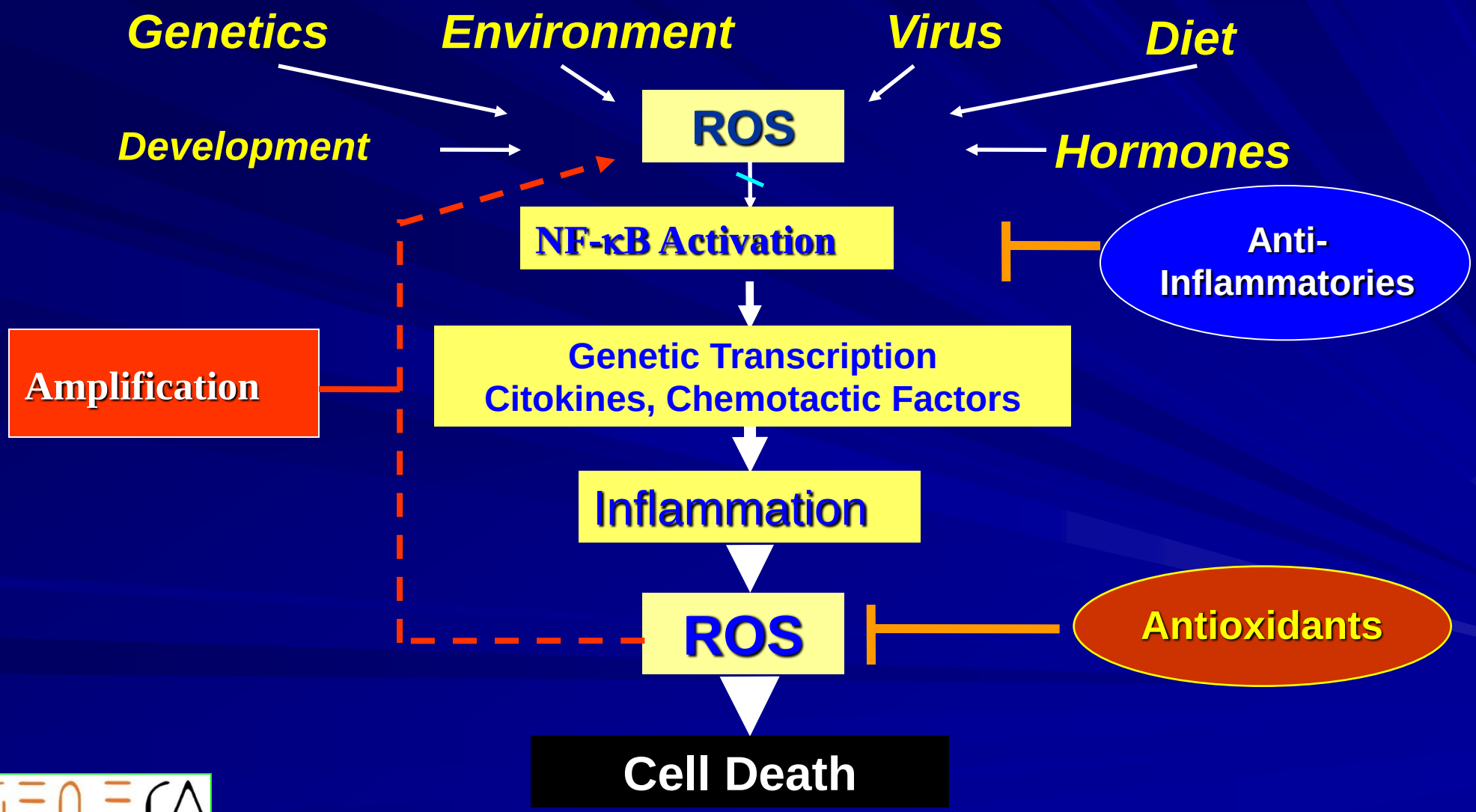




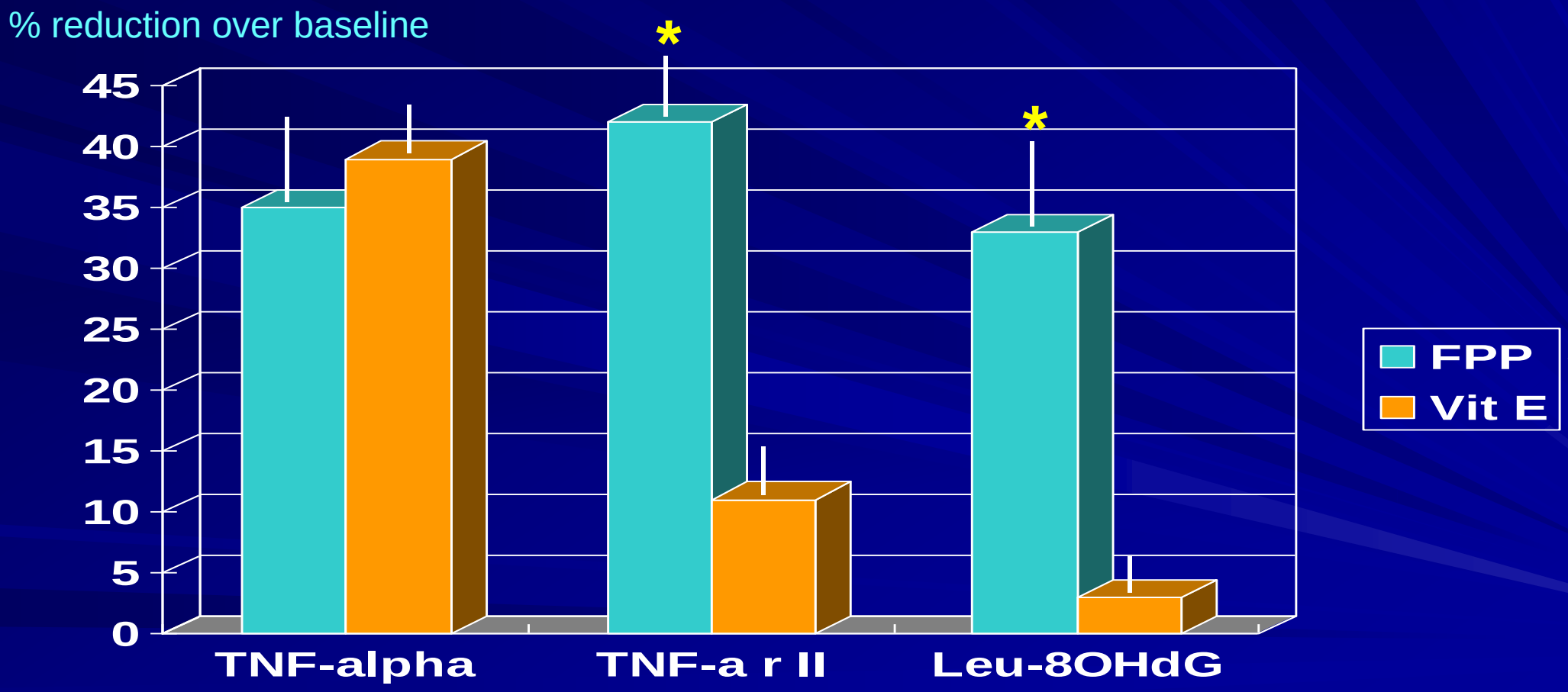
FPP intervention

Marotta et al NY Acad Sci 2006

Prevention of Chronic Diseases: the ox stress-inflammation network

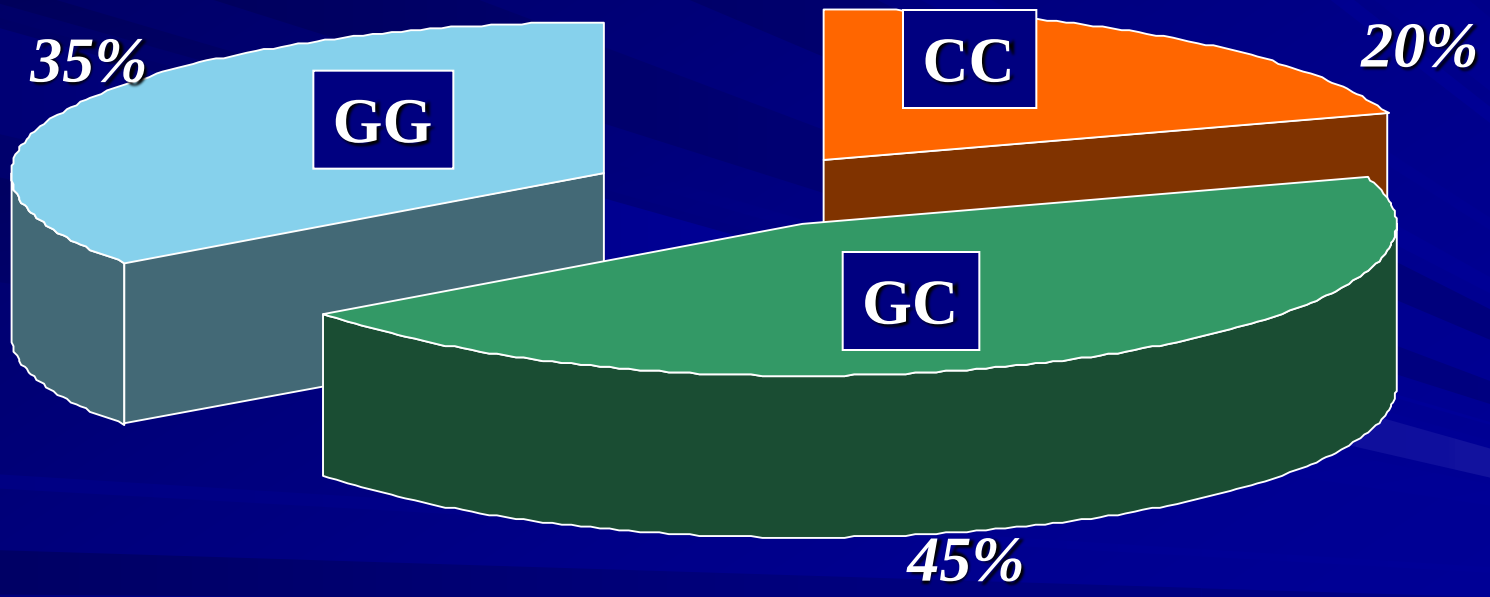


Modulating leukocyte DNA damage and cytokines by nutraceuticals in HCV-CLD: a fermented papaya preparation vs vitamin E

