Roberto Castello
Endocrinology and Metabolic Disease
Department, Azienda Ospedaliera, Verona, Italy

"Cascade"/Reflex Thyroid Testing

The endocrinologist approach

THYROID
Volume 13, Number 1, 2003

Preface

Thyroid Testing for the New Millenium

Carole A. Spencer, Ph.D., F.A.C.B.
Professor of Medicine
University of Southern California

TSH reference intervals

- 95% limits of the log-transformed values of at least 120 rigorously screened euthyroid volunteers with:
- No detectable thyroid autoAb
- No history of thyroid dysfunction
- No visible or palpable goiter
- No medications (except estrogen)

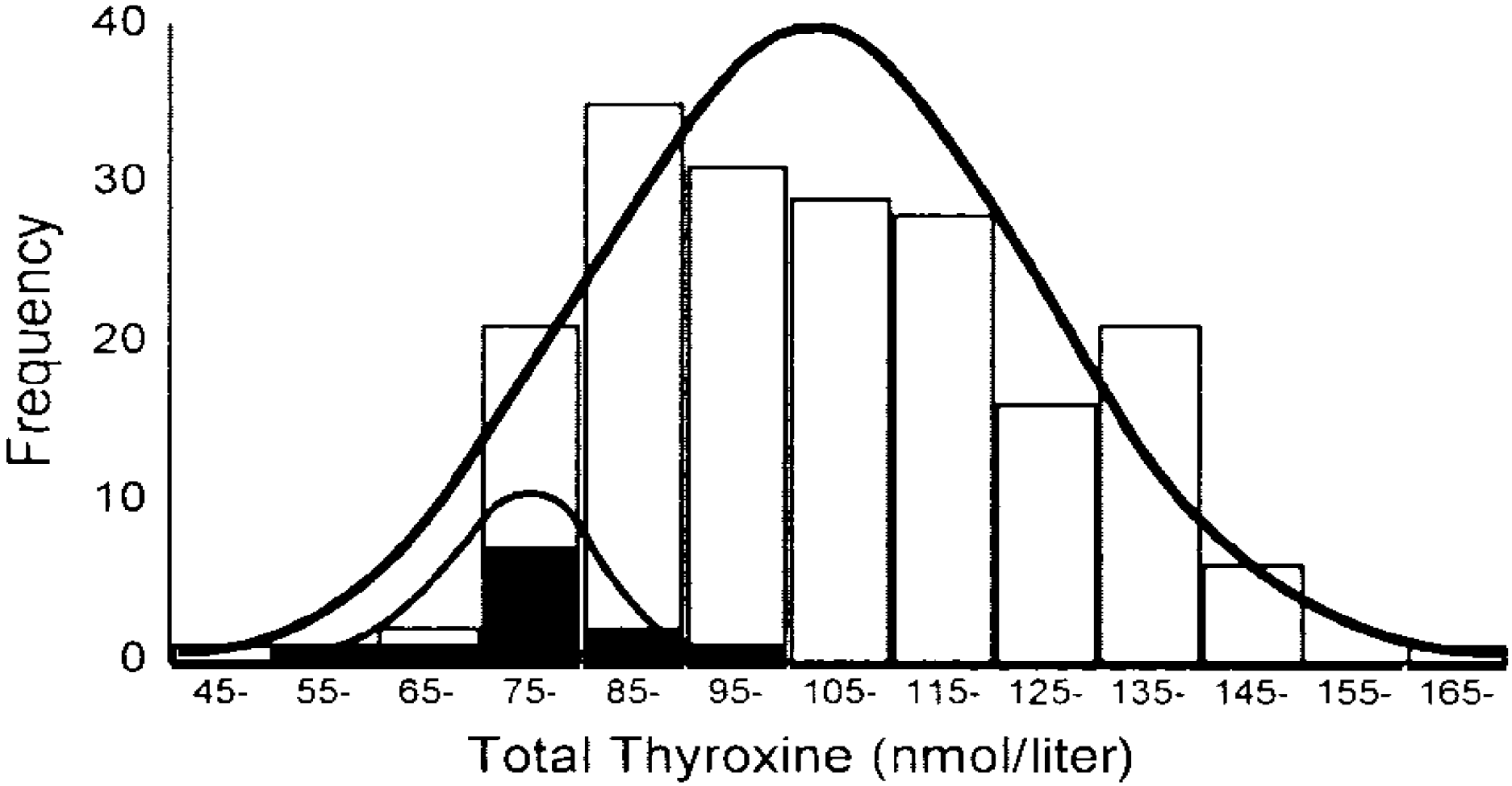
0013-7227/02/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 87(3):1068–1072

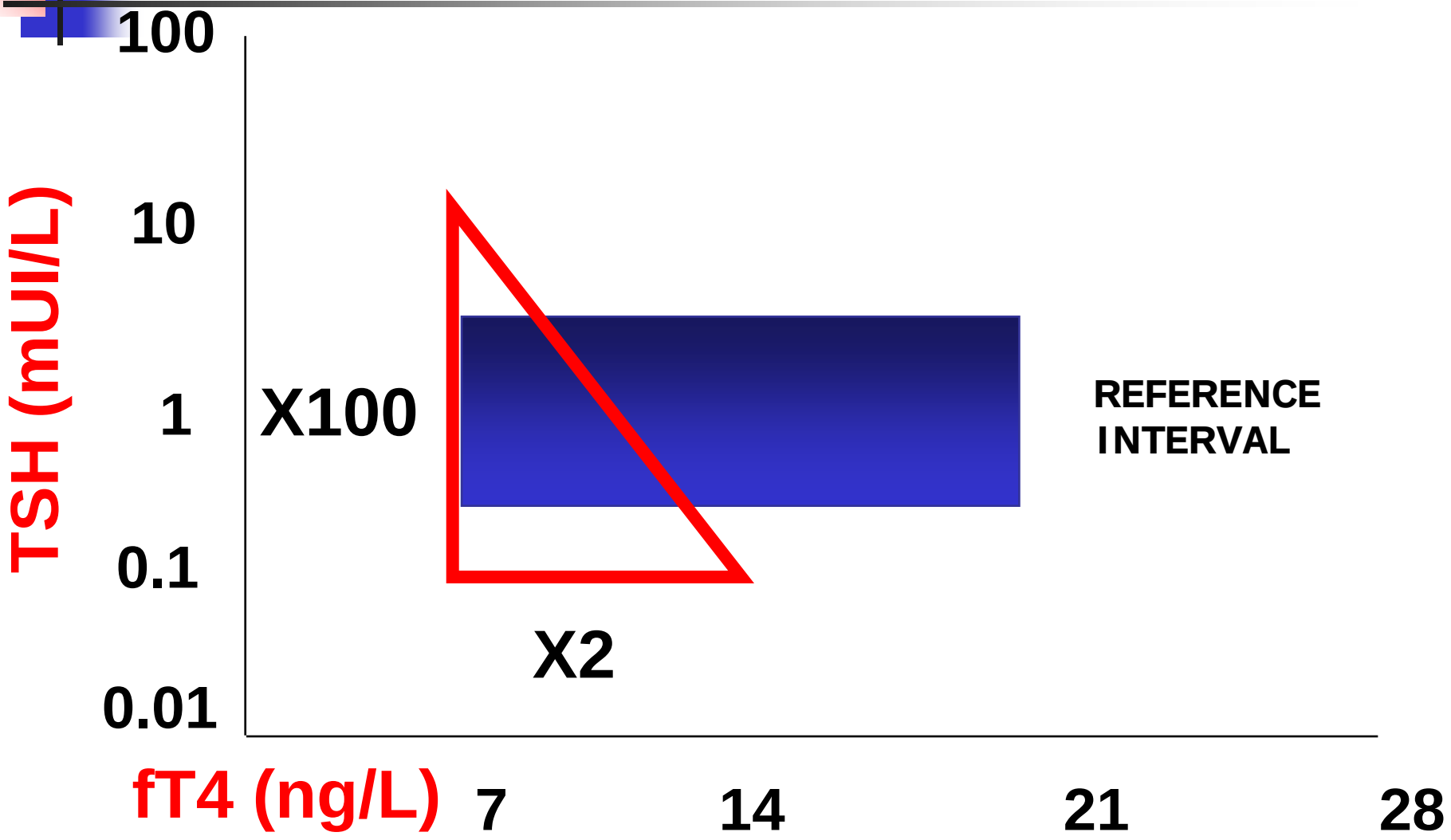
Narrow Individual Variations in Serum T_4 and T_3 in Normal Subjects: A Clue to the Understanding of Subclinical Thyroid Disease

STIG ANDERSEN, KLAUS MICHAEL PEDERSEN, NIELS HENRIK BRUUN, AND PETER LAURBERG

Department of Endocrinology (S.A., N.H.B., P.L.) and Clinical Biochemistry (K.M.P.), Aalborg Hospital, Aalborg, Denmark DK-9000



Log vs linear change



AACE Clinical Practice Guidelines

for the Diagnosis and Management of Thyroid Nodules 1996

- A sensitive TSH assay should be done
- T4 and T3 levels may be helpful
- Anti-TPO and antiTg levels are helpful

AMERICAN ASSOCIATION OF CLINICAL ENDOCRINOLOGISTS
AND ASSOCIAZIONE MEDICI ENDOCRINOLOGI
MEDICAL GUIDELINES FOR CLINICAL PRACTICE FOR THE
DIAGNOSIS AND MANAGEMENT OF THYROID NODULES

2.5.1. Assessment of Thyroid Function

Measurement of the serum TSH concentration is the single most useful laboratory test in the initial evaluation of thyroid nodules because of the high sensitivity of the before TSH assay

ENDOCRINE PRACTICE Vol 12 No. 1 January/February 2006

THYROID
Volume 14, Number 10, 2004

Serum Thyrotropin is a Better Predictor of Future Thyroid Dysfunction Than Thyroid Autoantibody Status in Biochemically Euthyroid Patients with Diabetes: Implications for Screening

Roderick E. Warren,[1] Petros Perros,[2] Moffat J. Nyirenda,[1] and Brian M. Frier[1]

TSH “FIRST TESTING” STRATEGY

Management of the Nontoxic Multinodular Goiter: A North American Survey

STEEN J. BONNEMA, FINN N. BENNEDBÆK, PAUL W. LADENSON, AND LASZLO HEGEDÜS

	ATA (n = 140)		ETA (n = 120)		*P* value
No. of blood tests[a]	3 (1–7)		4 (1–11)		<0.001
	n	%	n	%	
TSH	140	100.0	120	100.0	NS
Thyroid peroxidase antibodies	86	61.4	78	65.0	NS
Free T_4/index	76	54.3	89	74.2	<0.001
Tg antibodies	48	34.3	59	49.2	<0.02
Total T_3	32	22.9	28	23.3	NS
Total T_4	30	21.4	20	16.7	NS
Microsomal antibodies	24	17.1	12	10.0	NS
Free T_3 index	16	11.4	52	43.3	<0.001
Sedimentation rate	12	8.6	16	13.3	NS
Calcitonin	5	3.6	38	31.7	<0.001
Tg	3	2.1	9	7.5	NS
TSH-R antibodies	0	0.0	9	7.5	<0.005

J Clin Endocrinol Metab, January 2002, 87(1):112–117

Clinical Endocrinology (2003) 58, 20–21

Commentary

First-line thyroid function tests – TSH alone is not enough

G. J. Beckett and A. D. Toft

University Department of Clinical Biochemistry and,
Endocrine Clinic, Royal Infirmary, Edinburgh, Scotland

(Received 20 March 2002; returned for revision 15 April 2002;
finally revised 1 May 2002; accepted 2 September 2002)

The Evidence for a Narrower Thyrotropin Reference Range Is Compelling

Leonard Wartofsky and Richard A Dickey

TABLE 1. Possible reasons for elevated TSH values

Hashimoto thyroiditis
Medications (steroids; dopamine; iodine; amiodarone, *etc*.)
Family history of thyroid disease suggesting latent thyroid disorder
Nonthyroidal illness
Pregnancy[a]
Recovery phase of subacute thyroiditis
Other autoimmune conditions
Heterophilic antibodies
Bioinactive TSH secretion; TSH resistance syndromes
Germline mutations of TSH receptor
Thyroid hormone resistance
TSH-producing pituitary tumor

(*J Clin Endocrinol Metab* 90: 5483–5488, 2005)

0021-972X/00/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Circulating Thyrotropin Bioactivity in Sporadic Central Hypothyroidism*

LUCA PERSANI, ELISABETTA FERRETTI, STEFANO BORGATO, GIOVANNI FAGLIA, AND PAOLO BECK-PECCOZ

Institute of Endocrine Sciences, University of Milan, Istituto Auxologico Italiano Instituto di Ricovero e Cura a Carattere Scientifico (L.P.), and Ospedale Maggiore Instituto di Ricovero e Cura a Carattere Scientifico (S.B., G.F., P.B.-P.), 20145 Milan; and Department of Clinical Science, Endocrinology, University of Rome La Sapienza (E.F.), 00100 Rome, Italy

Clinical Chemistry 48:11
2023–2029 (2002)

Wrong Biochemistry Results: Two Case Reports and Observational Study in 5310 Patients on Potentially Misleading Thyroid-stimulating Hormone and Gonadotropin Immunoassay Results

Adel A.A. Ismail,[1,2*] Paul L. Walker,[1] Julian H. Barth,[2] Kryzsztof C. Lewandowski,[2] Rick Jones,[2] and William A. Burr[1]

Clinical Chemistry 50:12
2338–2344 (2004)

Performance Characteristics of Six Third-Generation Assays for Thyroid-Stimulating Hormone

MINDY L. RAWLINS[1] and WILLIAM L. ROBERTS[2*]

Is there a cut-off for TSH?

Lower TSH reference limit

There is general agreement that it lies between 0.2-0.4 mU/L

Canaris 2000
Parle 2001
Warren 2004

THYROID
Volume 13, Number 1, 2003
Mary Ann Liebert, Inc.

LABORATORY MEDICINE PRACTICE GUIDELINES
Laboratory Support for the Diagnosis and Monitoring of Thyroid Disease

2.5 mU/L

AACE Guidelines

AMERICAN ASSOCIATION OF CLINICAL ENDOCRINOLOGISTS
MEDICAL GUIDELINES FOR CLINICAL PRACTICE
FOR THE EVALUATION AND TREATMENT OF
HYPERTHYROIDISM AND HYPOTHYROIDISM

3 mU/L

ENDOCRINE PRACTICE Vol 8 No. 6 November/December 2002

Serum TSH, T_4, and Thyroid Antibodies in the United States Population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III)

JOSEPH G. HOLLOWELL, NORMAN W. STAEHLING, W. DANA FLANDERS, W. HARRY HANNON, ELAINE W. GUNTER, CAROLE A. SPENCER, AND LEWIS E. BRAVERMAN

4.2 mU/L

J Clin Endocrinol Metab, February 2002, 87(2):489–499

J Clin Endocrinol Metab, January 2005, 90(1):581–585

CONSENSUS STATEMENT: Subclinical Thyroid Dysfunction: A Joint Statement on Management from the American Association of Clinical Endocrinologists, the American Thyroid Association, and The Endocrine Society

Although our panel supports routine screening, the consensus panel did not; until additional information is available, we will just have to agree to disagree on this matter.

CONTROVERSY IN CLINICAL ENDOCRINOLOGY

The Evidence for a Narrower Thyrotropin Reference Range Is Compelling

Leonard Wartofsky and Richard A Dickey

Department of Medicine, Washington Hospital Center, Washington, D.C. 20010; Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814; and Georgetown University School of Medicine, Washington, D.C. 20006

(*J Clin Endocrinol Metab* 90: 5483–5488, 2005)

CONTROVERSY IN CLINICAL ENDOCRINOLOGY

The Thyrotropin Reference Range Should Remain Unchanged

Martin I. Surks, Gayotri Goswami, and Gilbert H. Daniels

(*J Clin Endocrinol Metab* 90: 5489–5496, 2005)

European Journal of Endocrinology (2006) **154** 633–637 ISSN 0804-4643

TOPIC FOR DISCUSSION

Is there a need to redefine the upper normal limit of TSH?

G Brabant, P Beck-Peccoz[1], B Jarzab[2], P Laurberg[3], J Orgiazzi[4], I Szabolcs[5], A P Weetman[6] and W M Wiersinga[7]

Abteilung Gastroenterologie, Hepatologie und Endokrinologie, Medizinische Hochschule, Hannover, Germany, [1]Institute of Endocrine Sciences, University of Milan, Ospedale Maggiore IRCCS, Padiglione Granelli, 20122-Milan, Italy, [2]Department of Nuclear Medicine and Endocrine Oncology, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland, [3]Department of Endocrinology and Medicine, Aalborg Hospital, Aarhus University Hospital, DK-9000 Aalborg, Denmark, [4]Service d'Endocrinologie Diabetologie, Centre Hospitalier Lyon Sud, Lyon, France, [5]Department of Internal Medicine, National Medical Center and Department of Dietetics, Semmelweis University, Budapest, Hungary, [6]Clinical Sciences Centre, Northern General Hospital, University of Sheffield, Sheffield, UK and [7]Academisch Medisch Centrum, Universiteit van Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

TSH value between 2 and 4 mU/l as abnormal..., is probably doing more harm than good.

CONTROVERSY IN CLINICAL ENDOCRINOLOGY

The Evidence for a Narrower Thyrotropin Reference Range Is Compelling

Leonard Wartofsky and Richard A Dickey

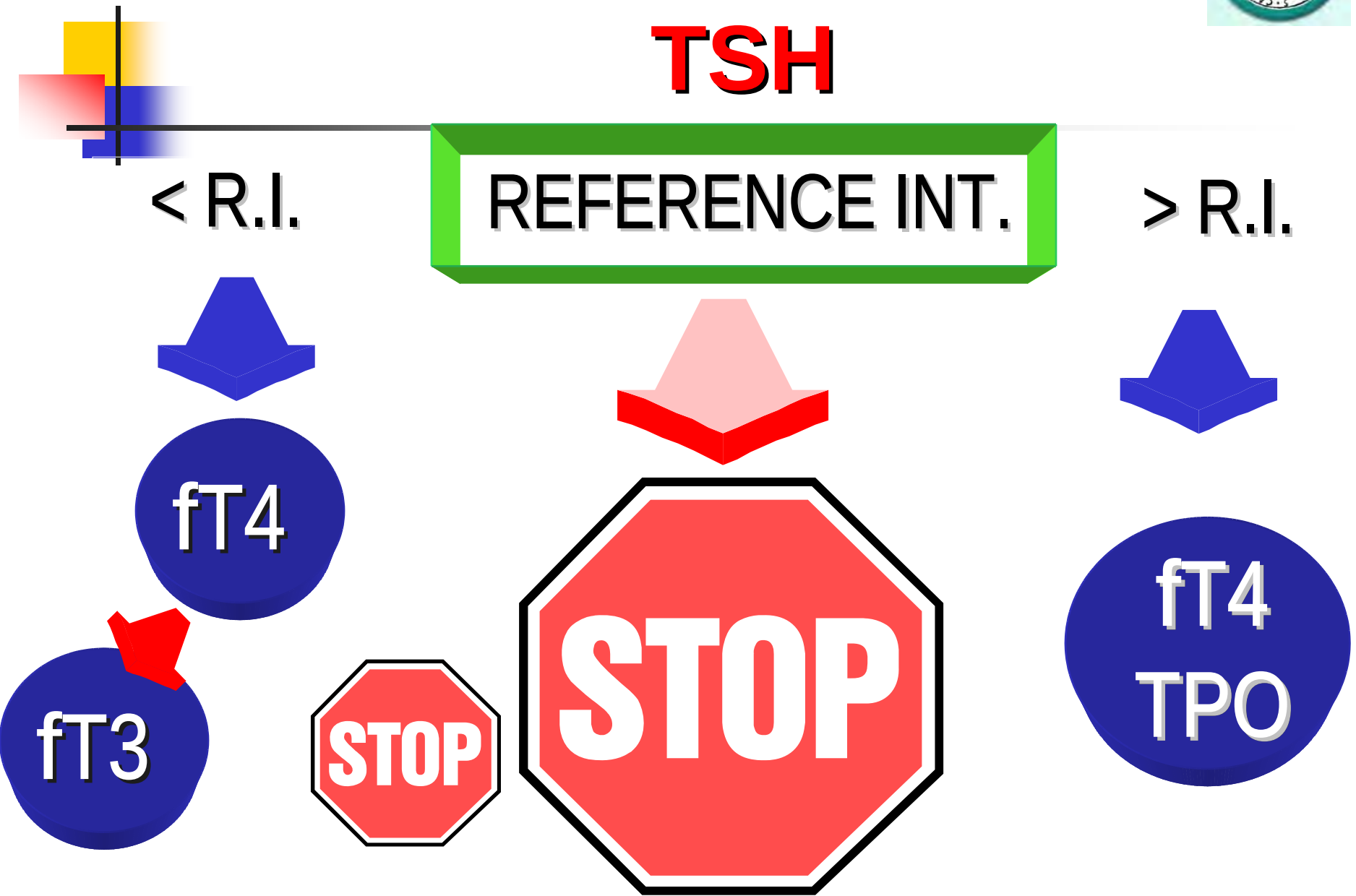
Department of Medicine, Washington Hospital Center, Washington, D.C. 20010; Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814; and Georgetown University School of Medicine, Washington, D.C. 20006

Grey Area

More judgment is required until more definitive data are available for the management of pts with TSH values between 2.5 and 5.0 mU/L

“Cascade”/ Reflex TSH

“Cascade”/Reflex TSH



“Cascade”/Reflex TSH

- Automated algorithm
- Single patient access
- All the test with a single sample
- The patient/NHS will pay only the carried out tests
- No delay in report

SURVEY OF INSTRUMENTS

Immunoassay market overflowing with change

Anne Ford

When is a negative actually a plus? When it's

product manager Mari Kelly. "The DiaSorin EBV assays will be the first automated EBV chemiluminescence assays on the U.S. market. The time

botic sample management system. DPC plans to launch an immunoassay workcell before AACC. Senior marketing manager Mark Smith says, "The

Bar-code placement per NCCLS standard Auto2A	yes
Onboard test auto inventory (determines vol. in container)	yes
Measures No. of tests remaining/Short sample detection	yes/yes
Auto detecti ecimen	yes
Clot detectio /Reflex testing capability	yes/yes
Hemolysis de ty detection-quantitation	yes/no
Dilution of patient samples onboard/Automatic rerun capability	yes/yes
Sample vol. can be increased to rerun out-of-linear range high results/ Increased to rerun out-of-linear range low results	no/no
Time between initial result & reaspiration of sample for rerun	seconds
Autocalibration or autocalibration alert	no
No. of calibrators required for each analyte	6 pt. or 2 pt.

PNLG

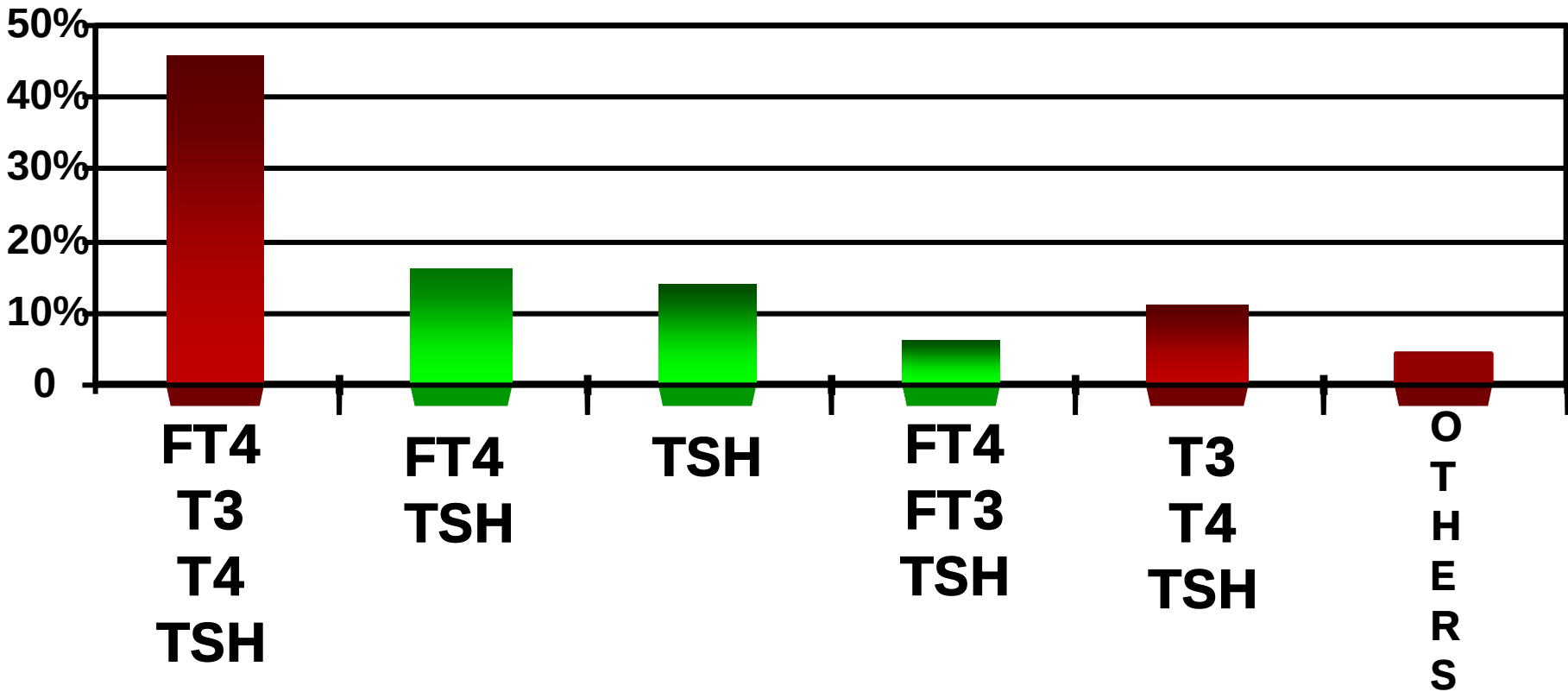
PROGRAMMA NAZIONALE LINEE GUIDA

ISTITUTO SUPERIORE DI SANITÀ

Documento di ausilio alle scelte decisionali
Raccomandazioni per la diagnostica delle malattie tiroidee

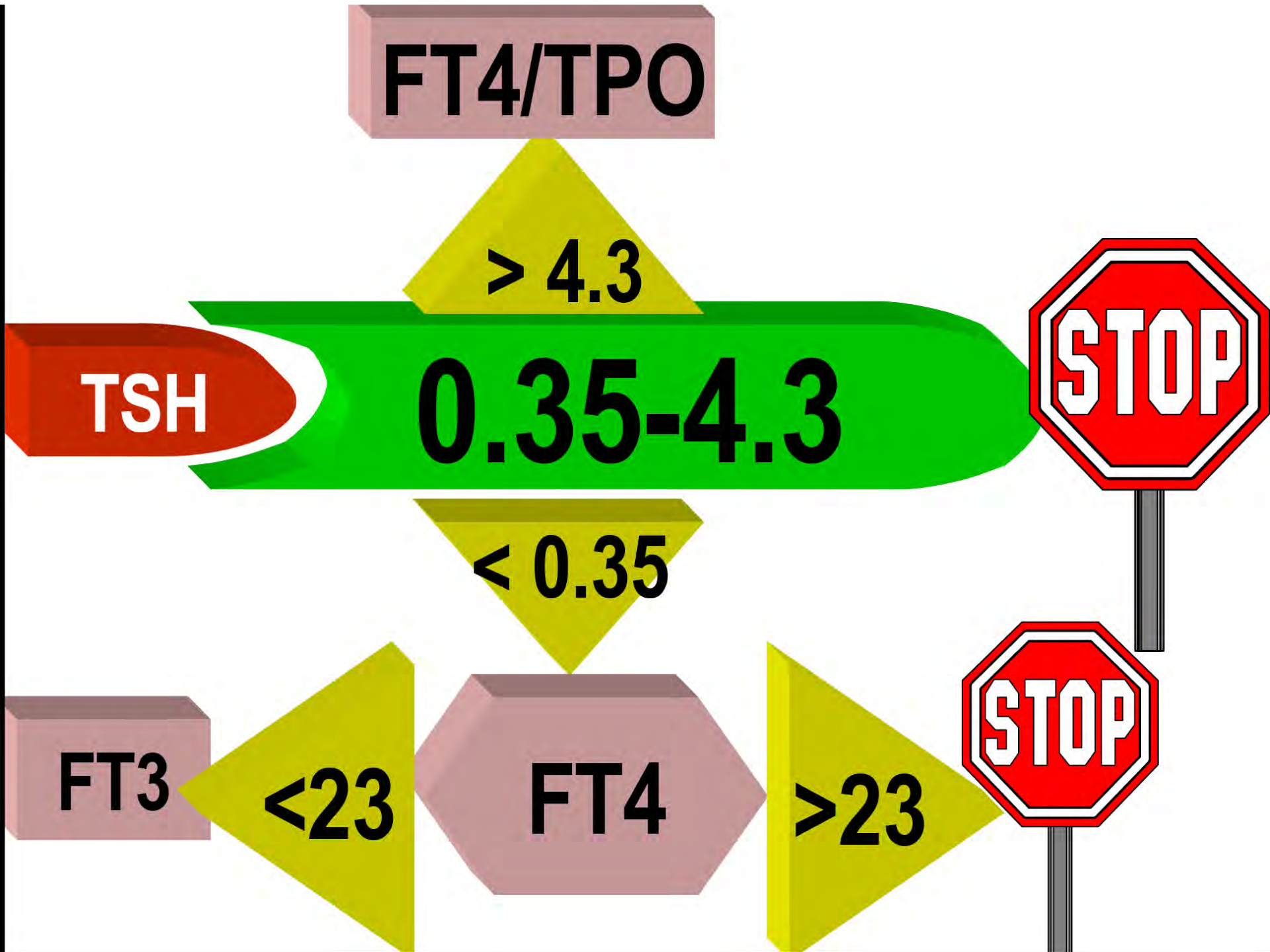
Autori

Thyroid tests requested for outpatients in summer 1995



Traditional TSH testing

- Higher risk of false positive/ clinically not important results
- Longer (sequential) reporting time
- Higher overall cost
- No clinical benefit for patient


FT4/TPO
> 4.3
TSH
0.35-4.3
STOP
< 0.35
STOP
FT3
<23
FT4
>23

Clinical Chemistry 52, No. 2, 2006

Davide Giavarina[1*]
Romolo Marco Dorizzi[2]
Giuliano Soffiati[1]

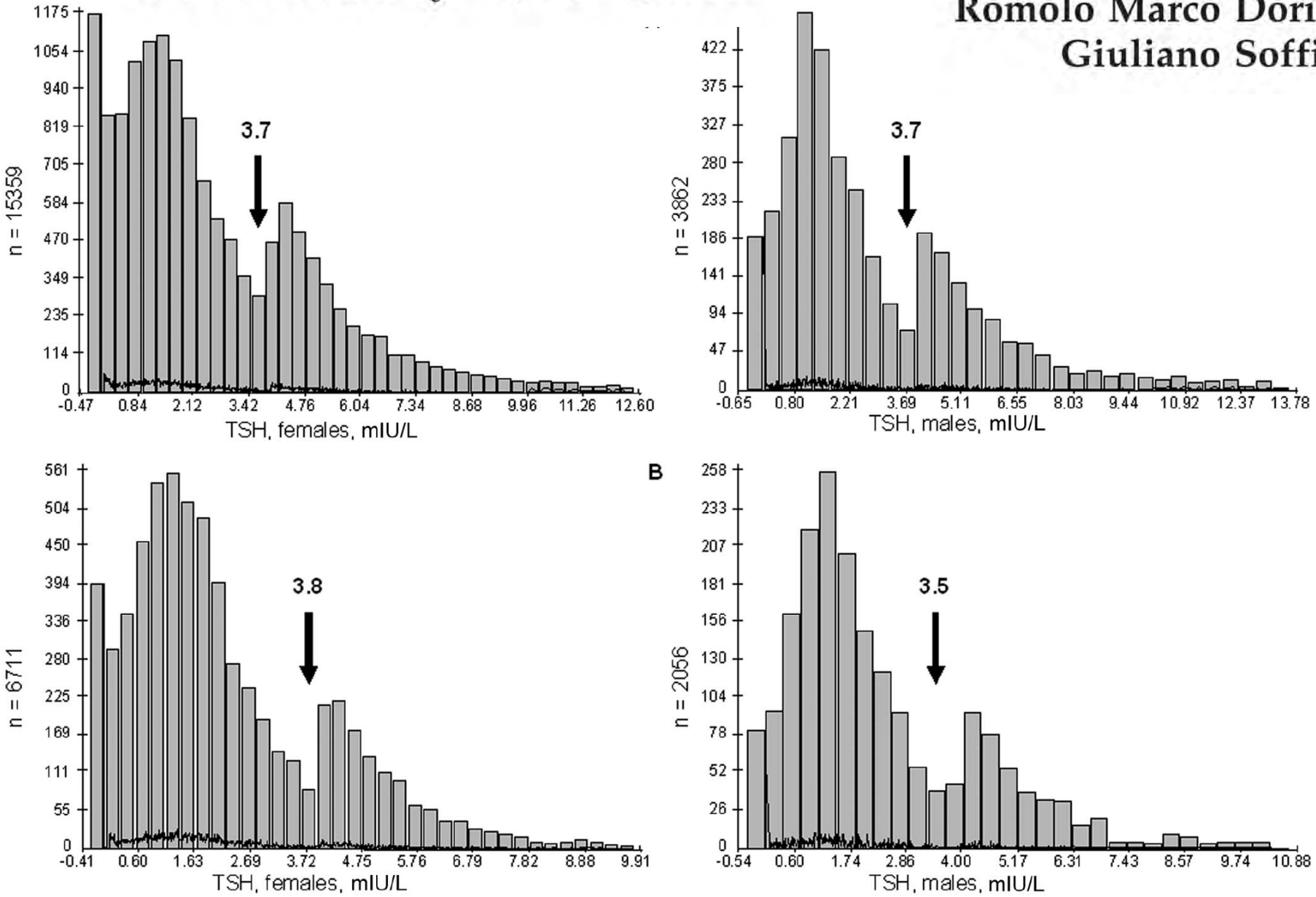
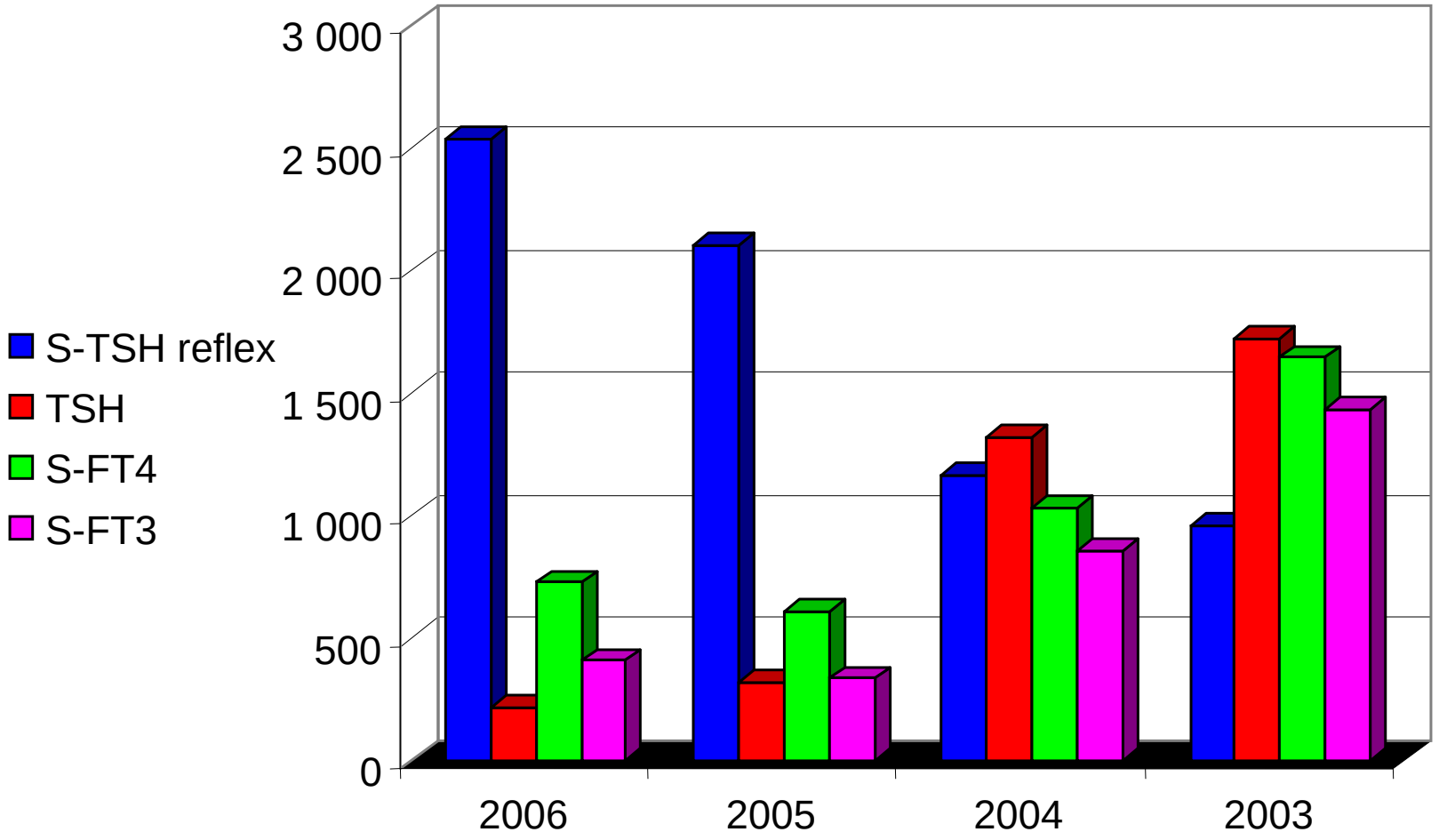
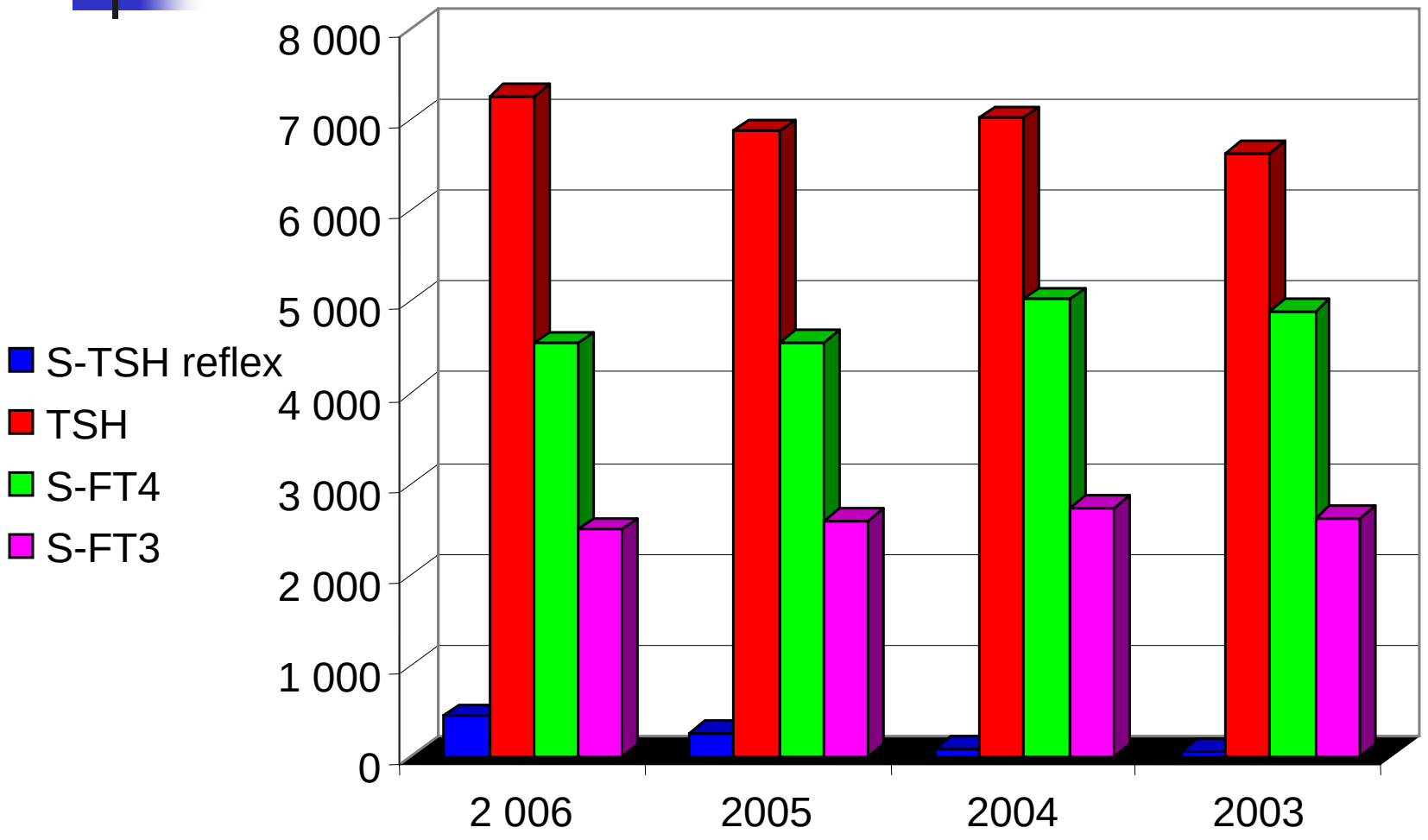


Fig. 1. HRLs for TSH calculated in the records retrieved before (*A*) and after (*B*) removal of repeat tests and results obtained in individuals positive for thyroid antibodies.