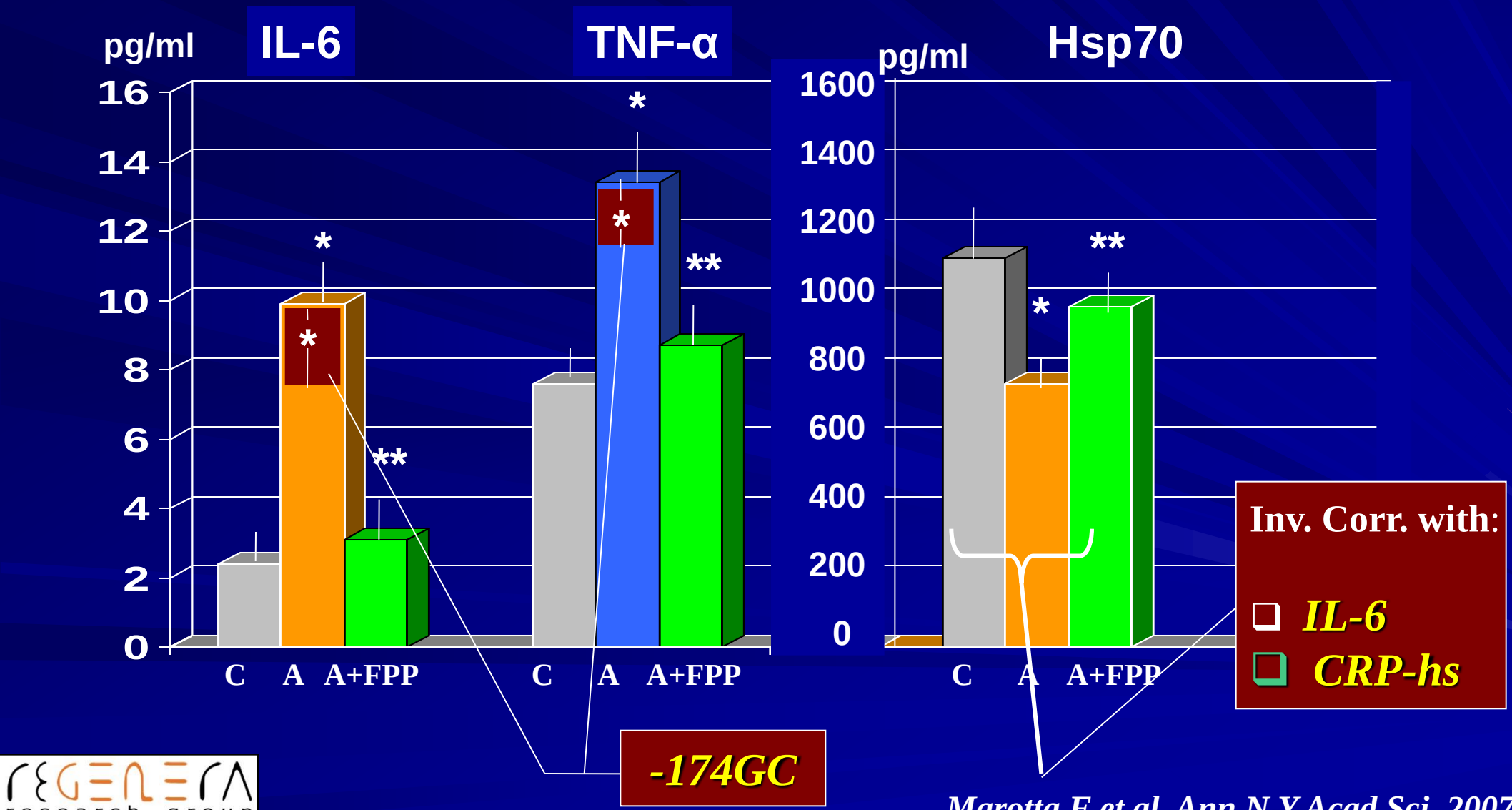


Marotta et al. J Gastroenterol Hepatol 2006

Interleukin-6 Promoter Polymorphism Analysis

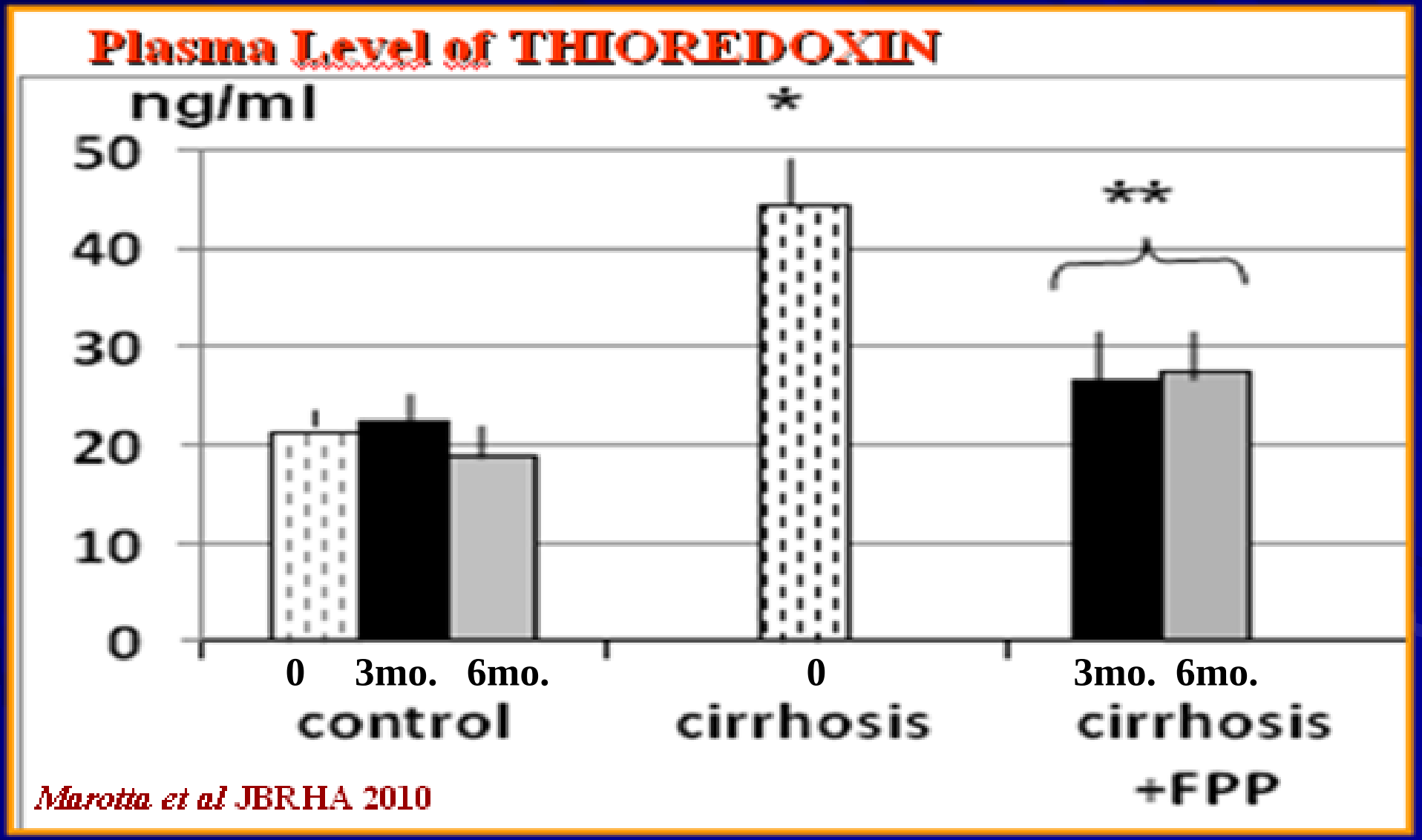


Effect of FPP supplementation on IL-6, TNF-α and Hsp70 in elderly population



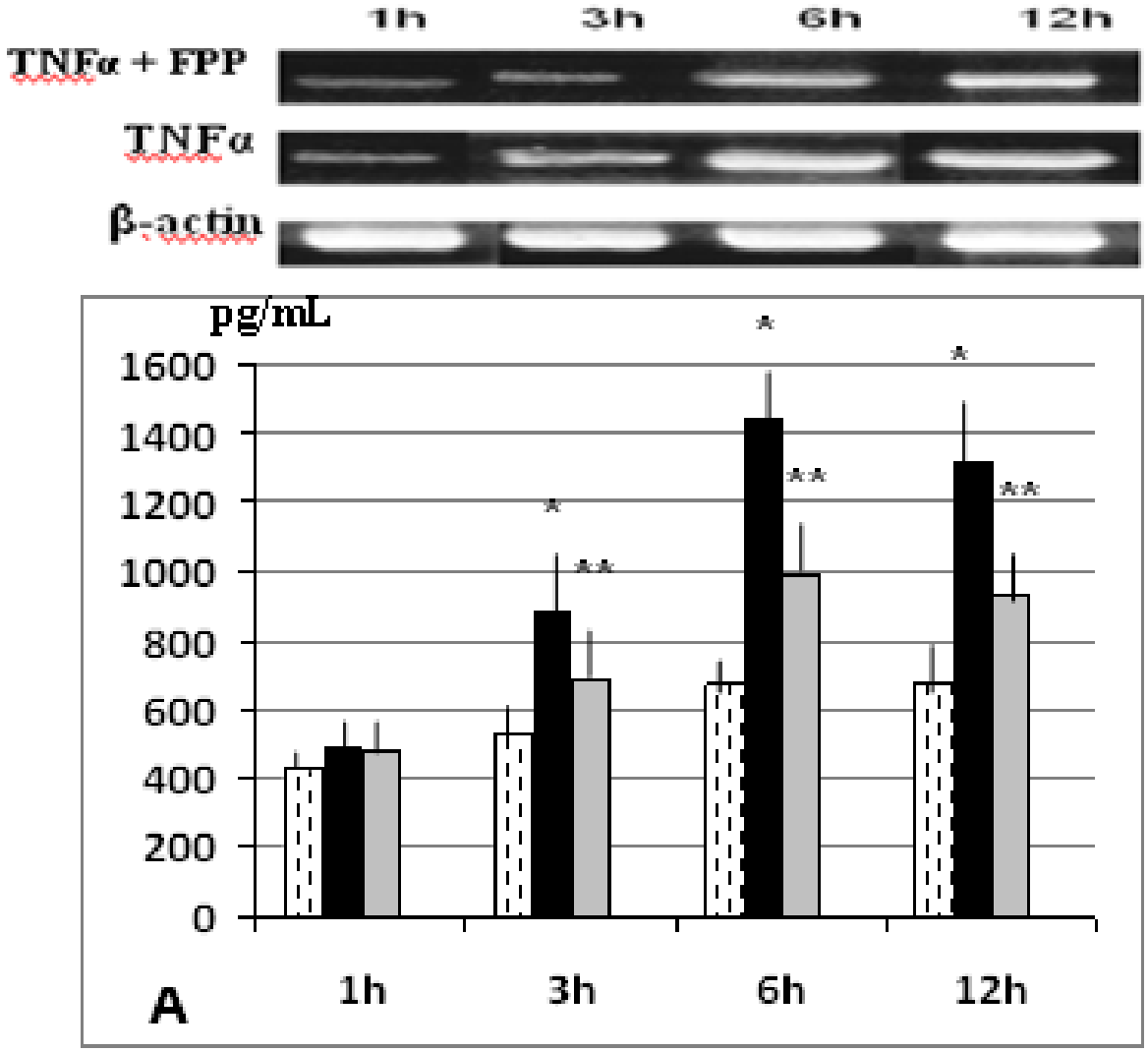
Marotta F et al. Ann N Y Acad Sci. 2007

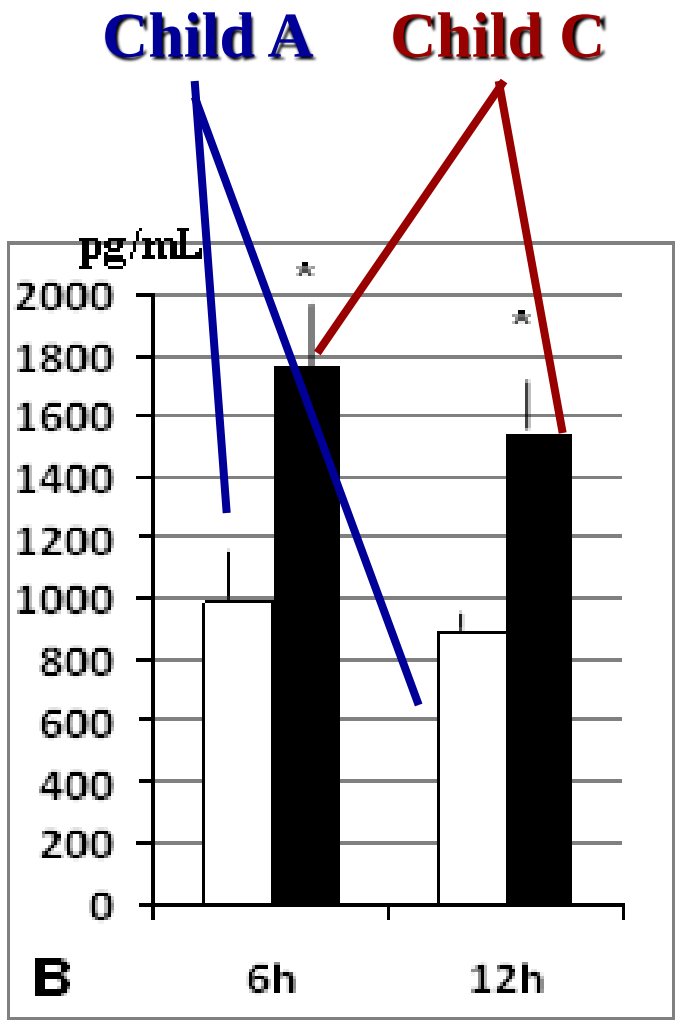
EFFECT OF A FERMENTED NUTRACEUTICAL ON THIOREDOXIN LEVEL AND TNF-α SIGNALLING IN CIRRHOTIC PATIENTS



EX-VIVO LPS-STIMULATION TEST OF TNF-α PRODUCTION FROM MONOCYTES: NUTRACEUTICAL MODULATION.

Marotta et al, JBRHA 2010





Vitamin Supplements

- Multivitamin without iron
  - **Excess iron** increases inflammation in the liver
  - Powder capsule formula is best for digestion
  - Can sometimes make people nauseated: take with food
- Fatty acids
  - Decreases muscle aching and fibromyalgia symptoms
  - Get refrigerated type to avoid rancidity

Vitamin Supplements

- Avoid Vitamin A unless you have been documented to be deficient
- Calcium with vitamin D two-three times daily
- Vitamin E: 400-1200 IU per day
  - Can help cell-mediated immune function, skin problems, memory loss
- Vitamin C: improves the immune function
- Lactobacillus acidophilus: aids with digestion

REDOX MODULATION IN OCCUPATIONAL STRESS : MODIFICATIONS BY NUTRACEUTICAL INTERVENTION

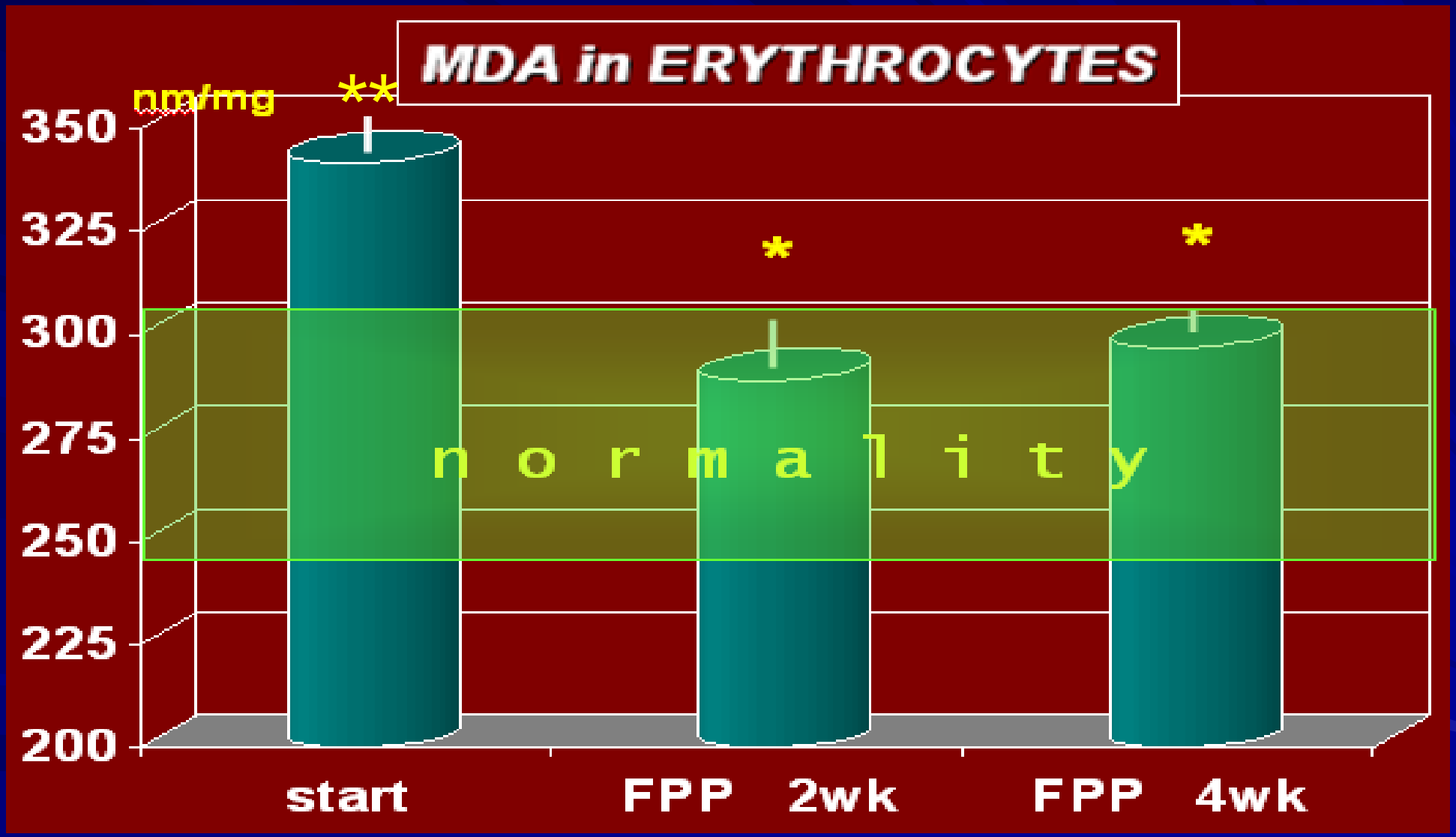
Marotta et al, JBRHA 2010

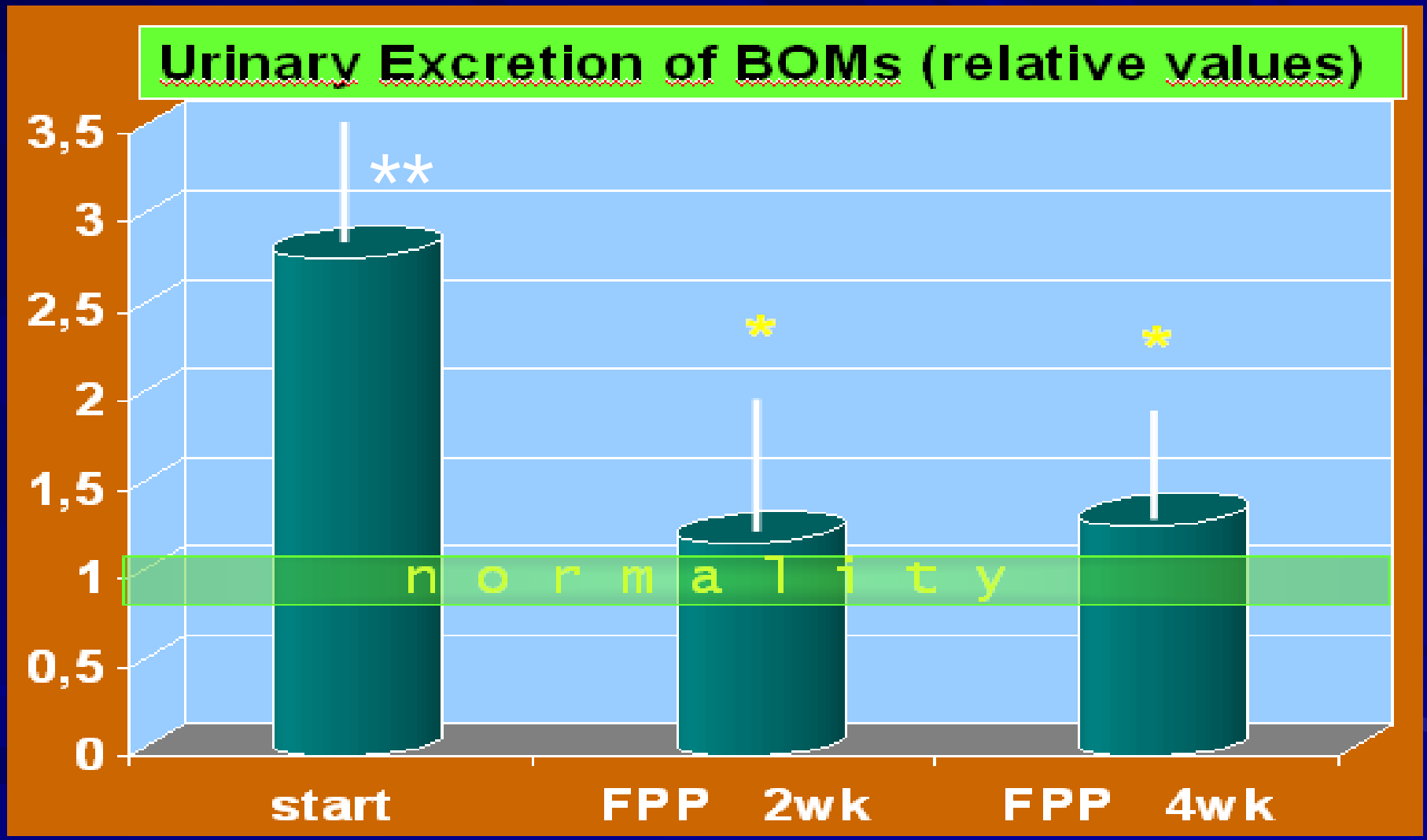
Patients and Methods

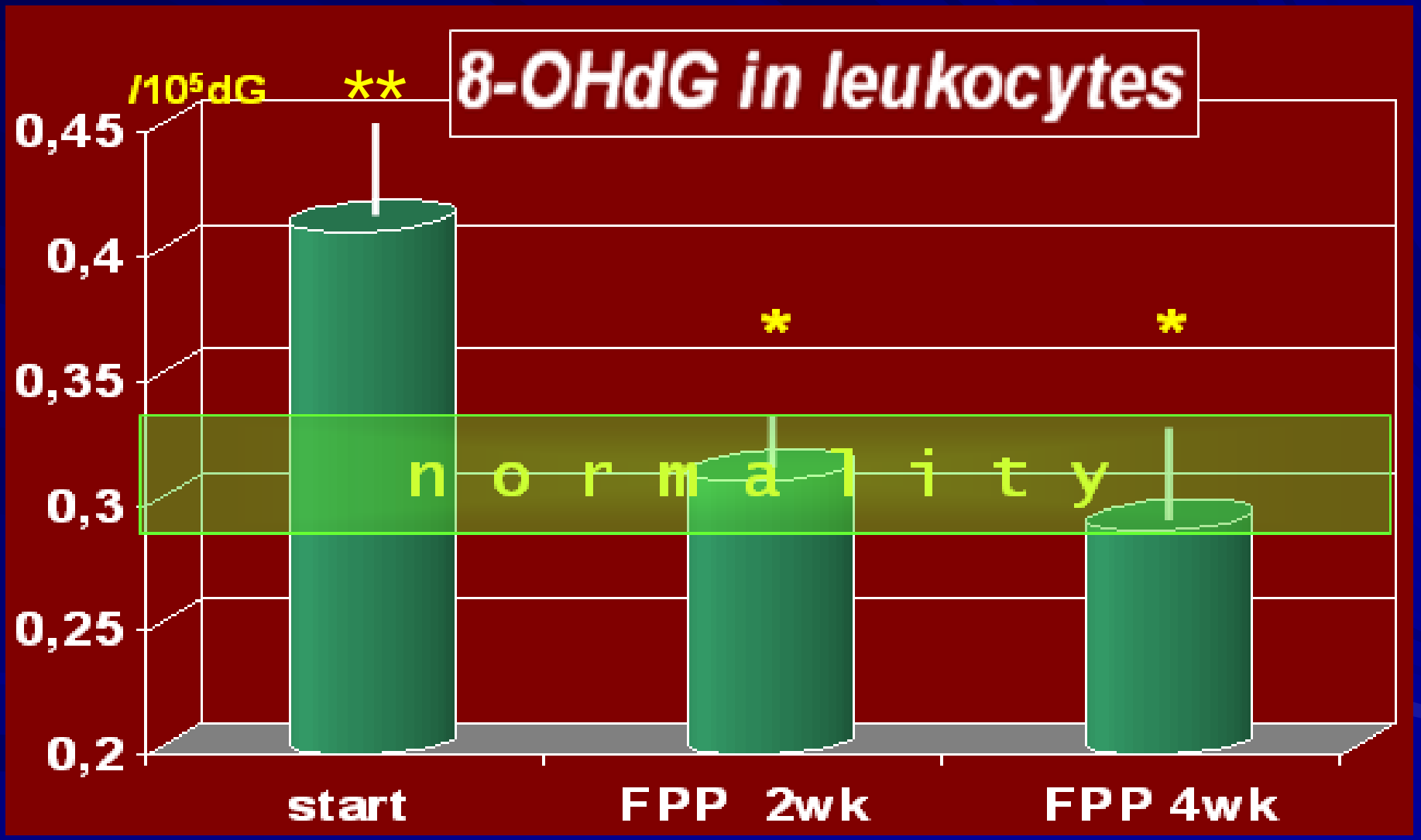
A) **39 healthy subjects, sedentary, teetotaller or <20g/day, non-smoking,**
B) **Stress questionnaire (State Trait Anxiety Inventory), Dr. Padrini's psycho-emotional questionnaire and Pittsburgh Sleep Quality Index**
C) **Dietary- and life-style questionnaire**

Treatment and controls

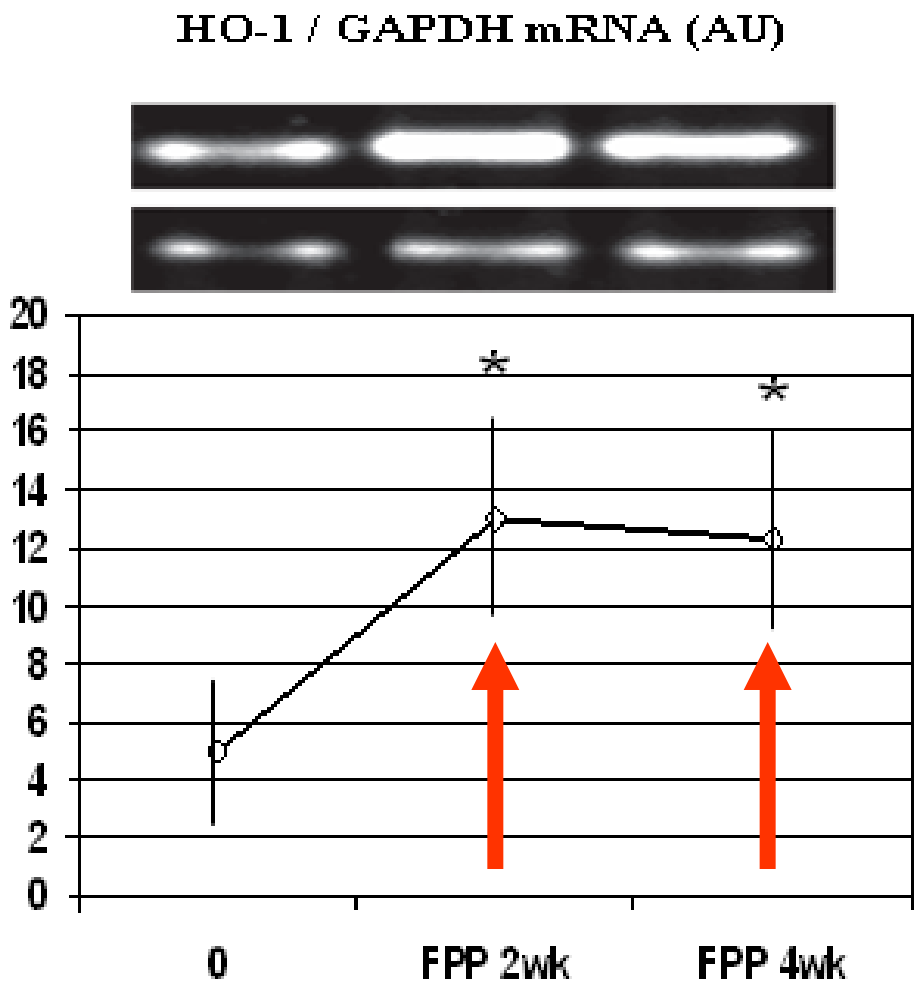
A) **FPP (Osato Res. Inst., Gifu, Japan) 9g/day (4.5g twice/day) for 1mo.**
B) Blood chemistry 2- and 4-wks afterwards