January-June requests: Inpatients



January-June requests: Outpatients



NEWSLETTER SCAP

Volume I, Numero I Settembre 2006

COMMISSIONE RAPPORTI TERRITORIO–UNIVERSITA'-OSPEDALE

SCAP

Prime attività della Sotto-Commissione SCAP

La Sotto-Commissione per l'Appropriatezza Prescrittiva dell'Ordine dei Medici-Chirurghi ed Odontoiatri della Provincia di Verona è stata costituita nel Dicembre 2005 e si posta come obiettivo quello di valutare, selezionare e verificare la trasferibilità nel Territorio della Provincia di Verona delle "Buone Pratiche Prescrittive" che Organizzazioni Scientifiche Nazionali ed Internazionali hanno messo a punto

- Disponibilità ad impegnare un pò del loro tempo in questa attività.

I primi tre temi che sono stati selezionati come meritevoli di valutazione sono stati:

- DIAGNOSTICA TIROIDEA
- INDICATORI (MARCATORI) DI NEOPLASIA
- SCREENING BIOCHIMICO PRE-SOMMINISTRAZIONE DI MEZZI DI

Outpatients (2006 vs 2003; +4%)

	€	2006	2005	2004	2003
TRAb	25.30	2479	1037	1417	1265
Tg	15.65	1768	1518	1549	1549
Ab Tg	12.70	5461	4661	8471	7912
Ab TPO	11.40	11503	9838	9607	9895
TSH reflex	30	5665	3243	1114	715
TSH	13.00	94383	89447	91377	86216
FT4	13.00	59141	59287	65299	63609
FT3	13.00	32279	33670	35568	34086
	TOTAL	212649	202681	214362	205248

€ 200.000/ year

TSH	13	2808	2499	17147	22360
FT4	13	9438	7837	13390	21359
FT3	13	5269	5355	11102	18629
.					
	TOTAL	57472	59239	62208	81632

Conclusions

- Rational and appropriate
- Quality of service maintained
- No further trouble for patients
- Follows international excellence endocrinology centers

Thanks:

Paolo Moghetti

Flavia Tosi

Giovanna Spiazzi

Michele Muggeo

Romolo M Dorizzi

Paolo Rizzotti

THE LANCET • Vol 357 • March 31, 2001

Research letters

Pitfalls in the use of thyrotropin concentration as a first-line thyroid-function test

Catherine A Wardle, William D Fraser, Christine R Squire

SURVEY OF INSTRUMENTS

Of all analyzers, immunoassay the trickiest

Raymond D. Aller, MD
David Smalley, PhD

ratory information system database to determine what assays are to be run on that tube, and proceed to run

ability to perform bound-versus-free immunoassay determinations. With the advances in homogeneous im-

tology assays.

◆ Much lower concentrations of analytes are being determined, so small

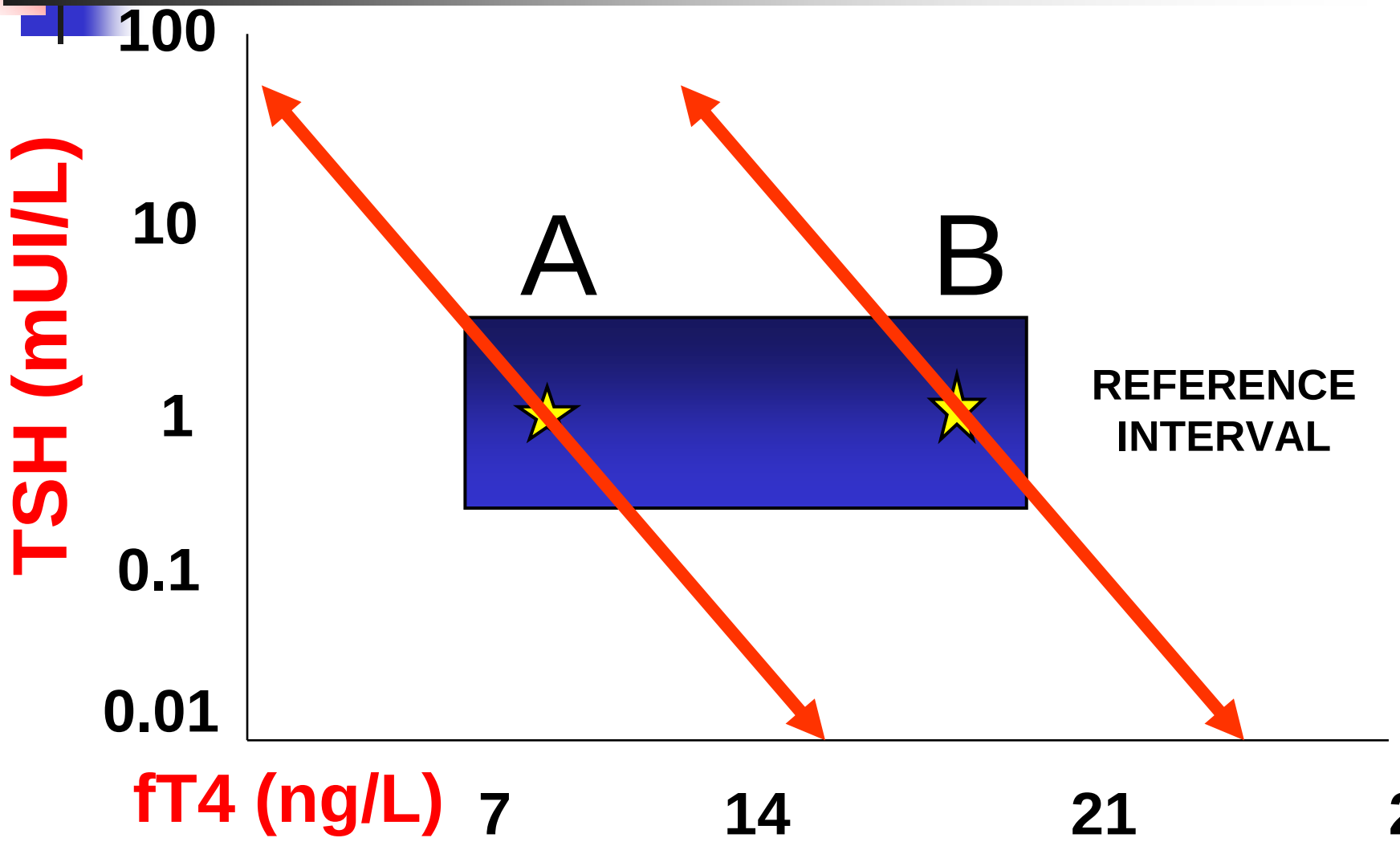
Advia Centaur/1998/U.S.
U.S./U.S.
>200/>600
Cont. random access/floor-standing/rack or direct track sampling
51.5 x 72.5 x 41.5 in./21 sq. ft.

Ability to access/change solutions, waste, disposables & reag. at any time w/o pausing sampling or processing; onboard automatic dilutions, repeats, & cascade reflex testing; disposable tips; 240 results/h

IMMULITE 2000/1998/U.S.
U.S./U.S.
650 worldwide
Cont. random access/floor
79 x 60 x 30/12.5 sq. ft.

Reflex testing; programmable out-of-range autodil.; sample-saver clot detection; customized, icon-driven, Windows-based SW; remote diag.; training-onboard, animated tutorial & interactive training CD; multirule QC SW with graphing capability; max. onboard reag. capacity for walkaway effic.; min. waste.; uninterrupted test processing when replacing or changing system reag., fluids, consumables

Different set-points in different subjects



Interpretation of thyroid function tests

Colin M Dayan

Panel 1: **Conditions in which TSH alone might be misleading**

Common

Recent treatment of thyrotoxicosis

Pituitary disease

Non-thyroidal illness

Rare

TSH-secreting pituitary tumour

Thyroid hormone resistance

Lancet 2001; **357:** 619–24

Interpretation of thyroid function tests

Colin M Dayan

Panel 4: **Low or normal TSH, low free T3 or free T4**

Common

Non-thyroidal illness

Recent treatment for hyperthyroidism (TSH remains suppressed)

Rare

Pituitary disease (secondary hypothyroidism)

Congenital TSH or thyrotropin-releasing hormone deficiency

Lancet 2001; **357:** 619–24

Interpretation of thyroid function tests

Colin M Dayan

Panel 6: **Raised TSH, normal free T4 or free T3**

Common

Subclinical autoimmune hypothyroidism

Rare

Heterophile (interfering) antibody
Intermittent T4 therapy for hypothyroidism
Drugs: amiodarone, sertraline, cholestyramine
Recovery phase after non-thyroidal illness

Congenital

TSH-receptor defects
Resistance to TSH associated with other (unspecified) defects
Pendred's syndrome—some cases (associated with sensineural deafness and goitre)

Lancet 2001; **357:** 619–24

Lancet 2001; **357:** 619–24

Conclusion

In most cases, interpretation of thyroid function tests with free hormone assays and TSH is straightforward. However, unusual conditions can generate common patterns of thyroid function, easily confused with more straightforward diseases (panels 2, 5, and 6), and unusual patterns of test results (panels 3, 4, and 7). One or more of the following features should prompt further investigation: (1) abnormal thyroid function in childhood; (2) familial disease; (3) thyroid function results inconsistent with the clinical picture; (4) an unusual pattern of thyroid function tests results—eg, widely discrepant free T4 and T3 results or a detectable TSH with raised free T3 or T4; and (5) transient changes in thyroid function.

Anno 5, Numero 20

marzo 2004

Lettera dal Laboratorio

NUMERO SPECIALE: IL TSH REFLEX

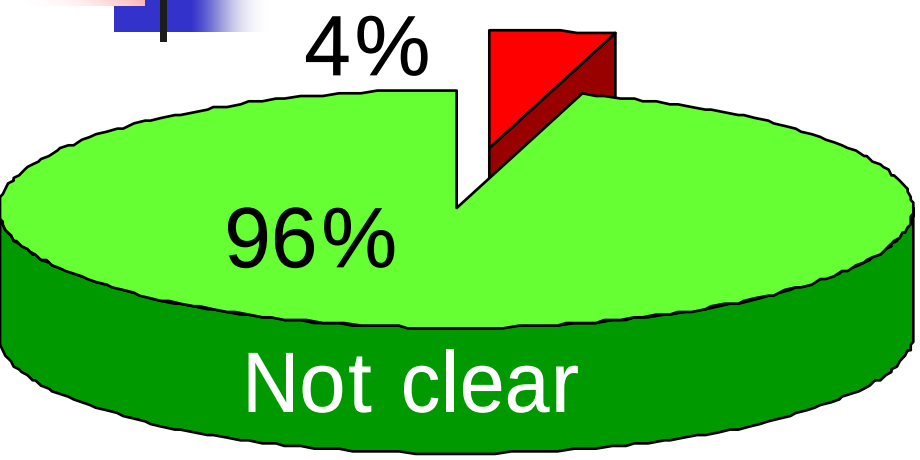
A distanza di 3 anni dall'introduzione del TSH reflex (o TSH a cascata) si porta alla Loro attenzione che a partire dal 31 marzo 2004 PER UN PERIODO SPERIMENTALE potranno essere richiesti (anche in regime di Ospedale Diurno, DH ed AMID) solo i seguenti esami relativi alla funzionalità tiroidea:

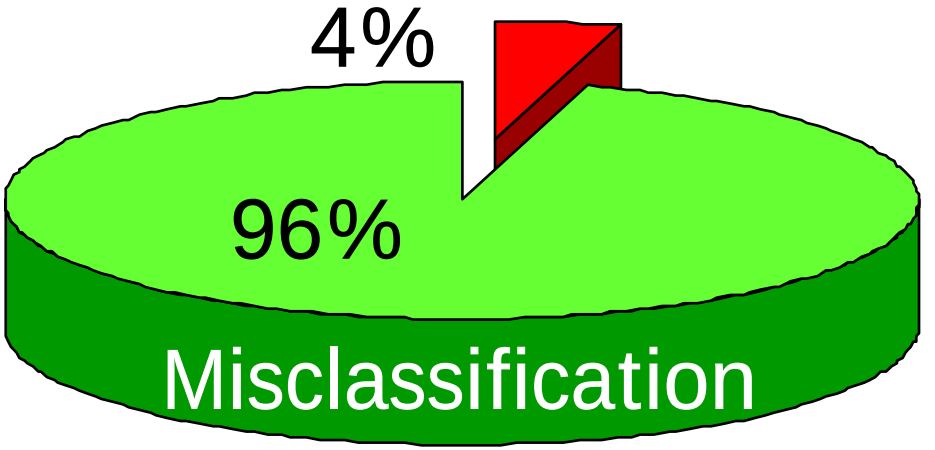
- TSH reflex (che comporterà l'esecuzione degli esami secondo lo schema proposto nel 2001 e ricordato a pag.4)
- TSH
- Anticorpi anti-TPO

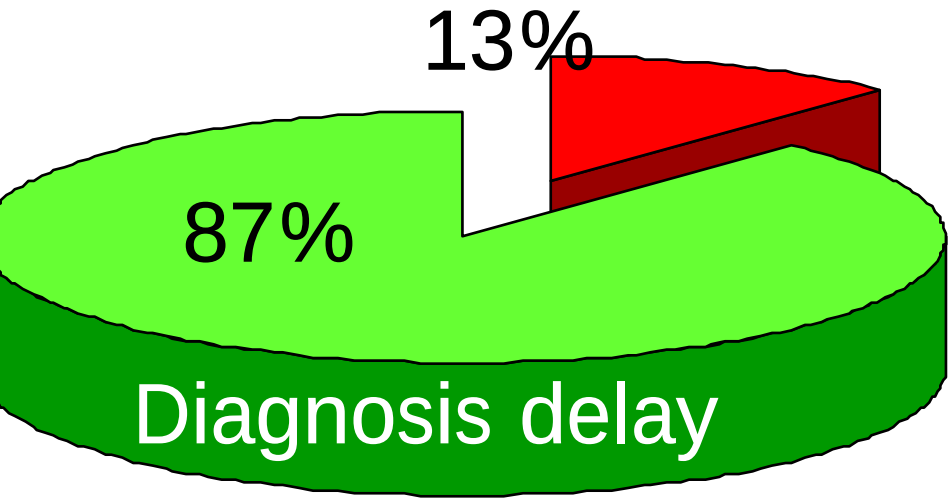
TALE MODALITA' DI RICHIESTA CONTINUERA' FINO AL 1 OTTOBRE 2004 QUANDO SARANNO VALUTATI I RISULTATI E, TENUTO CONTO DI TUTTE LE OSSERVAZIONI DEI REPARTI, SI DECIDERA' SE CONTINUARE O MENO CON L'ESPERIMENTO

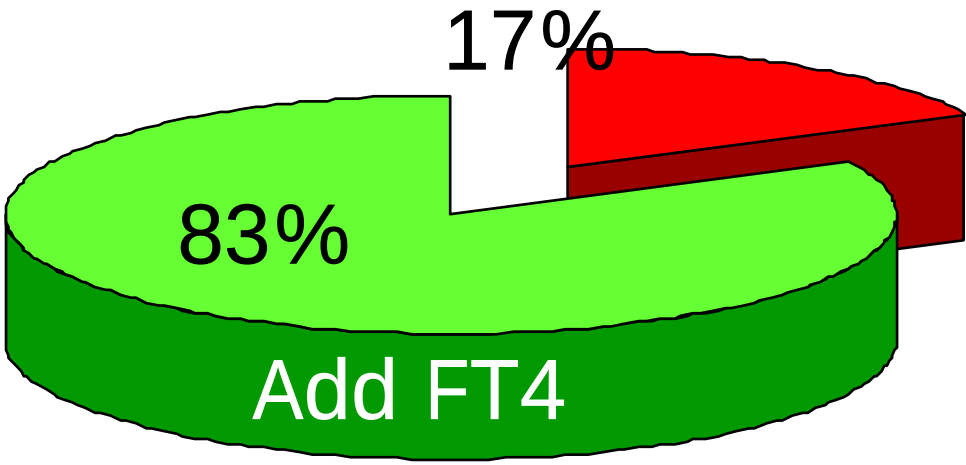
ATOLOGICHE
AOLO RIZZOTTI)
torio

Survey results

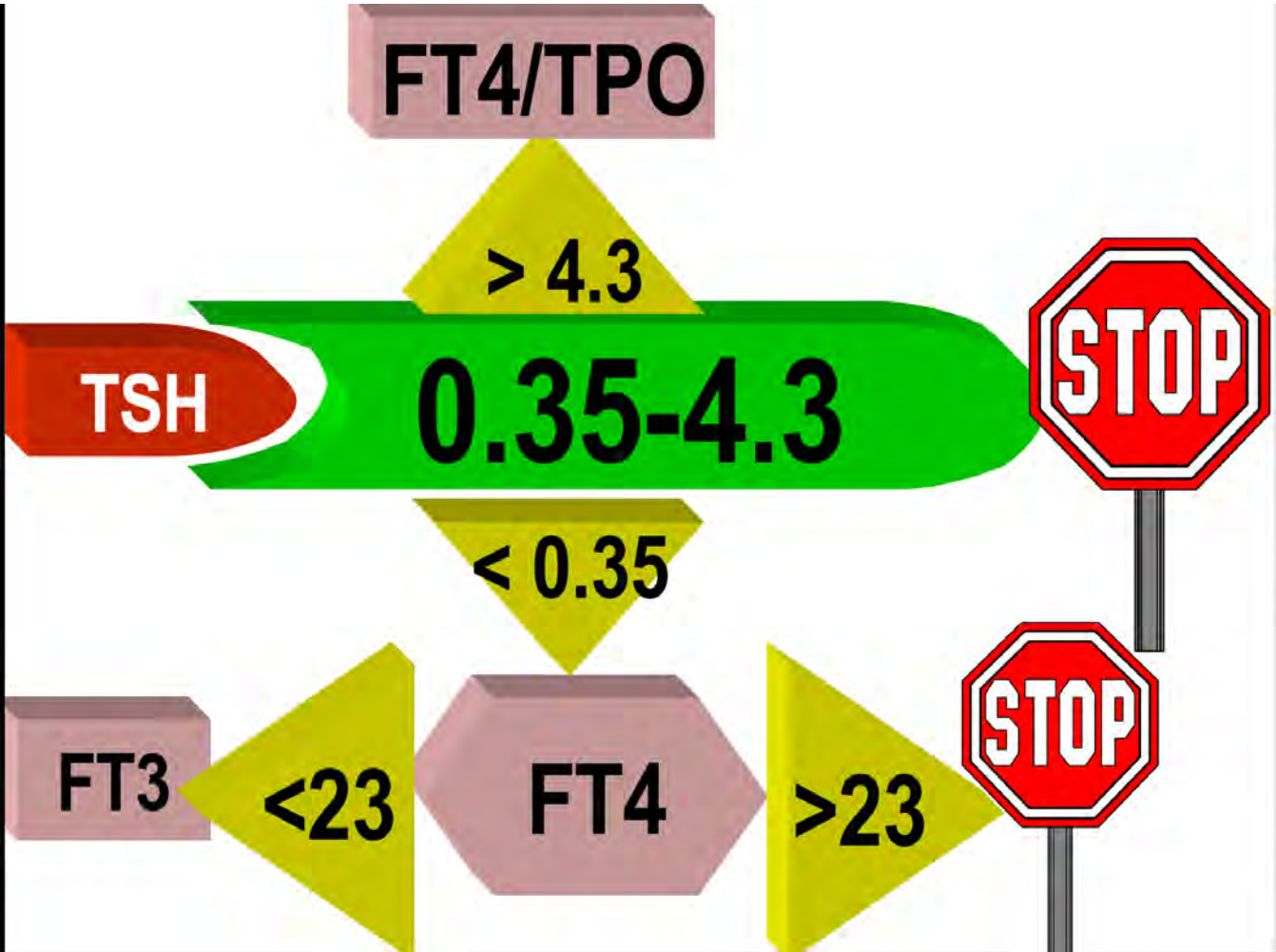








SCAP



TSH "riflesso" diagnostico.

IITSH riflesso monitoraggio **NON** prevede TPO

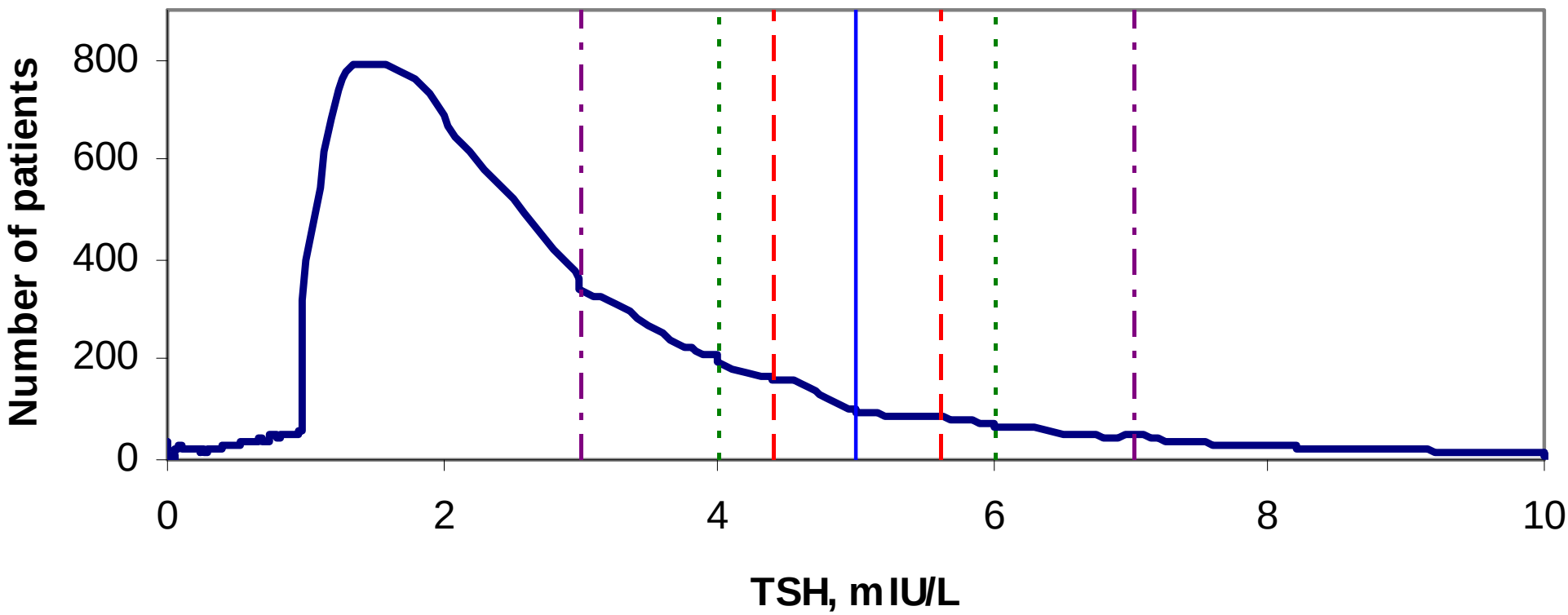
TSH Reference Range Controversy

George G. Klee, M.D., Ph.D.
Mayo Clinic
Rochester, Minnesota, USA

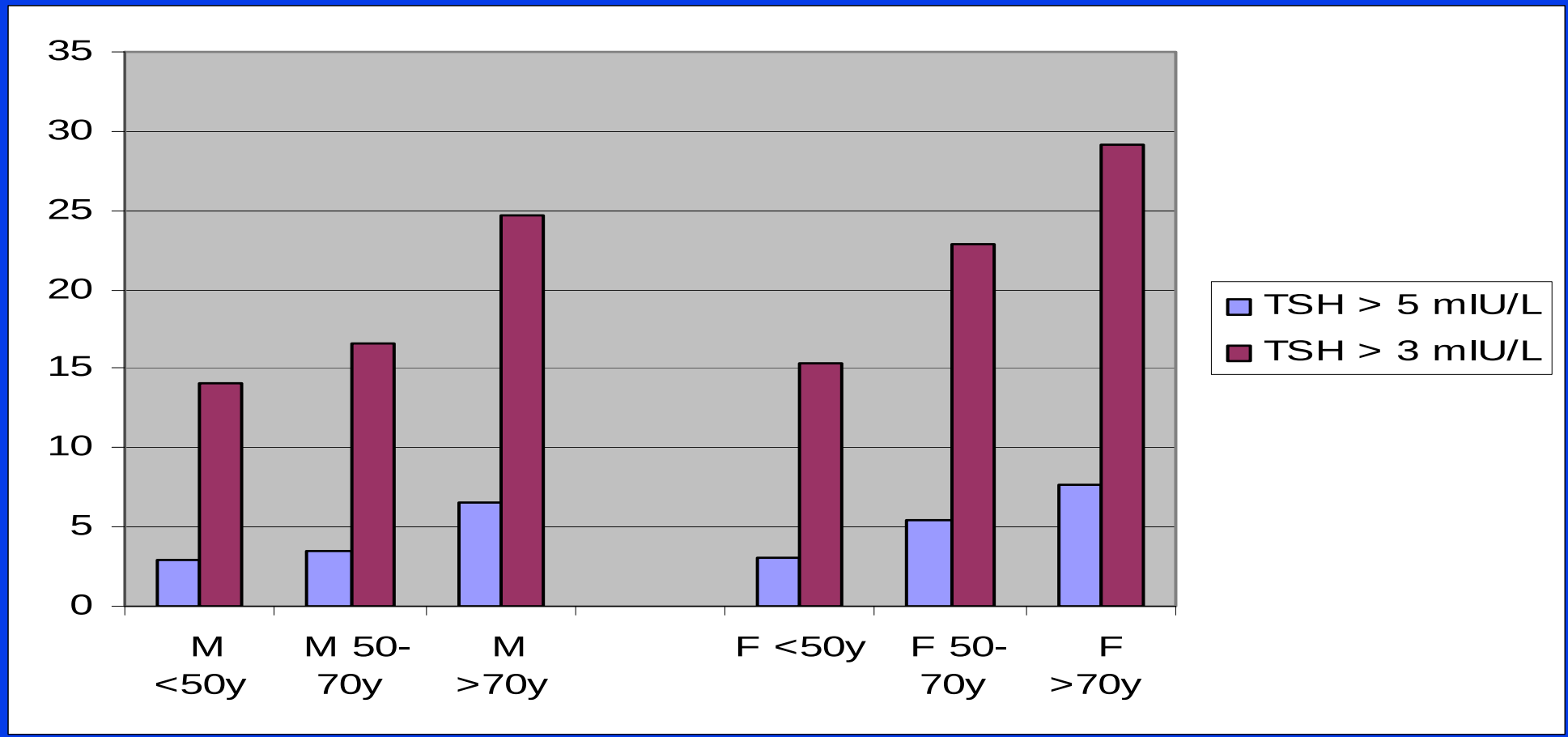
Reference Range For TSH

- TSH is Log-normally distributed
- The 95% range for TSH is ~0.3 - 5.0 mIU/L
- When patients with goiter, family history and positive TPO antibodies are excluded, the range in the literature is ~ 0.3 to 3.0 mIU/L
- A reference range of 0.3 to 3.0 mIU/L would cause a four fold increase in number of abnormal cases

Frequency Distribution of TSH Values



Percentage of Clinically Healthy Patients with Elevated TSH



TSH in Patients Without Clinical Record of Thyroid Disease

	Males			Females		
Age, years	<50	50-70	>70	<50	50-70	>70
TSH >5.0 mIU/L	2.9%	3.5%	6.5%	3.1	5.4	7.7
TSH >3.0 mIU/L	14.1%	16.6%	24.7%	15.3	22.8	29.2
Fold Increase	4.9	4.7	3.8	4.9	4.2	3.8

TSH Reference Range Controversy

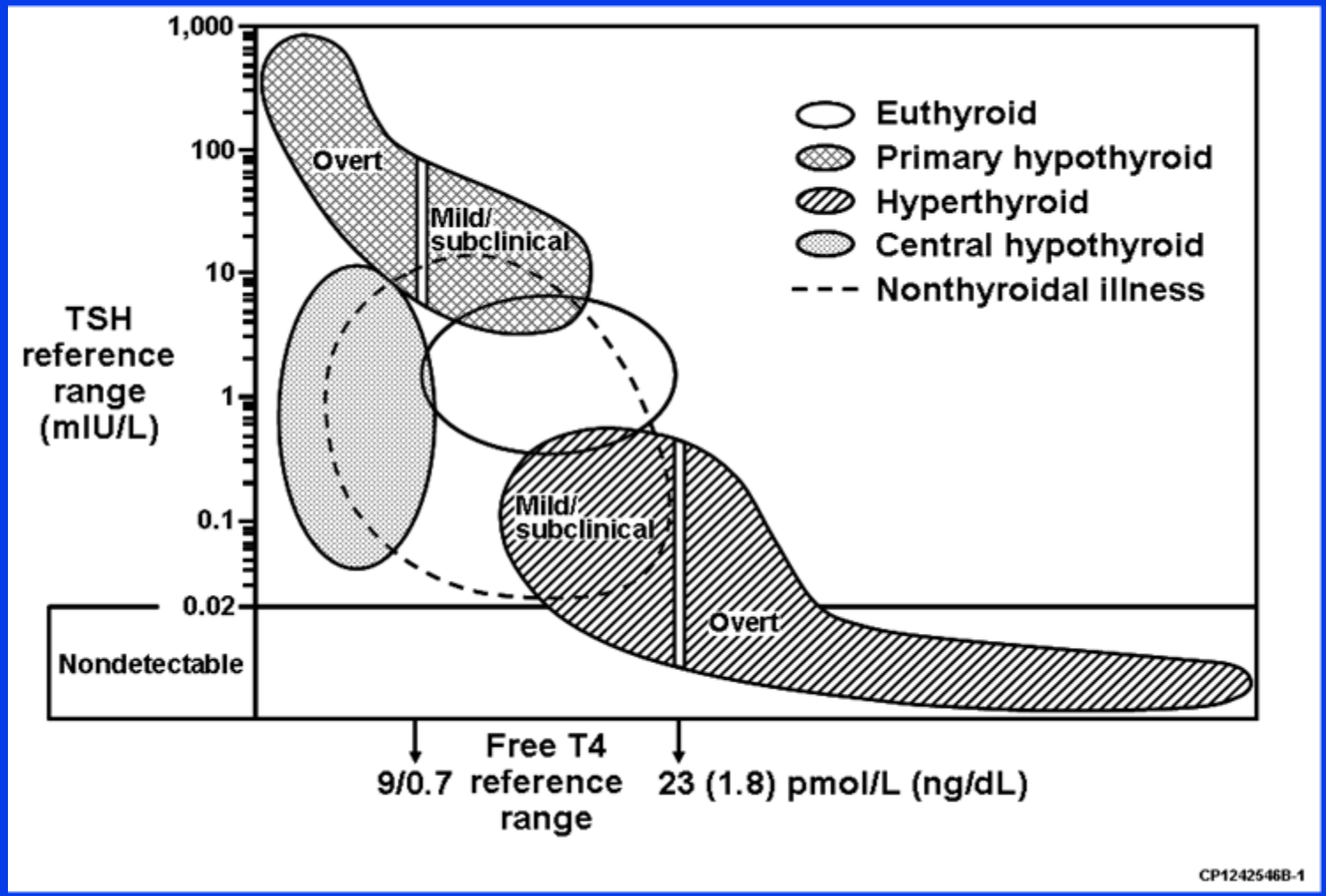
- The choice of “Normal” Reference Range can substantially effect the number of patients targeted for thyroxine replacement therapy.
- Clinical Thyroid Examination and Thyroid Ultrasound, as well as Thyroid antibody measurements, may be needed to define range.
- Randomized Clinical trials may be needed to determine efficacy of thyroxine treatment at lower TSH levels.