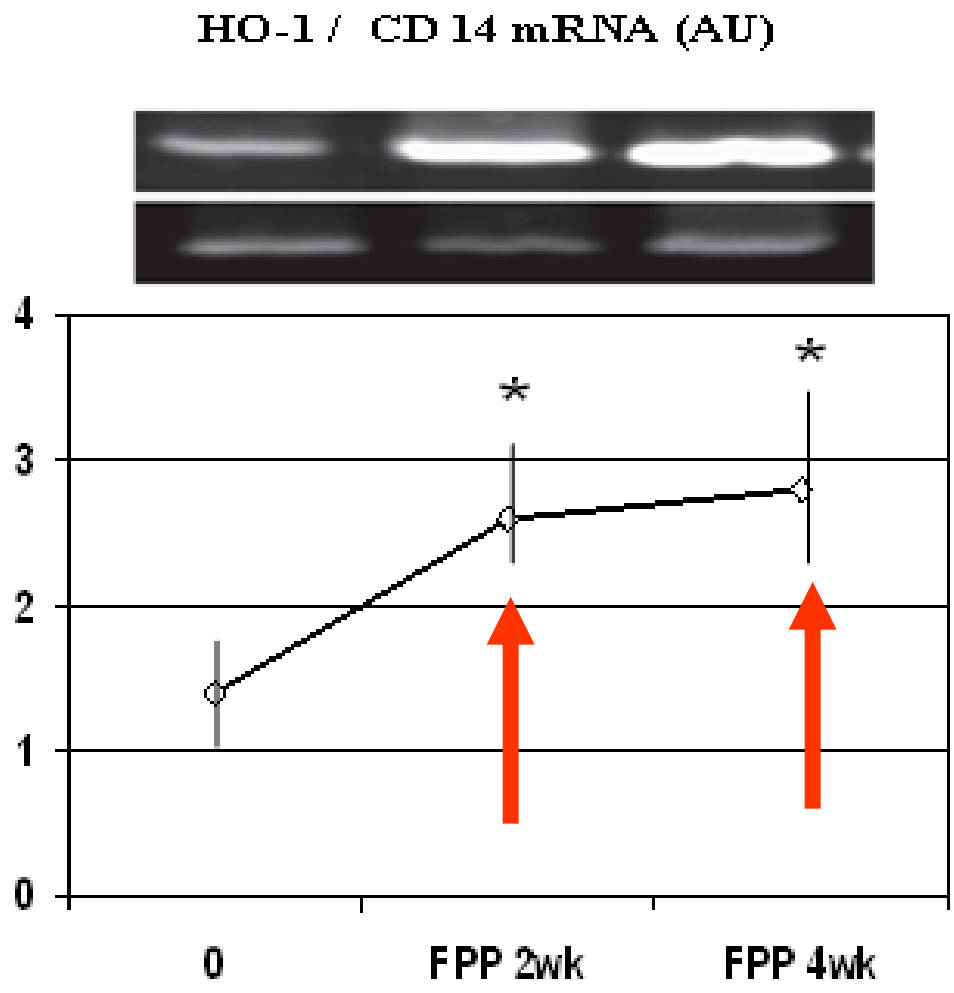






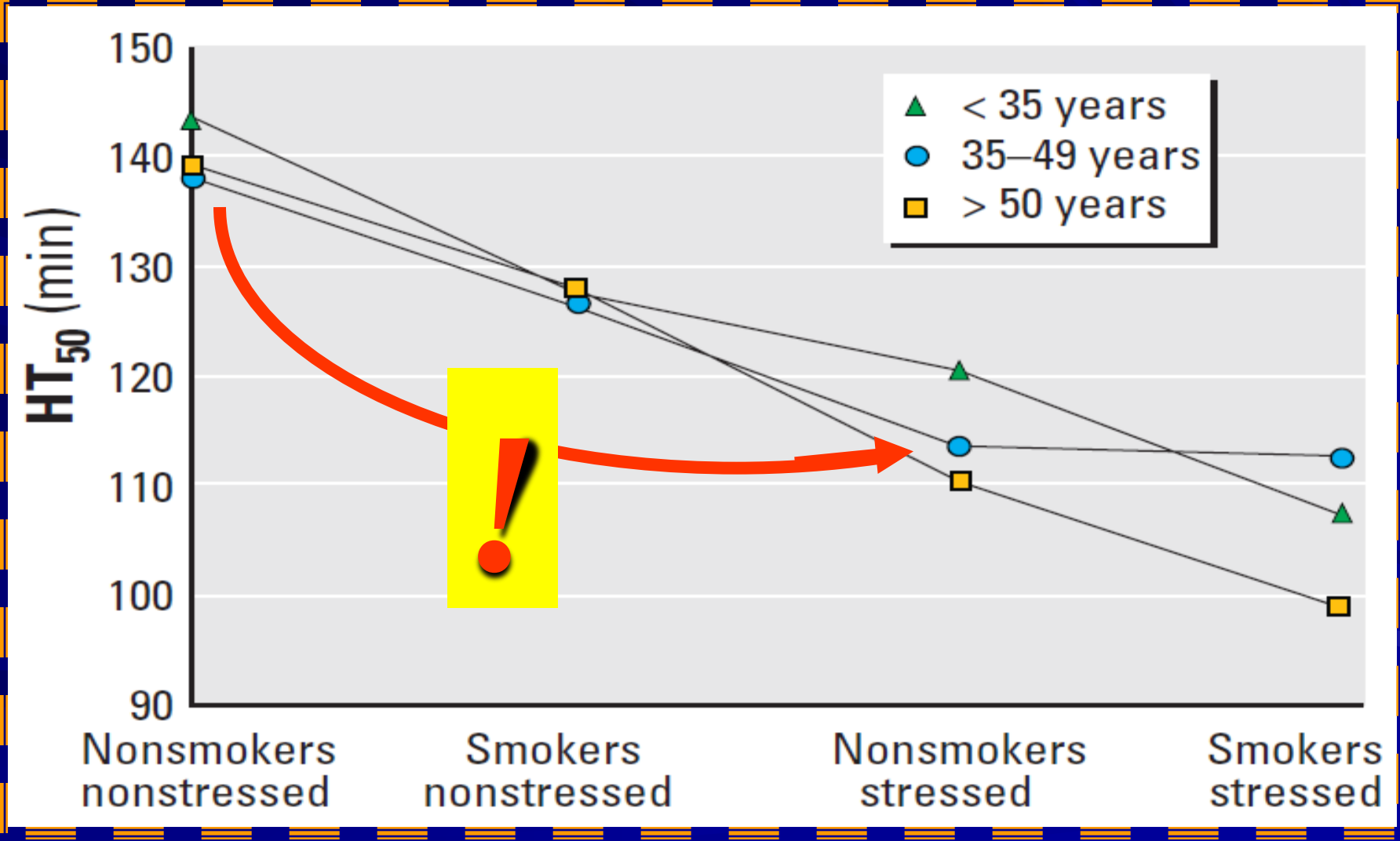
CAN WE IMPROVE OUR ADAPTATIVE RESPONSE THROUGH "GOOD" GENES ACTIVATION?



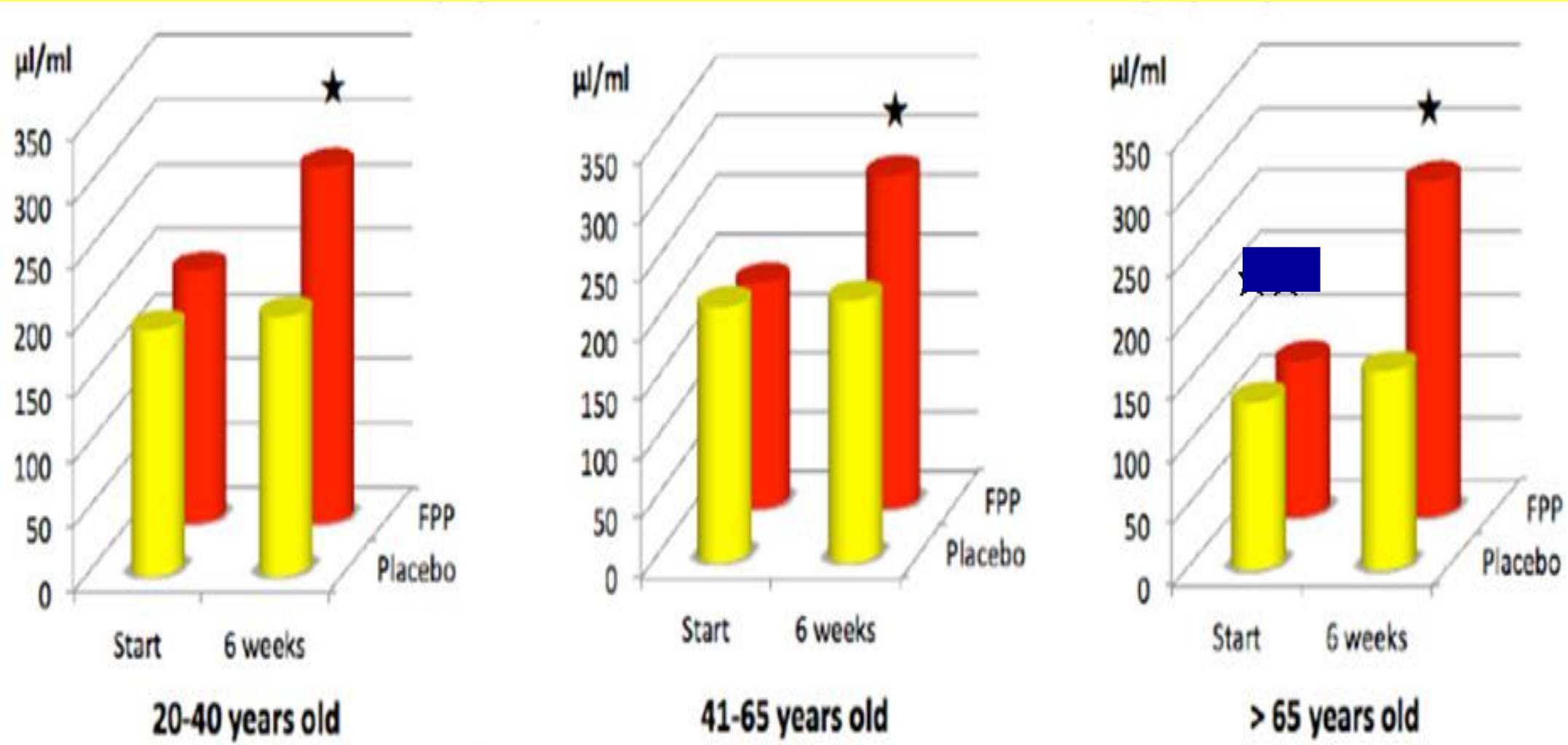


Assessment of Lifestyle Effects on the Overall Antioxidant Capacity of Healthy Subjects

Jean-François Lesgards, *Environ Health Perspect* 110:479–487 (2002)

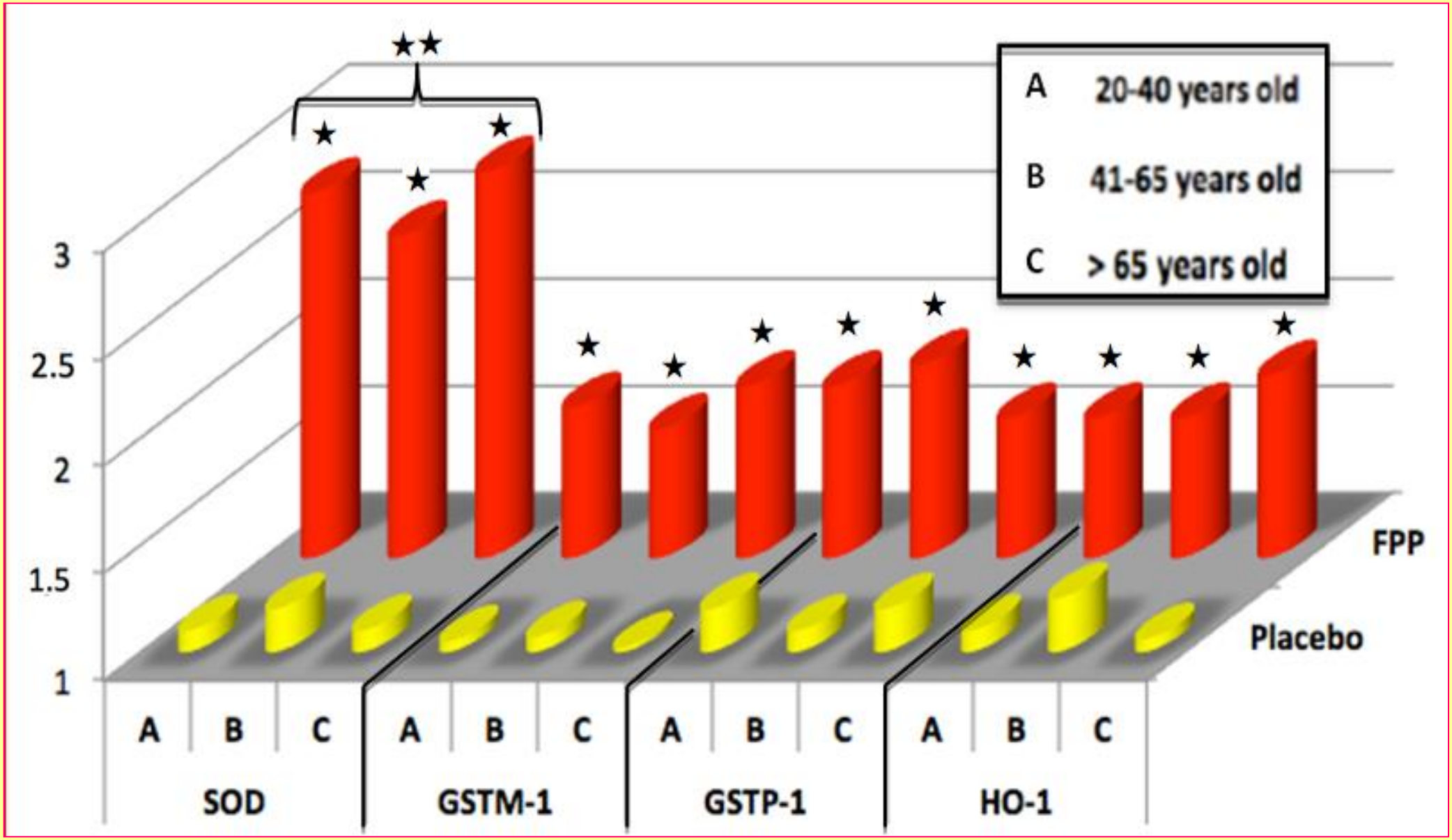


SALIVARY SECRETION OF IgA: *EFFECT OF FPP SUPPLEMENTATION IN DIFFERENT HEALTHY AGE GROUPS*



F. Marotta et al, JBRHA, 2012

Potenziation of Phase II detoxification and Antioxidant Gene Expression in epithelial cells from nasal lavage: *effect of FPP*



F. Marotta et al, JBRHA, 2012

Can Nutrition and Nutraceutical Supplementation affect gene expression of our genes?

Pincemail J et al. **Dept. Cardiovascular Surgery, Diabetology, University of Liege, Belgium**

600g of fruit & vegetables/day for 2 months to diabetics :

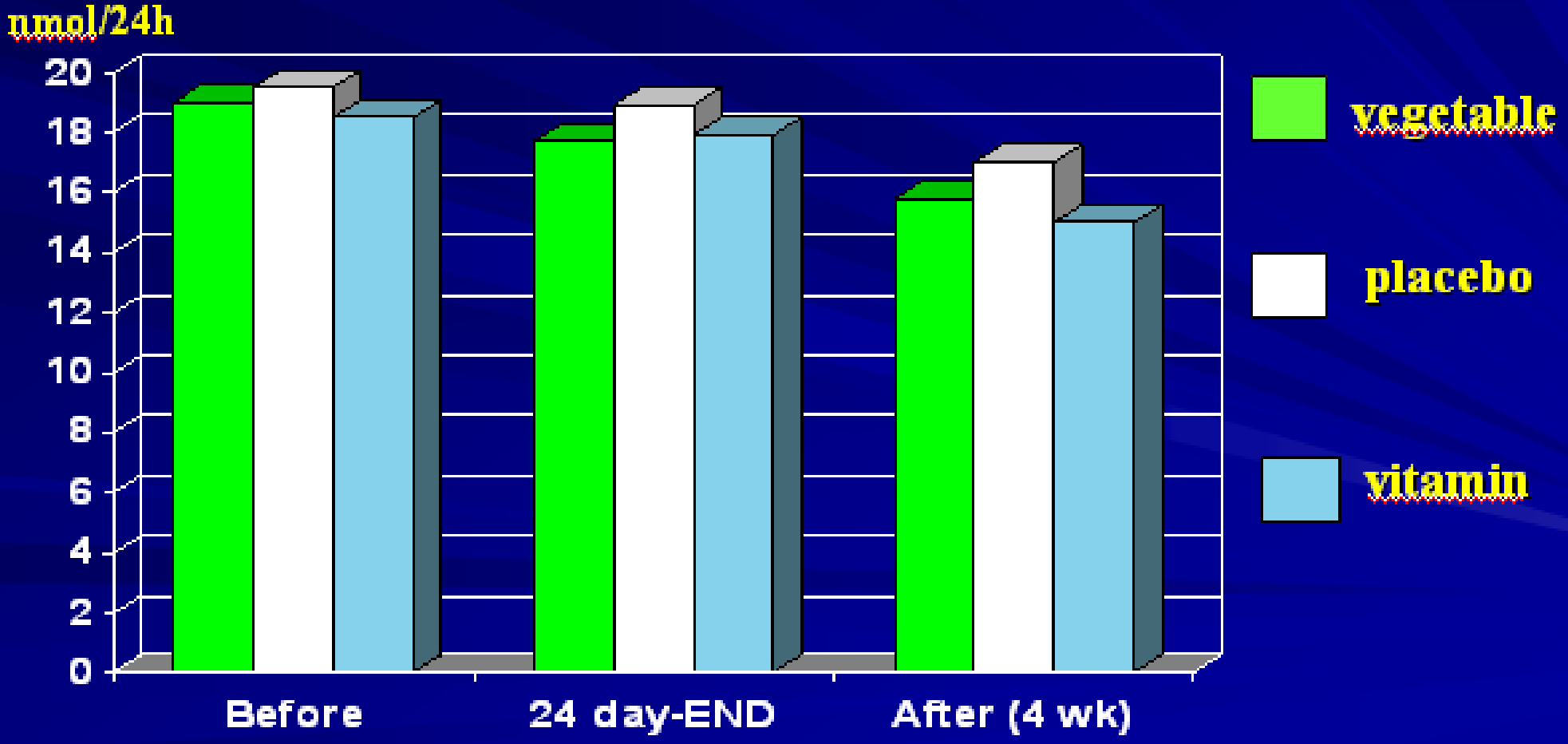
a) Reduction of some lipoperoxidation markers