A TSH-Driven Ordering Cascade for Thyroid Function Testing

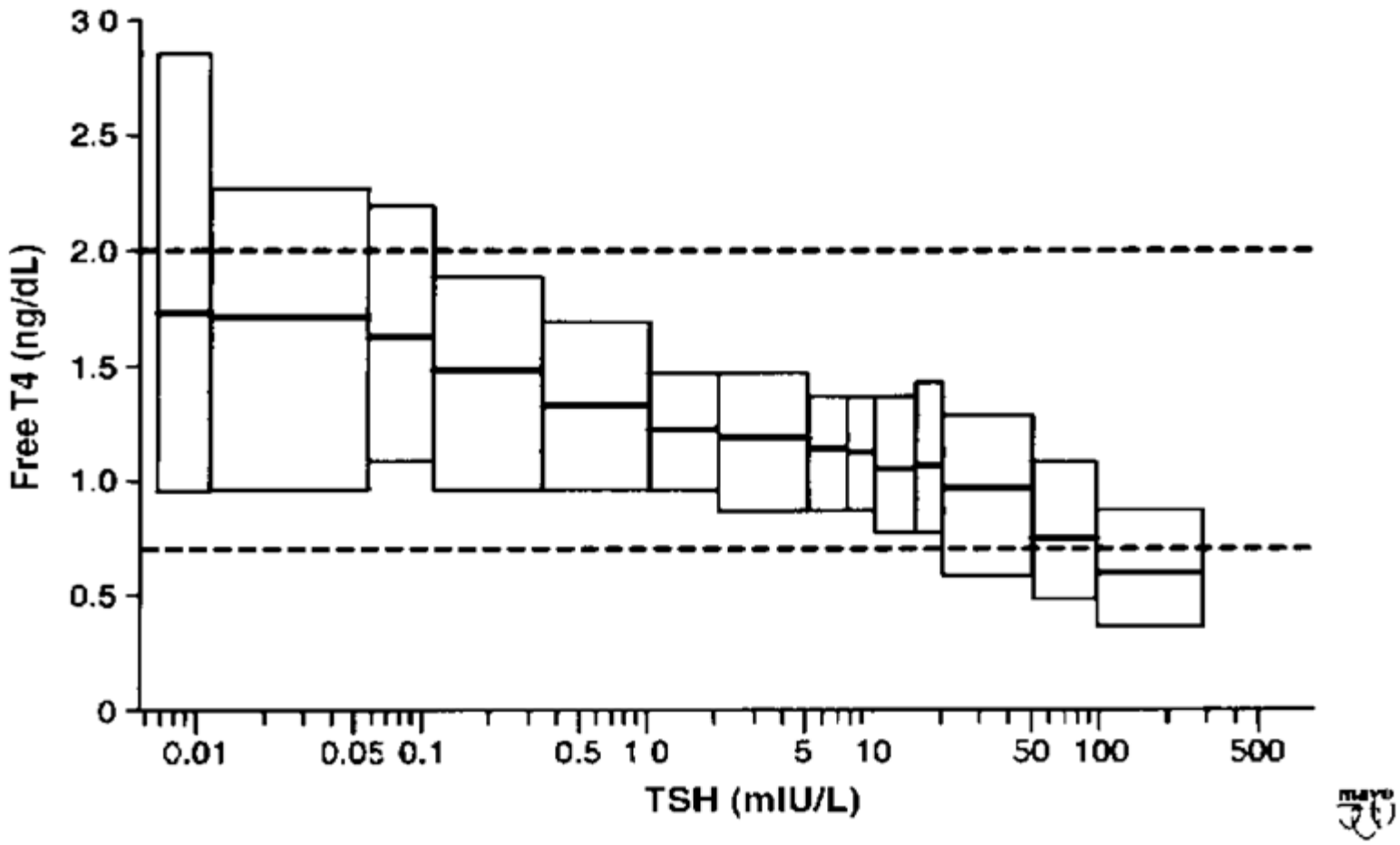
George G. Klee, M.D., Ph.D.
Mayo Clinic
Rochester, Minnesota, USA

Classification of Thyroid Disorders

Modified from NACB-Practice Guideline (Thyroid 13:3-126, 2003)



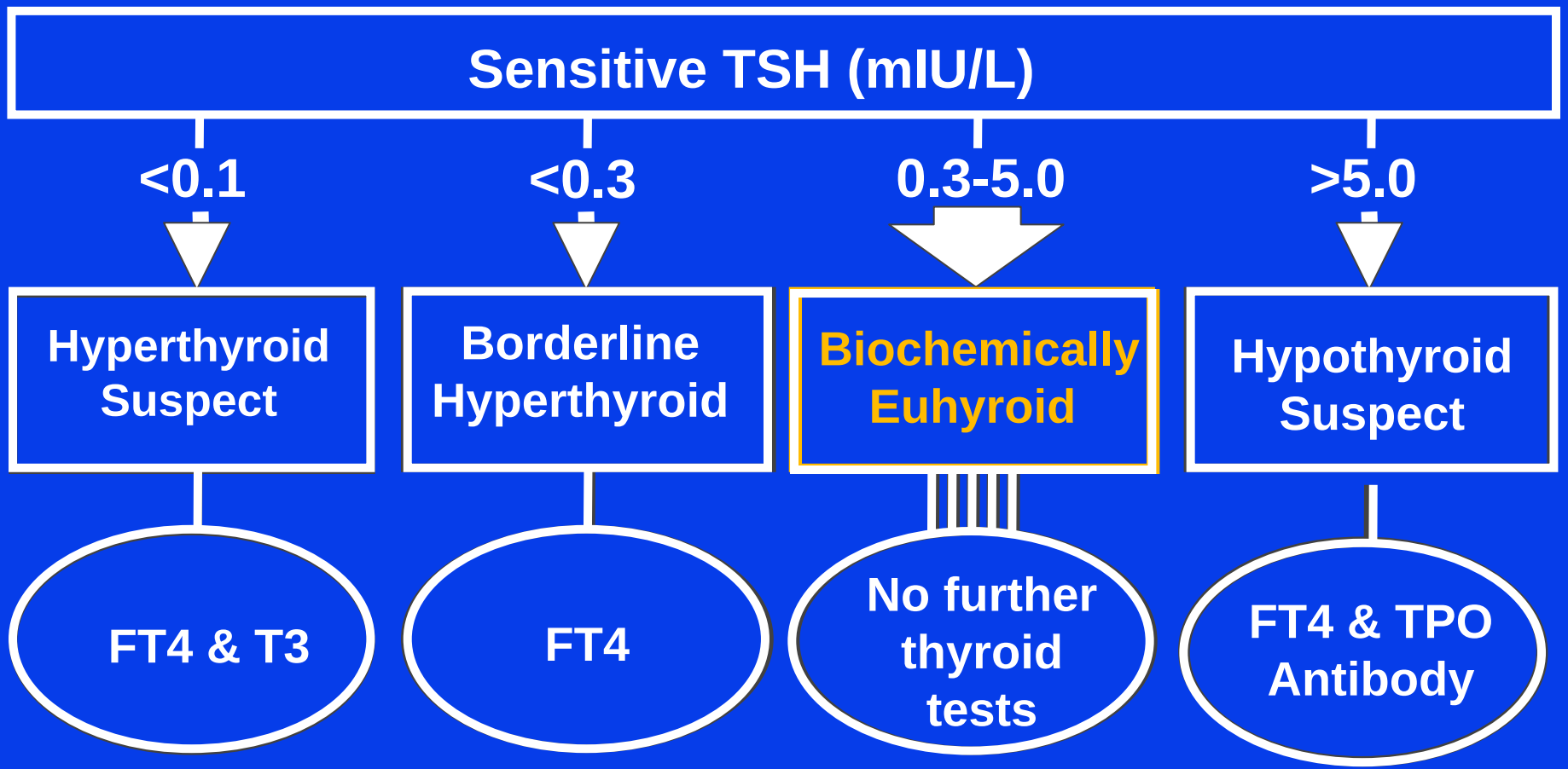
Relationship of Free T4 with TSH



Thyroid Function Testing Cascade Goals:

- Reduce turnaround time for follow-up tests
- Reduce number of concurrent orders for sTSH, FT4, T3, and TPO antibody
- Encourage sTSH-based biochemical thyroid evaluations
- Provide a more uniform follow-up of cases with abnormal sTSH

Thyroid Function Testing Cascade



Thyroid Function Testing Cascade Test Request Form

- ☐ sTSH
- ☐ Thyroxine, free (FT4)
- ☐ Thyroxine, total (TT4)
- ☐ Thyroperoxidase (TPO) antibody
- ☐ **T3** assay
- ☑ sTSH with FT4, T3, and TPO antibody when indicated

January to June 2006 Ordering Patterns: Mayo Clinic versus Mayo Medical Lab

- Mayo Clinic:
  - 29,424 TSH
  - 24,701 Cascade

Cascade orders =

45.6%

- Mayo Medical Lab:
  - 2963 TSH
  - 908 Cascade

Cascade orders =

23.5%

Mayo Thyroid Cascade-Test Distributions

58% Female - Median Age 59 years

42% Male - Median Age 63 years

12.7% ↑ TSH > 5.0 mIU/L

4.4% ↓ TSH < 0.3 mIU/L

82.9% → TSH 0.3 - 5.0 mIU/L

Distribution of TPO Antibody in Patients with TSH >10 mIU/L

- 6122 Patients with TSH > 10 mIU/L
- 2867 (46.8%) had Positive TPO Antibody
- 3255 (53.1%) had Negative TPO Antibody

Distribution of TSH in Patients with Negative TPO Antibody

28,025 Patients with Negative TPO Antibody

– 3,255 (11.6%) had TSH > 10 mIU/L Initially

11,126 Patients with NEG TPO & TSH<10 , had follow-up TSH measurements

– 1,095 (9.8%) developed TSH >10 in 5 years

- RISK of TSH > 10 mIU/L with NEG TPO =1:10

Distribution of TSH in Patients with Positive TPO Antibody

12,603 Patients with Positive TPO Antibody

- 2,867 (22.7%) had TSH > 10 mIU/L Initially

5,541 Patients with Initial POS TPO & TSH<10 had follow-up TSH Measurements

- 1,050 (18.9%) developed TSH >10 in 10 years

- RISK of TSH > 10 mIU/L with POS TPO = 1:5

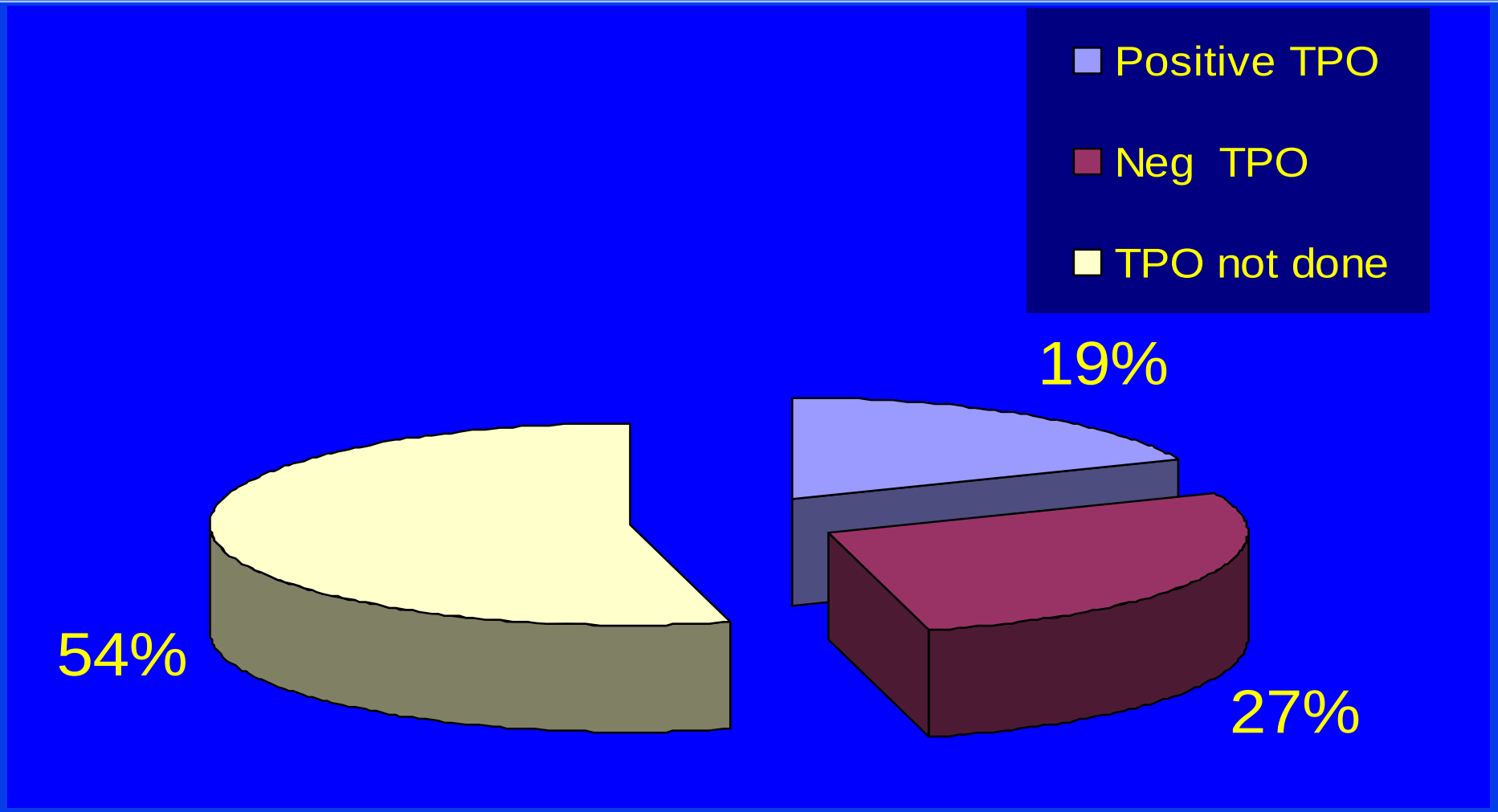
Effect of TPO on Risk of TSH > 10 mIU/L

- At Time of Initial Measurement:
  - 11.6 % had TSH > 10 mIU/L
    when TPO antibody is Negative
  - 22.7 % had TSH >10 mIU/L
    when TPO antibody is Positive
- In Subsequent 10 years:
  - 9.8% Develop TSH >10 mIU/L if TPO Negative
  - 18.9% Develop TSH >10 mIU/L if TPO Positive

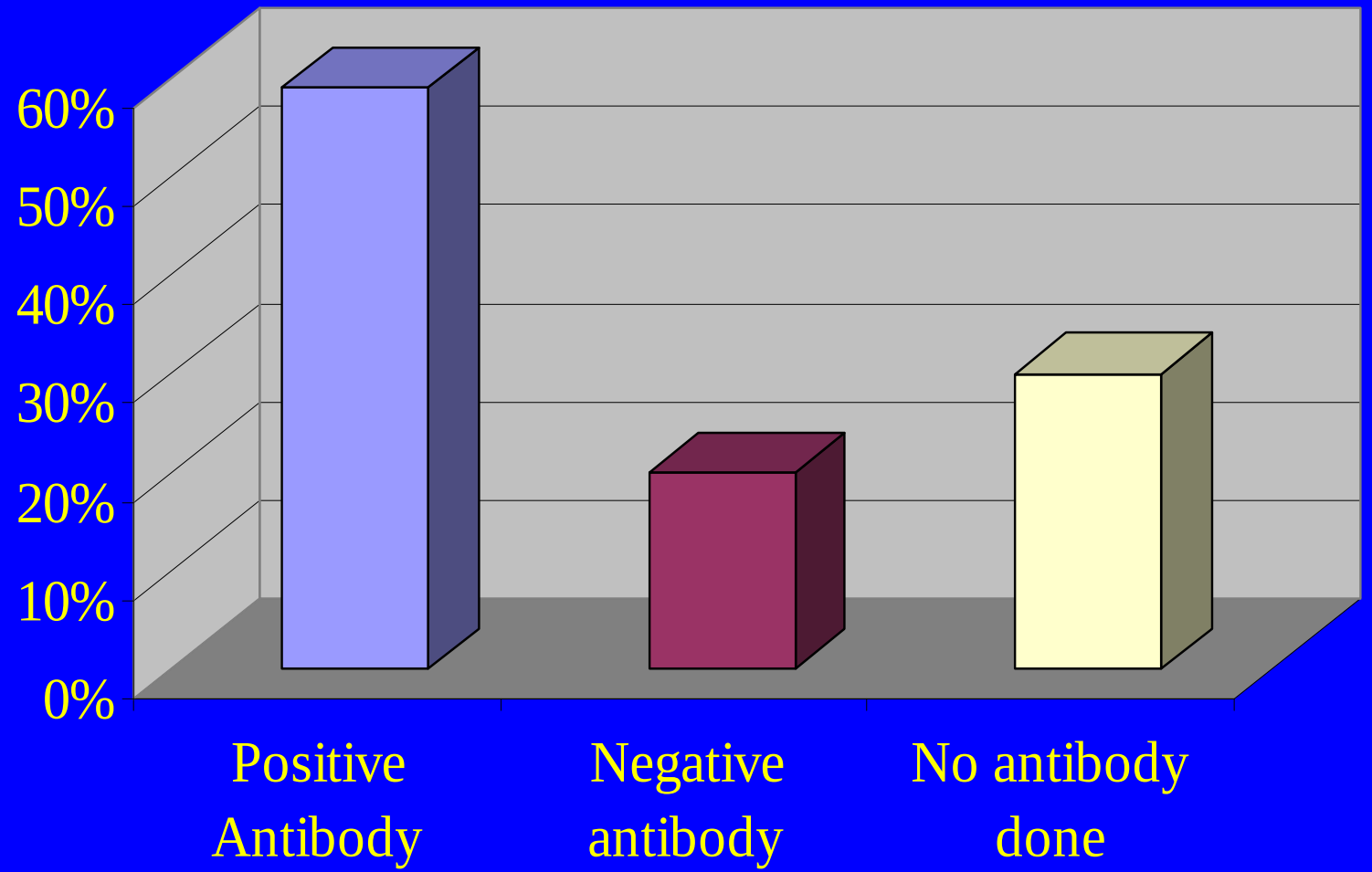
Effect of Thyroid Cascade on Patient Care

- More TPO antibody tests are measured
- More T3 tests are measured
- Many patients (especially men) with positive TPO antibody and elevated TSH have thyroid replacement therapy prescribed by their physicians

TPO Antibody Status in 6802 Patients with Serum TSH 5-10 mIU/L



Effect of TPO Antibody Status on Decision to Initiate Thyroxine Therapy (TSH 5-10)



Effect of Gender and TPO ATB on Decision to Treat For TSH 5-10 mIU/L

Gender	Anti-TPO Antibody	
	Positive	Negative
Females	45%	18%
Males	61%	27%

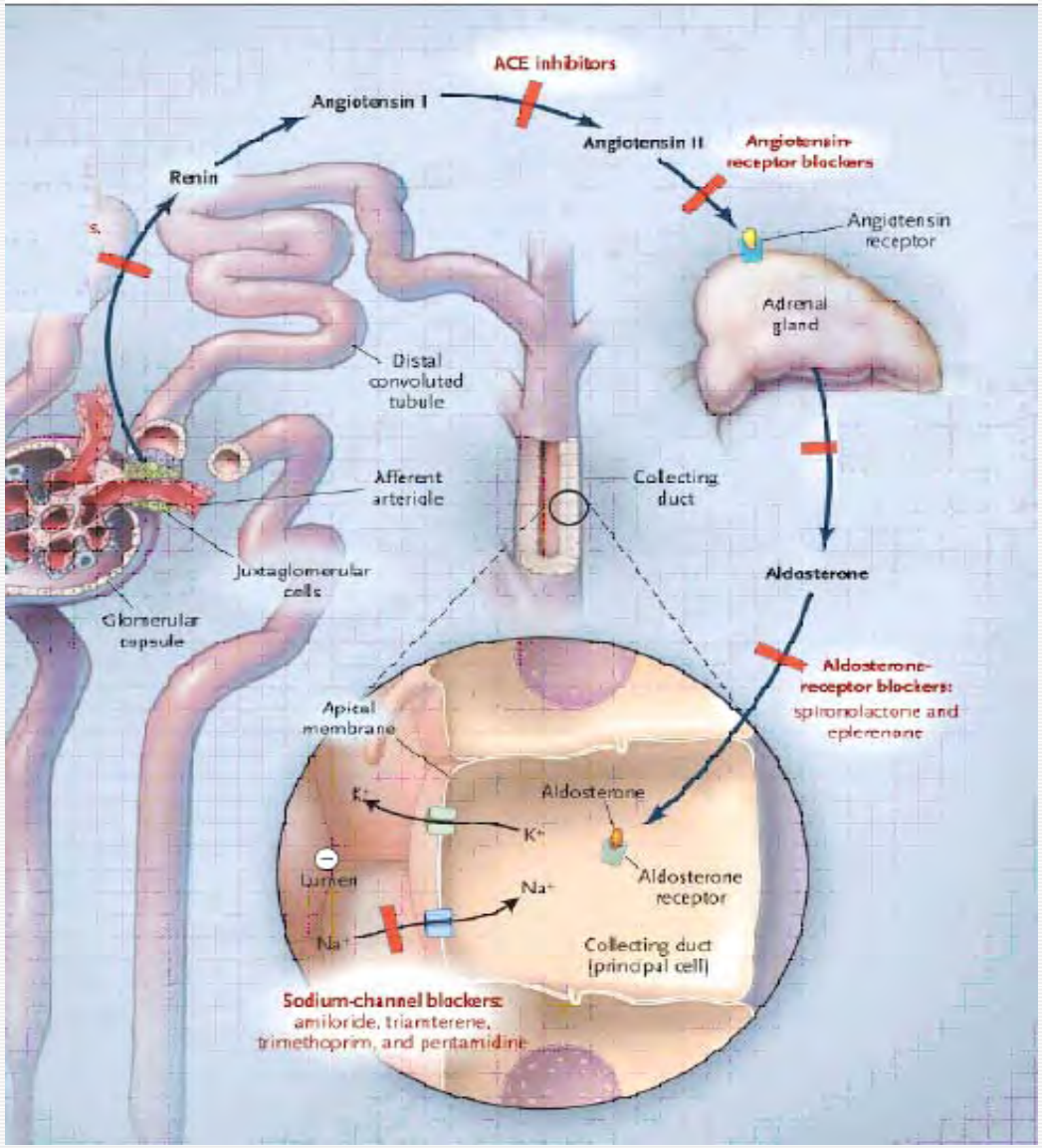
Thyroid Cascade Summary

- TSH-First Testing works well for most patients.
- FT4 is less sensitive than TSH for early changes, but this test provides additional information when TSH is abnormal.
- Thyroperoxidase (TPO) antibodies correlate with risk of elevated of TSH and with the decision to treat.

Thyroid Cascade- Recommendations

- TSH-High Cascade to Thyroperoxidase antibody test is mainly useful for thyroid disease case-finding. Therefore, separate cascades may be better for case-finding versus thyroid monitoring.
- TSH-Low Cascade to T3 for cases with normal FT4 identifies few cases with T3-Toxicosis. Therefore, we recommend not cascading to FT3 unless TSH $\leq$0.1 mIU/L and FT4 is normal.

The Renin–Angiotensin–Aldosterone System



Aldosterone binds to a cytosolic receptor (Mineralocorticoid Receptor, MR) in the principal cell and stimulates sodium reabsorption across the luminal membrane through a well-defined sodium channel.

Palmer M, N Eng J Med; 2004 351:585

Aldosterone: Epithelial and Nonepithelial Effects

Aldosterone

Epithelial actions:

Kidneys

- reabsorption of sodium and water;
- excrection of potassium;

Colon, sweat and salivary glands

- reabsorption of sodium
- excrextion of potassium

Nonepithelial actions:

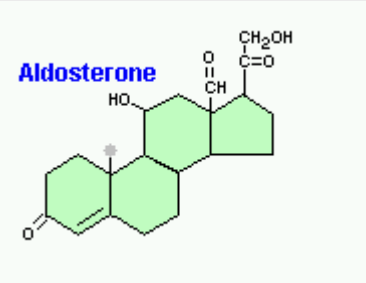
Cardiovascular system

- stimulates perivascular and interstitial cardiac fibrosis;
- promotes collagen deposition in blood vessels;
- modulates vascular tone;

CNS

- effects ?

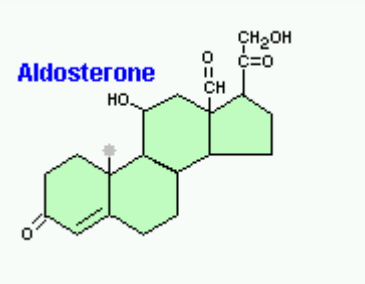
Prevalence of Primary Aldosteronism



The prevalence of primary aldosteronism has increased since:

- the Plasma aldosterone (PAC)/plasma renin activity (PRA) ratio has been accepted as a screening test for PA
- it has been recognized that most patients with PA are not hypokalemic

Common questions



- How common is PA?
- Which patients should be screened for PA?
- Which are the most common causes of PA?
- How PA can be diagnosed ?
- How should the clinician distinguish between the different causes of PA?
- What is the best treatment for PA?

Which patients should be screened for primary aldosteronism?

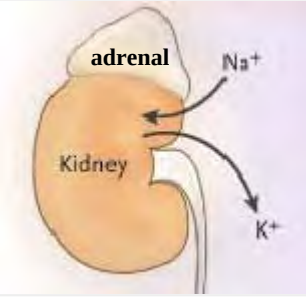
- Patients with spontaneous or unprovoked hypokalemia, especially if the patient is also hypertensive
- Patients who develop severe and/or persistent hypokalemia in the setting of low-to-moderate doses of potassium-wasting diuretics
- Patients with refractory hypertension
- In hypertensives with a family history of hypertension or stroke at young age (< 50 ys)
- Patients with an adrenal incidentaloma and hypertension

Clinical characteristics of patients with PA

- Hypertension is almost invariable
- Muscular weakness
- Abdominal distension
- Ileus from hypokalemia
- Findings related to complications of hypertension
- not edema despite the volume-expanded state due to spontaneous natriuresis and diuresis (aldosterone escape) that appears to be mediated by atrial natriuretic peptide.

Causes of primary aldosteronism

Aldosterone-producing adenoma (APA)	45 %
Bilateral adrenal hyperplasia or idiopathic hyperaldosteronism (IHA)	50 %
Unilateral hyperplasia or primary adrenal hyperplasia	<2 %
Aldosterone producing carcinoma	1 %



Familial hyperaldosteronism

Type I (glucocorticoid-remediable aldosteronism: GRA)	<2 %
Type II (aldosterone-producing adenoma or idiopathic hyperaldosteronism)	<2 %

How is primary aldosteronism diagnosed?

Screening tests

Morning blood sample in seated ambulant patient for

K+

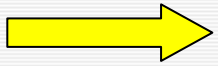
Plasma renin activity (PRA)

Plasma aldosterone concentration (PAC)

PAC/PRA after captorpil suppression

PAC/PRA ratio: > 40 ng/dl/ng/ml/h

PAC > 15 ng/dl



Primary aldosteronism

Confermatory tests

Plasma aldosterone suppression after

Saline Infusion Test (2 lt 0.9% isotonic saline over 4 h)

Fludrocortisone suppression test (0.1 mg every 6 h for 4 days)

Captopril supression test (25-50 mg)

Screening Test

PAC / PRA Ratio in the Upright Posture

	Cut-Off	Sensitivity %	Specificity %	PPV %	NPV %
Upright Posture	**40**	100	84,4	80,3	100
After Captopril	**20**	81,2	91,2	92,8	77,5

PPV: Positive Predictive Value. NPV: Negative Predictive Value.

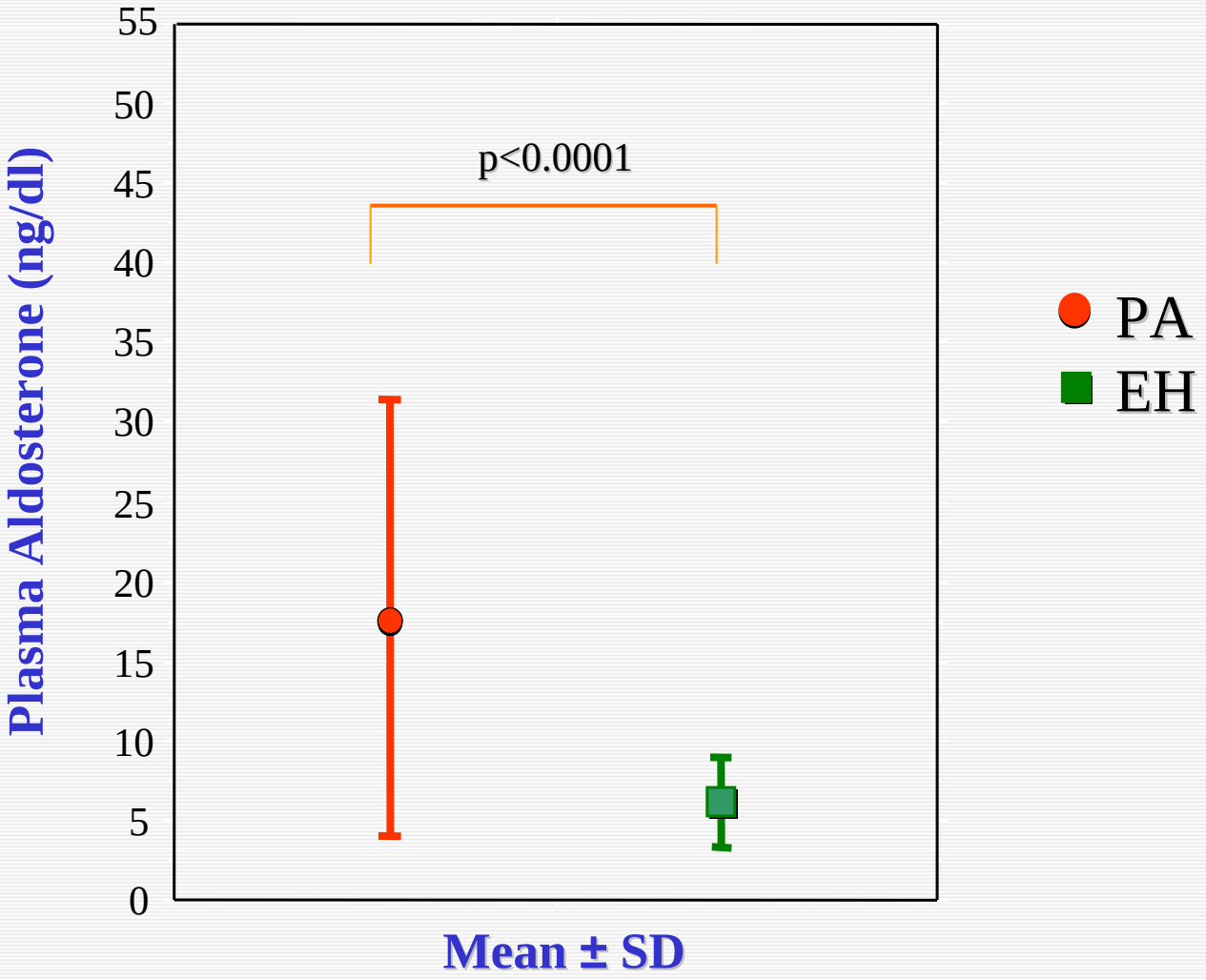
Confermatory test

Plasma aldosterone after Saline Infusion Test

Cut-Off	Sensitivity	Specificity	PPV	NPV
5,0 ng/dl	97,56 %	45,65 %	61,53 %	95,45 %
7,5 ng/dl	85,37 %	80,43 %	79,54 %	86,05 %
10 ng/dl	53,66 %	93,48 %	88,00 %	69,35 %

PPV: Positive Predictive Value. NPV: Negative Predictive Value.

Plasma Aldosterone after Saline Infusion Test in the differential diagnosis of primary aldosteronism (PA) and essential hypertension (EH)



Determining the subtype of PA
Radiological test

Radiological investigations:

- Computed tomography (CT)
- Magnetic resonance imaging (MRI)

Adrenal venous sampling (AVS):

Cortisol-corrected PAC lateralization ratio > 4.0:

Sensitivity: 95.2% Specificity: 100%

The PAPY
(Primary Aldosteronism Prevalence in ItalY) Study

Gian Paolo Rossi[1], Giampaolo Bernini, Chiara Caliumi, Giovanbattista Desideri, Bruno Fabris, Claudio Ferri, Chiara Ganzaroli, Gilberta Giacchetti, Claudio Letizia, Mauro Maccario, Francesca Mallamaci, Massimo Mannelli, Gaetana Palumbo, Enzo Porteri, Damiano Rizzoni, Ermanno Rossi,
Franco Mantero[2]

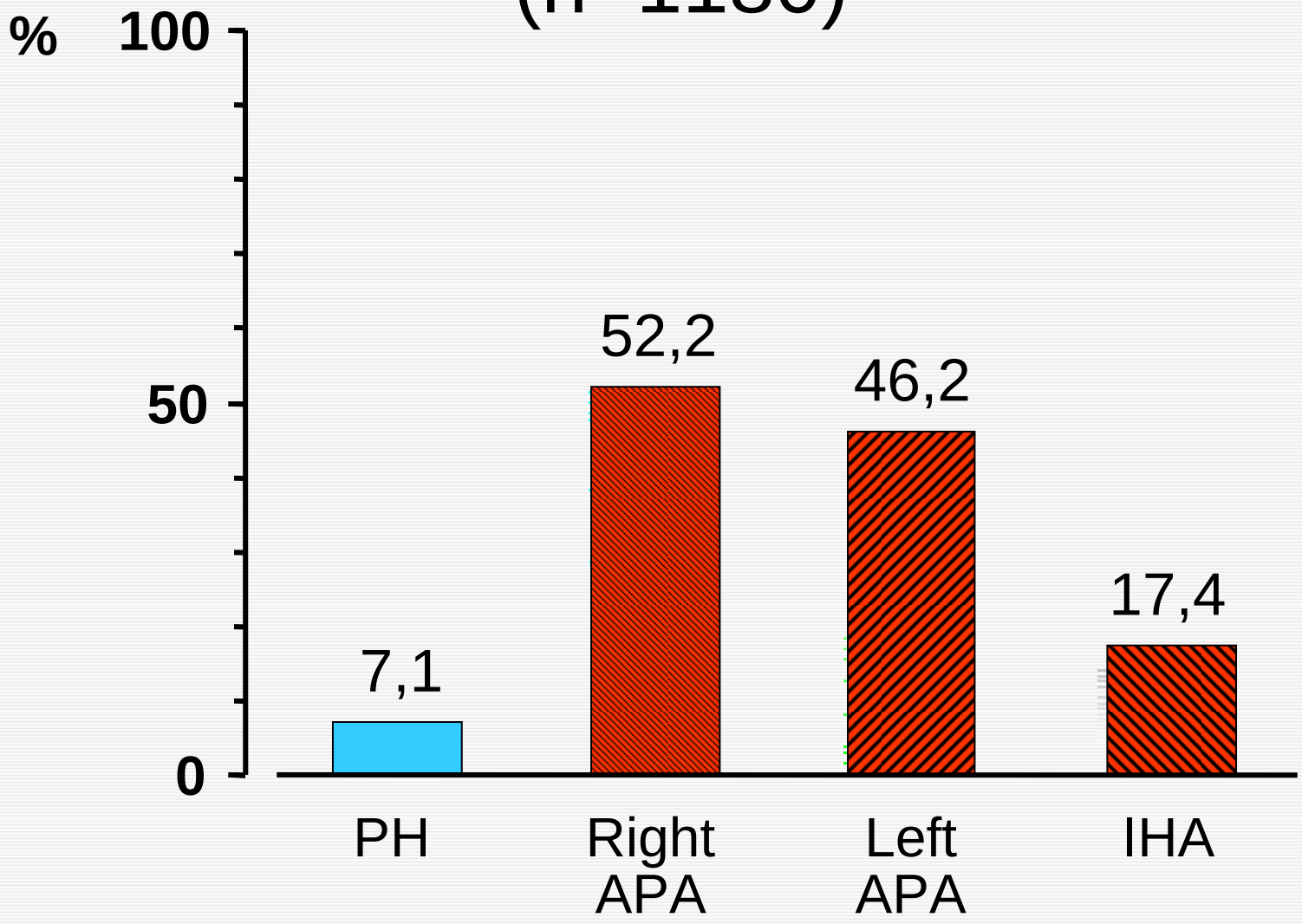
for the Working Group on Primary Aldosteronism of the Italian Society of Arterial Hypertension

[1]DMCS -Clinica Medica 4, [2] Chair of Endocrinolgy
University of Padua Medical School

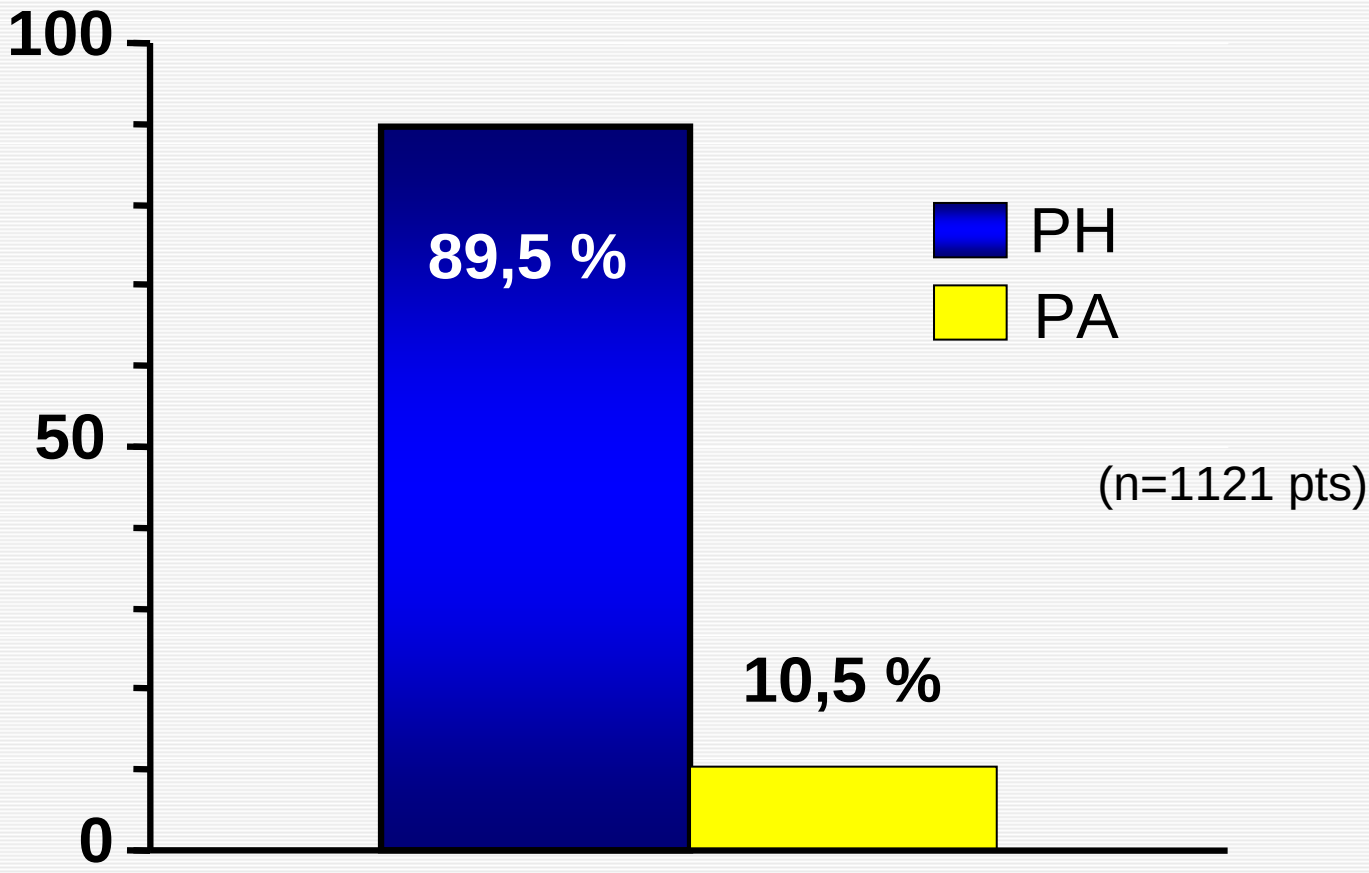
The PAPY (Primary Aldosteronism Prevalence in ItalY) Study:

A Multicentre Study of The Italian Society of Arterial Hypertension

Prevalence of Hypokalemia by Underlying Adrenal Pathology (n=1180)



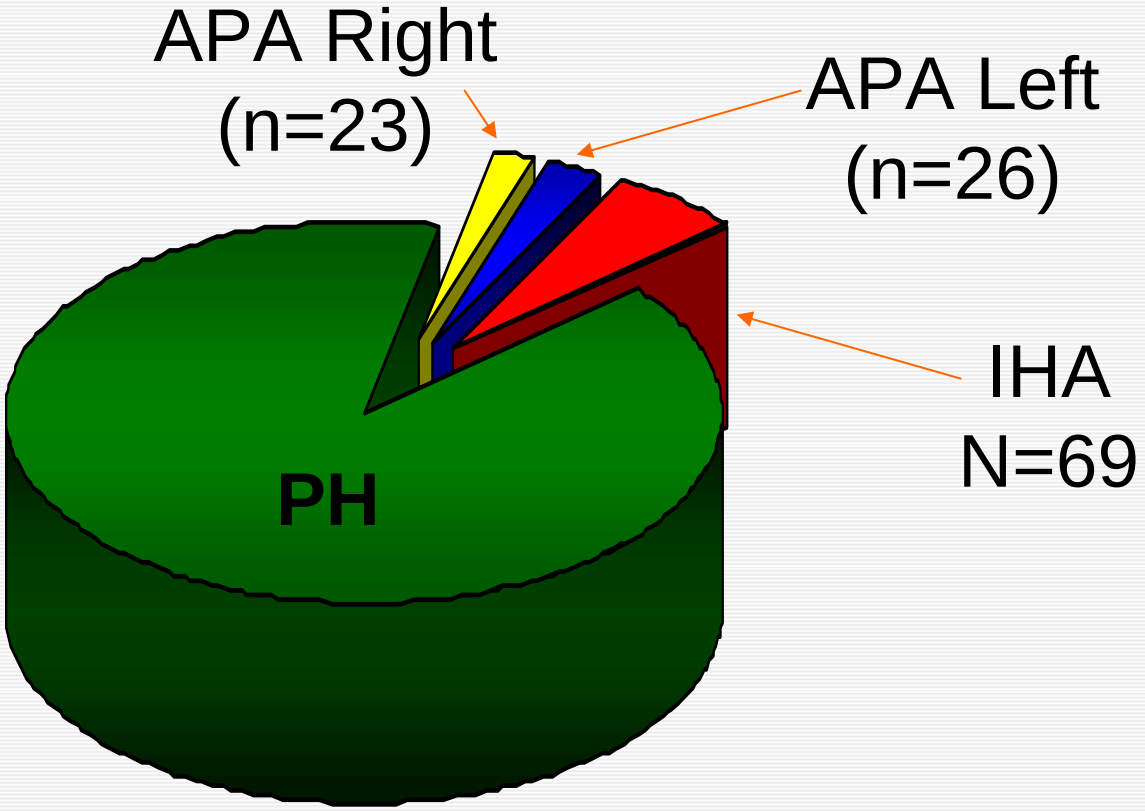
Prevalence of PA and Primary Hypertension (PH) in Patients with Conclusive Diagnosis



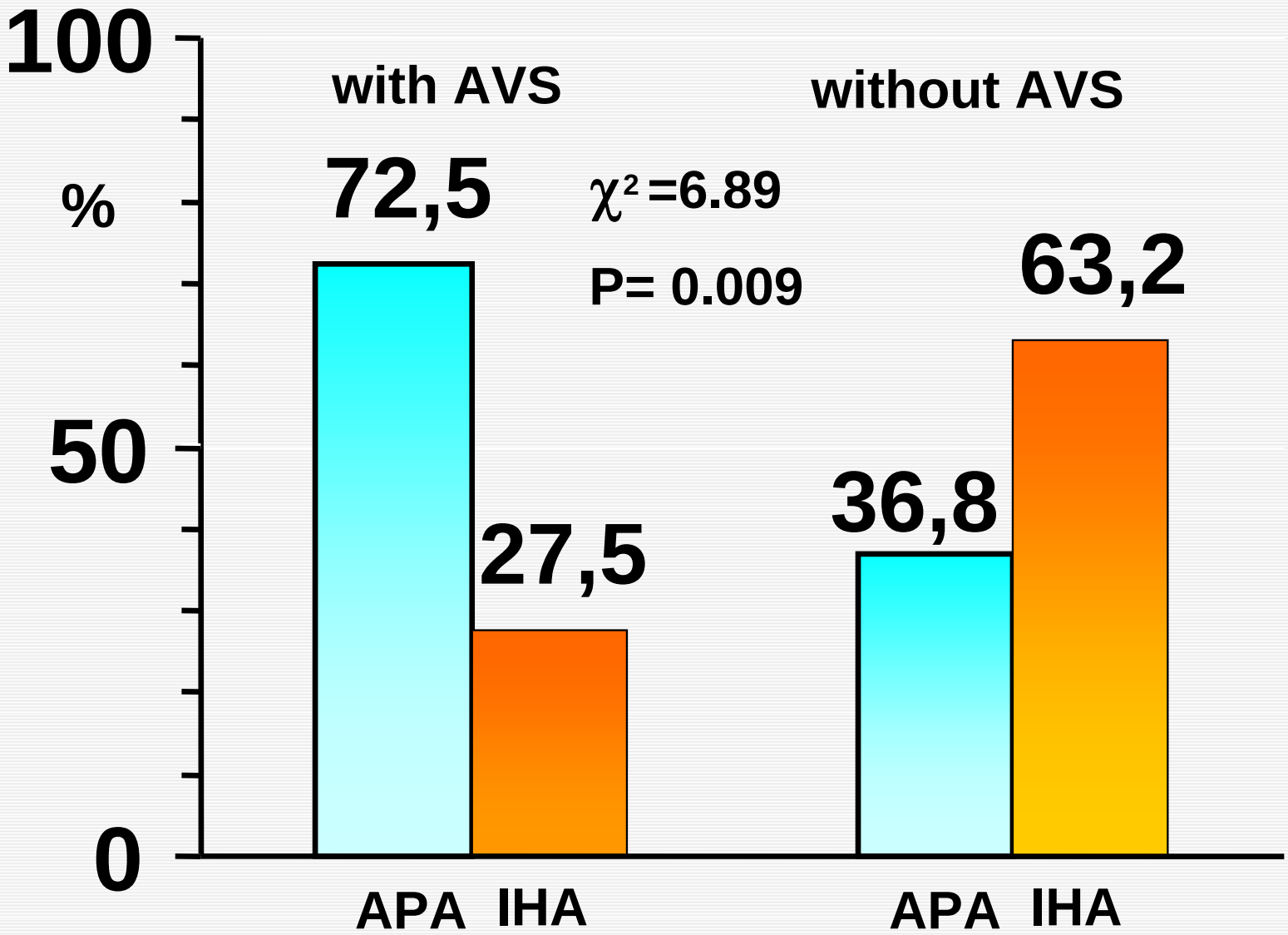
PAPY Study

Final diagnosis PA=118

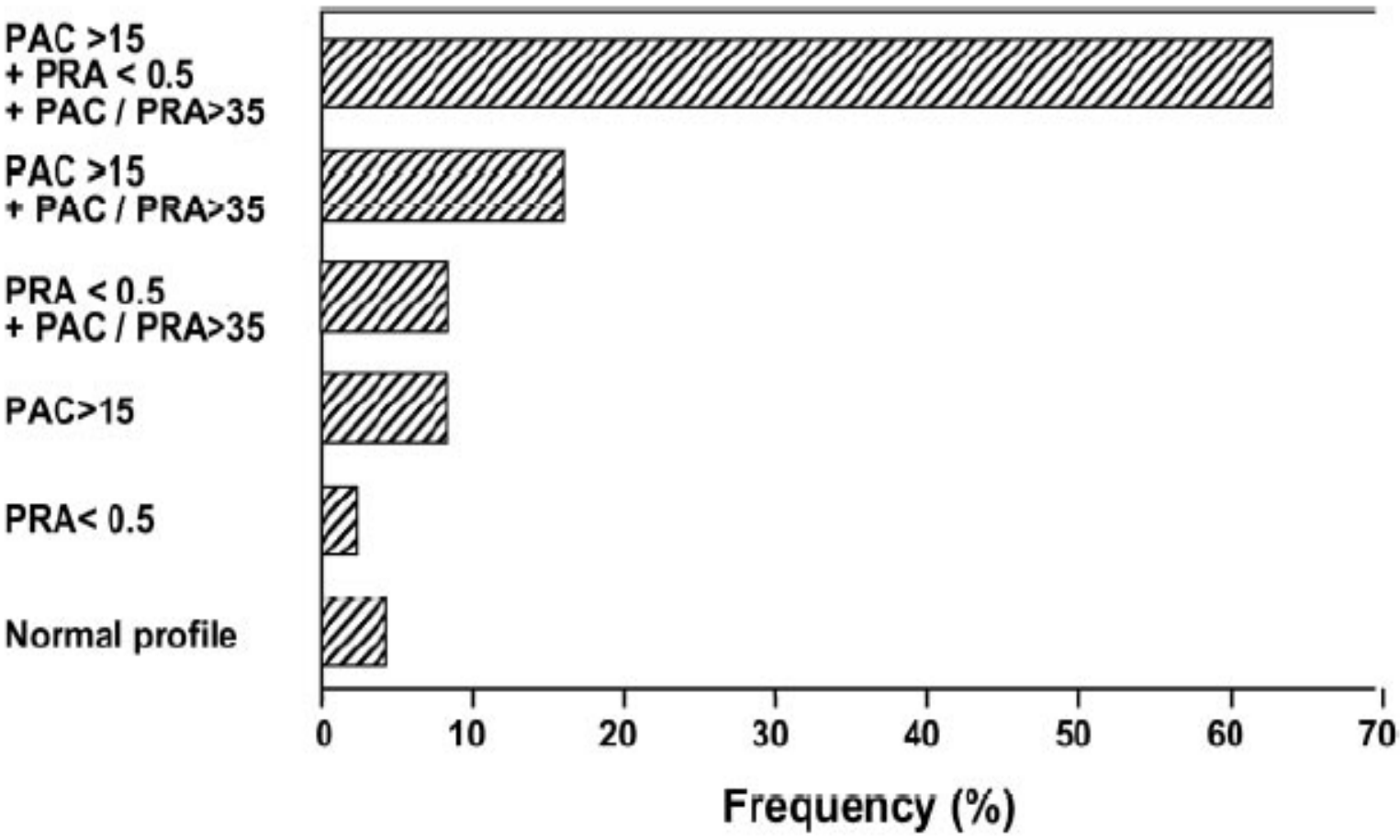
(n=1121 pts)



Prevalence of the Different Pathologies Underlying PA according to Availability of AVS

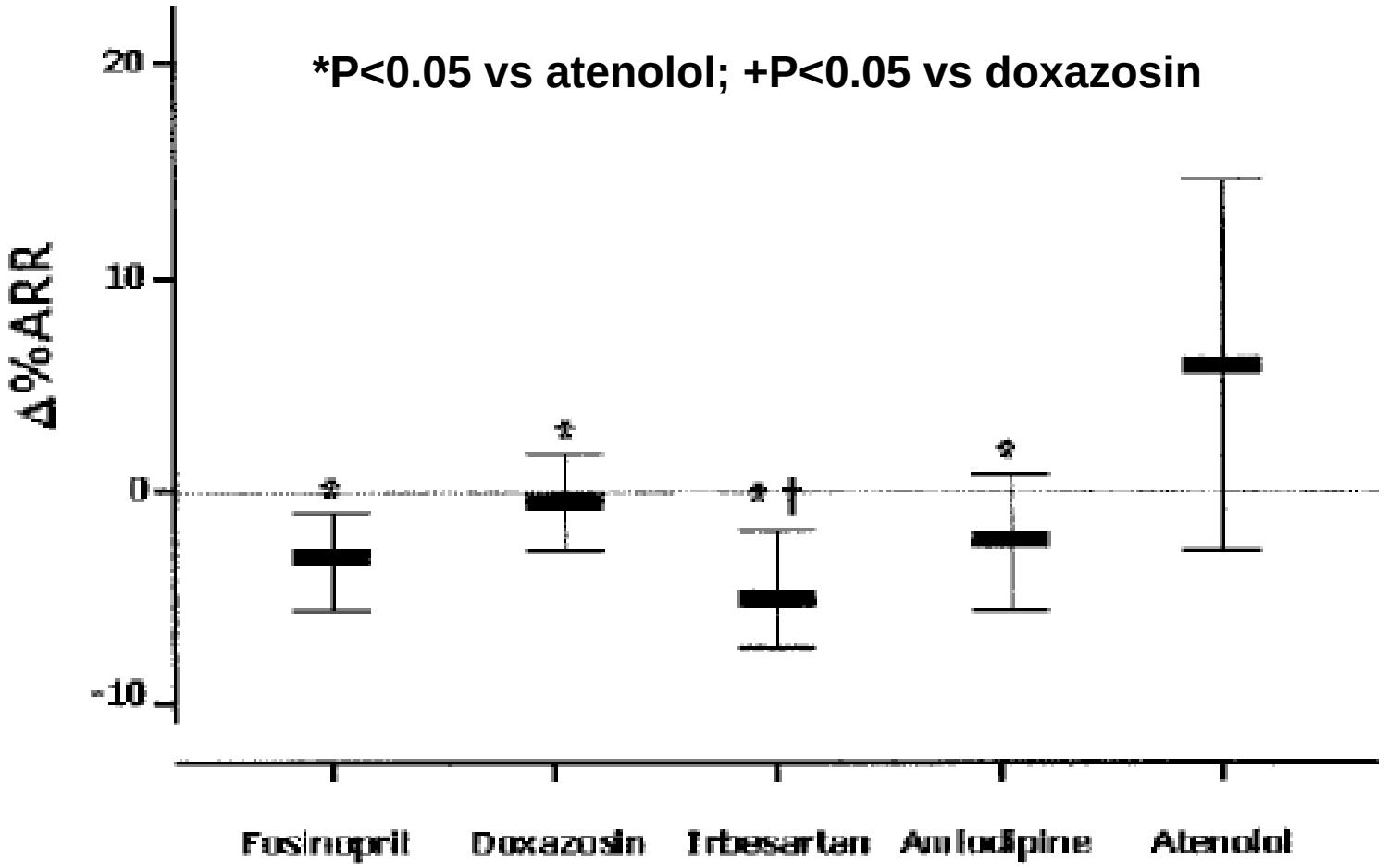


Frequency of patterns in 385 blood samples



Tanabe A *et al*, 2003

Percentage change in the ARR (PAC/PRA) after 2 month therapy with single drug



Mulatero *et al*, 2002

Cut-off values for the aldosterone/renin ratio using plasma renin activity or active renin measurements

	Plasma renin			
	Activity		Immunoreactive	
	ng / ml / h	pmol / l / min	mU / l	ng / l
Plasma aldosterone				
ng/dl	**> 27**	**> 2.1**	**> 3.3**	**> 5.4**
pmol/l	**> 750**	**> 59**	**> 90**	**> 150**

Ferrari et al, J Hypertension, 2004

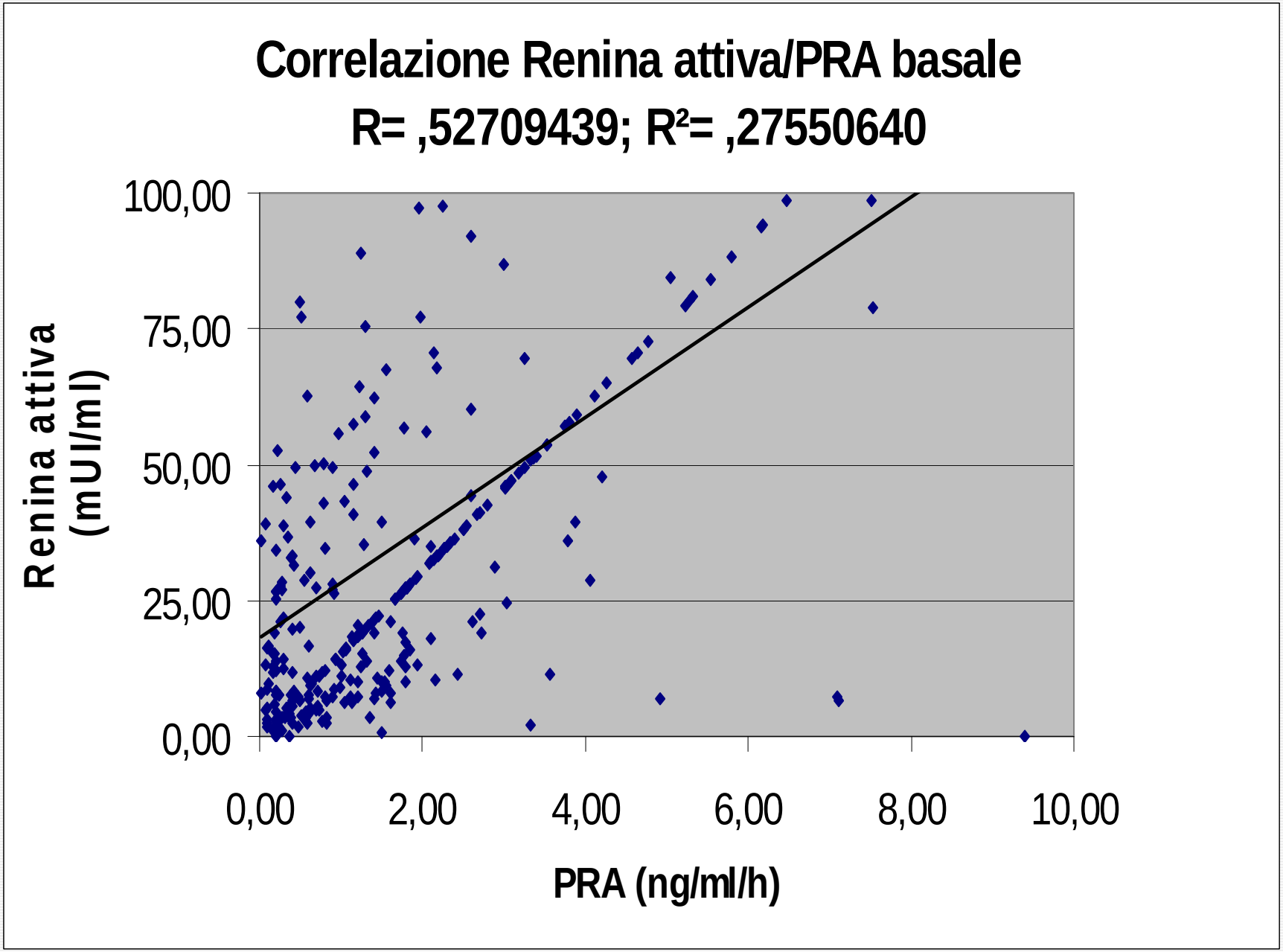
GUIDLINES FOR APPLICATION OF ALDO/PRA RATIO (ARR) IN SCREENING FOR PRIMARY ALDOSTERONISM

1) Repeat measurement of Aldo/PRA ratio before going on to a suppression test
2) Consider influences (age, diet, drugs, posture, method of collection, sK and sCreatinine levels)

RECOMMENDED CONDITIONS FOR DETERMINATION OF ARR

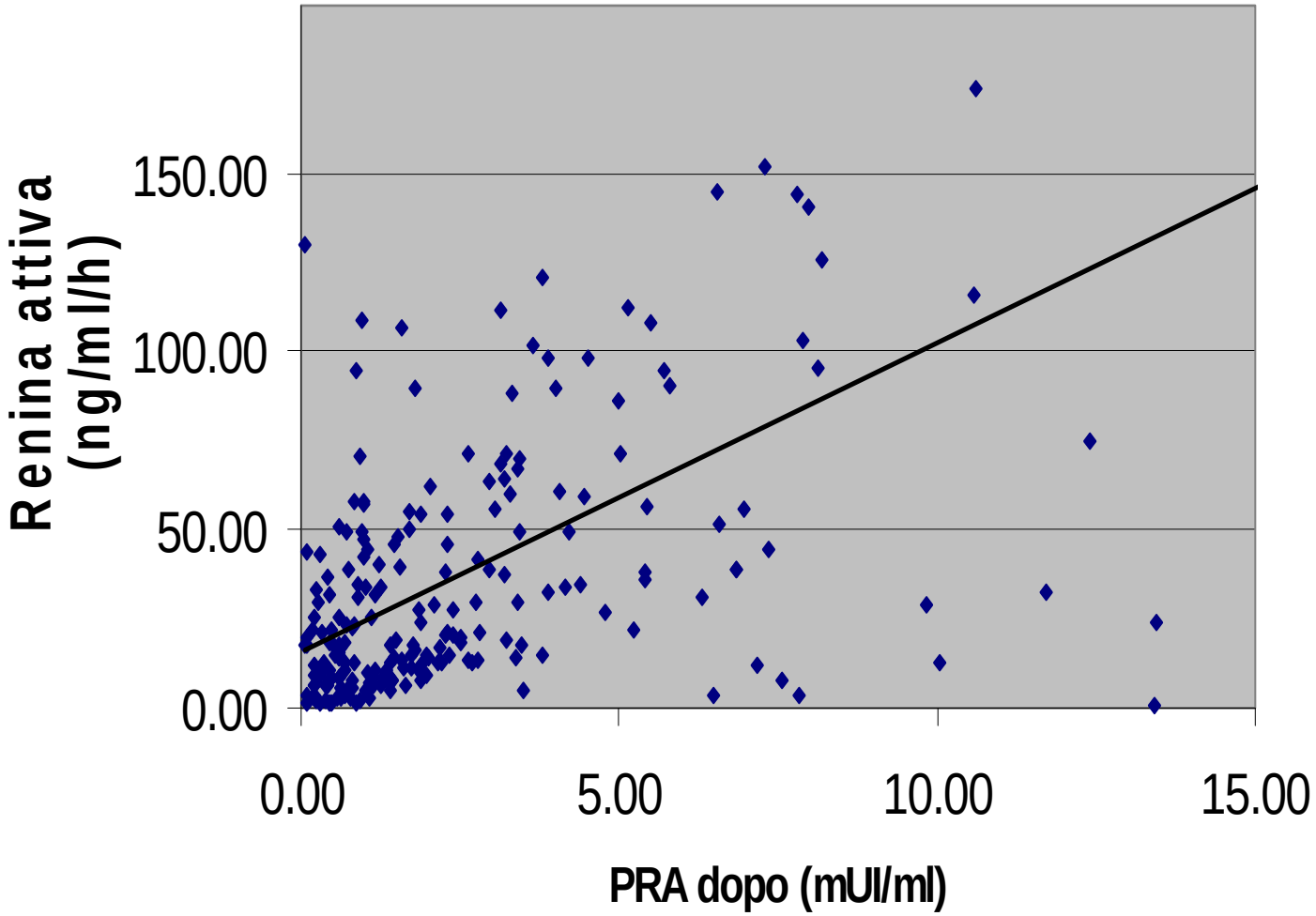
1) Mid-morning, seated for 5 – 15 min
2) If antihypertensives required, use doxazosin, prazosin, or CCB, singly or in combination for 1 month before sampling

Adapted from GORDON RD, 2004


Correlazione Renina attiva/PRA basale
R= ,52709439; R²= ,27550640
Renina attiva (mUI/ml)
100,00
75,00
50,00
25,00
0,00
0,00
2,00
4,00
6,00
8,00
10,00
PRA (ng/ml/h)

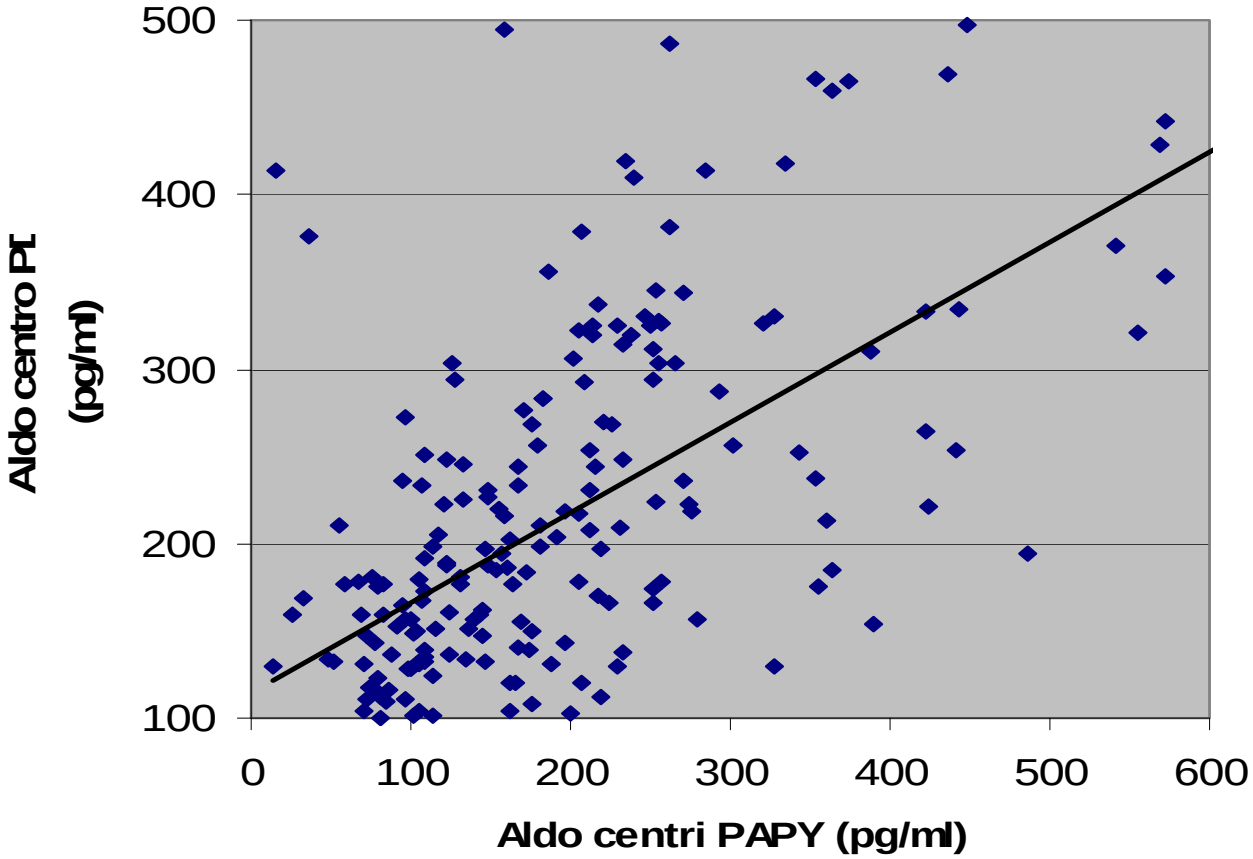
Correlazione Renina attiva/PRA dopo Capoten

R= ,68936042; R^2= ,4729616



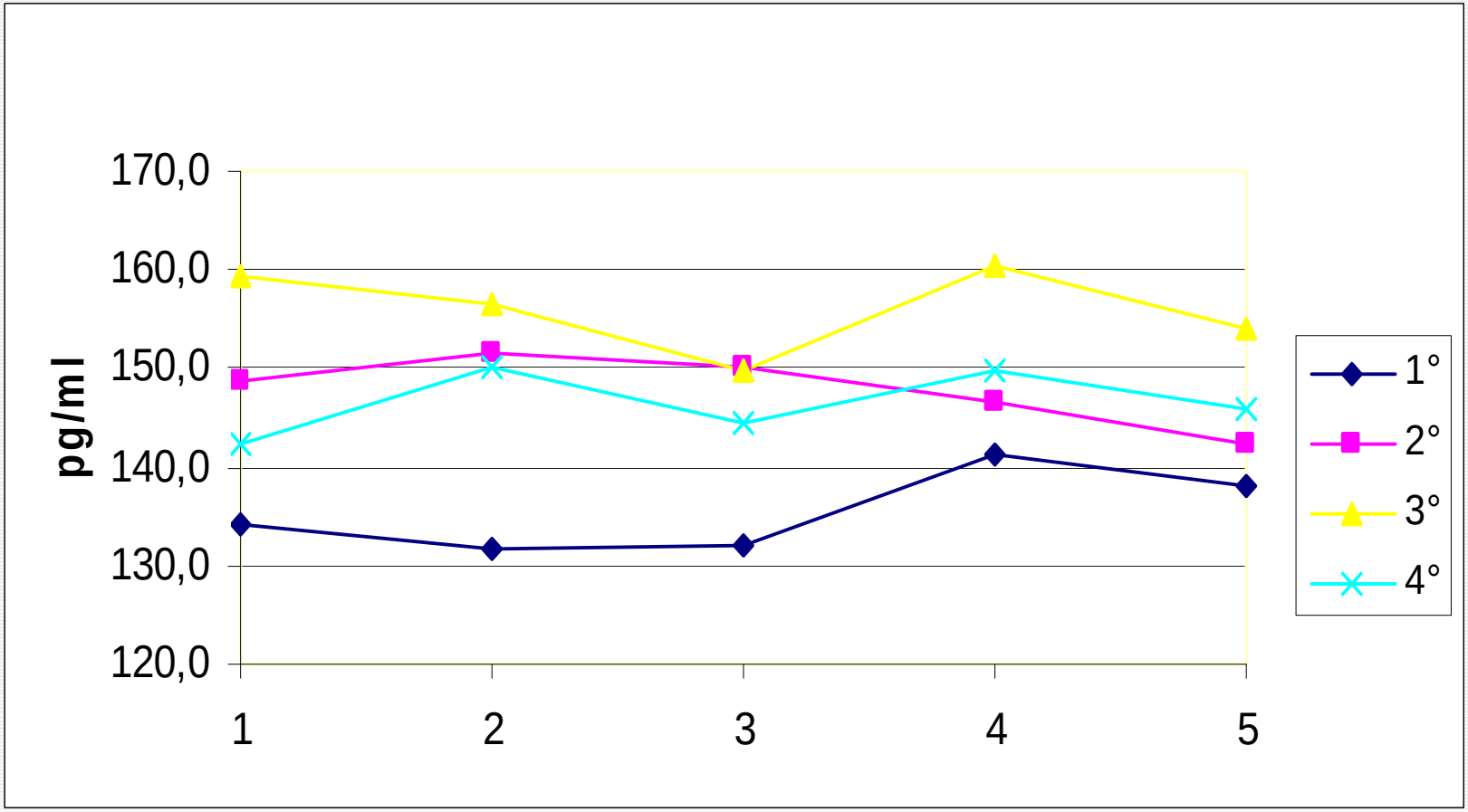
Correlazione aldosterone Centro di PD/aldo centri PAPY

R= ,47196374; R²= ,21935566



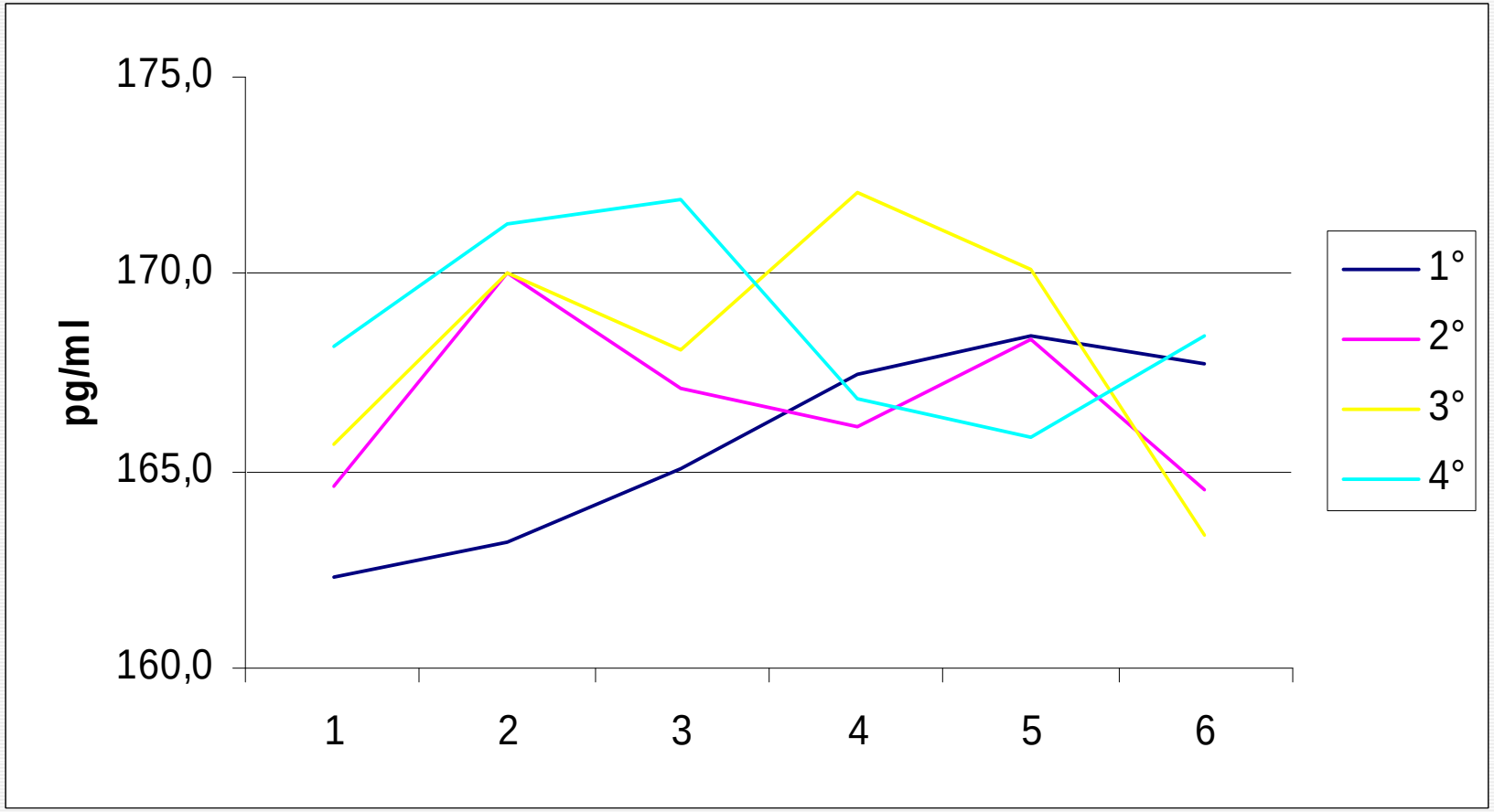
Intra ed inter-assay con siero di controllo

(Aldosterone Diasorin)

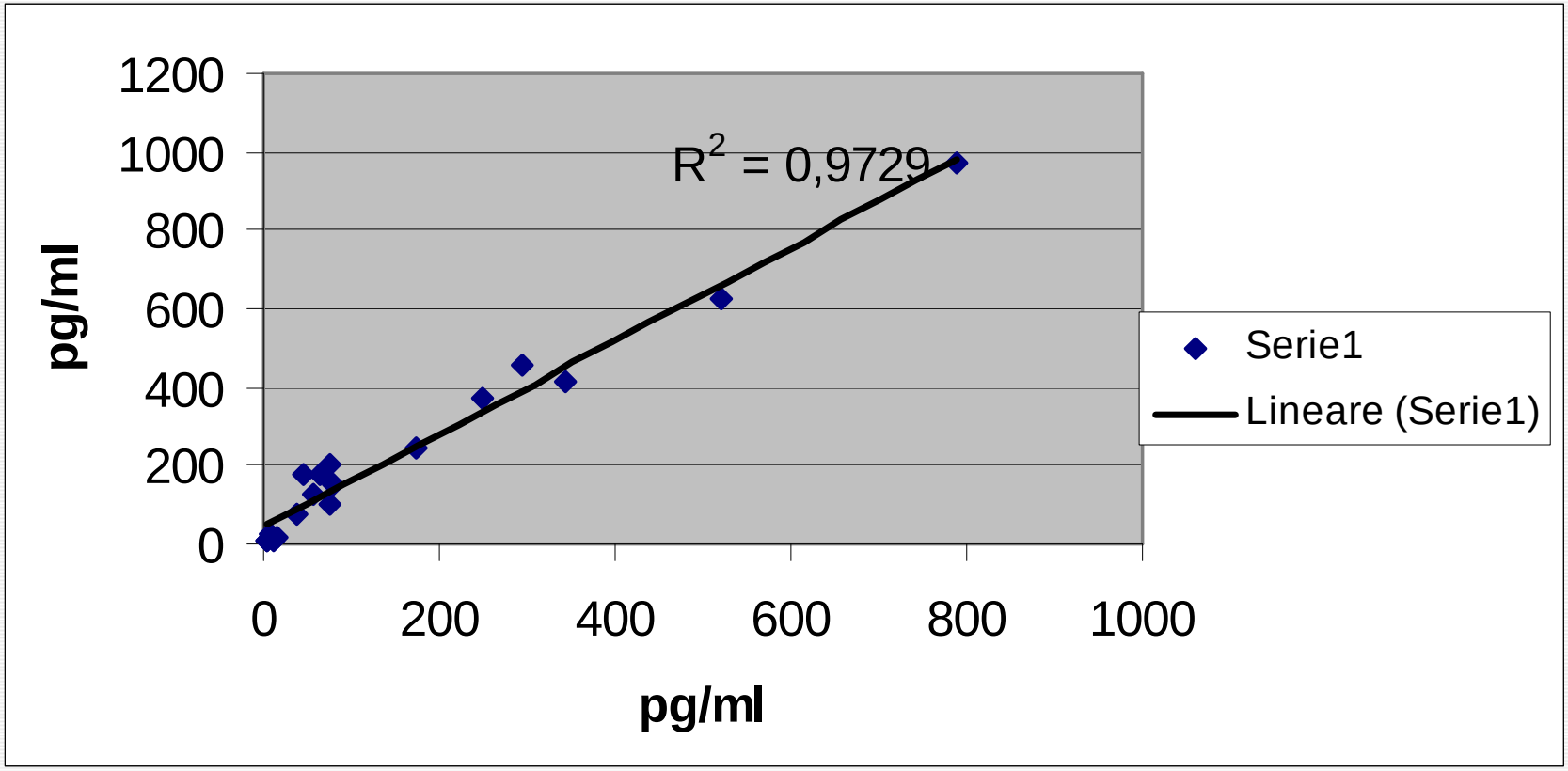


Intra ed inter-assay con siero di paziente

(Aldosterone Diasorin)

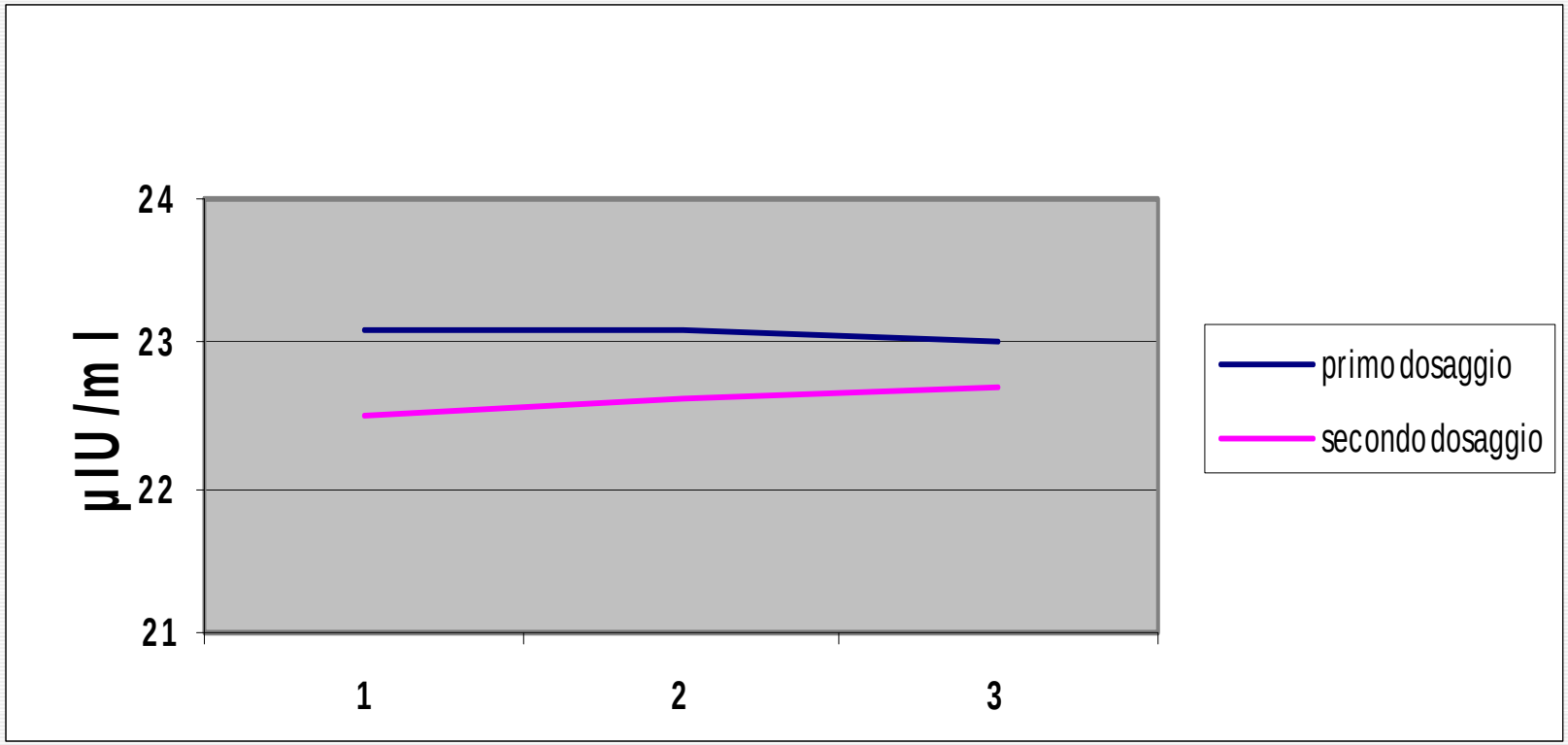


Correlazione Aldosterone Adaltis/diasorin



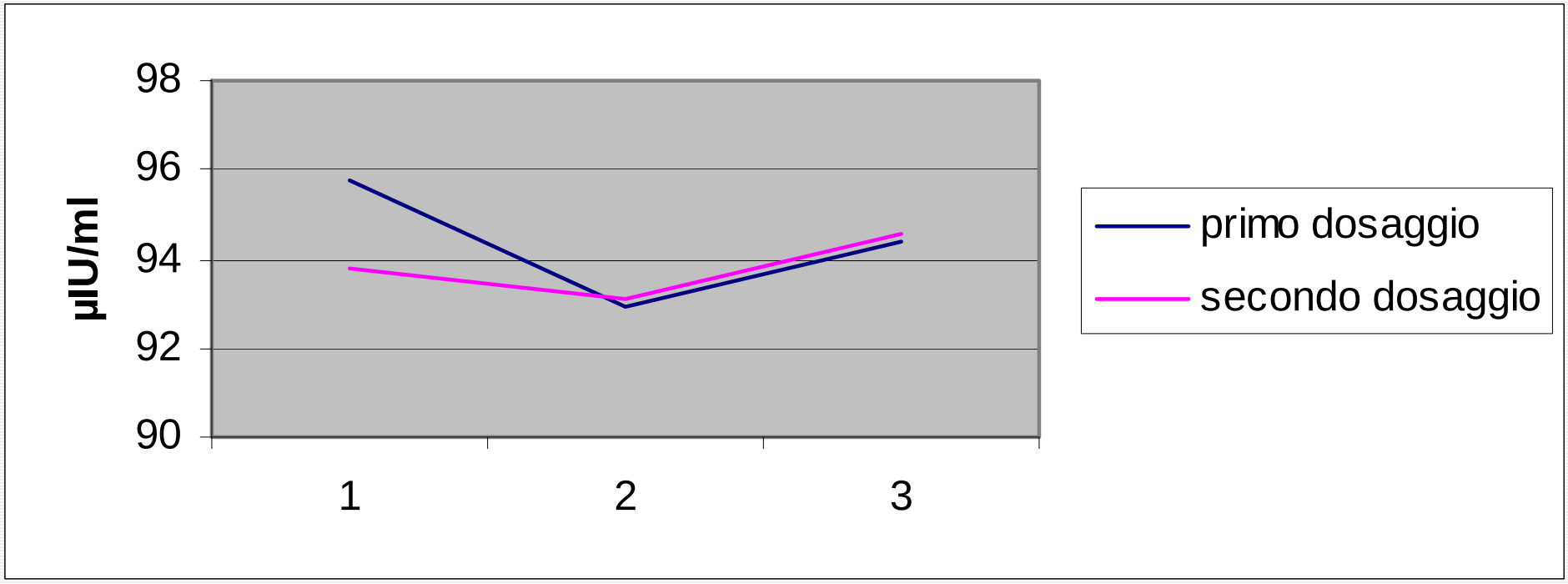
RENINA: intra ed inter- assay siero controllo 1°