b) No variation of plasma level of vitamin C and β-carotene *!!*

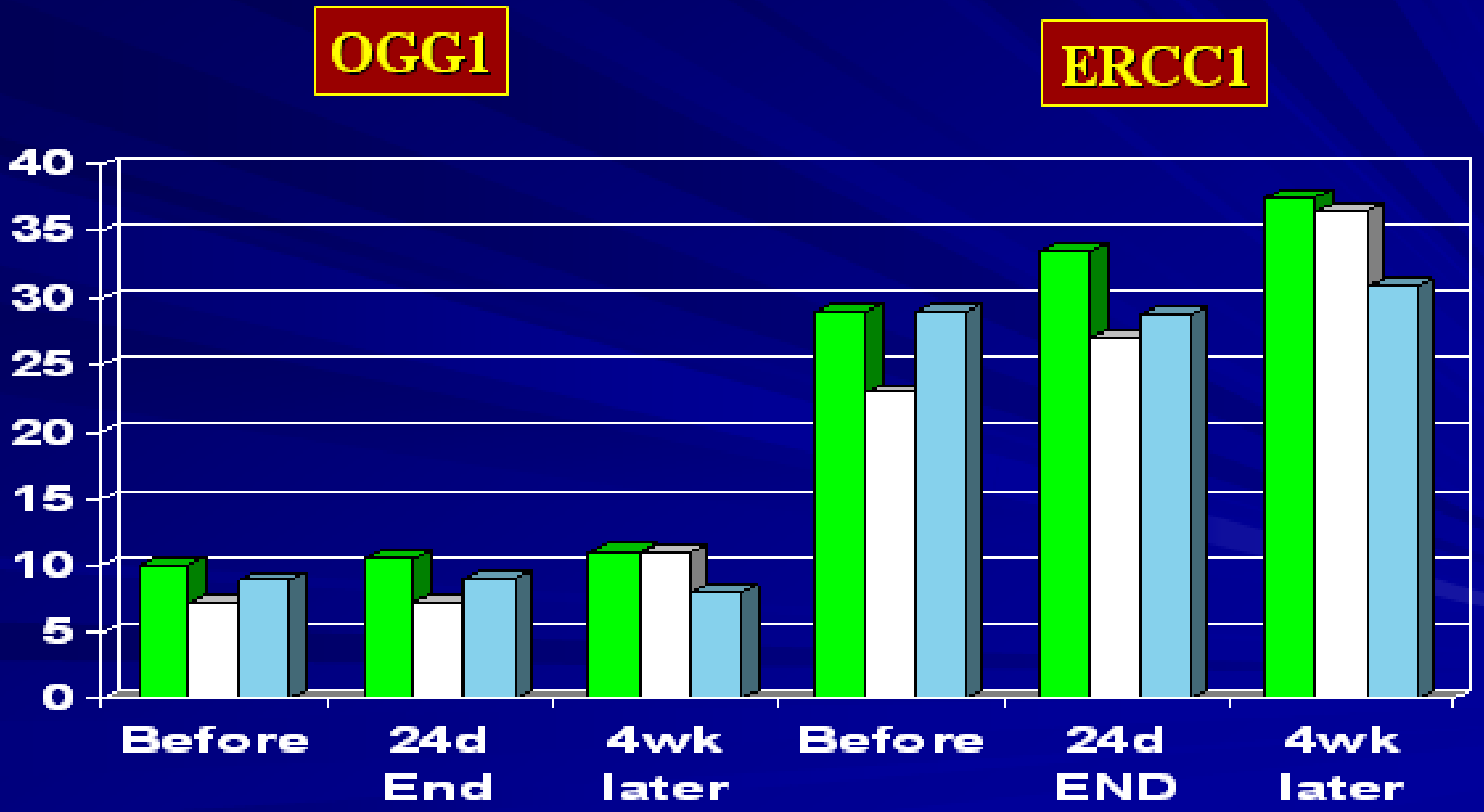
No Effect of 600 Grams Fruit and Vegetables Per Day on Oxidative DNA Damage and Repair in Healthy Nonsmokers[1]

Canc Epidem Prev, 2003

Level of 24 h urinary 8-oxodG excretion (mean and SD)



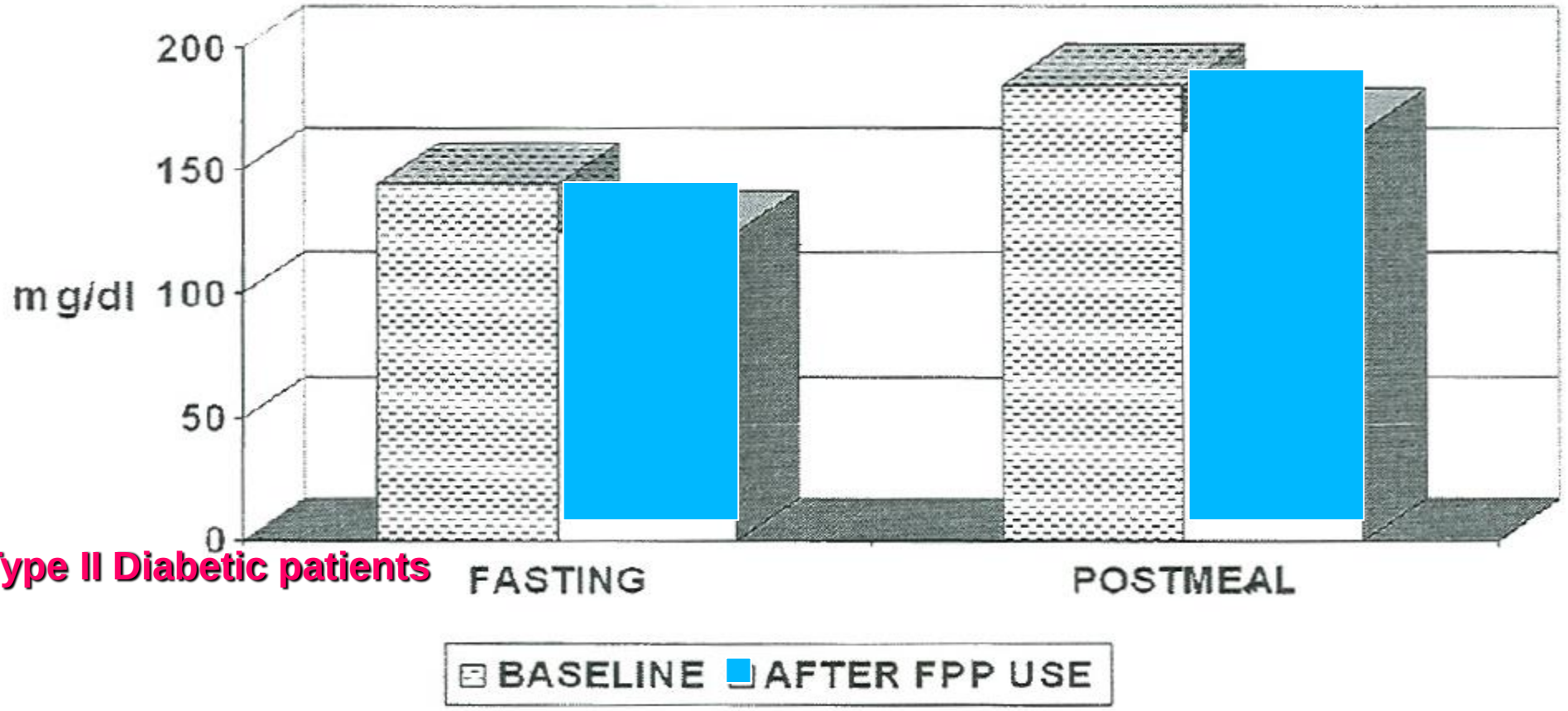
Expression of *OGG1* and *ERCC1* mRNA relative to 18S in leukocytes isolated from whole blood



Liver disease & Diabetes

Plasma glucose level decreases as collateral effect of fermented papaya preparation use

Danese et al La Clin Ter, 2006

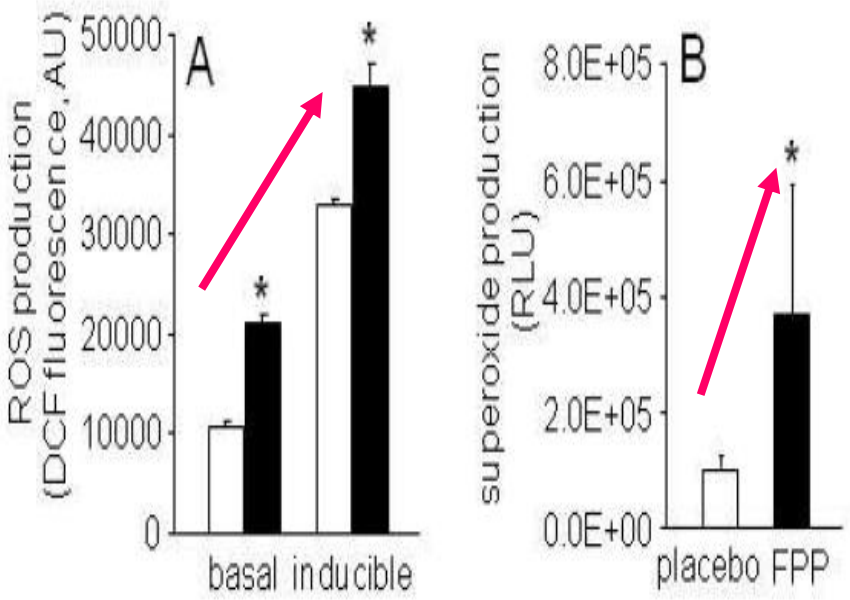


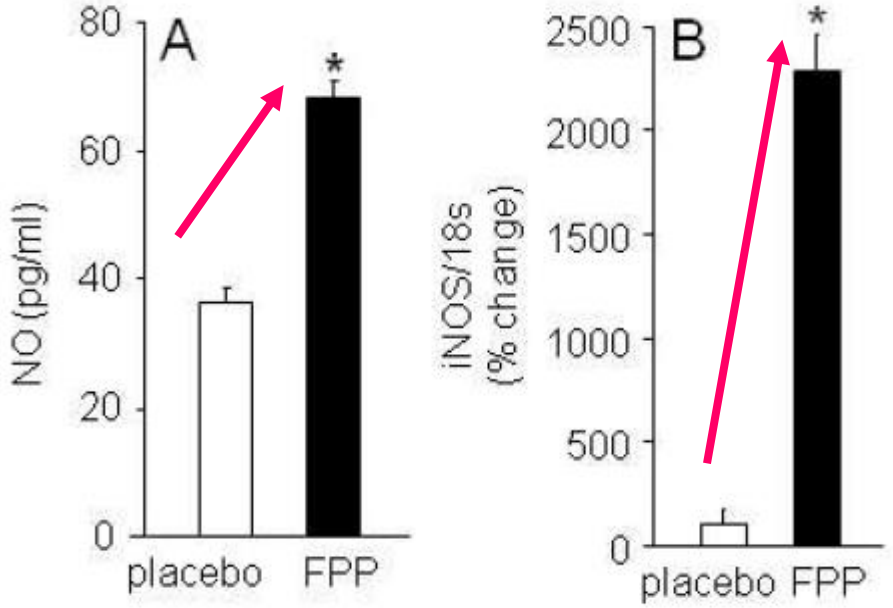
Type II Diabetic patients

Liver disease & Diabetes

Improved function of diabetic wound-site macrophages and accelerated wound closure in response to oral supplementation of a fermented papaya preparation

Antiox & Redox Sign, 2009





Improved function of diabetic wound-site macrophages and accelerated wound closure in response to oral supplementation of a fermented papaya preparation