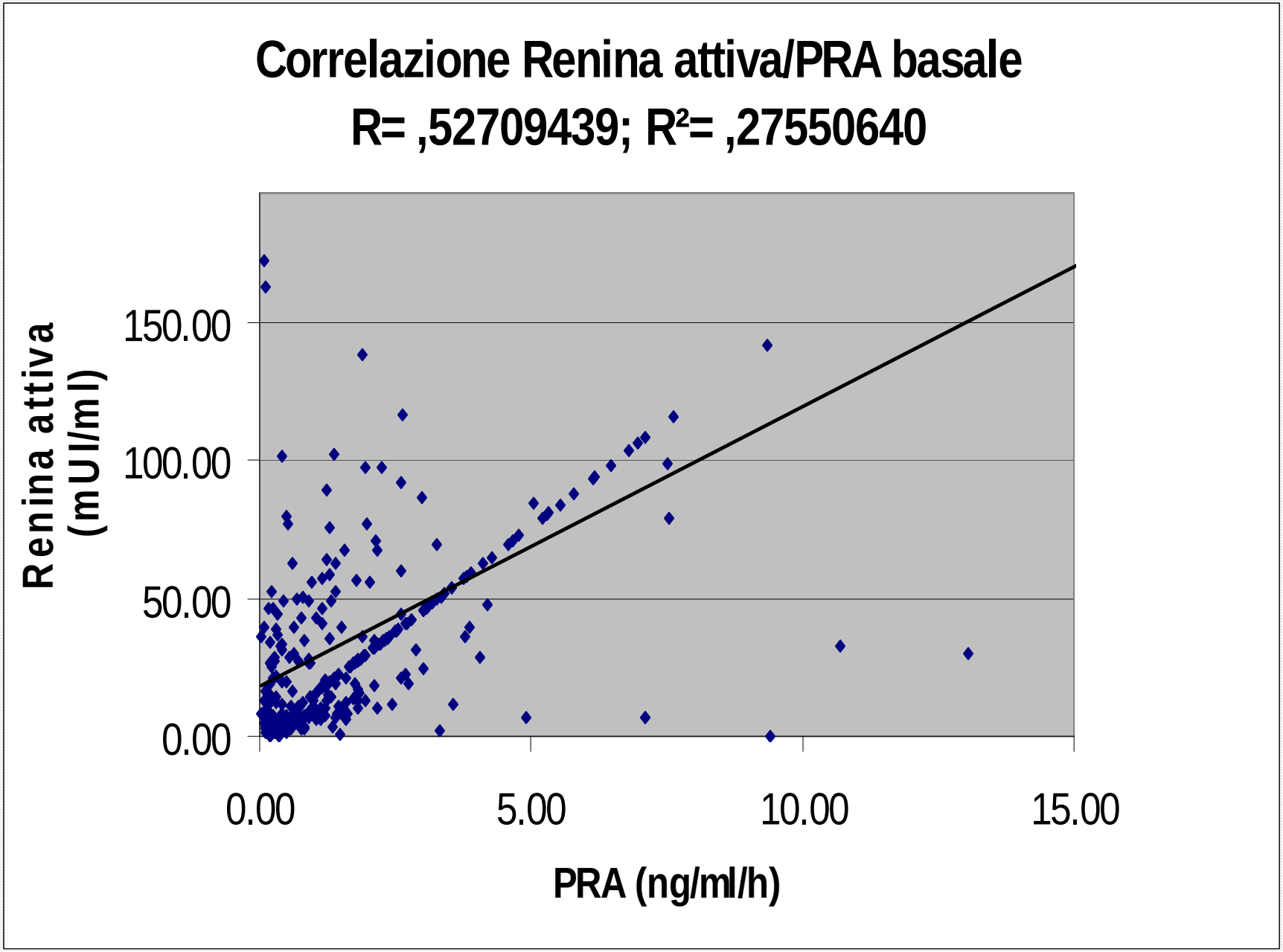
(Diasorin- LIAISON)



RENINA: intra ed inter- assay siero controllo 2°

(Diasorin- LIAISON)

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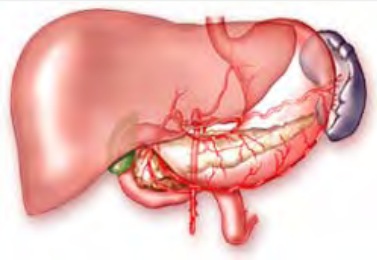
R= ,52709439; R²= ,27550640
Renina attiva (mUI/ml)
150.00
100.00
50.00
0.00
0.00
5.00
10.00
15.00
PRA (ng/ml/h)

Nonepithelial Mechanisms of Aldosterone Action



Angiotensinogen

Renin

Angiotensin I

Angiotensin II

Vasoconstriction

Increased sympathetic activiry

Aldosterone

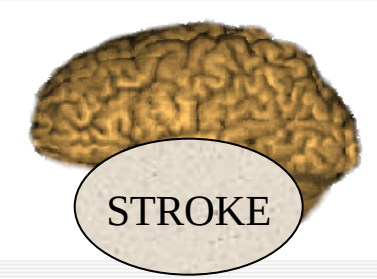
Genomic/non genomic effects

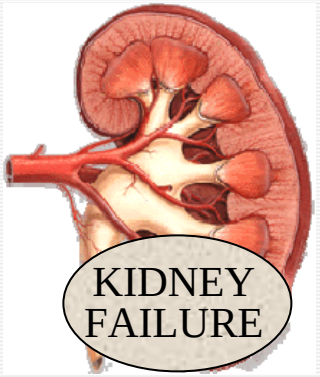
Increased Blood Pressure

Increased inflammation and atherosclerosis

myocardial fibrosis
hypertrophy
ischemia
incresed QT-interval
Decreased RR variability

Renal fibrosis
nephropathy





McFarlane, JCEM 2003

New Concepts of Aldosterone Biology

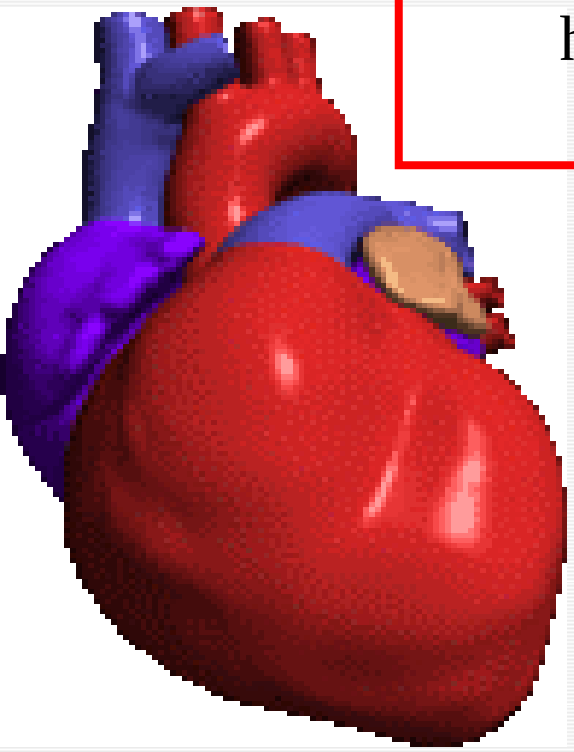
Preclinical studies show:

- **Aldosterone has physiological and patho-physiological effects in nonepithelial tissues including heart, vasculature, and brain.**

- **Mineralocorticoid receptors are also located in non epithelial tissues including cardiomyocytes, brain, and blood vessel wall (VSCM and endothelial cells) and circulating monocytes, role of these receptors is less well understood.**

- **Aldosterone is proinflammatory and has pathologic effects including cerebral vascular disease, myocardial fibrosis, nephrosclerosis1**

Aldosterone Actions in the Heart

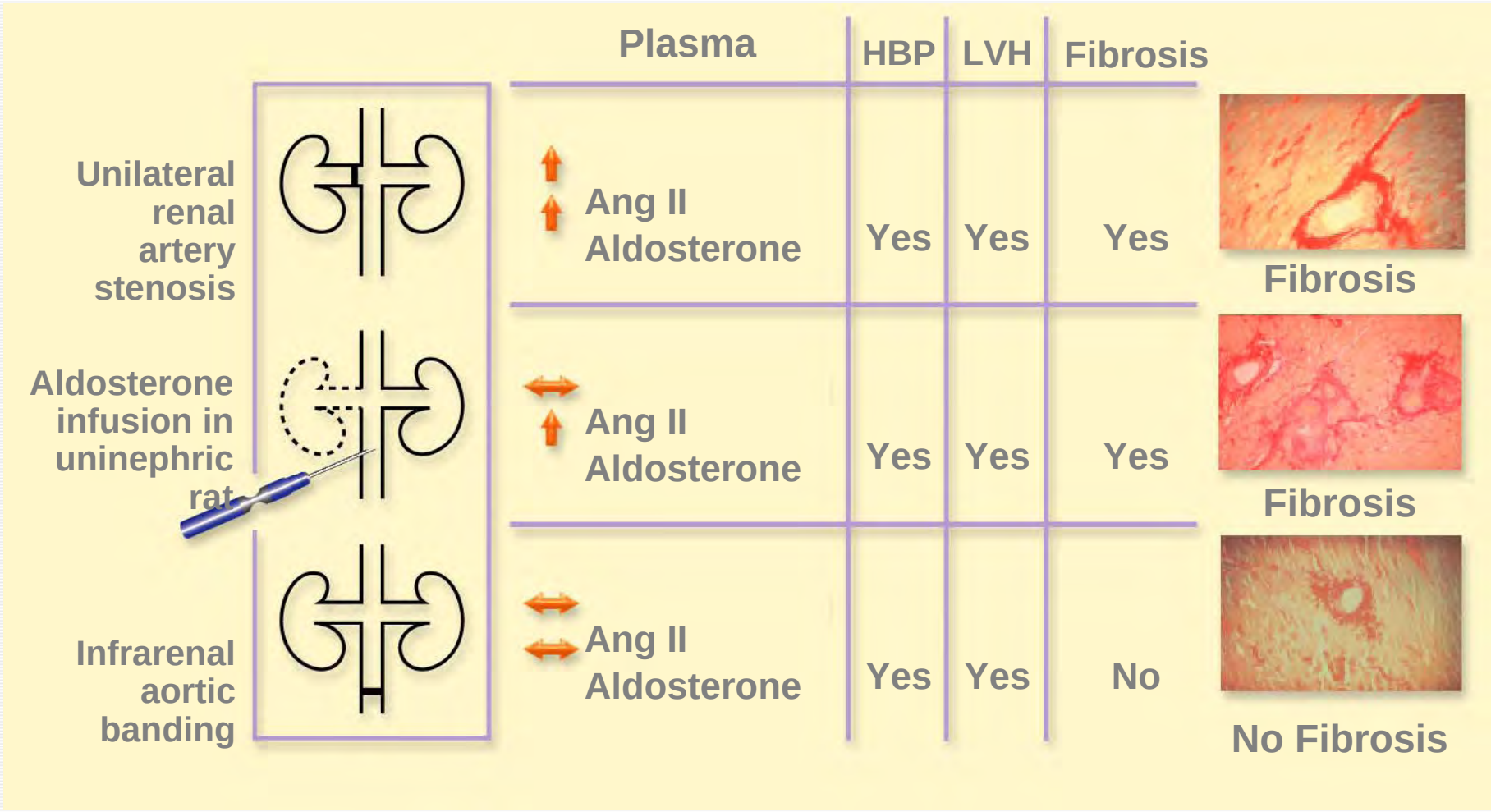


In animal models aldosterone in conjunction with a high-salt diet produces hypertension, cardiac hypertrophy, and cardiac fibrosis.

Pathological actions of aldosterone are independent of systolic bloood pressure , hypokaliemia, and cardiac hypertrophy, demonstrating a direct cardiovascular effect of aldosterone

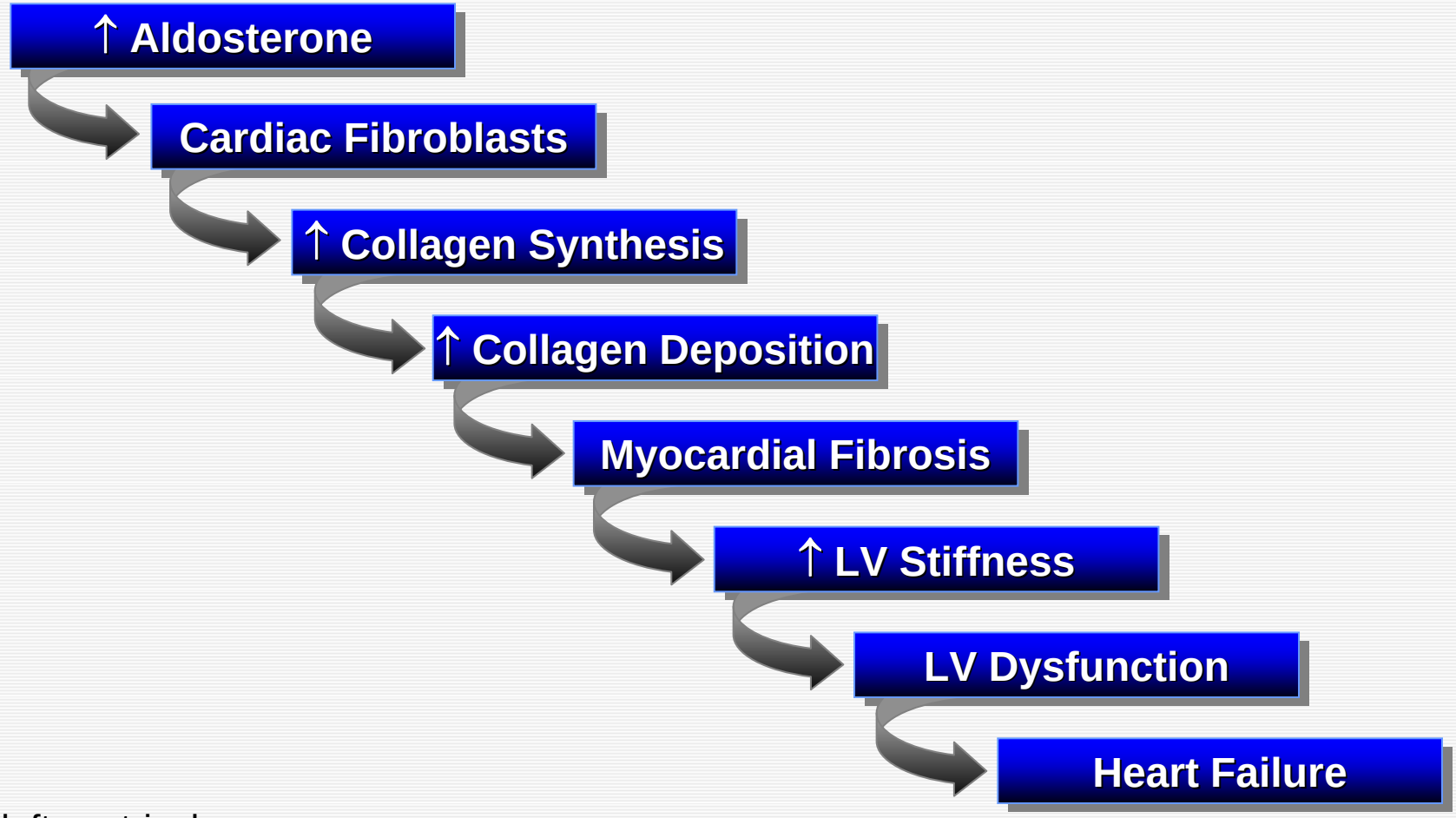
Aldosterone has direct effects, activating an inflammatory cascade, leading to cardiac fibrosis.

Aldosterone Contributes to *Myocardial Fibrosis* and *Left Ventricular Hypertrophy*



Ang II, angiotensin II; HBP, high blood pressure; LVH, left ventricular hypertrophy
Reprinted with permission from Brilla CG et al. *Circ Res.* 1990;67:1355

Myocardial Fibrosis in Heart Failure: Classic Aldosterone Hypothesis

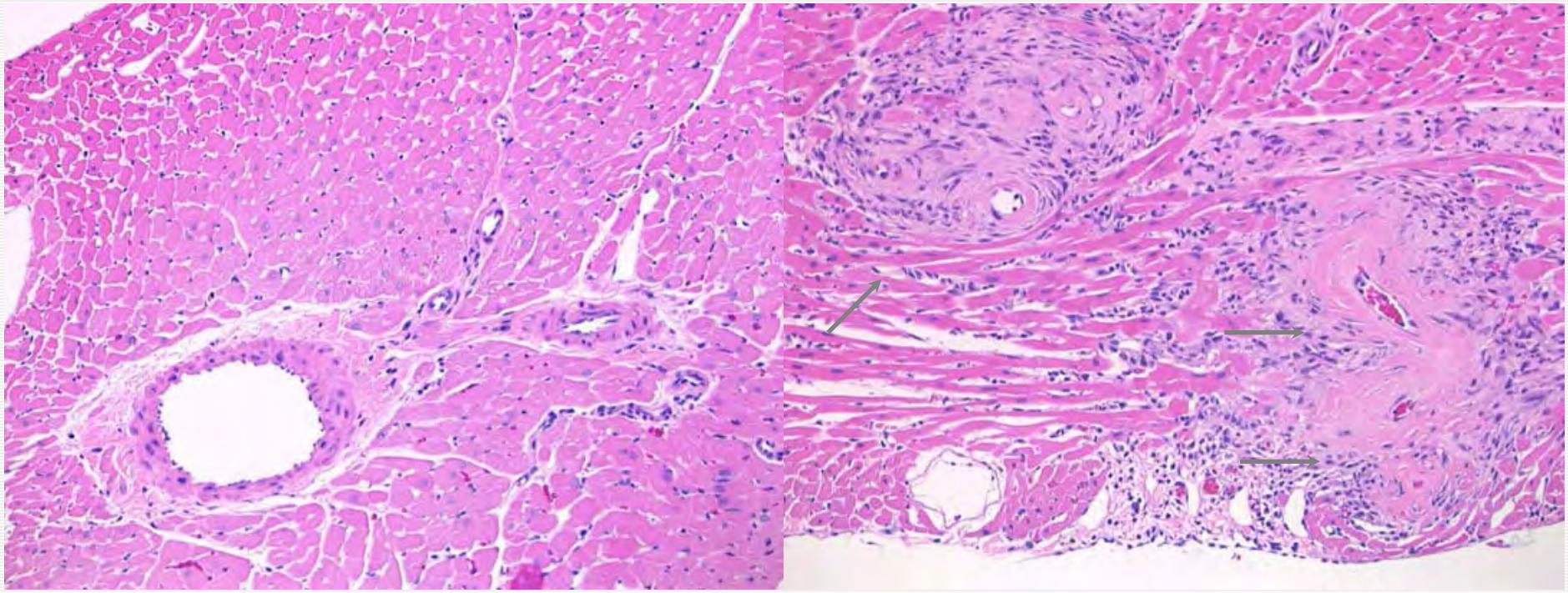


LV, left ventricular

Weber KT. *N Engl J Med.* 2001;345:1689

Aldosterone-Induced Cardiac Vascular Inflammatory Lesions

Ang II/Salt-Hypertensive Rat Model



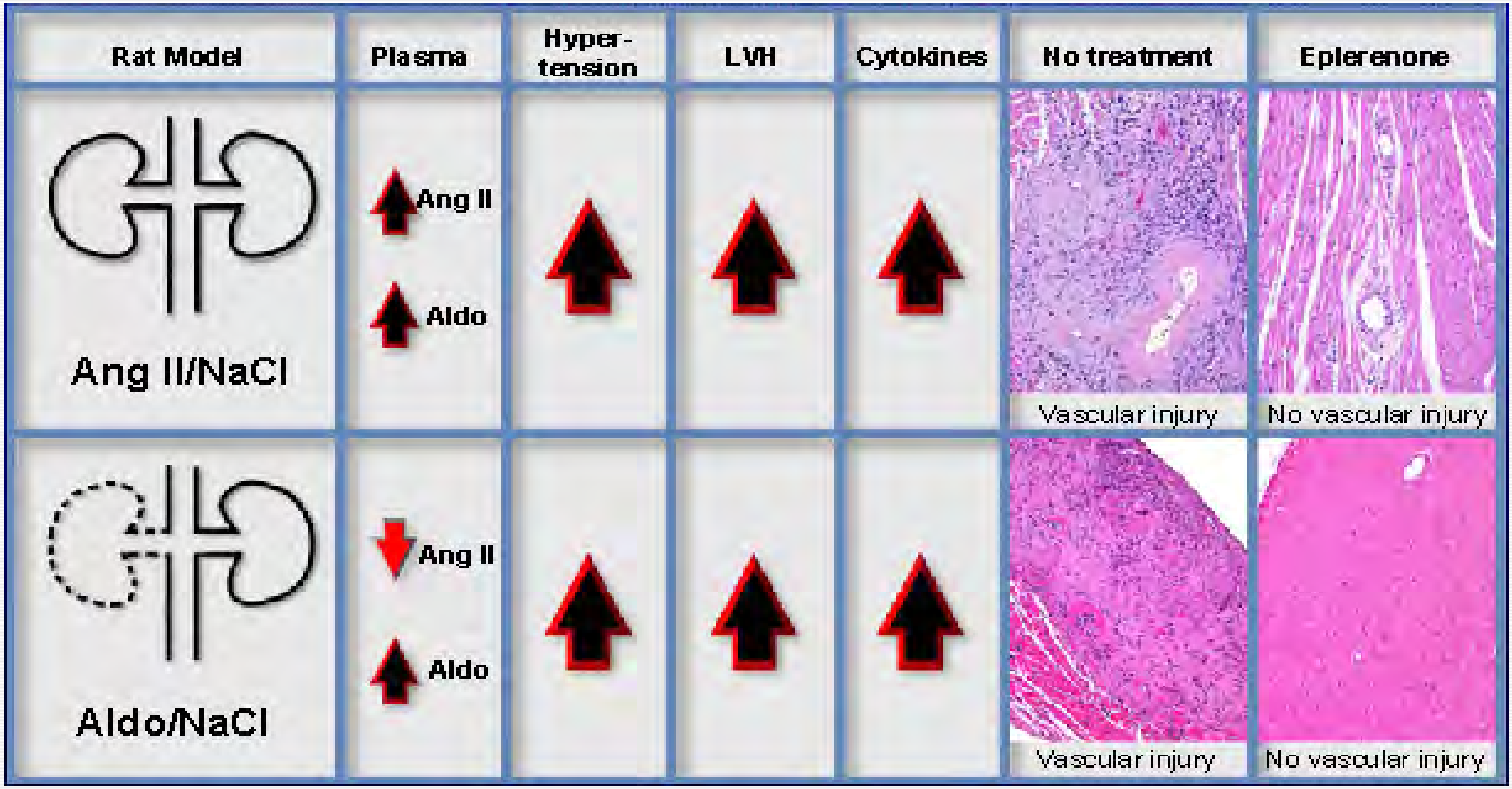
Ang II + NaCl + ADX

Ang II + NaCl + ADX + Aldosterone

Ang II: angiotensin II; NaCl: salt; ADX: adrenalectomy

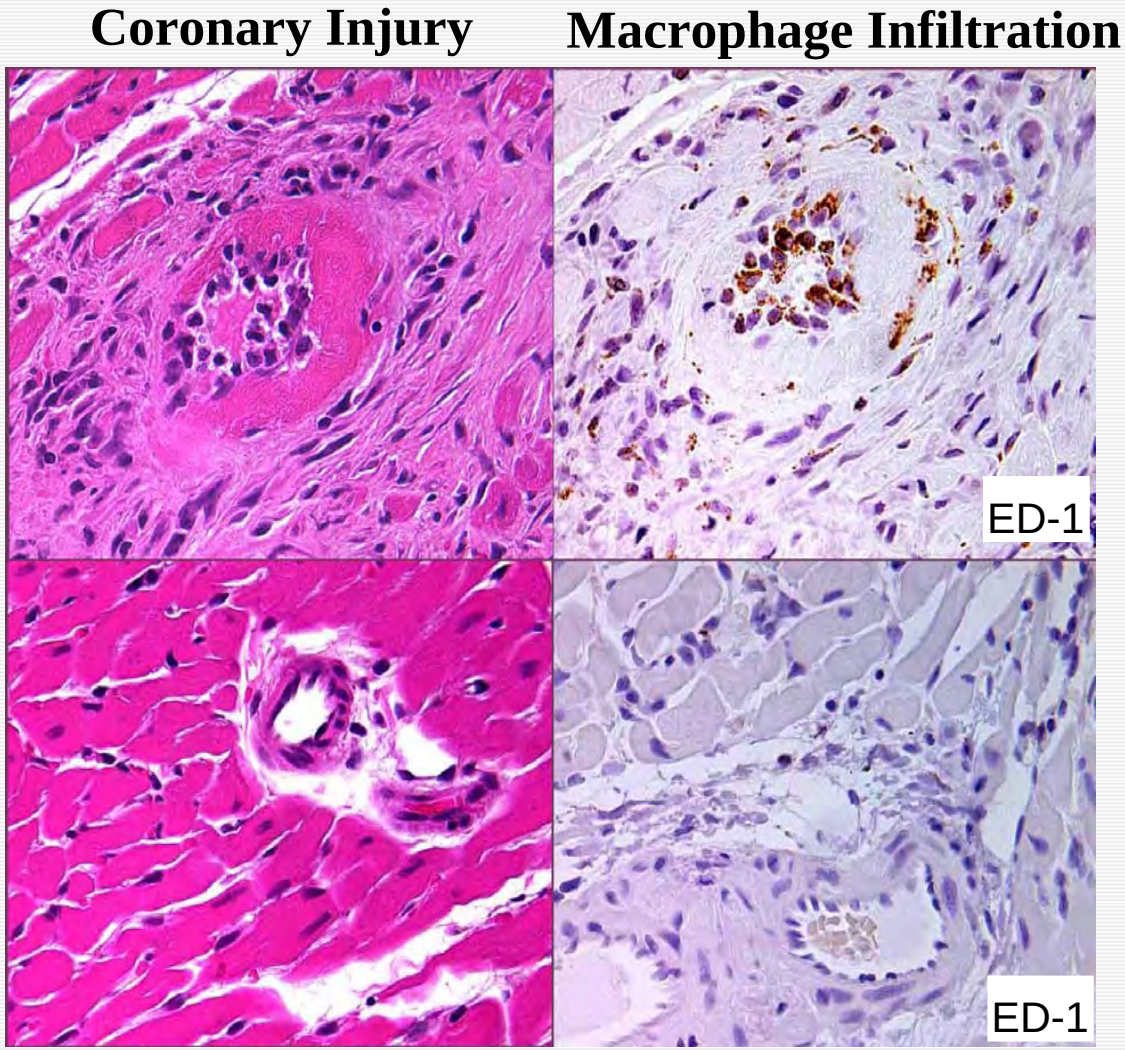
Martin-Berger CL et al. *Hypertension.* 2001; 38:500

Current Concepts of Aldosterone-induced Myocardial Injury



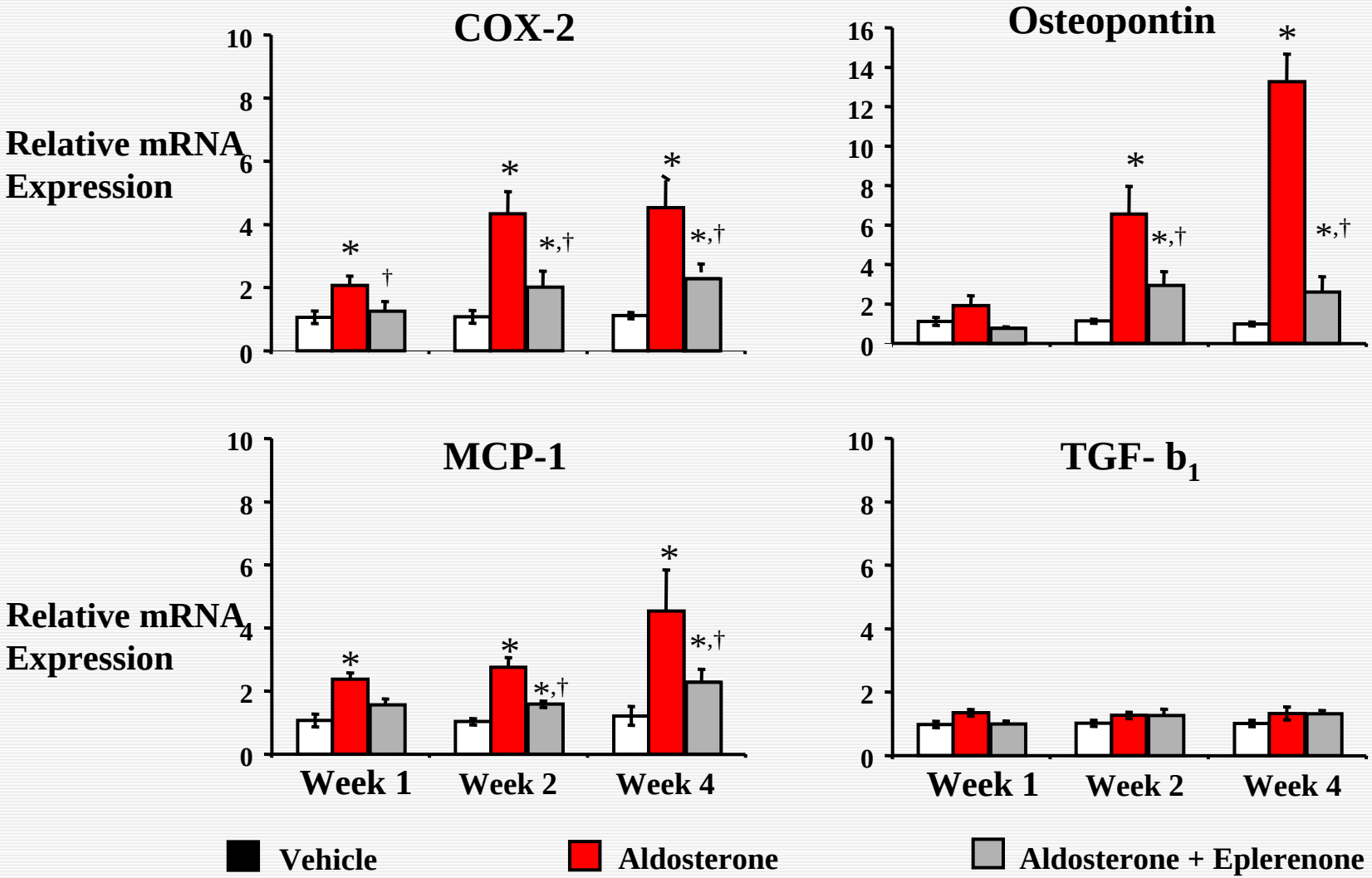
Rocha R, et al. Endocrinology. 2000;141:3871. Rocha R, et al. AJP. 2002;283:H1802.

Aldosterone Induces Coronary Vascular Inflammatory Damage : Effect of MR Antagonism



Rocha R, et al. *Am J Physiol Heart Circ Physiol*. 2002 Nov;283(5):H1802-1810

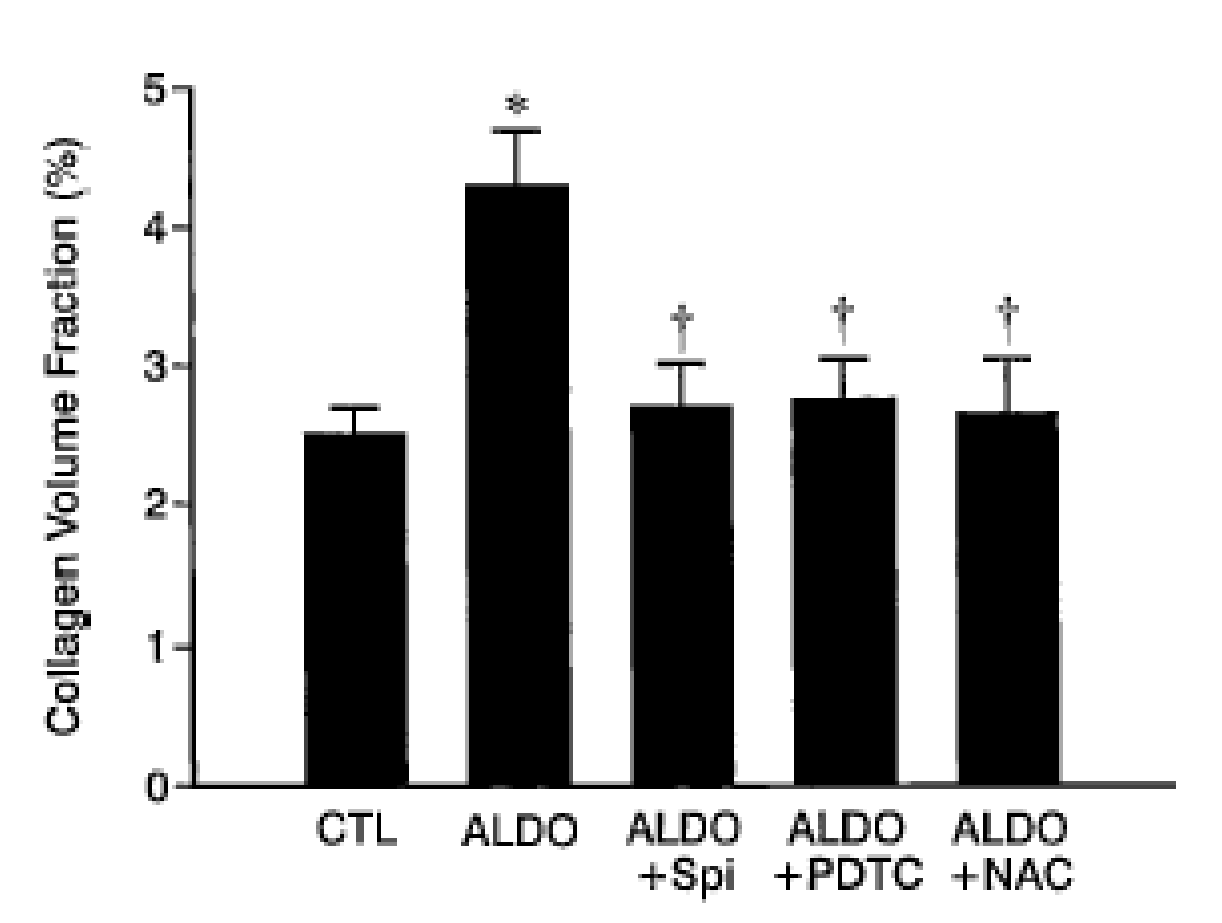
Time Course of Proinflamatory Molecule Expression in the Heart of Aldosterone/NaCl Hypertensive Rats



Rocha R et al. *Am J Physiol.* 2002;283(5):H1802-H1810.

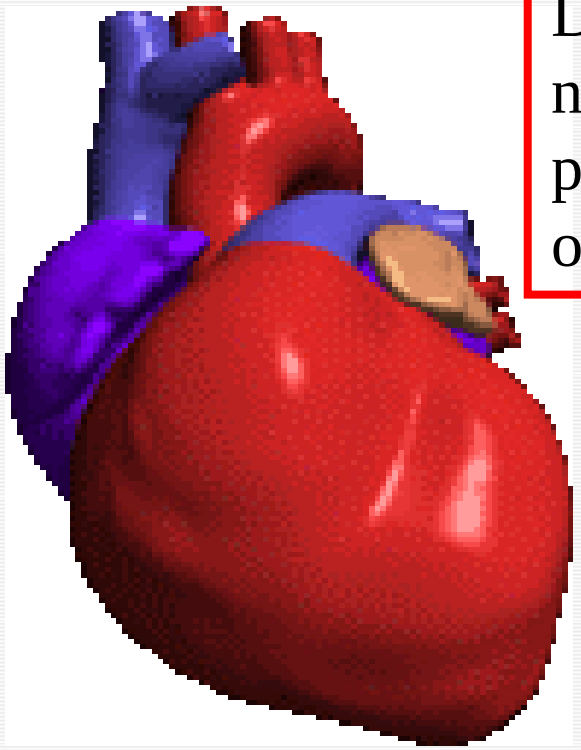
*$P < 0.05$ vs NaCl †$P < 0.05$ vs Aldo/NaCl.