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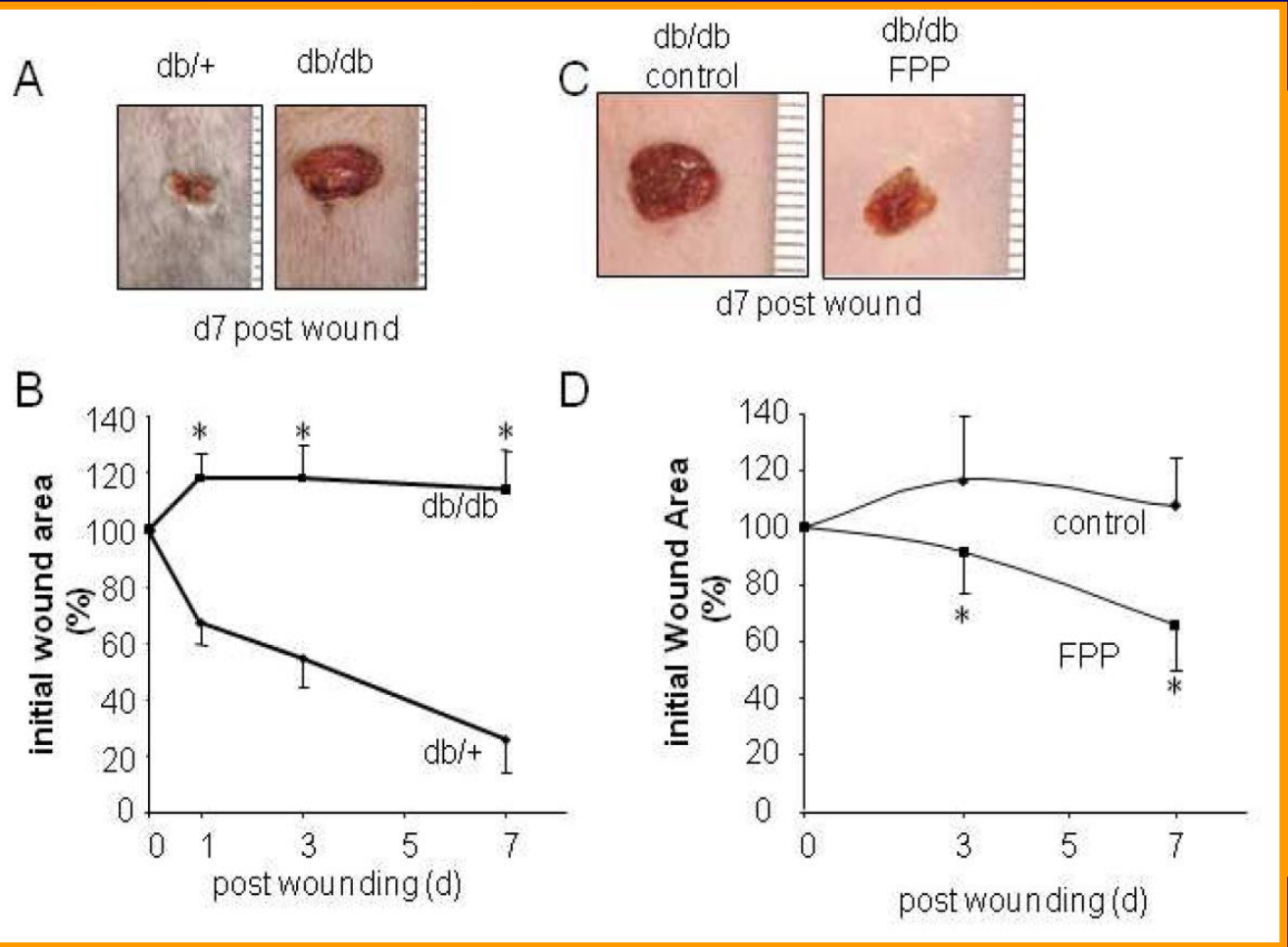
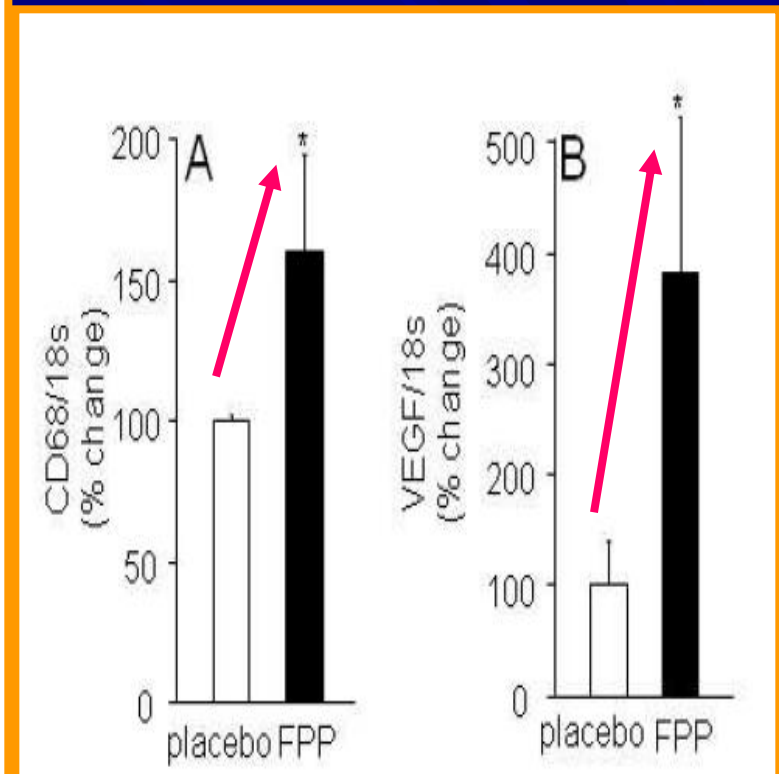


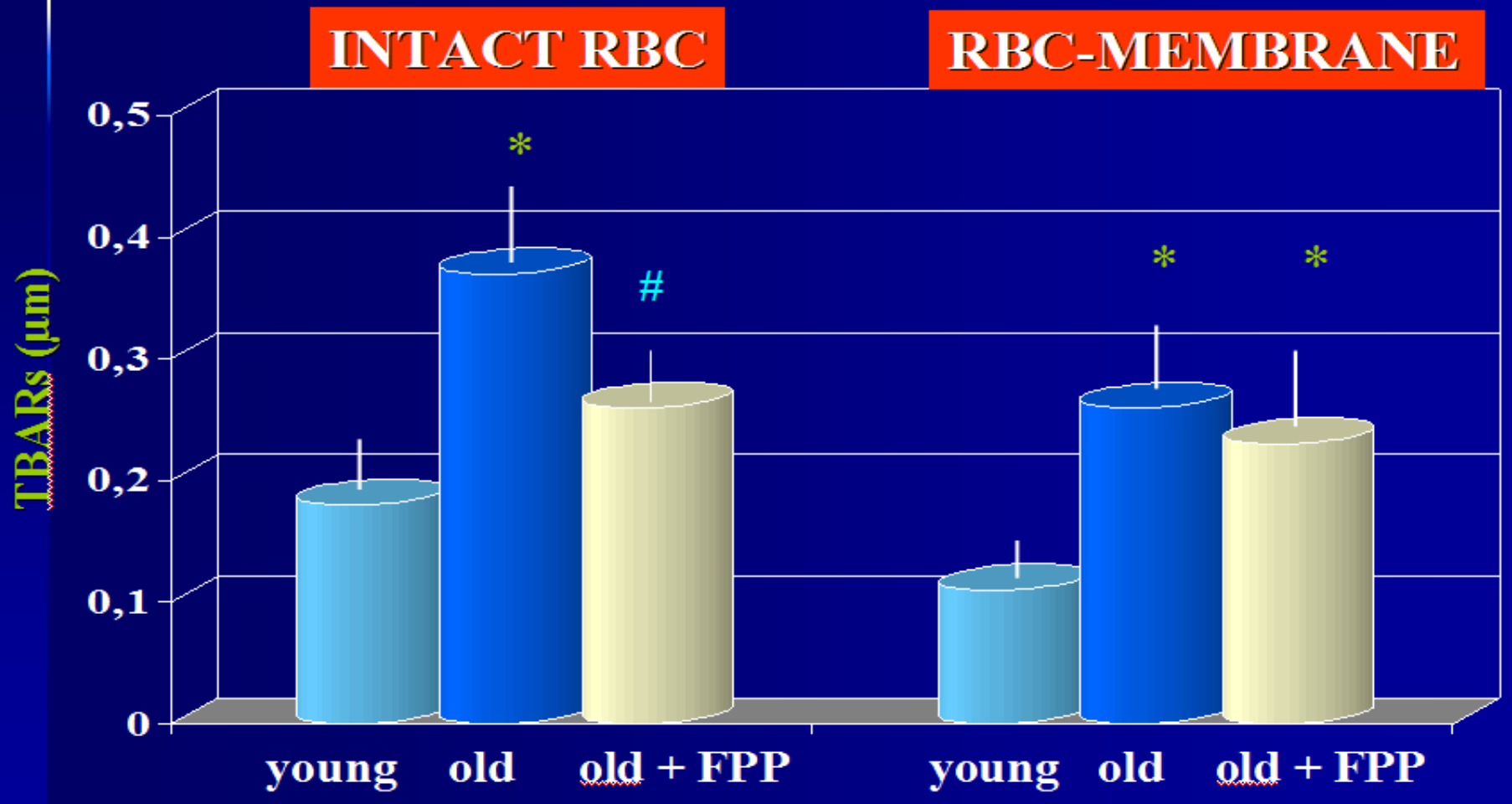


Age-related susceptibility of erythrocytes to oxidative stress: A preliminary nutraceutical approach

F. Marotta, K Pavasuthipaisit, C. Yoshida, F. Albergati, P. Marandola

ReGenera Res. Group for Aging Intervention, Milano
Institute of Science & Technology, Mahidol University, Thailand
ORI Bioscience Lab., Gifu, Japan

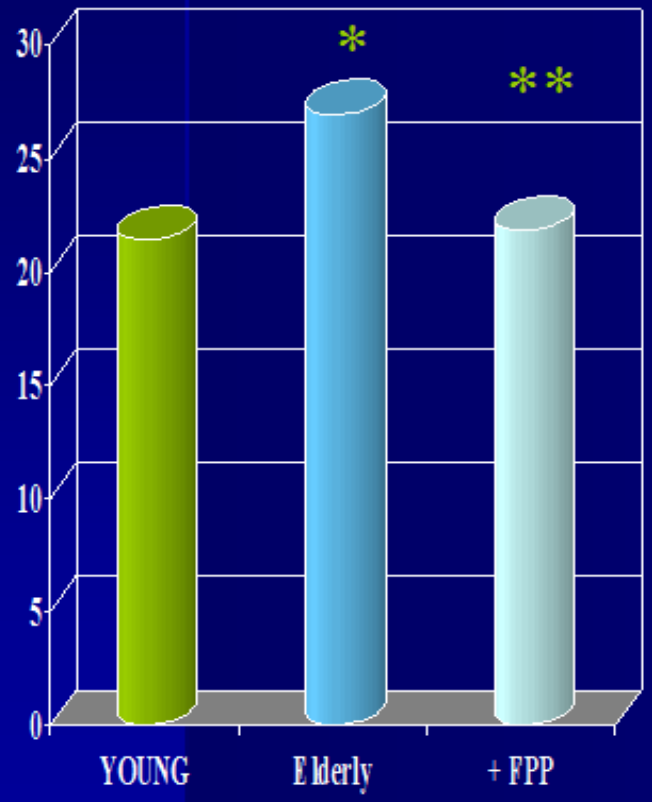
Generation of TBARs in Erythrocyte in vitro: Effect of Aging and FPP Administration



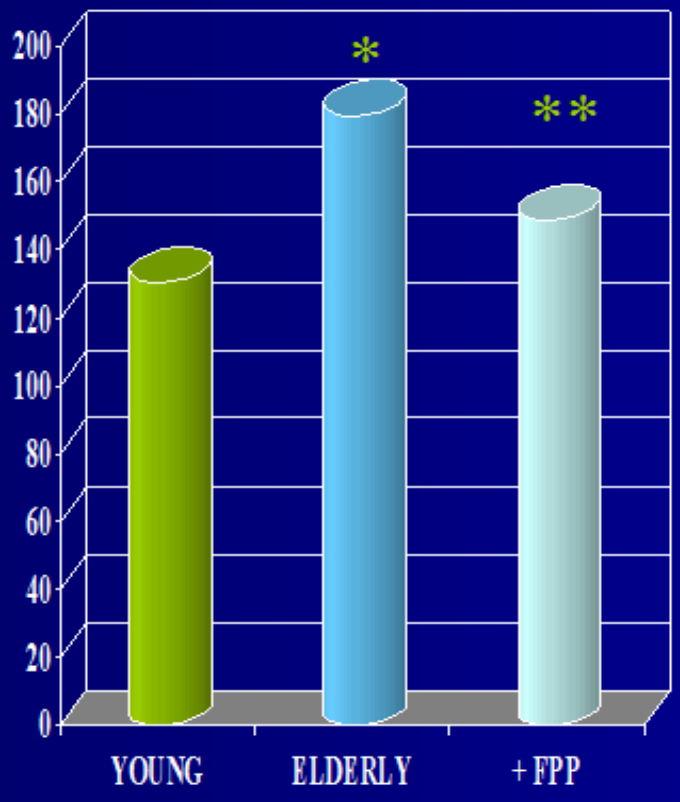
Marotta et al.Rejuvenation Res. 2006

Peroxidation Profile in young and elderly subjects: role for a nutraceutical?

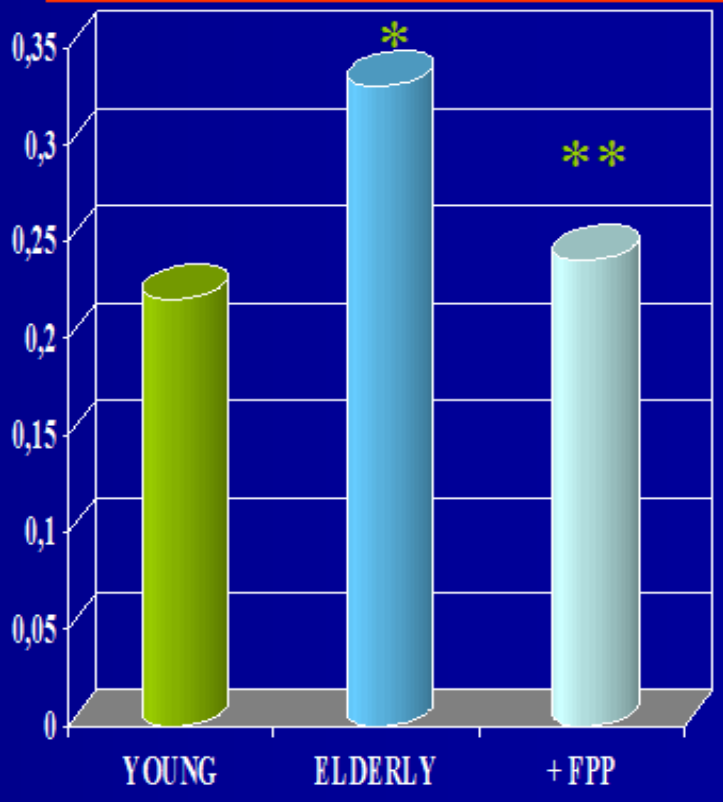
Hydroperoxides
(H_2O_2 mg/dl of plasma)



Plasma Antiox. Capacity
(lag time needed: min)