Collagen volume fraction in rat hearts
A marker of fibrosis



Weber AM J Patol, 2002

Effects of Aldosterone on Heart: Summary



Data from hypertensive rat model contribute to new hypothesis about aldosterone-dependent pathologic process that is independent of the level of angiotensin II[1]

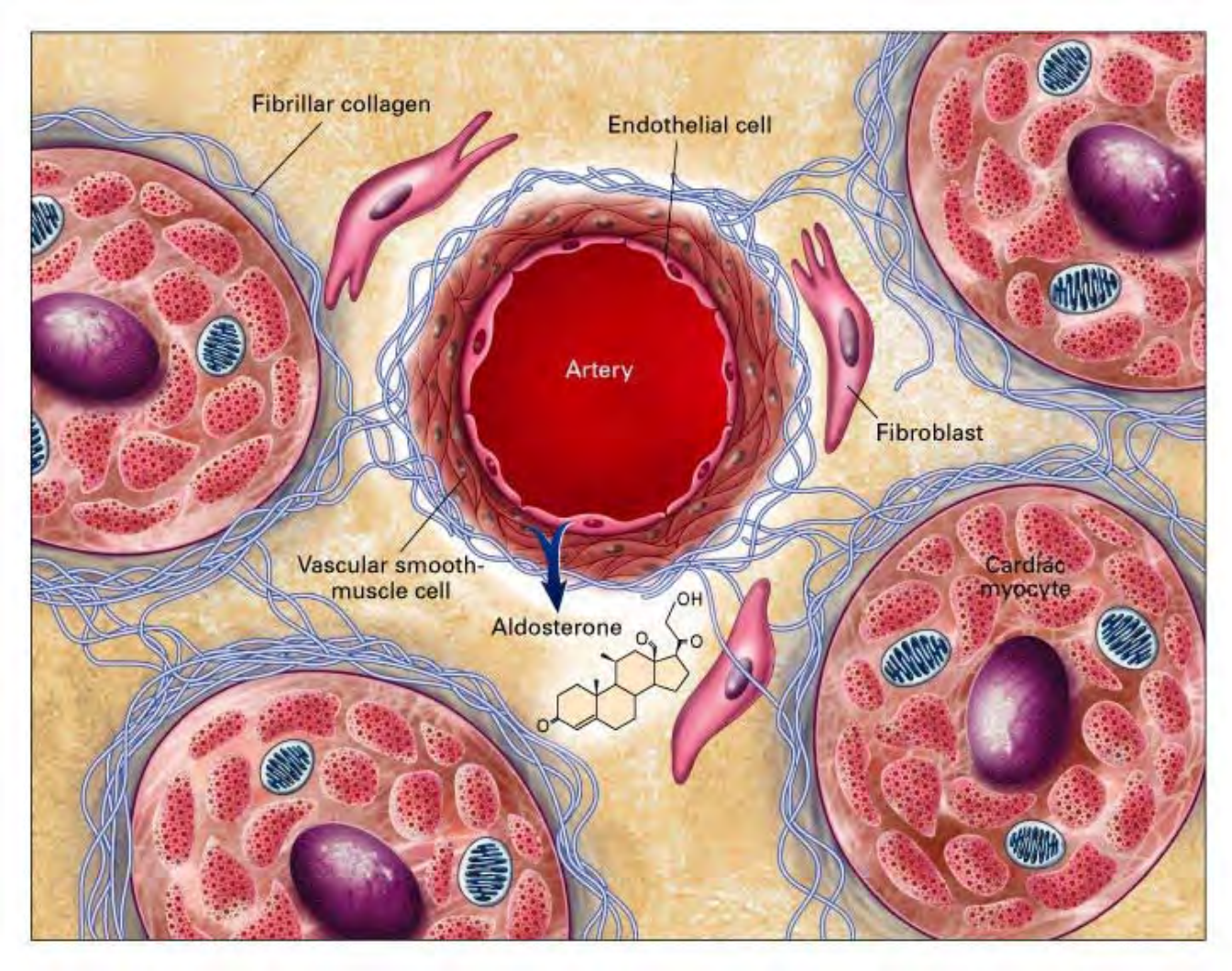
Process progresses from localized inflammation to vascular and myocardial fibrosis, ultimately leading to myocardial injury[1]

Localized vascular injury can generate focal areas of ischemia and ultimately lead to infarction[2]

[1]Rocha R et.al. *Endocrinology.* 2000;141:3871 [2]Rocha R et al. *Hypertension.* 2001;38:479

Aldosterone actions in the Blood Vessel Wall

Extraadrenal Production of Aldosterone by Endothelial and Vascular Smooth-Muscle Cells in an Intramyocardial Coronary Artery.



Weber KT et al. *N Eng J Med* 2001;345:23

Aldosterone actions in the Blood Vessel Wall

Aldosterone-salt administration in rats is able to increase large artery stiffness associated with fibronectin (Fn) accumulation, these changes were reversed if rats were treated with eplerenone

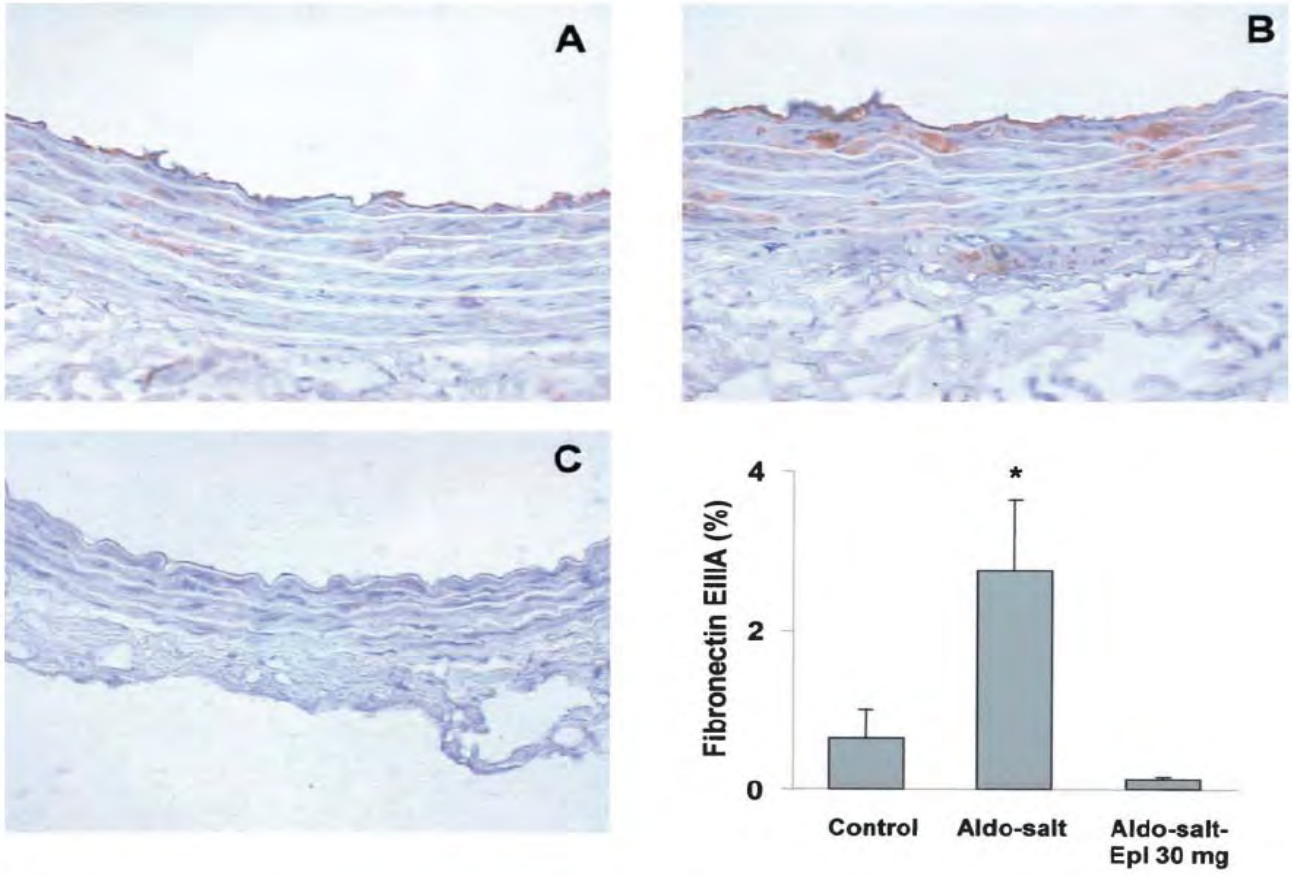
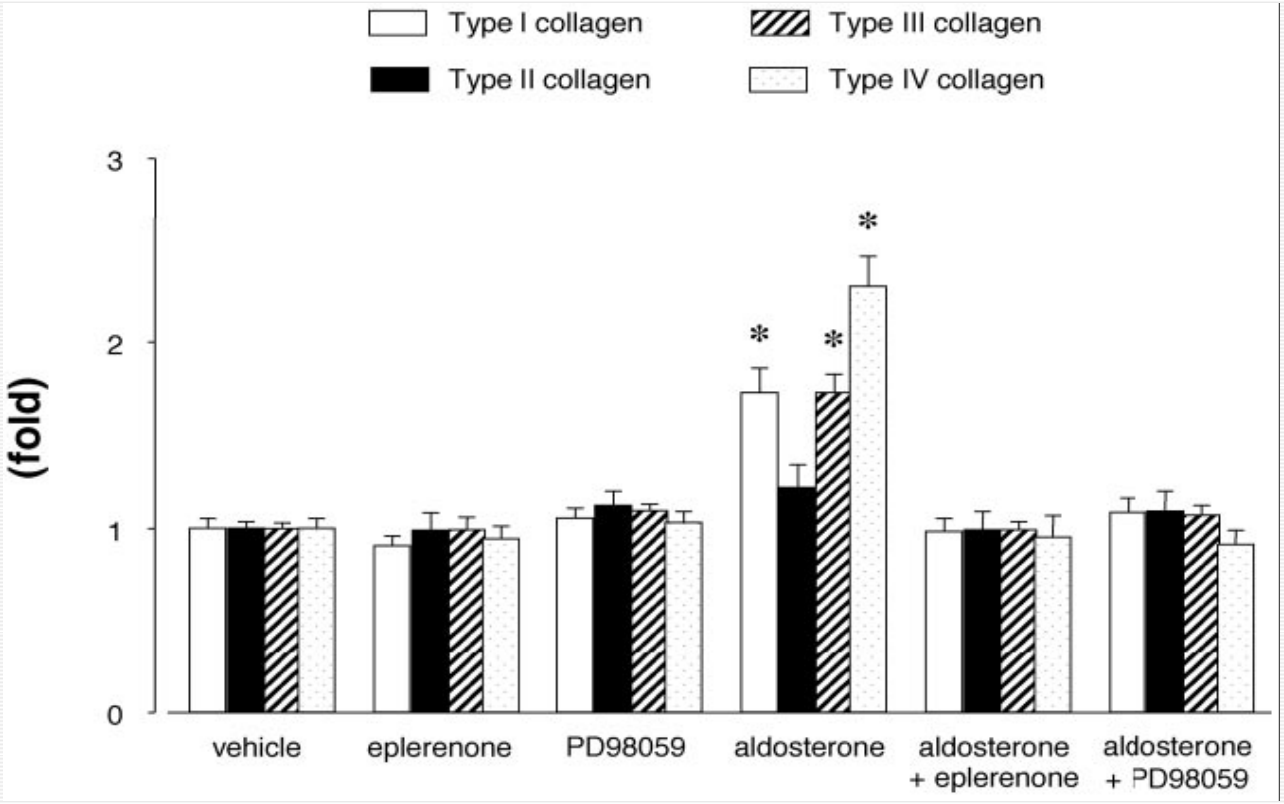


Figure 3. EIIIA Fn immunohistological staining of the abdominal aorta in control (A), Aldo-salt–treated (B), and Aldo-salt-Epl–treated rats (30 mg/kg^{-1} · d^{-1}) (C). EIIIA Fn was significantly increased in Aldo-salt rats vs control rats and Aldo-salt-Epl rats; *$P<0.05$.

Lacolley etal. *Circulation* . 2002;106:2848

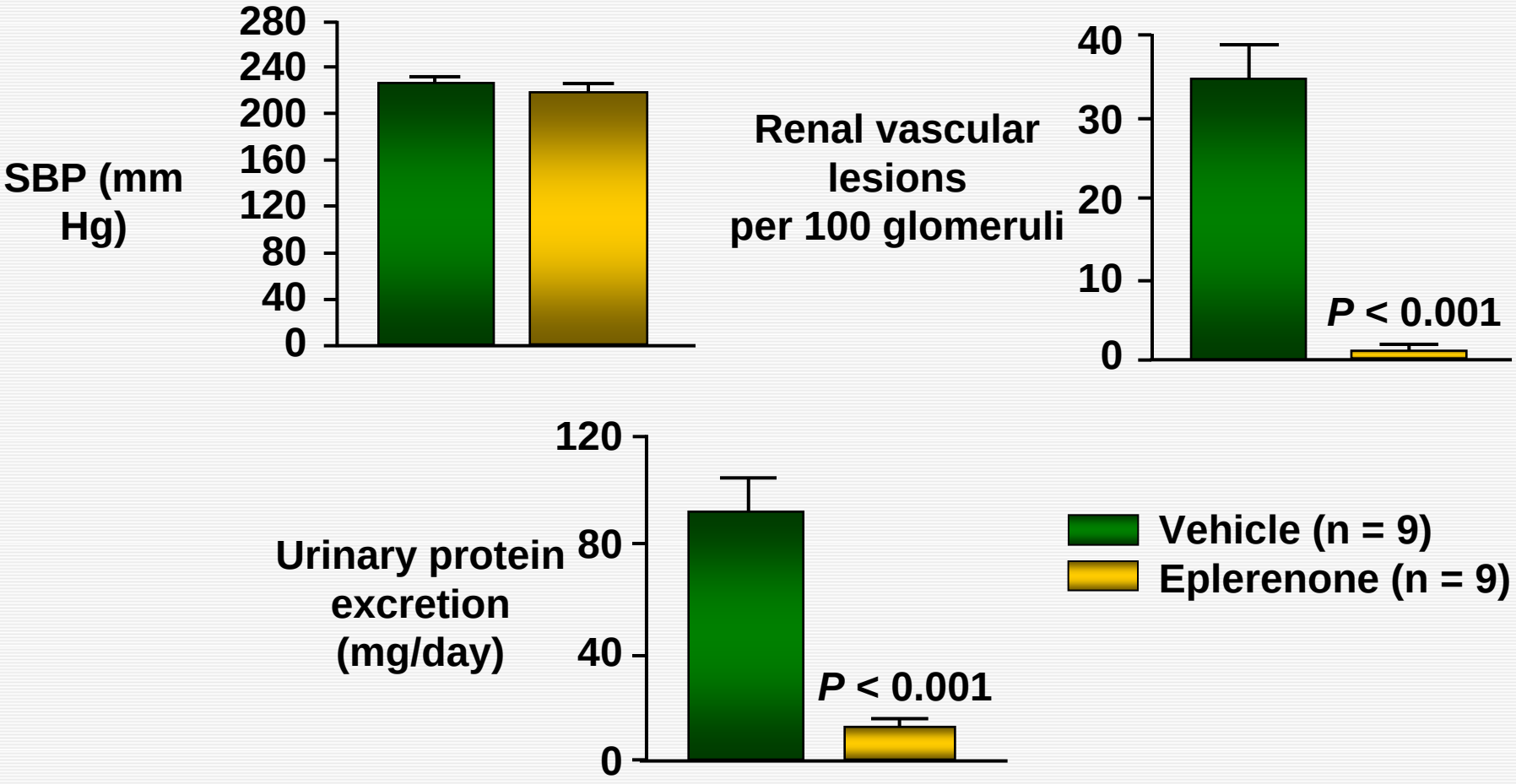
Effect of Aldosterone and Mineralocorticoid Receptor Blockade on the Kidney

Aldosterone-salt administration in rats resulted in severe tubulointerstitial fibrosis with an increased renal collagen content, and these were prevented by concurrent treatment with eplerenone



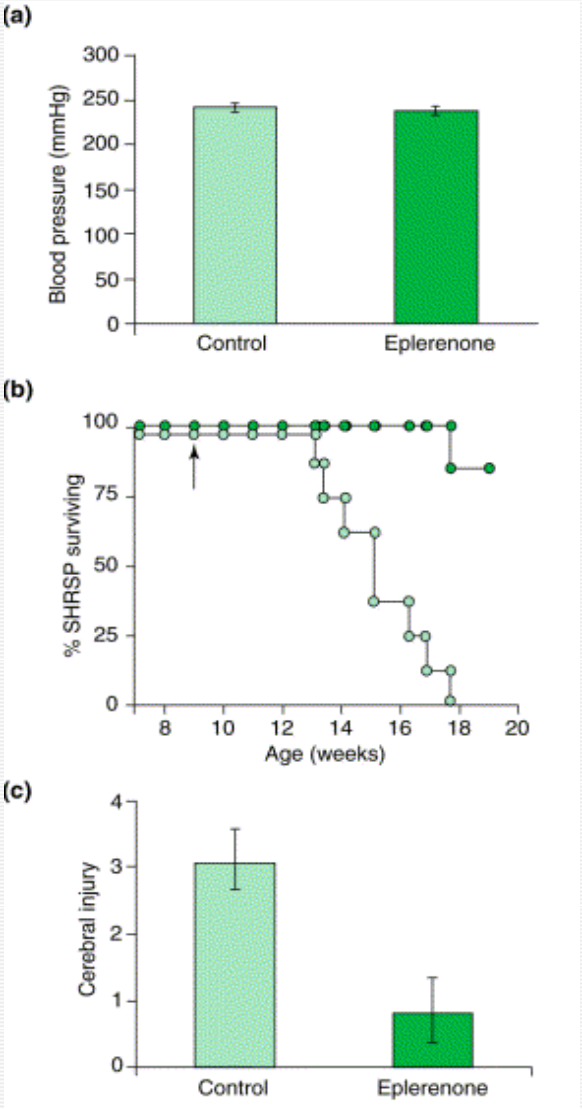
Nagai Y et al *Hypertension*. 2005;46:1039

Effects of Aldosterone Blockade on Proteinuria and Renal Injury in SHRSP



Rocha and Stier, Unpublished data 2003

Effect of Aldosterone and Mineralocorticoid Receptor Blockade on the Brain

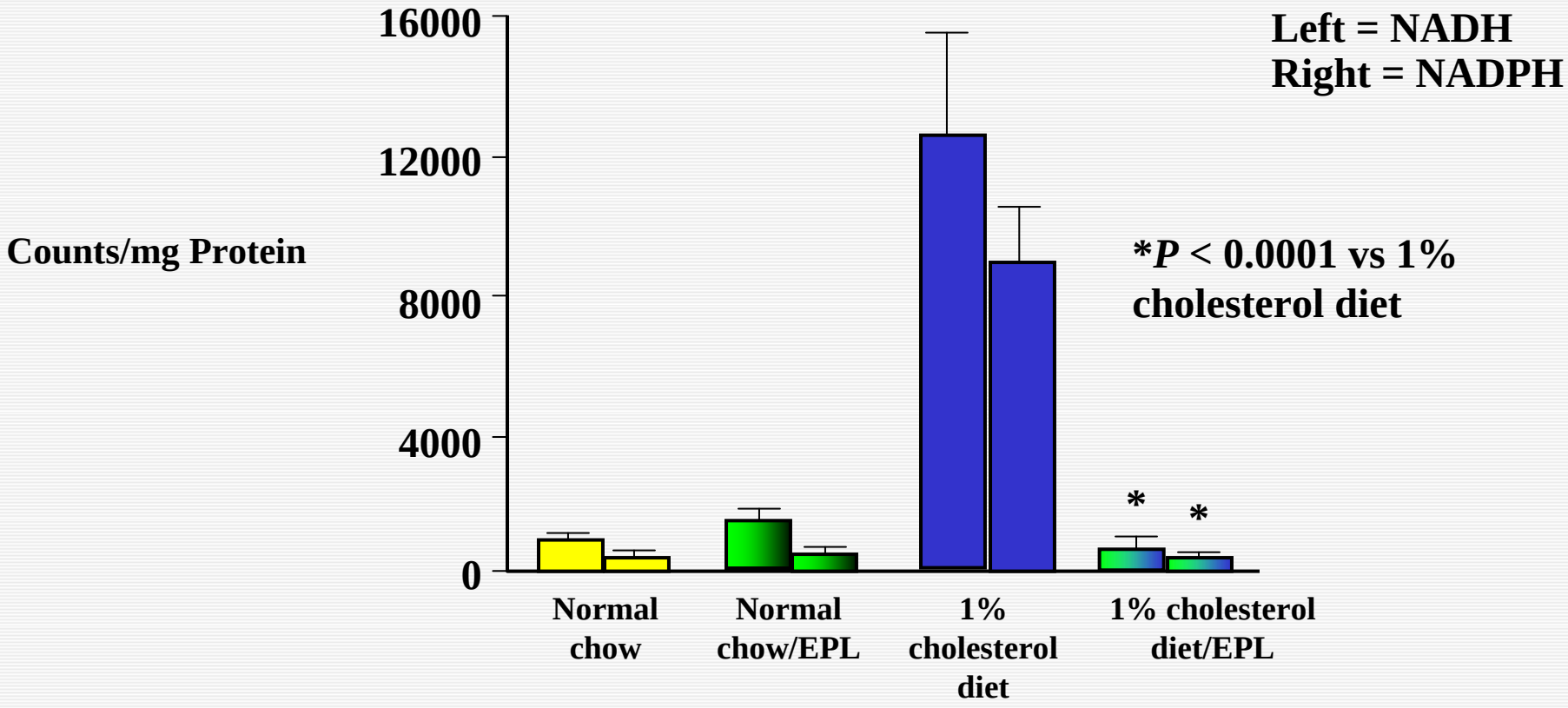


Cerebral injury and survival with eplerenone. (a) Tail-cuff systolic blood pressure in saline-drinking, stroke-prone, spontaneously hypertensive rats (SHRSP) measured before death. (b) Survival curves forSHRSP treated with vehicle (light green circles, $n = 8$) or eplerenone (dark green circles, $n = 7$; 100 mg kg−1d−1; $P < 0.001$). Arrow indicates when treatment began. (c) Histopathological scores for cerebral injury in SHRSP receiving either vehicle or eplerenone treatment ($P < 0.001$).

Aldosterone and Oxidative stress

- Aldosterone is closely associated with vascular disease of all sorts, notably atherosclerosis, diabetic induced vasculopathy and hypertension-induced organ damage.
- The generation of reactive oxigen species, particularly via NADPH oxidase is important in mediating the effects

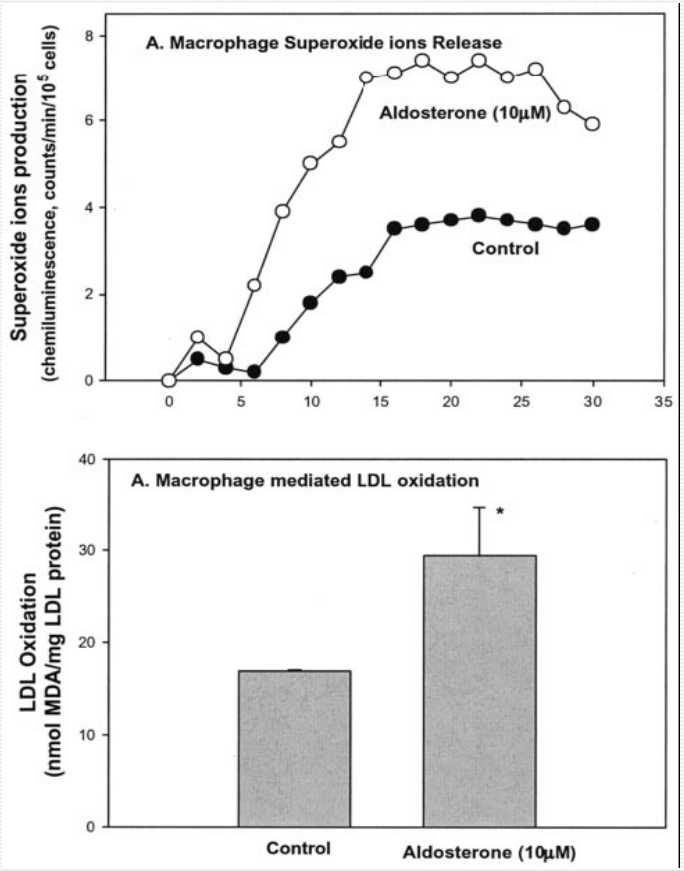
NADH/NADPH Oxidase Activity is Markedly Reduced by Aldosterone Antagonism



Rajagopalan S, et al. *Circulation*. 2002;105:2212-6.

Eplerenone 50mg/kg twice daily

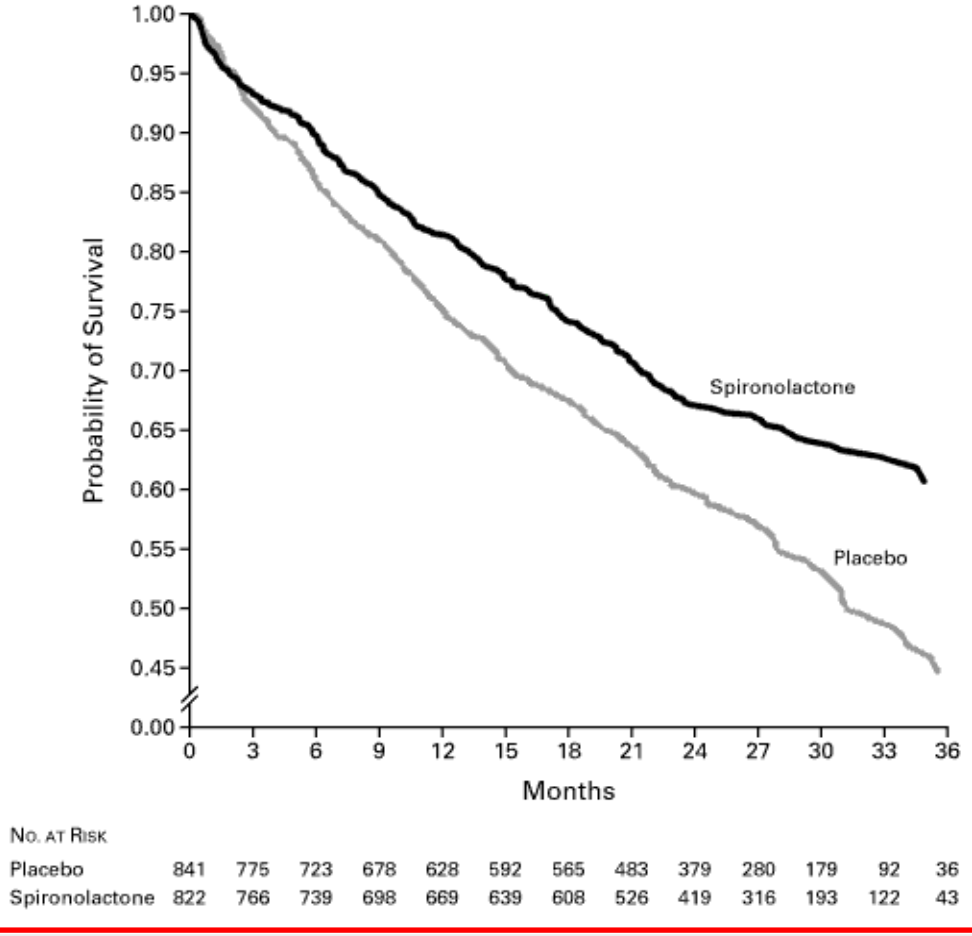
Aldosterone Administration to Mice Stimulates Macrophage NADPH Oxidase and Increases Atherosclerosis Development



Blocking of the mineralocorticoid receptor and inhibition of tissue ACE and/or the angiotensin receptor-1 reduced aldosterone deleterious pro-oxidative and proatherogenic effects.

Clinical Implications

Randomized Aldactone Evaluation Study (RALES)



Blockade of MR in moderate to severe heart failure reduces morbidity and mortality

Rales trials demonstrated a major role for aldosterone in the progression of heart failure

Patients with moderately severe herat failure given low-dose spironolactone in addition to best practice therapy showed a 30% reduction in mortality and a 35% reduction in morbidity

Pitt B. NEJM, 1999

Eplerenone Post Heart Failure Efficacy and Survival Study (EPHESUS)

AMI, Rales, LVEF ≤ 40%
Standard therapy

Eplerenone
25-50 mg daily
N=3313

Randomized
3-14 days
Post AMI

1012 deaths

Placebo
N= 3319

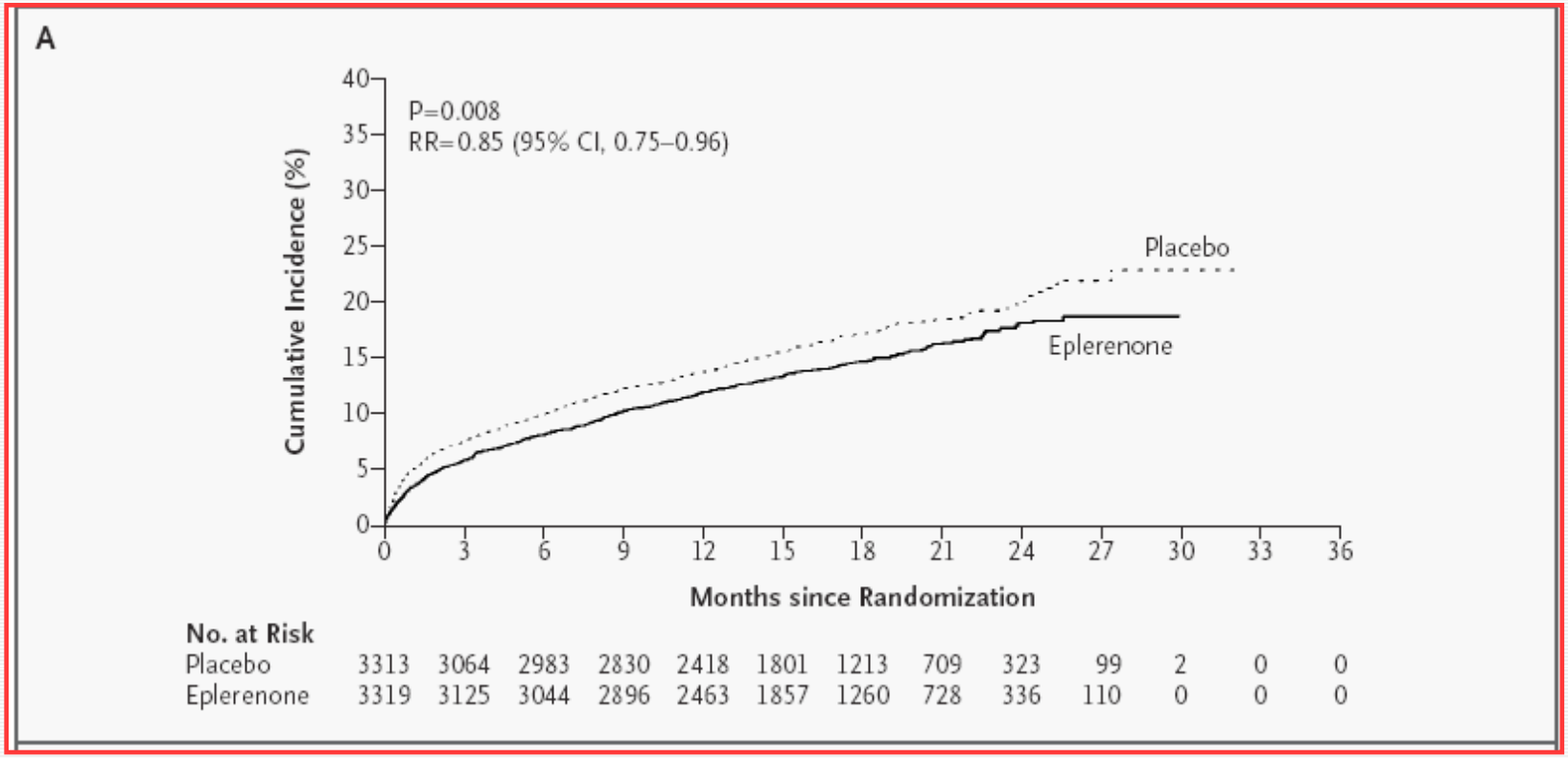
Primary endpoints: All cause mortality
CV mortality + CV hospitalizations

Secondary endpoints: CV mortality
CV hospitalizations
All cause mortality + all cause hospitalizations

Other endpoints: new onset of atrial fibrillationflutter
NYHA functional class
Quality of life

Pitt B. N Engl J Med. 2003

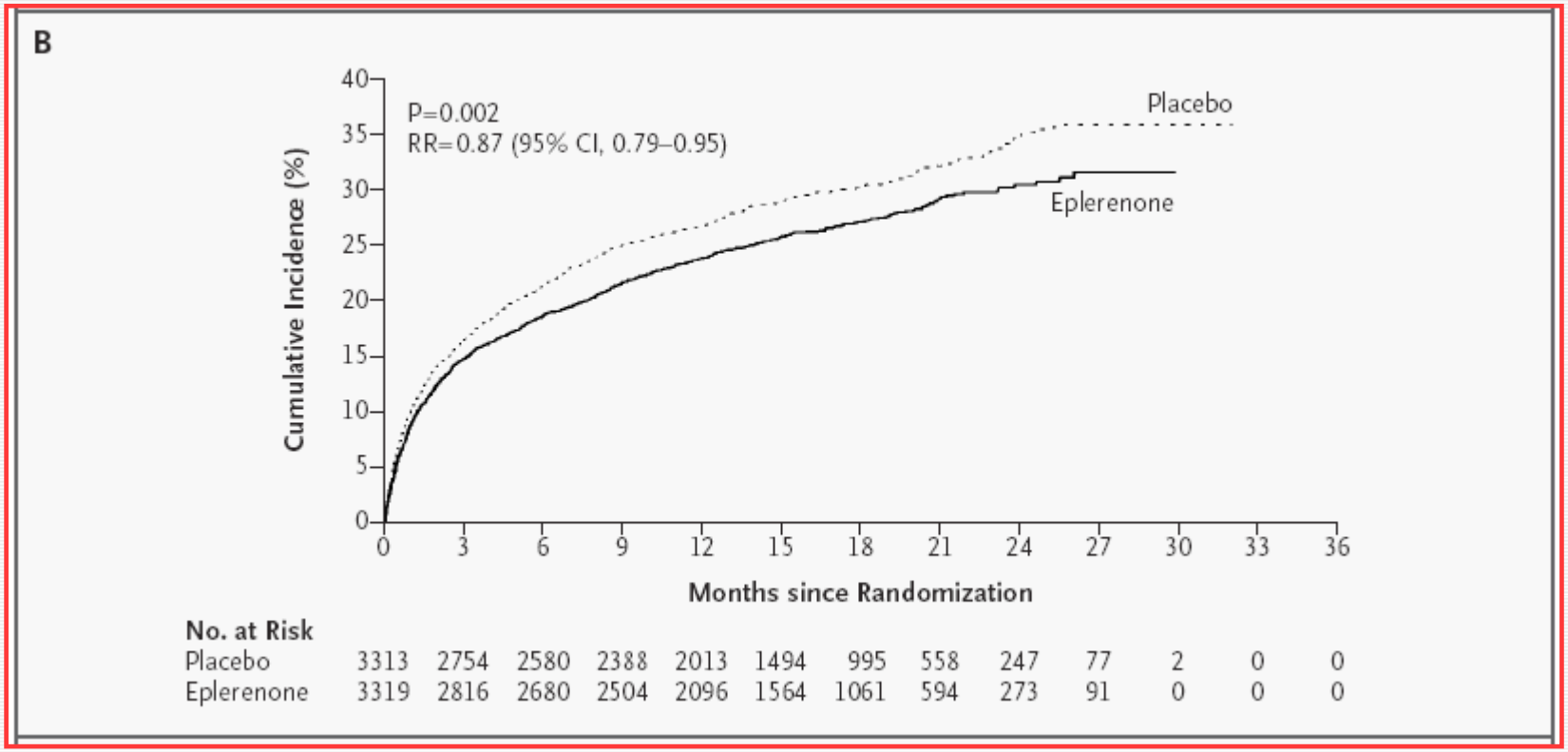
Kaplan-Meir stimates of the Rate of Death from Any Cause



RR: relative risk. CI: confidence interval

Pitt B. N Engl J Med. 2003

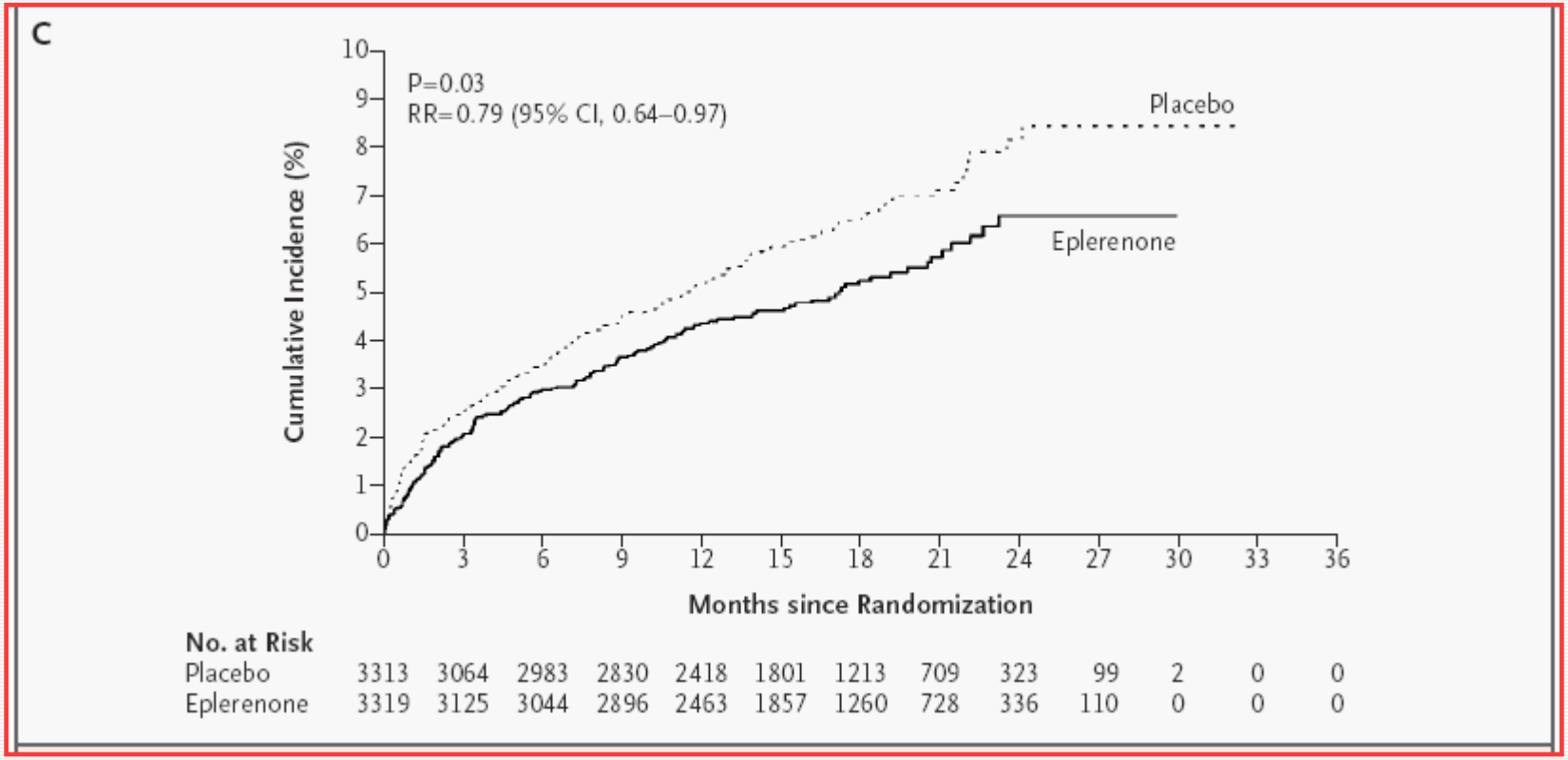
Kaplan-Meir stimates of the Rate of Death from Cardiovascular Causes or Hospitalization for Cardiovascular Events



RR: relative risk. CI: confidence interval

Pitt B. N Engl J Med. 2003

Kaplan-Meir stimates of the Rate of Sudden Death from Cardiac Causes

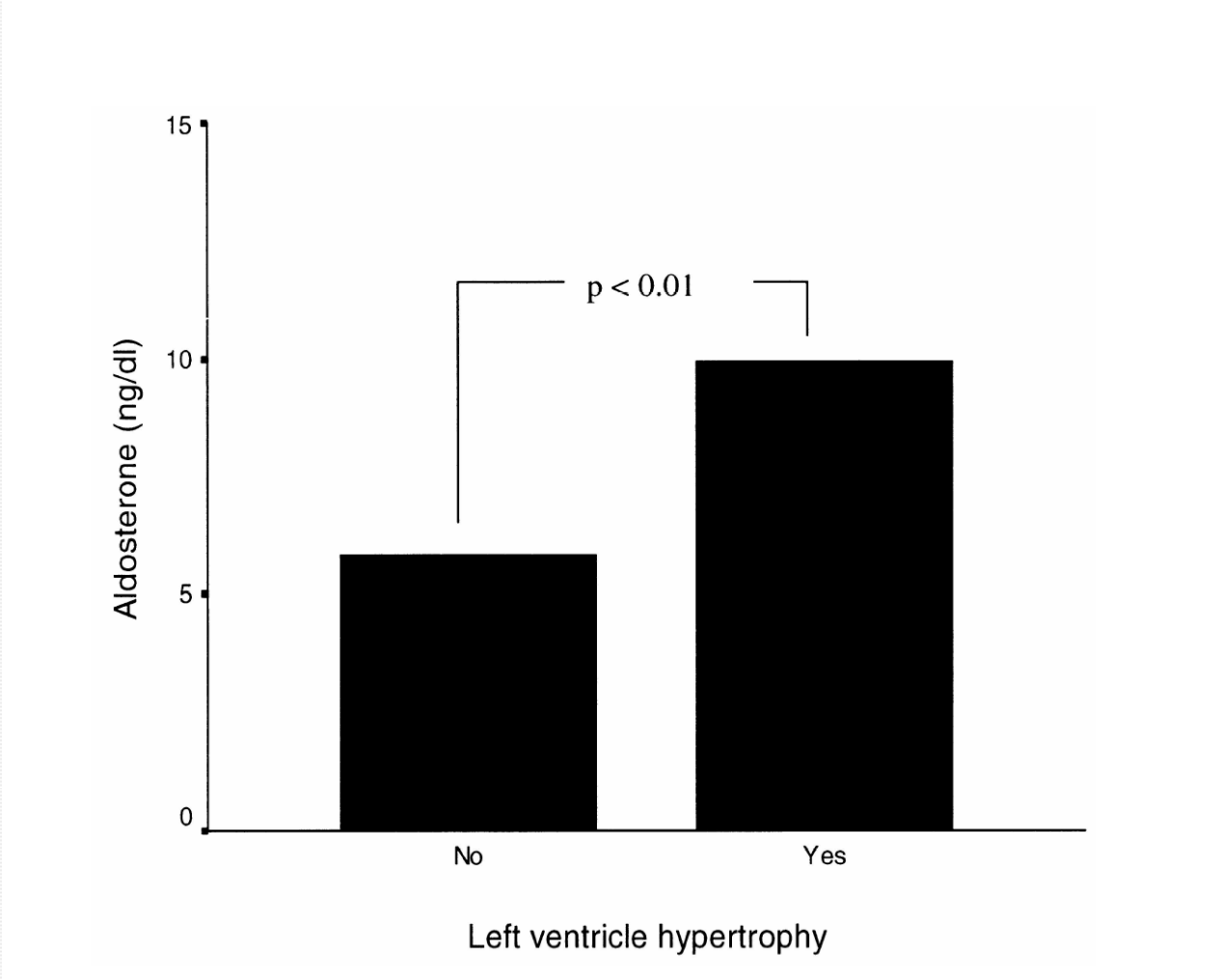


RR: relative risk. CI: confidence interval

Pitt B. N Engl J Med. 2003

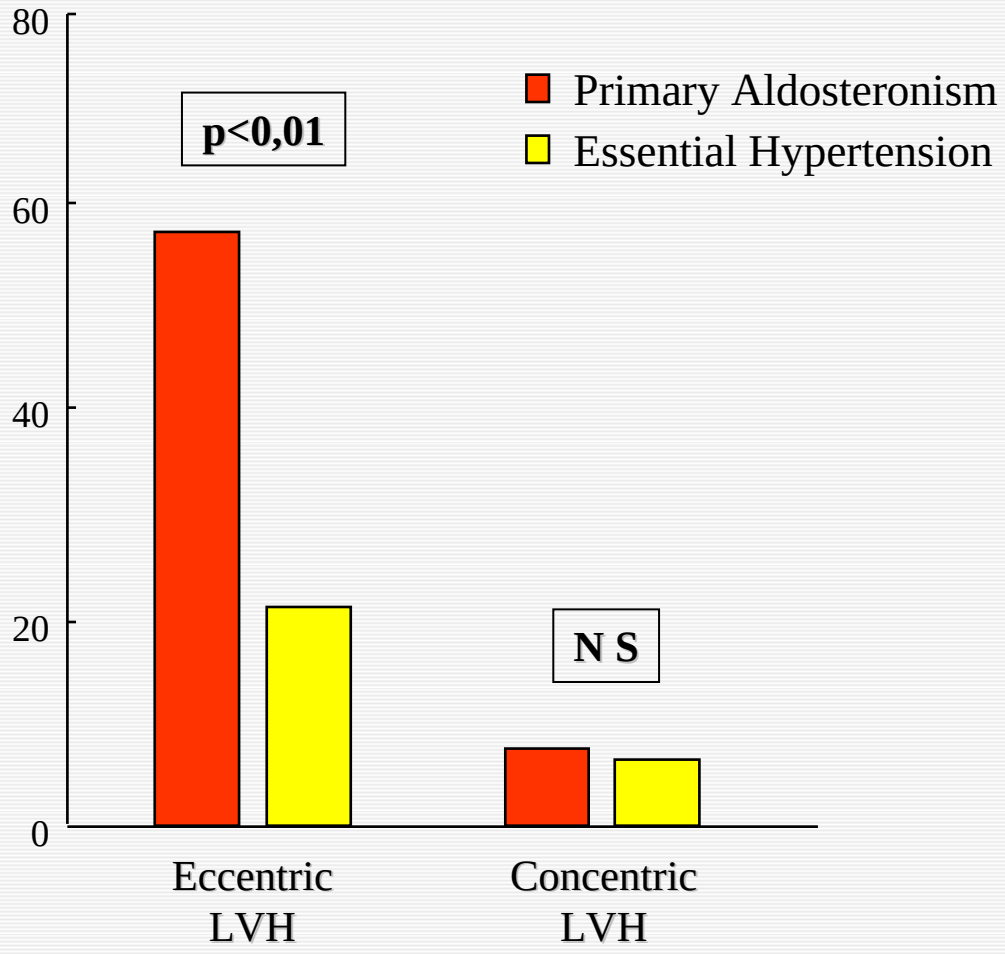
Clinical Implications

The influence of Aldosterone on the development of left ventricular geometry and hypertrophy in patients with essential hypertension



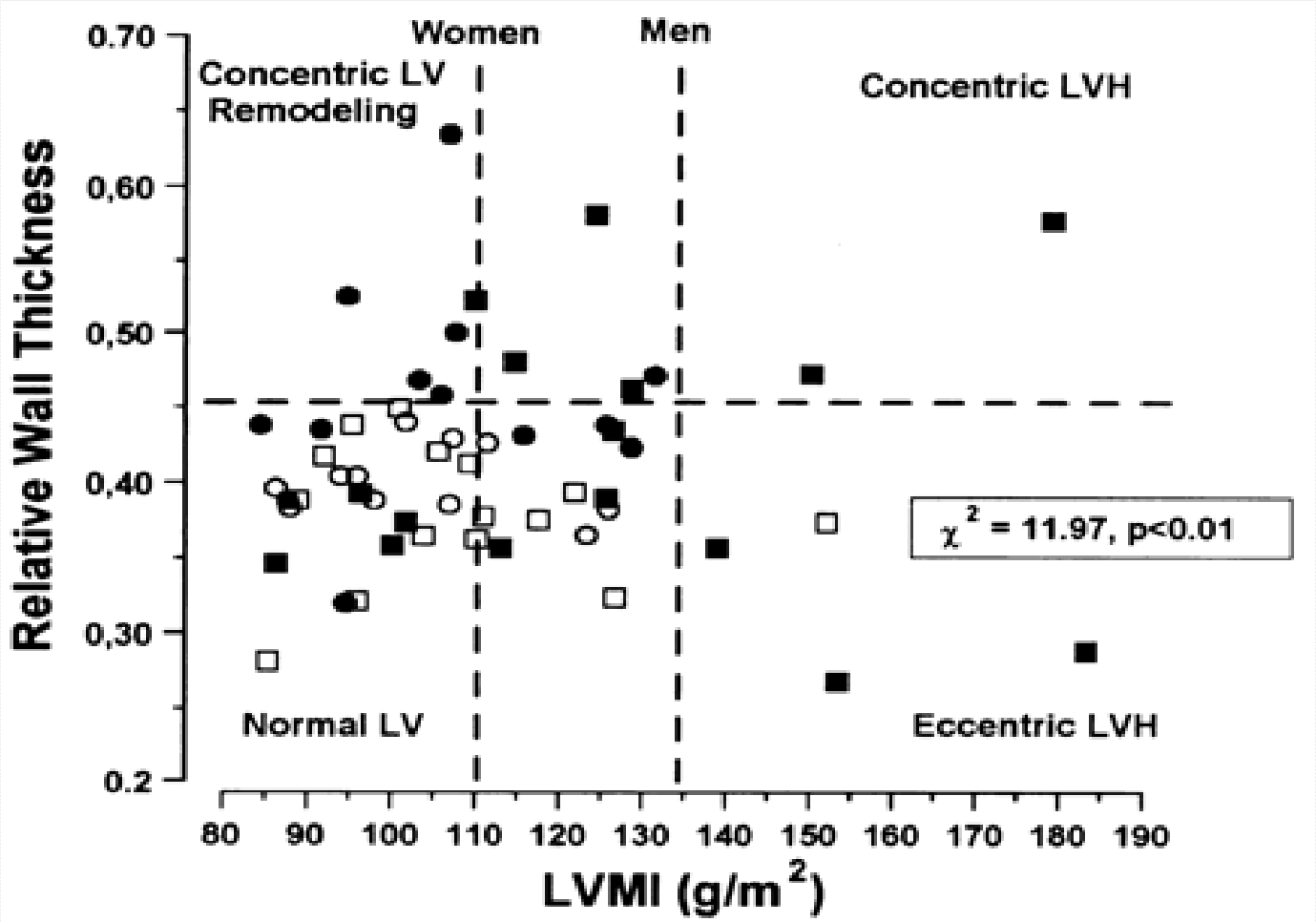
Soylu, Jpn Heart J, 2004

Prevalence of Eccentric and concentric Left Ventricular Hypertrophy (LVH) in Primary Aldosteronism and Essential Hypertension



Shigematsu Y: Hypertension, 1997

Changes in left ventricular anatomy in essential hypertension (EH) and primary aldosteronism (PA)



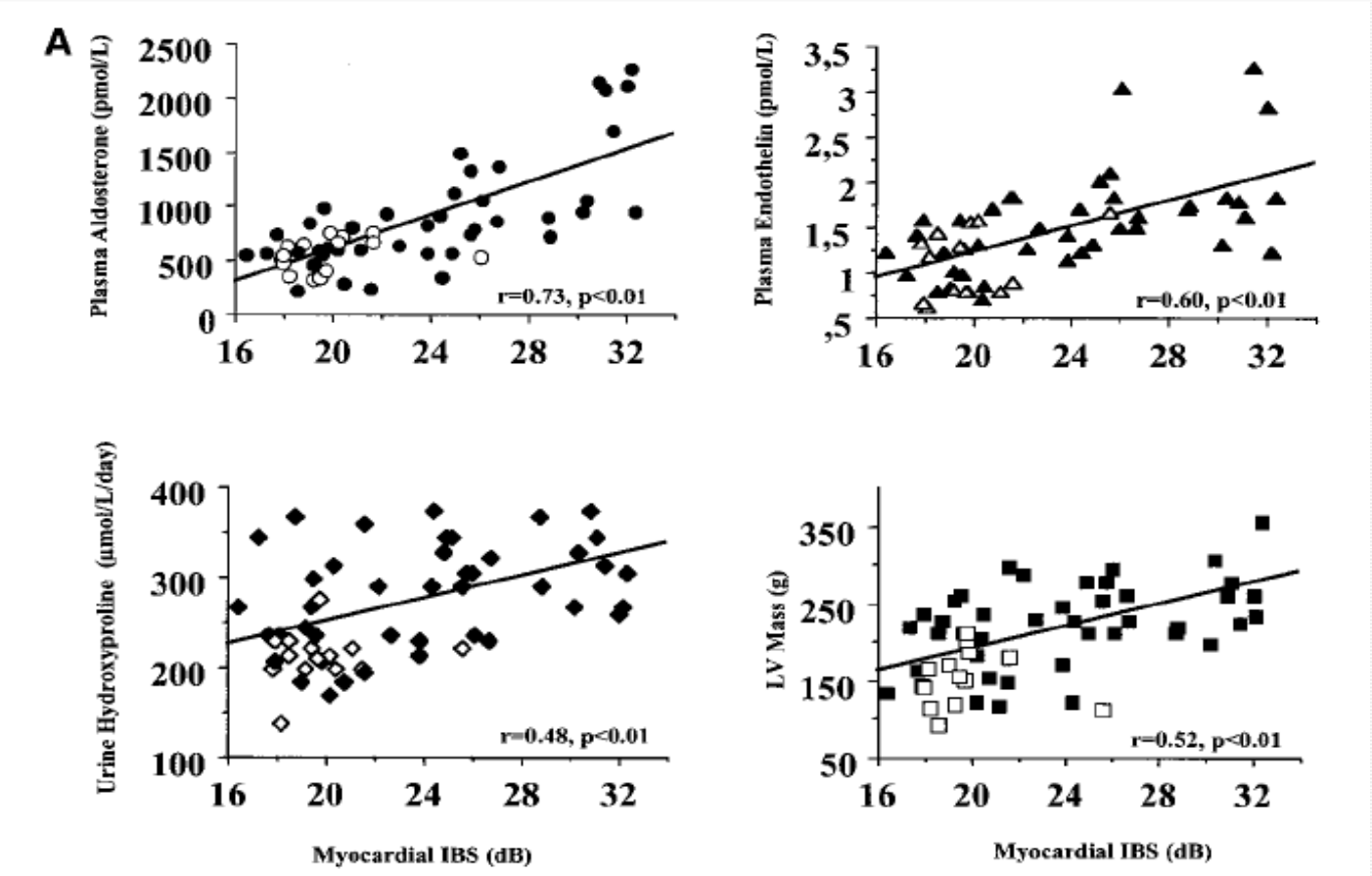
PA (black) , EH (white)

- **Men**
- **Women**

A significantly higher proportion of patients with LVH and LV concentric remodeling was found in the PA patient groups.

Rossi GP et al. *Hypertension* 1996

Myocardial ultrasonic backscatter in hypertension: relation to aldosterone and endothelin

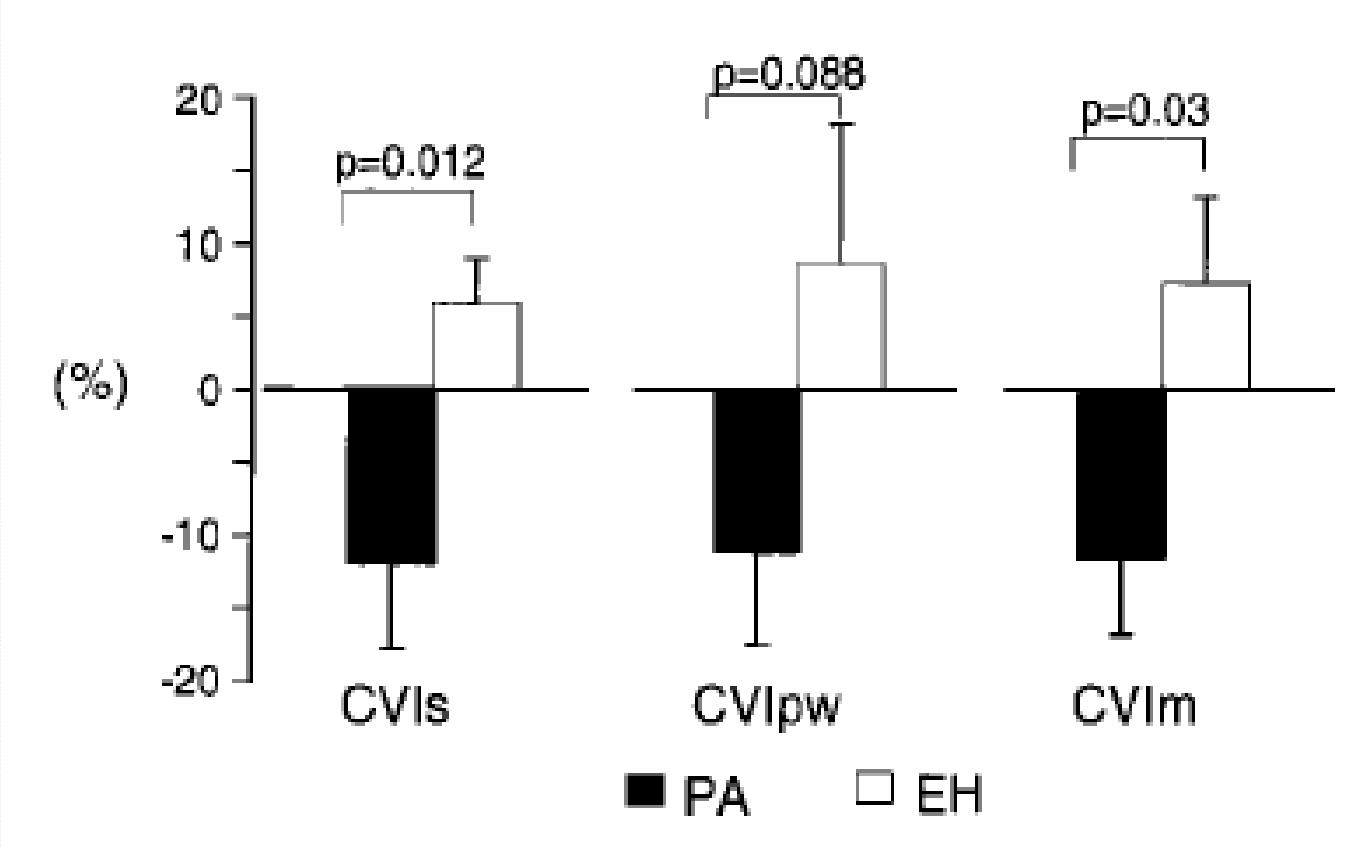


ultrasonic integrated backscatter signal (IBS)

The data of this study suggest that in human hypertension, circulating aldosterone and immunoreactive endothelin may induce alterations in left ventricular myocardial texture, possibly related to increased myocardial collagen content.

Kozakova M et al, *Hypertension. 2003 41:230-6.*

Videodensitometric analysis of the Left Ventricle Myocardial Texture



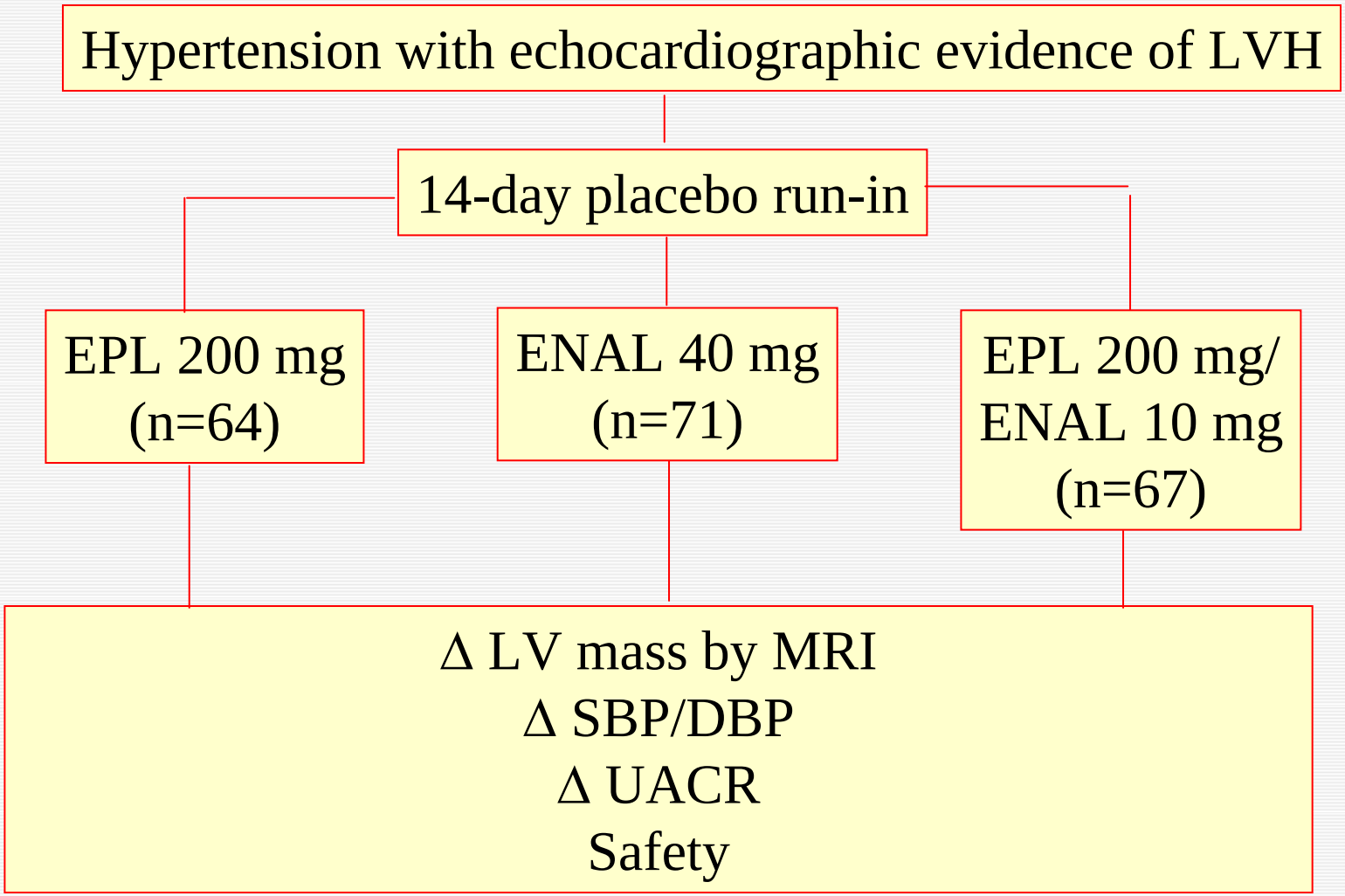
CVIs, septum ventricular; **CVIpw**, LV posterior wall; **CVIm**, mean of the two indexes

These parameters are significantly lower in primary aldsoteronism than in essential hypertension

Rossi et al. *Hypertension*, 2002

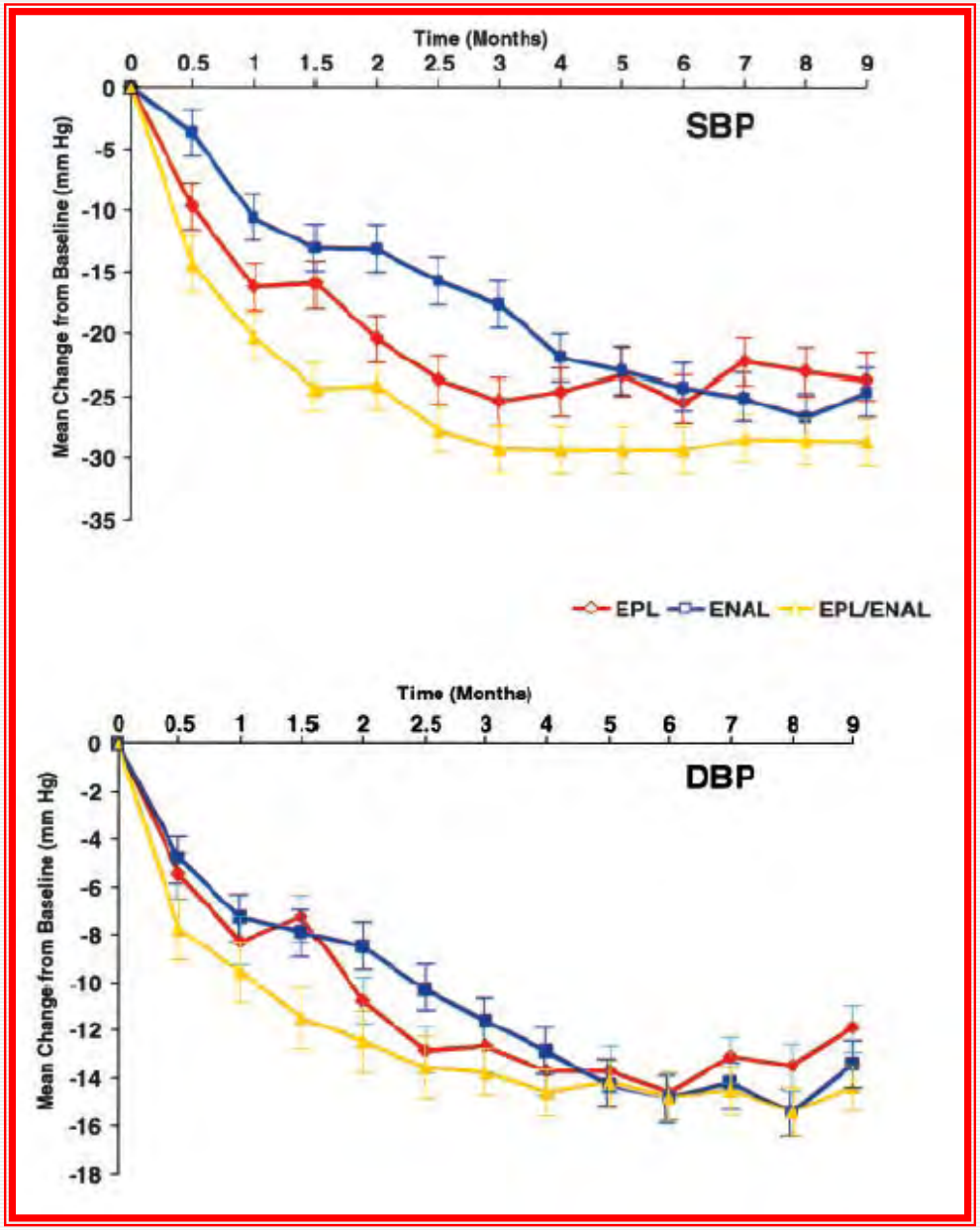
The 4E–Left Ventricular Hypertrophy Study

This study compared LVH regression during treatment with eplerenone, enalapril, and their combination in patients with hypertension.



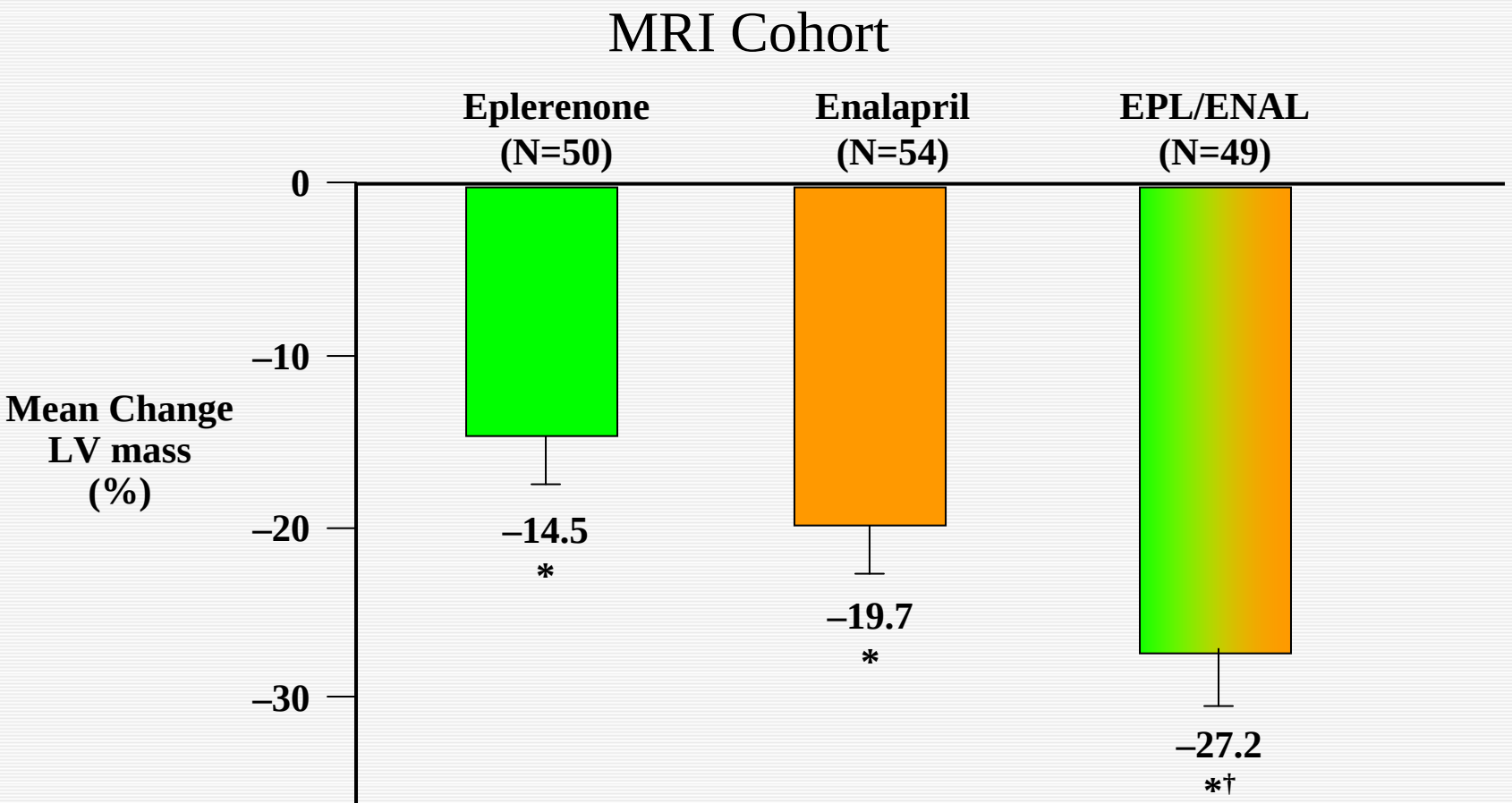
Pitt B, et al. *Circulation.* 2003;108:1831

4E-LVH- Mean Changes from Baseline in SBP/DBP



Pitt B, et al. *Circulation.* 2003;108:1831

4E LVH- Mean Changes from Baseline in LV Mass

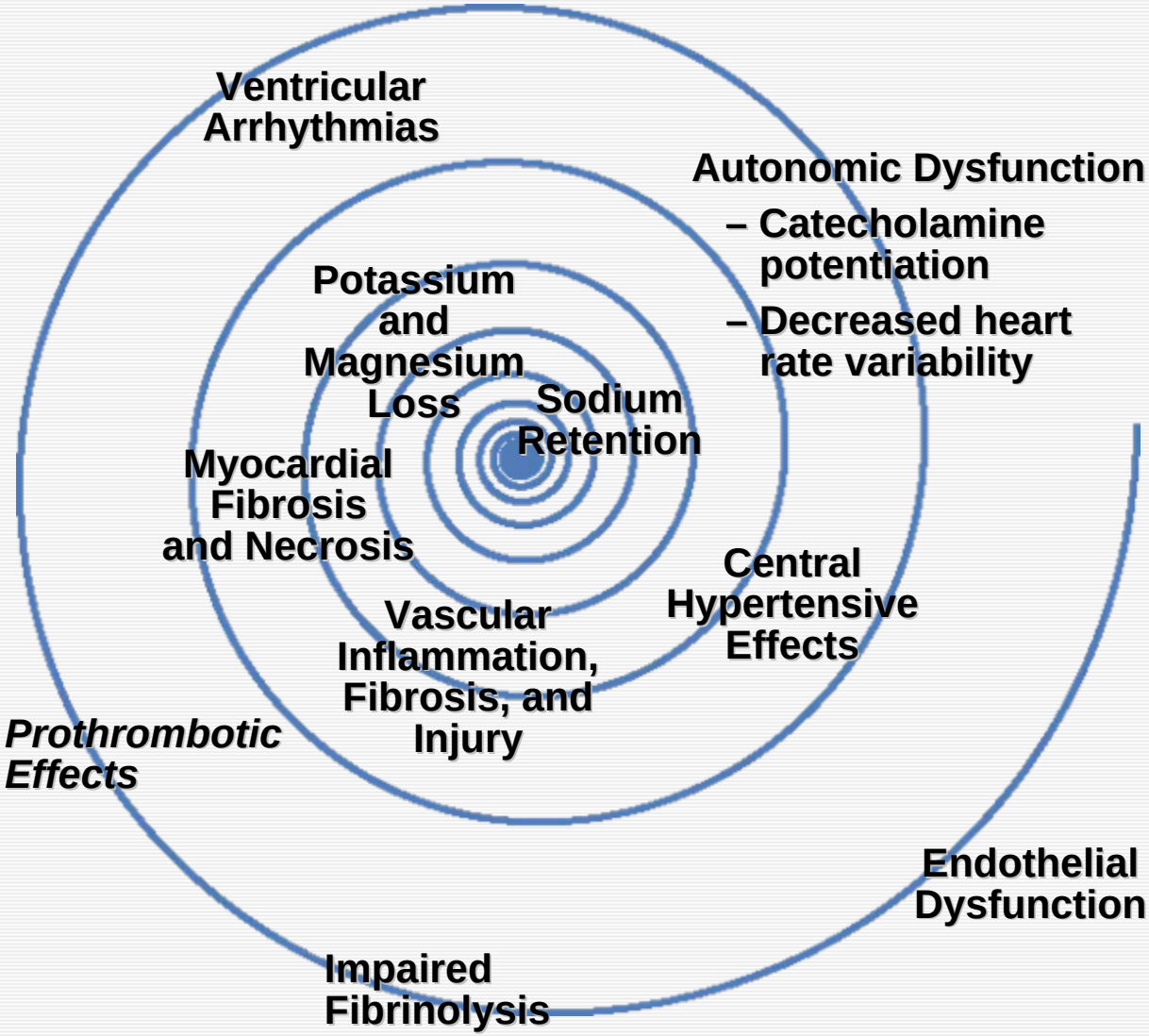


*P < 0.05 vs baseline within each treatment group

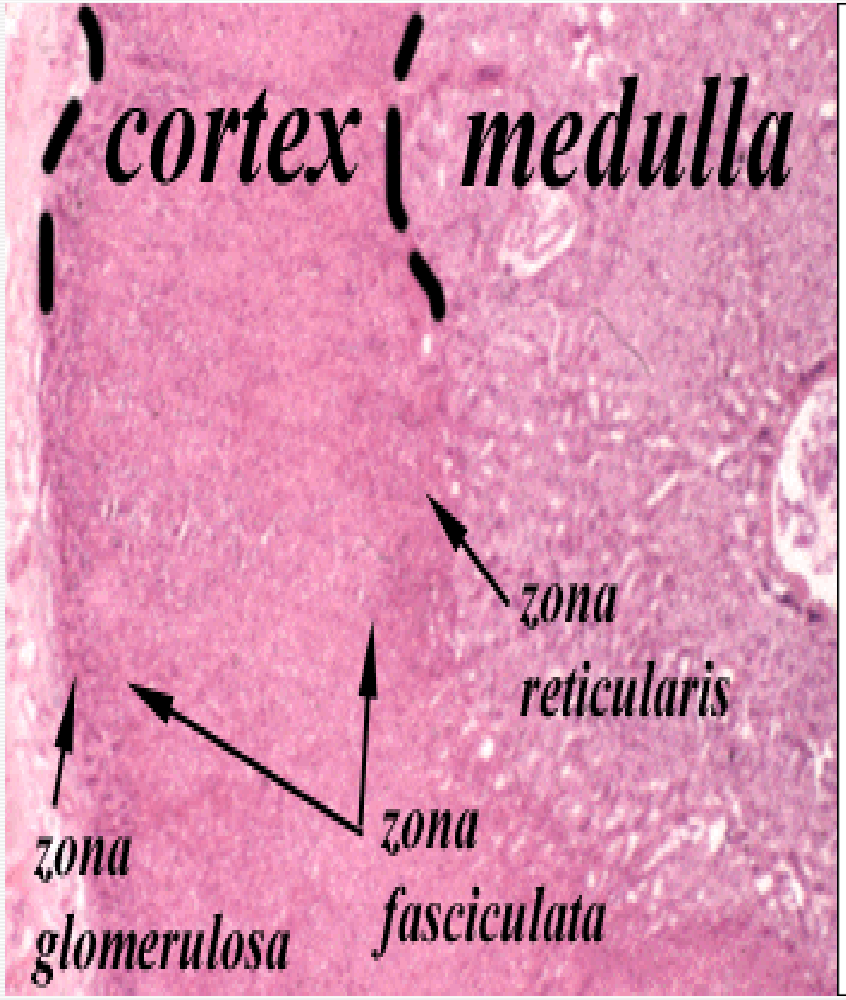
†P = 0.007 vs eplerenone alone

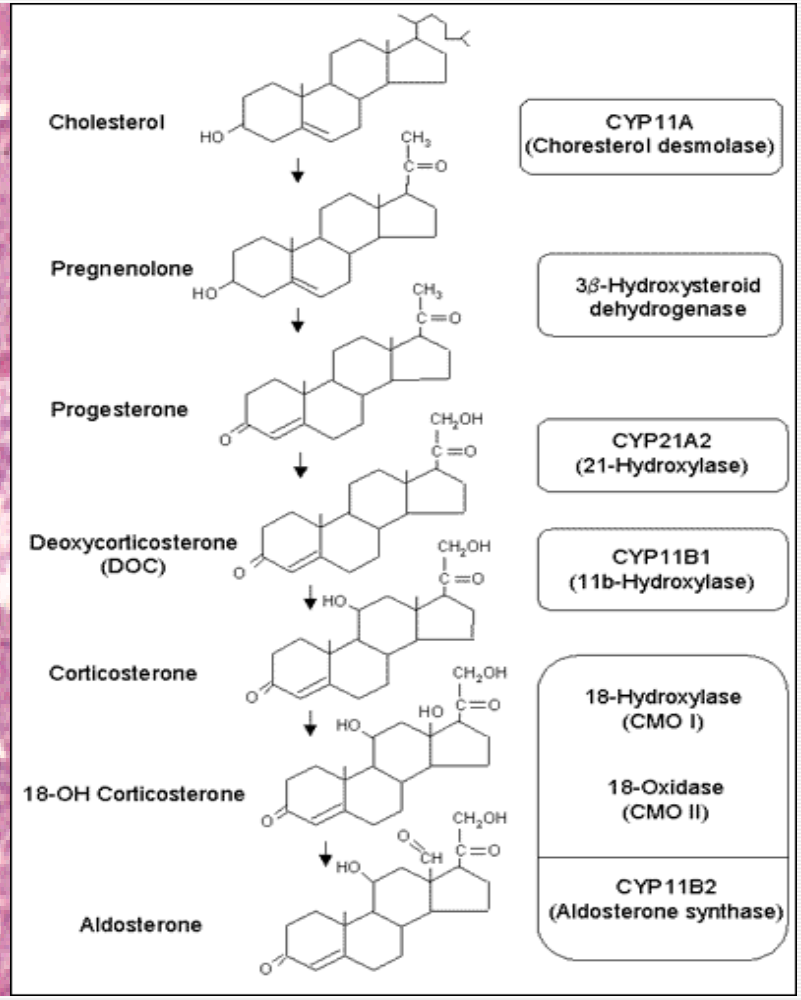
Pitt B, et al. *Circulation.* 2003;108:1831