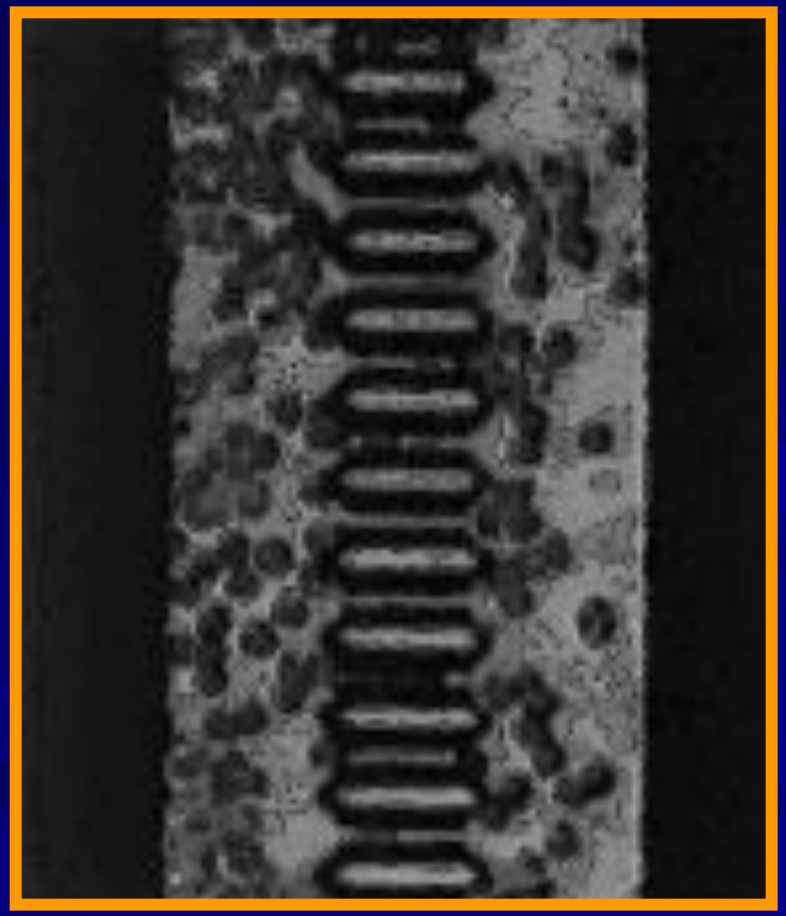


Fluoresc. Anisotropy
(rs= Iv-Ih/Iv+2Ih)

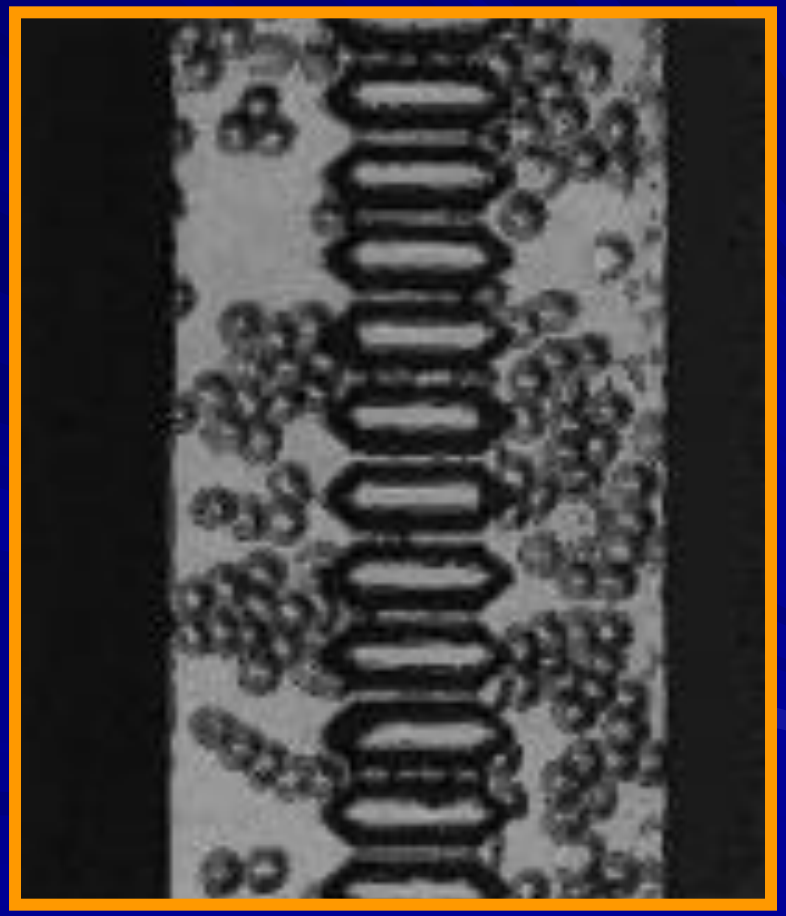


Marotta et al.Rejuvenation Res. 2006

Micro-Channel array Flow Analyzer: old RBC deformability under placebo and FPP treatment



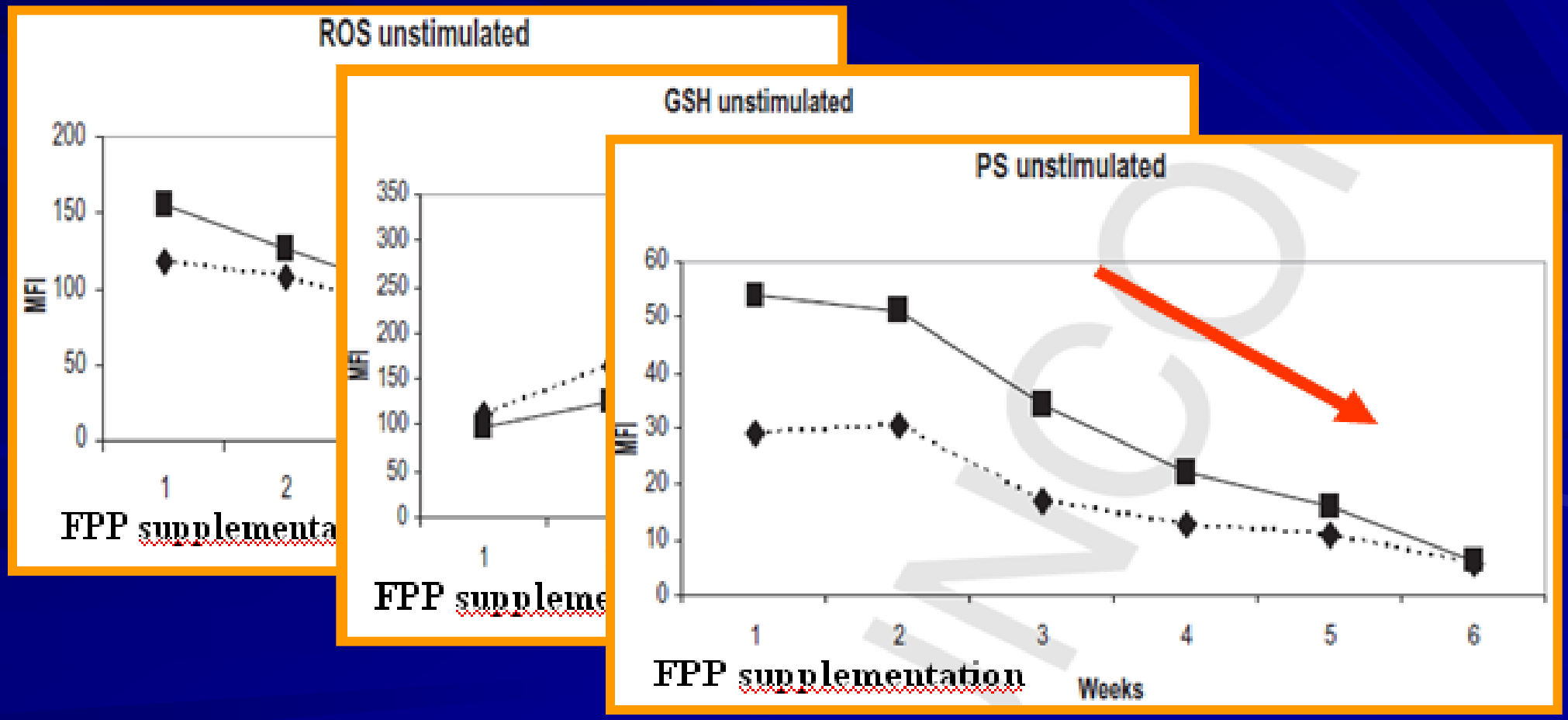
Placebo



FPP

Amelioration of Oxidative Stress in RBC from Patients with β-thalassemia Major and Intermedia and E-β-thalassemia Following Administration of Fermented Papaya Preparation

Eliezer A. Rachmilewitz *Phytother. Res.* 2010

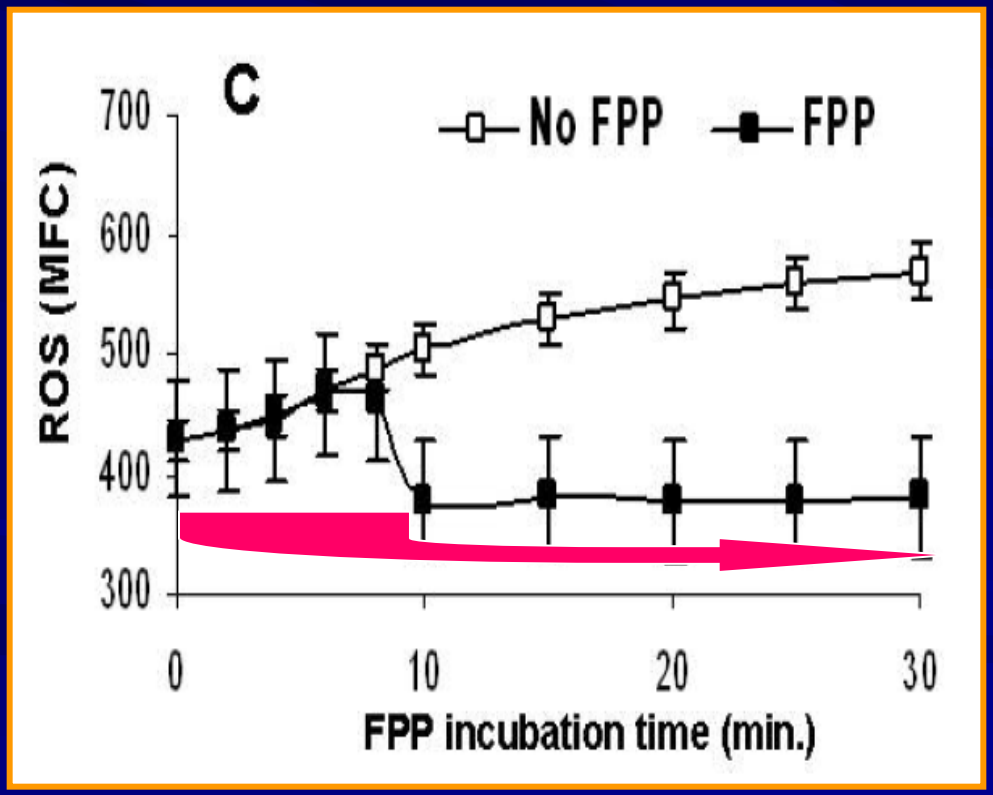


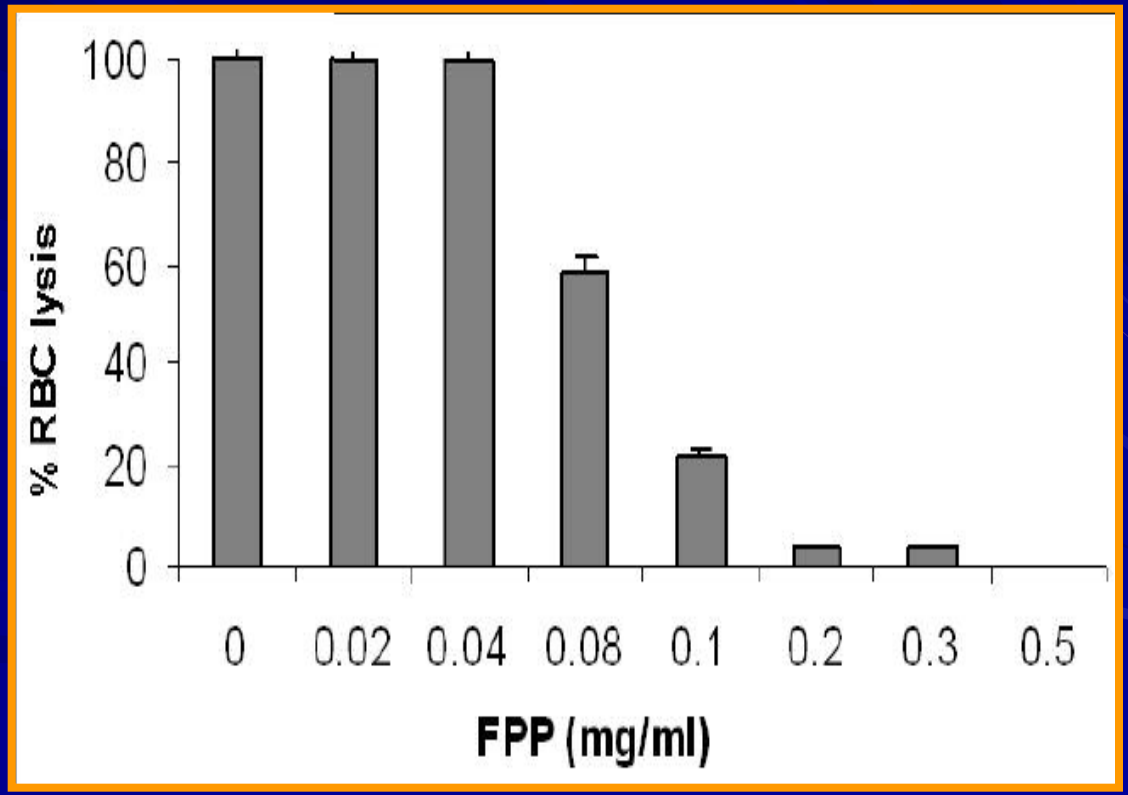
Fermented Papaya Preparation as Redox Regulator in Blood Cells of β-Thalassemic Mice and Patients

Phytother. Res. **22**, 820–828 (2008)

Johnny Amer[1], Ada Goldfarb[1], Eliezer A. Rachmilewitz[2] and Eitan Fibach[1]

[1]Department of Hematology, Hadassah – Hebrew University Medical Center, Jerusalem, Israel
[2]Department of Hematology, The E. Wolfson Medical Center, Holon, Israel





Overcoming Barriers: Antioxidants for Steatosis and Metabolic Syndrome

- Few studies evaluating impact of improved oxidation on SVR
- Phase I trial: antioxidant cocktail* given for 20 weeks (N = 50)[1]
  - ALT normalization: 44%; ≥ 2-point improvement in HAI score: 36%
- Ursodeoxycholic acid given with HCV therapy did not improve SVR rates (N = 52)[2]
- Patients received IFN or IFN + vitamin E for 24 weeks (N = 24)[3]
  - Greater response, reduction in viral load with vitamin E

*__Cocktail__ included glycyrrhizin, schisandra, silymarin, ascorbic acid, lipoic acid, L-glutathione, alpha-tocopherol, glycyrrhizin, ascorbic acid, L-glutathione, and B-complex

1. Melhem A, et al. J Clin Gastro. 2005;39:737-742. 2. Tanaka K, et al. J Gastroenterol Hepatol. 1996;11:1155-1160. 3. Look MP, et al. Antiviral Res. 1999;43:113-122.

Overcoming Barriers: Weight Loss for Steatosis and Metabolic Syndrome

- 3-month weight-loss program resulted in reduced steatosis and liver enzymes, improved fibrosis (N = 19)[1]
  - Mean weight loss: 5.9 kg
  - Mean fasting insulin reduced ($P < .002$)
  - Reduced steatosis ($P < .005$) and Knodell fibrosis score ($P = .04$)
- 3-month low calorie diet (n = 15) vs controls (n = 17) before pegIFN/RBV therapy in GT 1 patients[2]
  - Reduced insulin resistance in weight-loss group
  - Response 60% for weight-loss group vs 17.6% for controls

1. Hickman IJ, et al. Gut. 2002;51:89-94. 2. Tarantino G, et al. Gut. 2006;55:585-586.

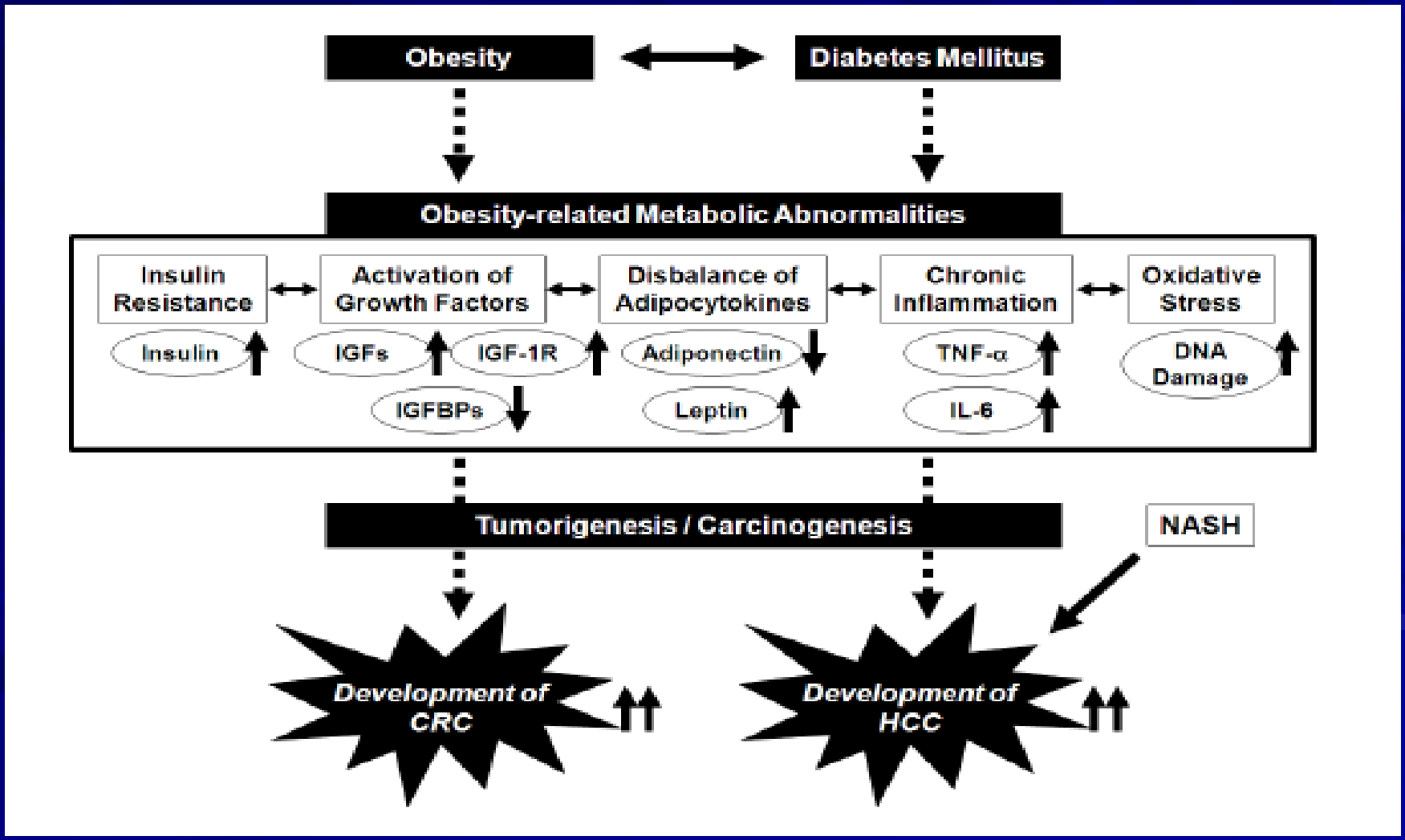
Overcoming Barriers: Insulin Sensitizers for Steatosis, Metabolic Syndrome

- Thiazolidinediones: pioglitazone, rosiglitazone
  - Pioglitazone improved insulin sensitivity through SOCS 3 suppression in mouse model[1]
  - 55 NASH patients with impaired glucose metabolism received hypocaloric diet + pioglitazone or placebo for 6 months[2]
    - Diet + pioglitazone superior at improving glucose tolerance, ALTs
    - Diet + pioglitazone led to greater drop in liver fat content, improved steatosis, reduced inflammation
- Biguanide: metformin reduces glucose production in liver
  - Effects in NASH less robust than thiazolidinediones[3]

1. Kanatani Y, et al. Diabetes. 2007;56:795-803. 2. Belfort R, et al. N Eng J Med. 2006,355:2297-2307. 3. Harrison SA. J Clin Gastroenterol. 2006;40:68-76.

Nutraceutical Approach for Preventing Obesity-Related Colorectal and Liver Carcinogenesis

Int. J. Mol. Sci. **2012**,



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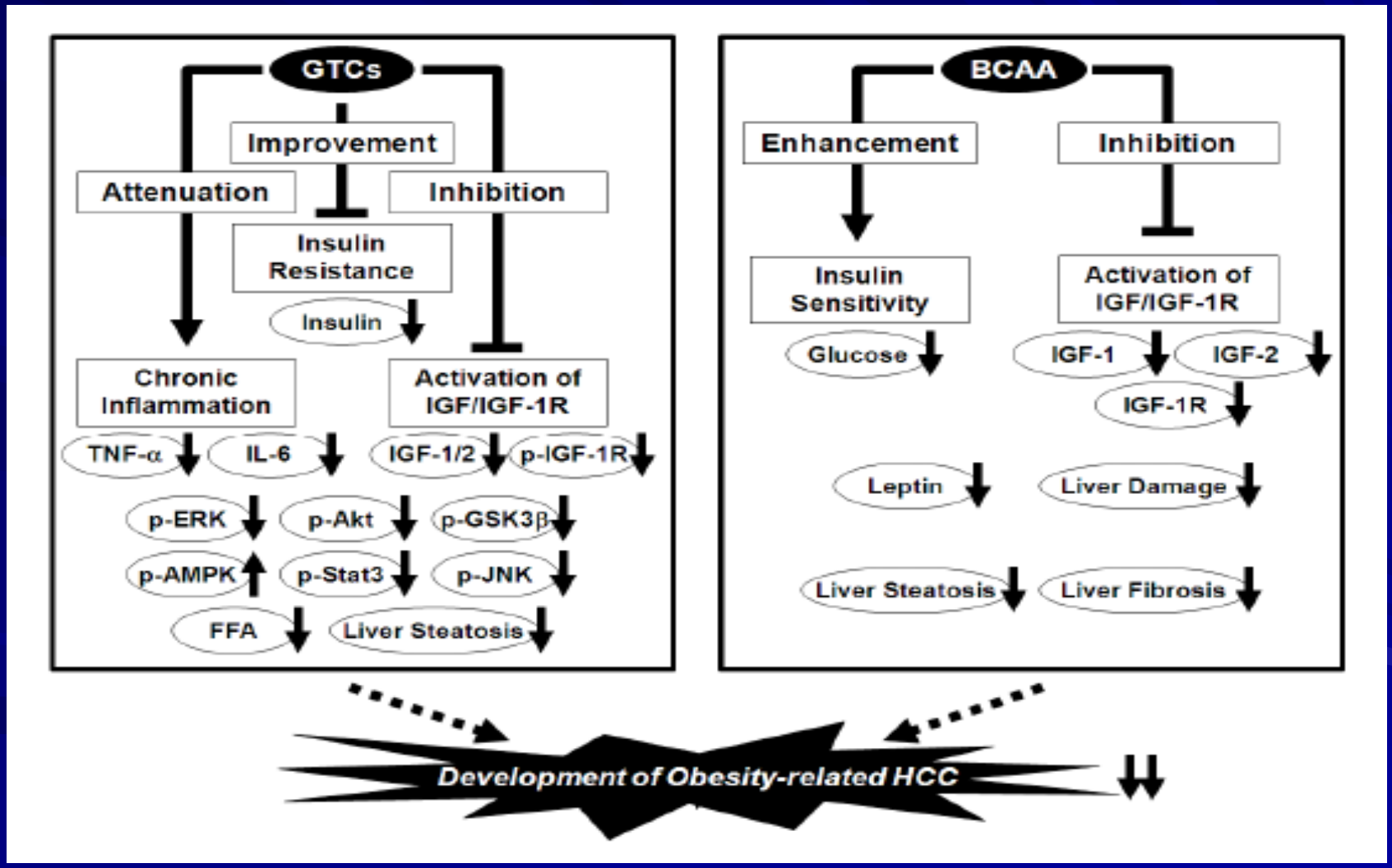

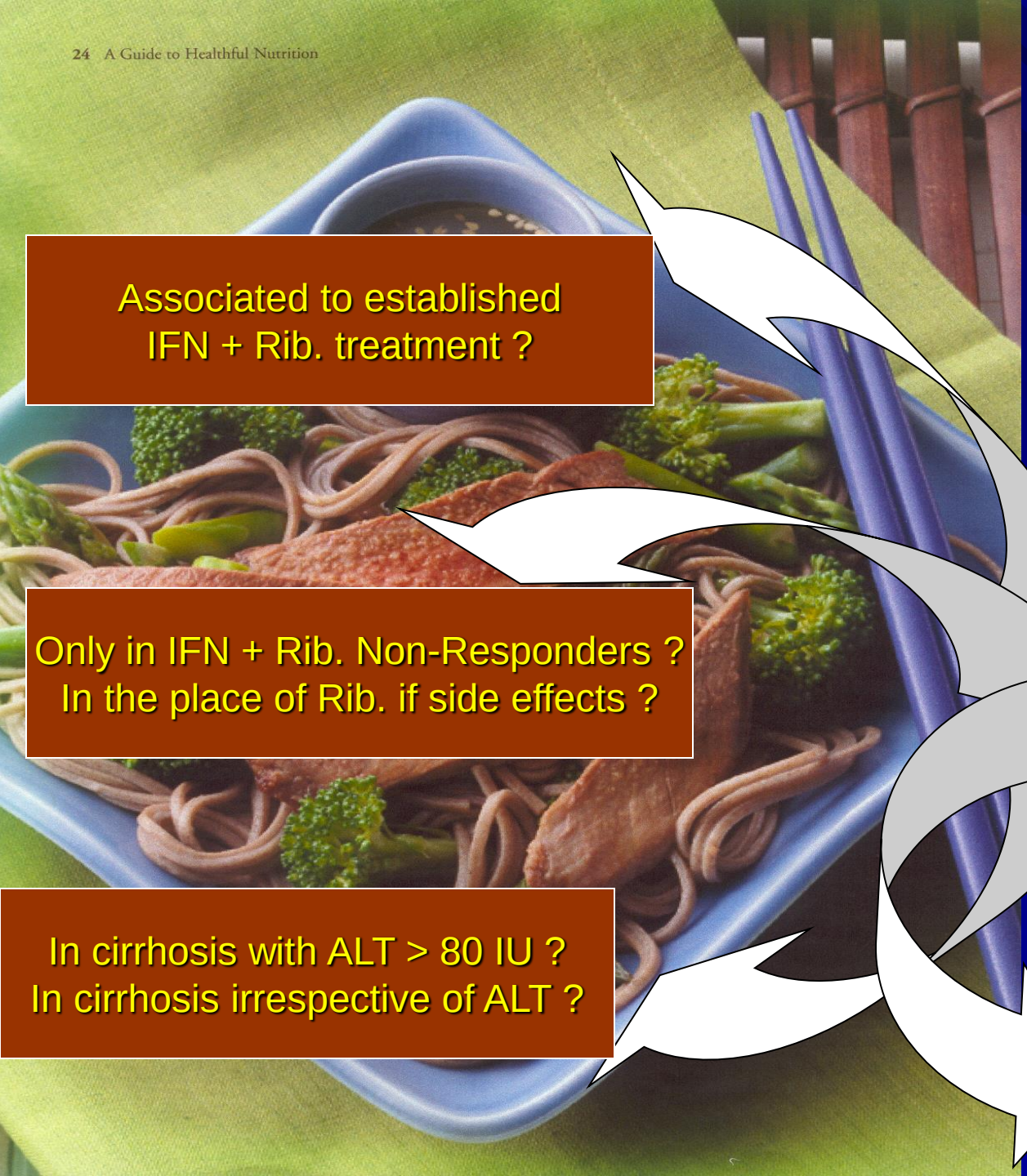

- Macronutients-CHD, fats, and proteins
- Micronutrients-vitamins and minerals
- Dietary fiber

Associated to established IFN + Rib. treatment ?

Only in IFN + Rib. Non-Responders ?
In the place of Rib. if side effects ?

In cirrhosis with ALT > 80 IU ?
In cirrhosis irrespective of ALT ?

- ***EB-Phytochemicals (FPP,***
- ***high-quality silibin,***
- ***modified-YHK)***

In associated NASH Tx

Post-op liver surgery ? etc.