Aldosterone: Important Contributor to Cardiovascular Disease

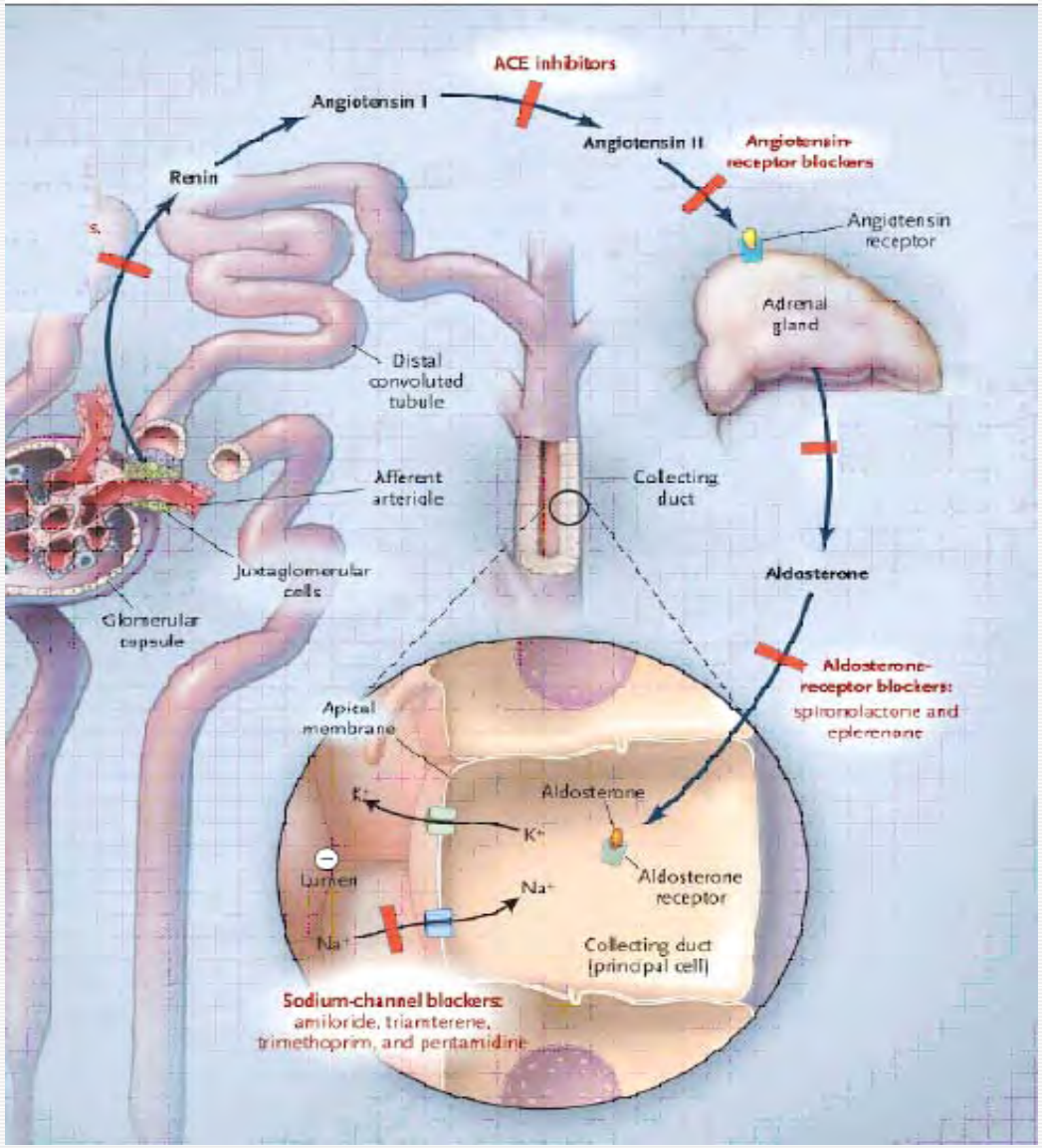


The Pathways of Biosynthesis of Aldosterone by the Adrenal Cortex





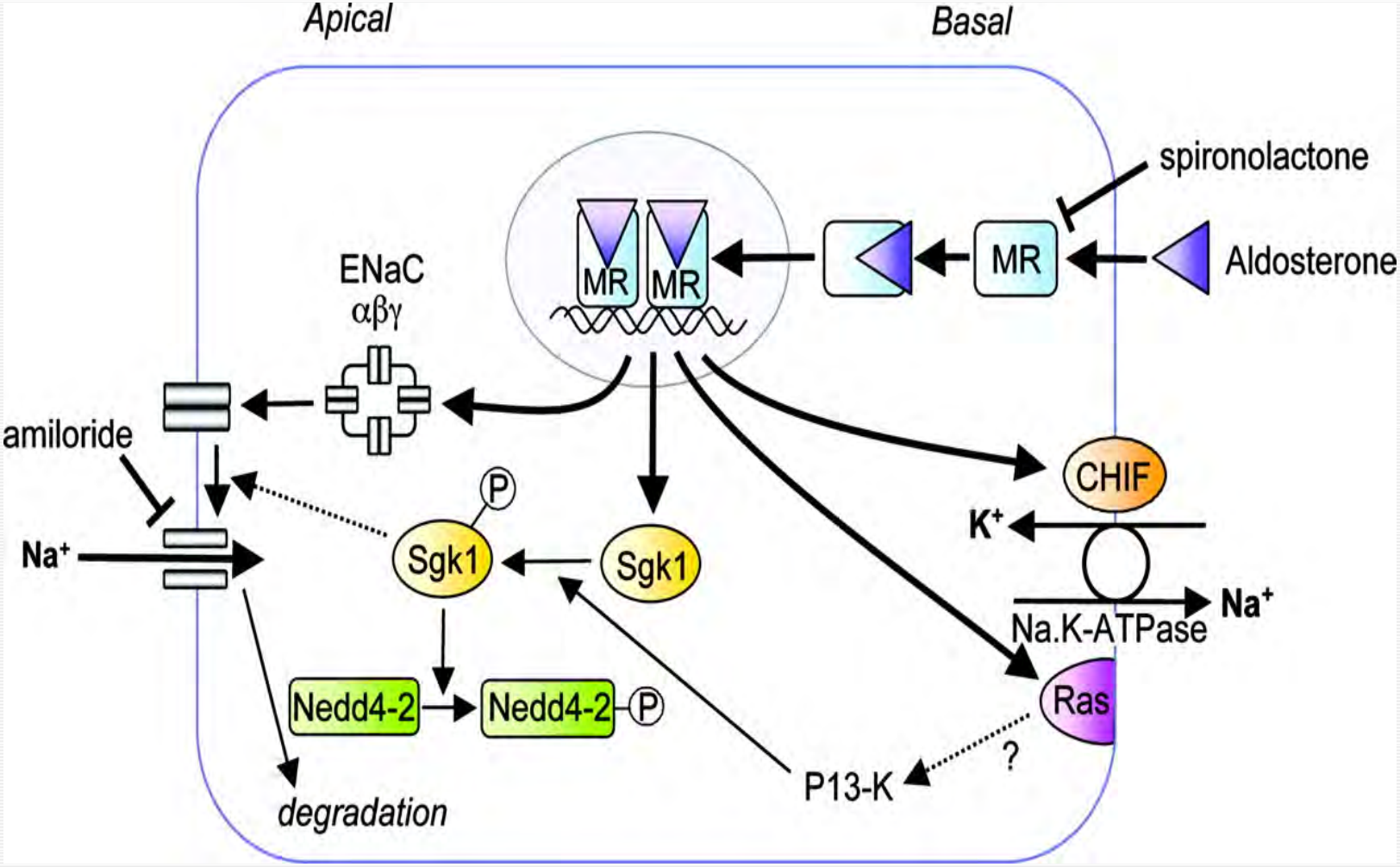
The Renin–Angiotensin–Aldosterone System



Aldosterone binds to a cytosolic receptor (Mineralocorticoid Receptor, MR) in the principal cell and stimulates sodium reabsorption across the luminal membrane through a well-defined sodium channel.

Palmer M, N Eng J Med; 2004 351:585

Representation of an Aldosterone-Responsive Epithelial Cell

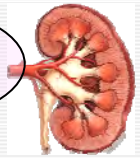


Fuller P et al. Hypertension 2005; 46: 1227

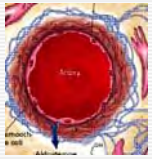
The Nongenomic Actions of Aldosterone

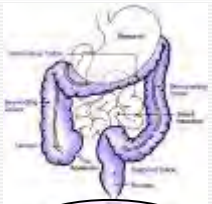
Rapid Nongenomic Effects of Aldosterone on:

Kidney

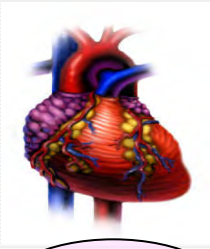


Vascular wall

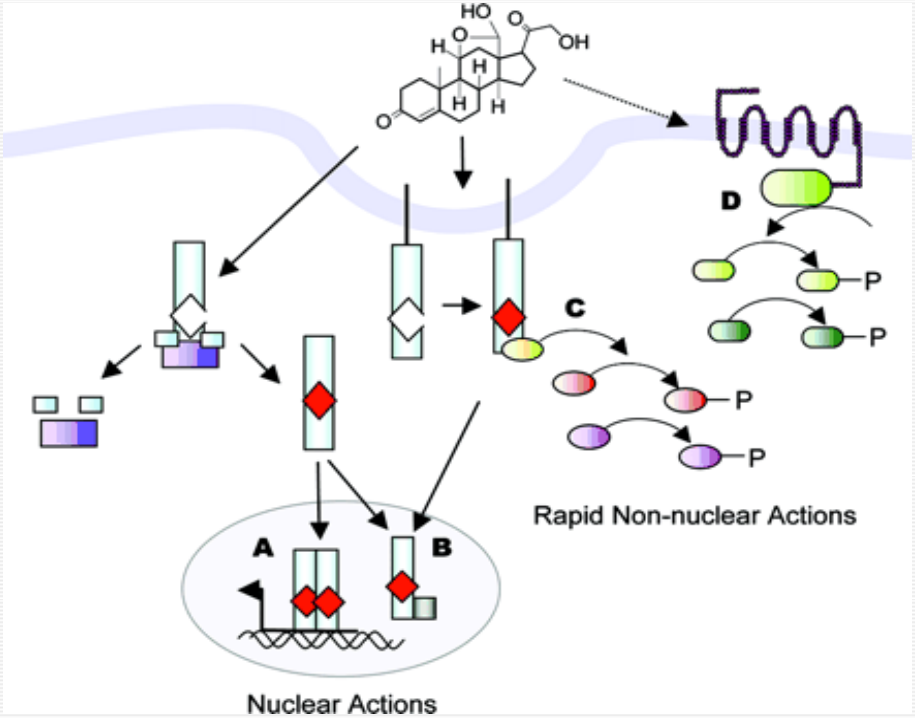




Colon



Heart



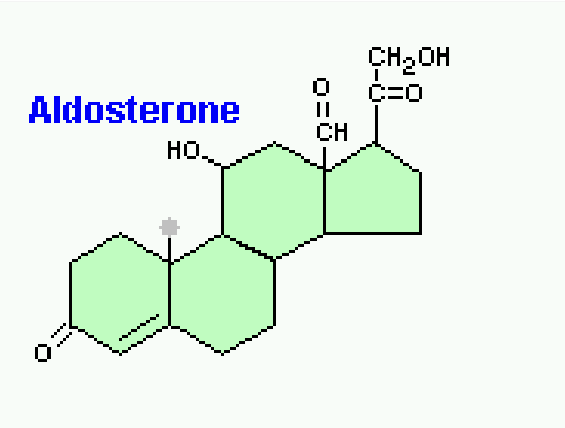
Mechanisms by which aldosterone actions may be mediated at cellular level

The classic genomic pathway:

the ligand (♦) bounds directly the receptor with DNA (A-B)

Rapid effects may be mediated by the MR (C) or by a putative novel transmembrane receptor (D).

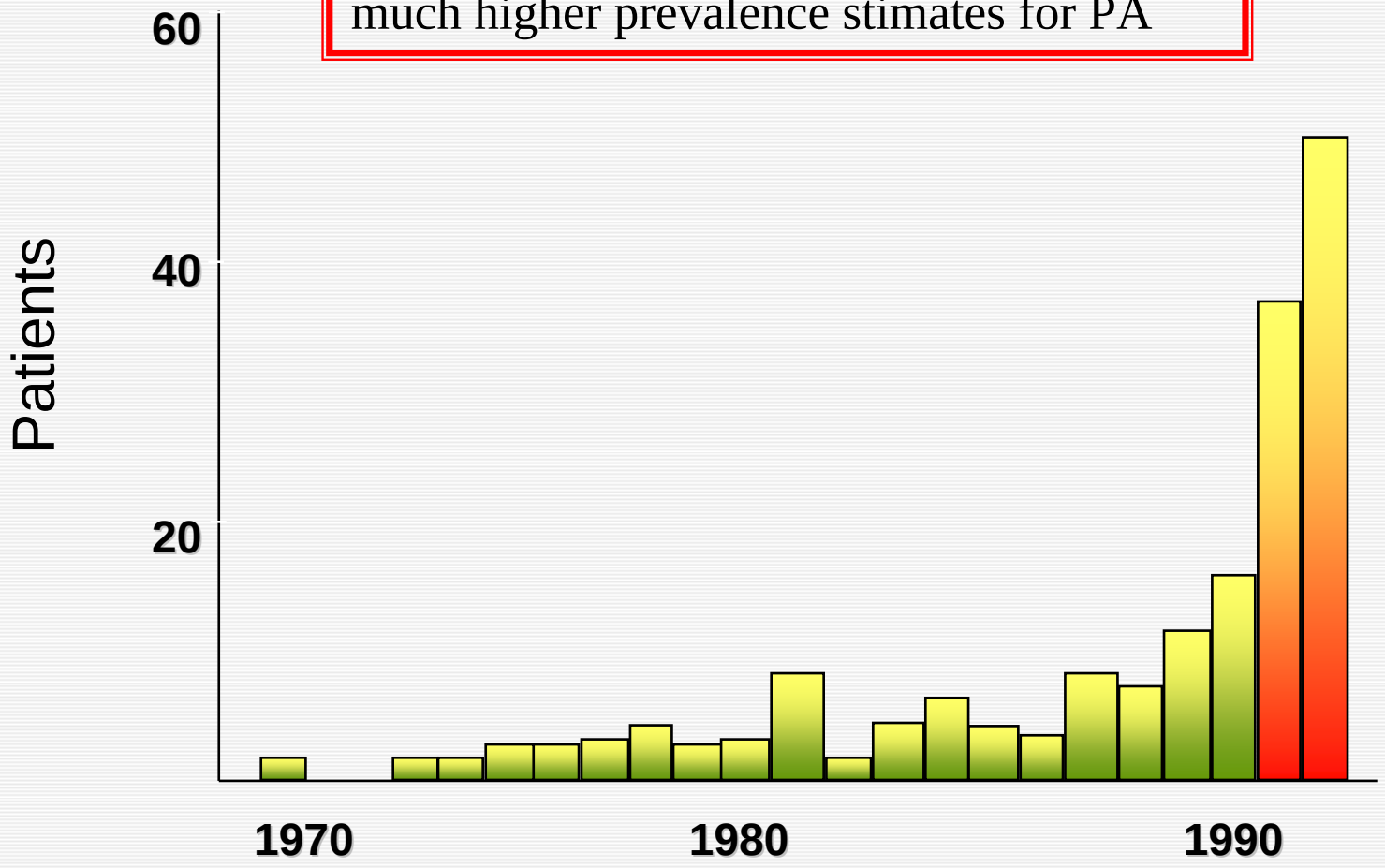
Primary Aldosteronism (PA)



In 1955 Jerome Conn described the syndrome of primary aldosteronism characterized by hypertension, hypokalemia, suppressed plasma renin activity, and increased aldosterone excretion

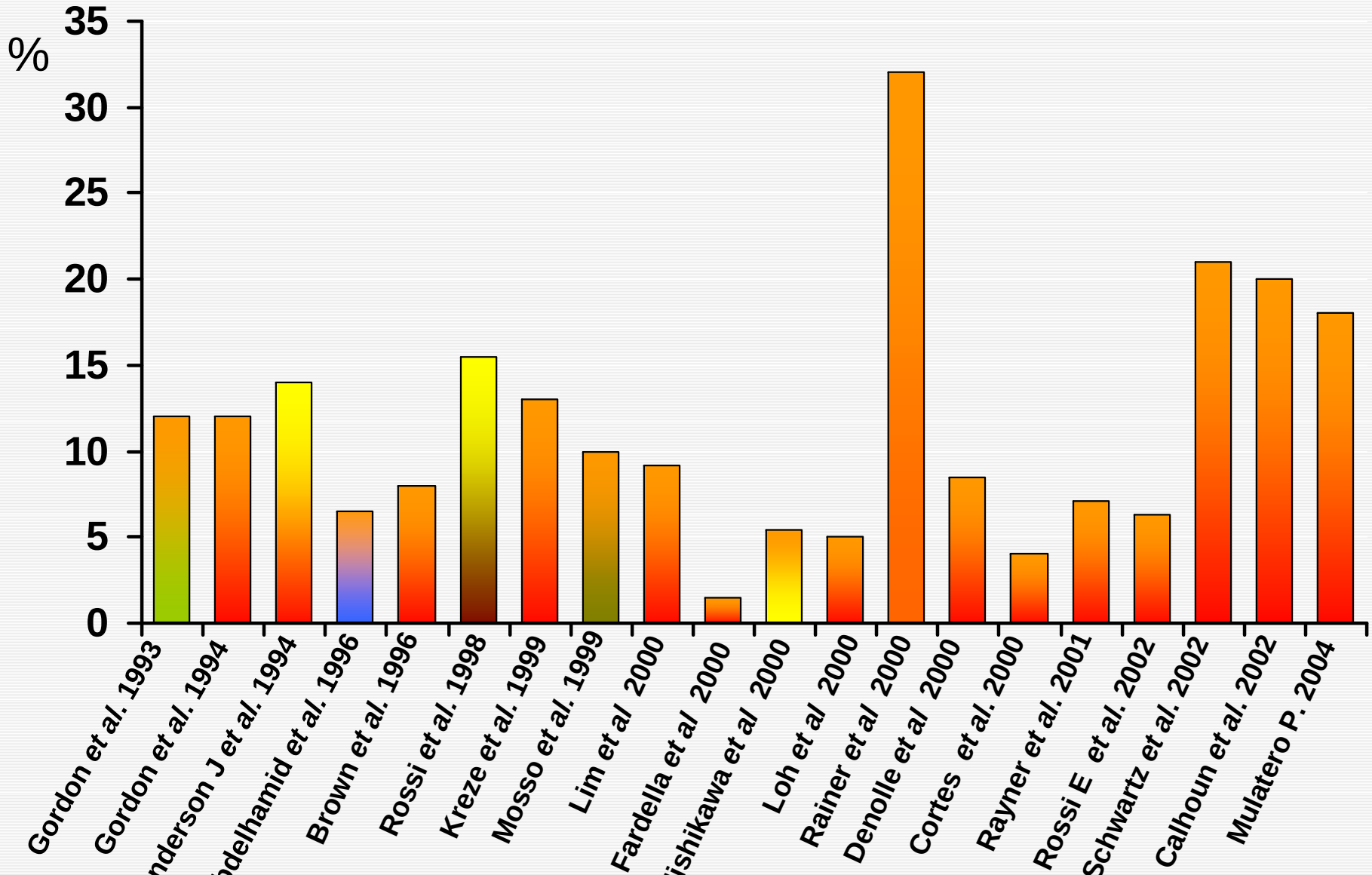
PAC/PRA Ratio in the screening of Primary Aldosteronism

Using the PAC/PRA ratio has resulted in much higher prevalence stimates for PA

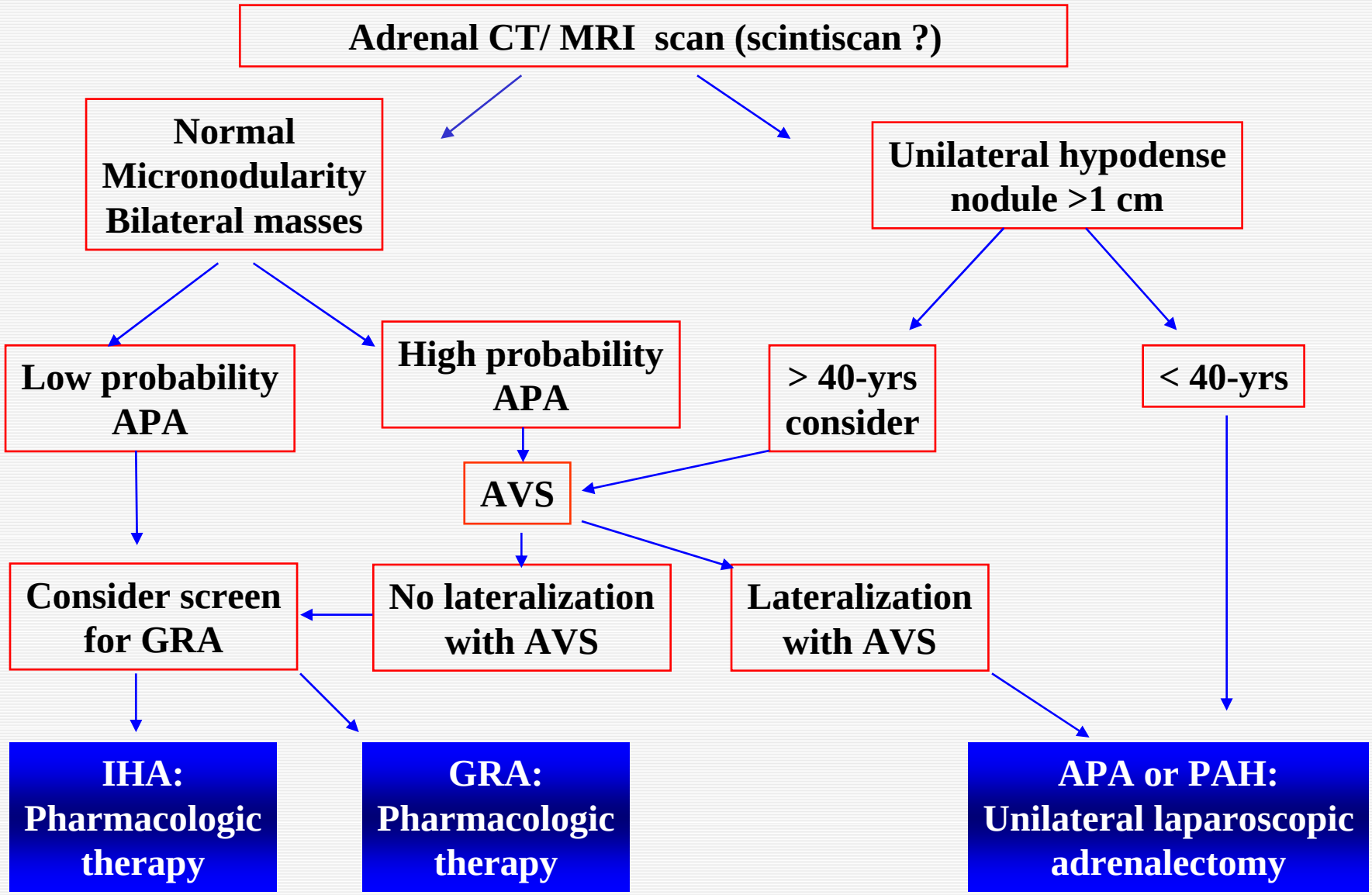


Greenslopes Hospital

Prevalence of Primary Aldosteronism in studies published in the last decade

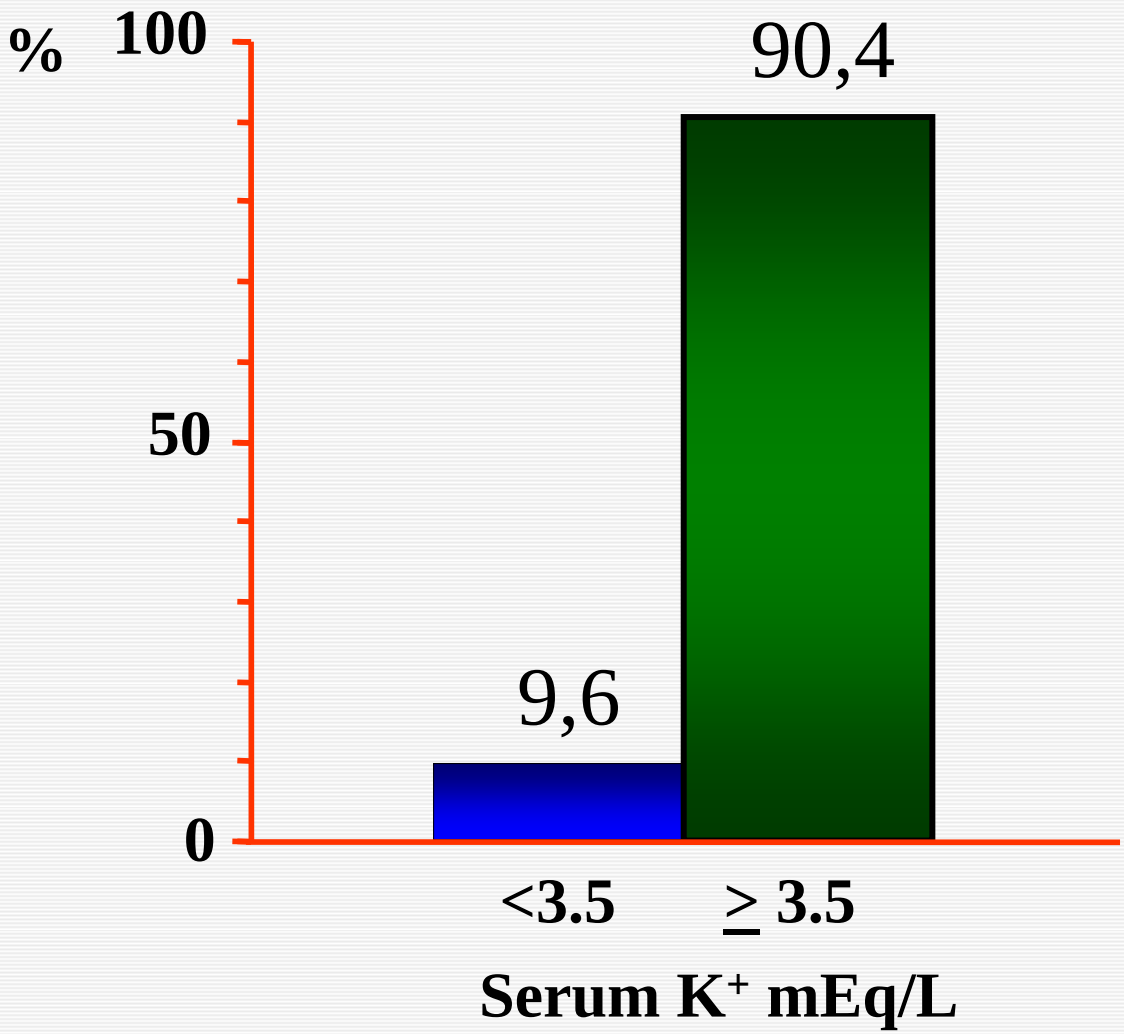


Determining the subtype of PA in order to define therapy



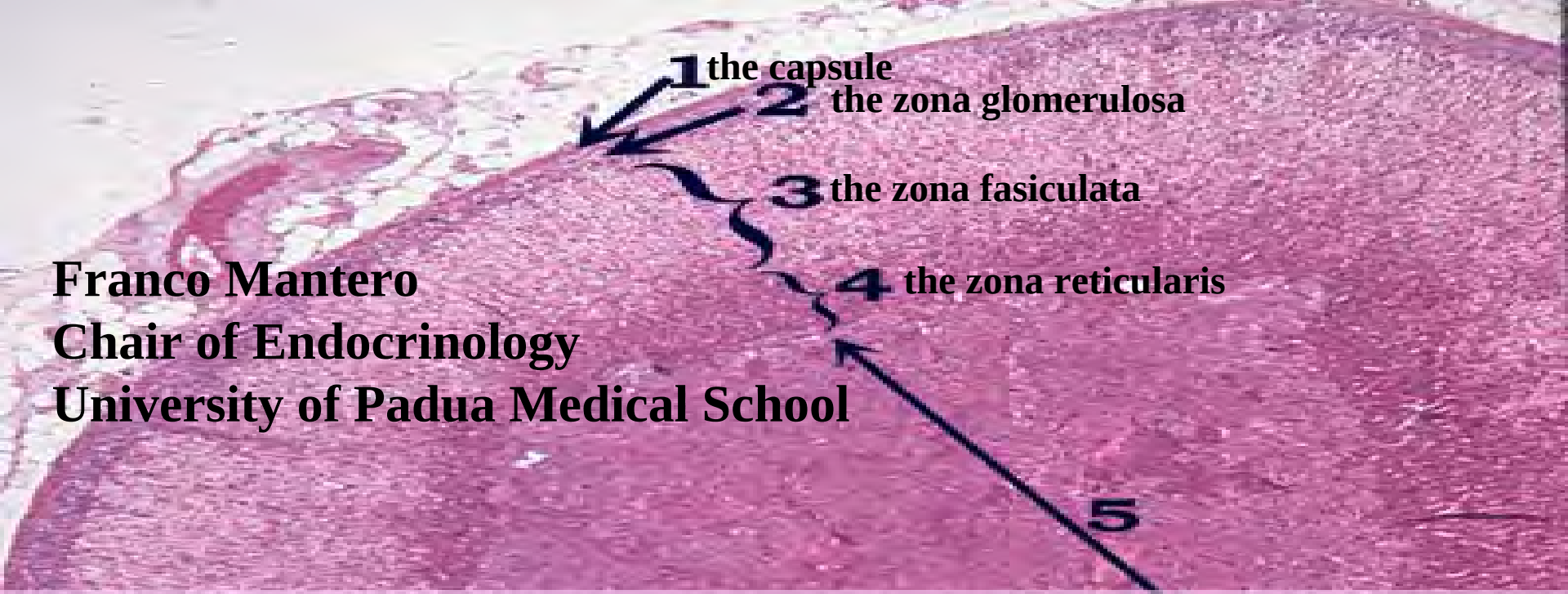
Modified from Young WF. Trends Endocrinol Metab, 1994

Most patients with primary aldosteronism are normokalemic



(n=1174)

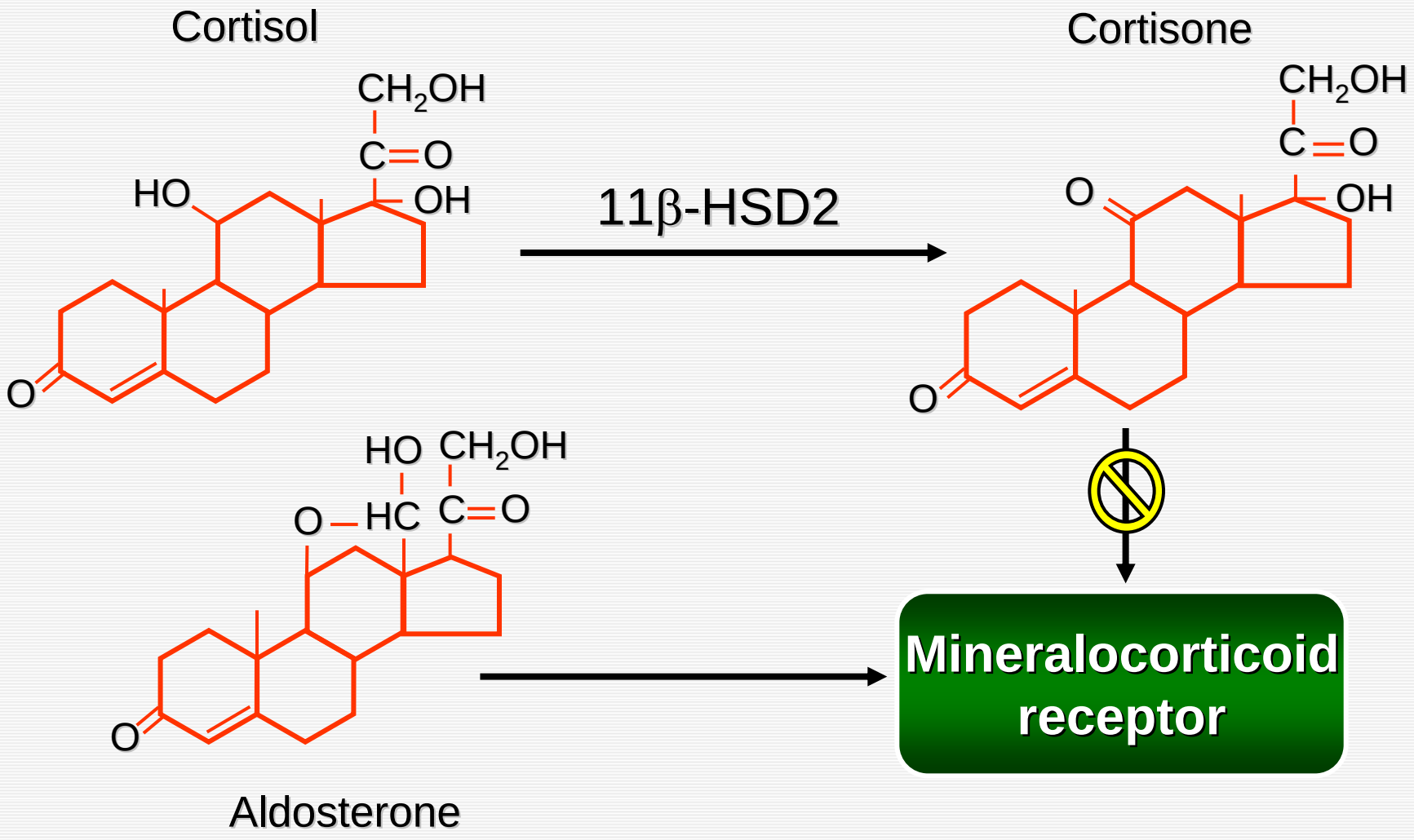
PAPY STUDY



Franco Mantero
Chair of Endocrinology
University of Padua Medical School

Aldosterone: pathophysiology and clinical aspects in hypertension

Mineralocorticoid Receptor
"Protection" by 11β-HSD2 against Occupation by Glucocorticoids



Glucocorticoid Remediable Aldosteronism (GRA)

- First described in 1966 (DJ Sutherland; *Can Med Assoc J 1966)*
- Autosomal dominant inheritance
- Severe hypertension in infancy or adulthood
- Family history of:
  - ✓Hypertension
  - ✓Cerebrovascular Accidents
- Severe hypokalaemia (50% of cases) especially with diuretics
- Resistent Hypertension
- Suppression of the Renin Angiotensin System
- Normal or elevated plasma Aldosterone
- Response to low doses of dexamethasone

Determining the subtype of PA
Dynamic tests

Posture test: increase of >50% of PAC after 2 h supine position predicts IHA or Angiotensin-II responders APA

Angiotensin-II stimulation test [1]

Captopril supression test [1]

Dexamethasone supression test: a PAC value < 4 ng/dl after 0.5 mg oral dexamethasone for 4 days is highly predictive of GRA [2]

[1] Mantero F. J Endocrinol Invest 2003; 26: 92; [2]Litchfield WR et al. 1997; 82: 3570

Romolo M Dorizzi

Laboratorio Analisi Chimico Cliniche ed Ematologia

Ospedale Civile Maggiore – Verona

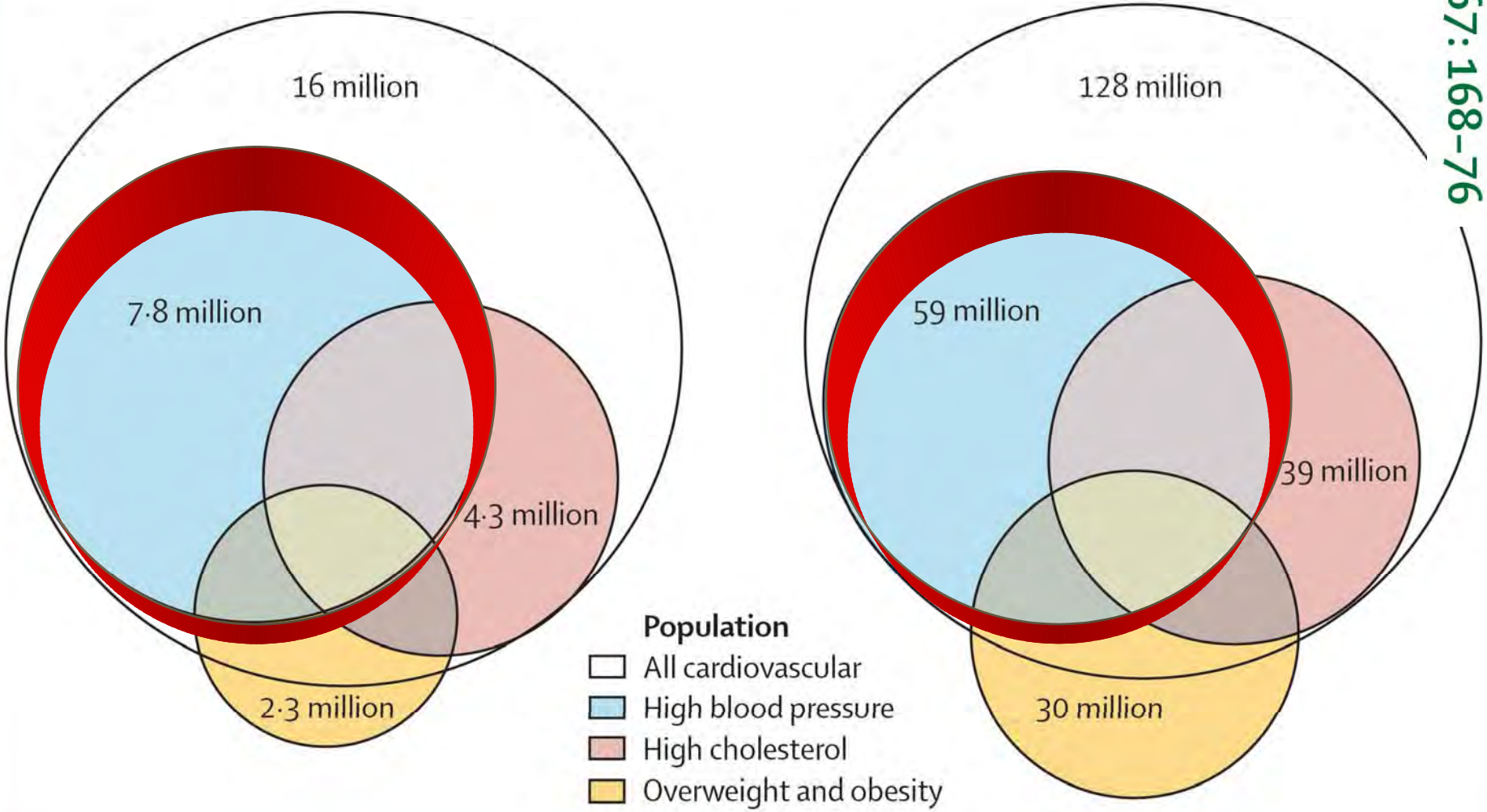
Renin and Aldosterone measurement in Hypertension screening

romolo.dorizzi@azosp.vr.it

Controversies in hypertension

Norman M Kaplan, Lionel H Opie

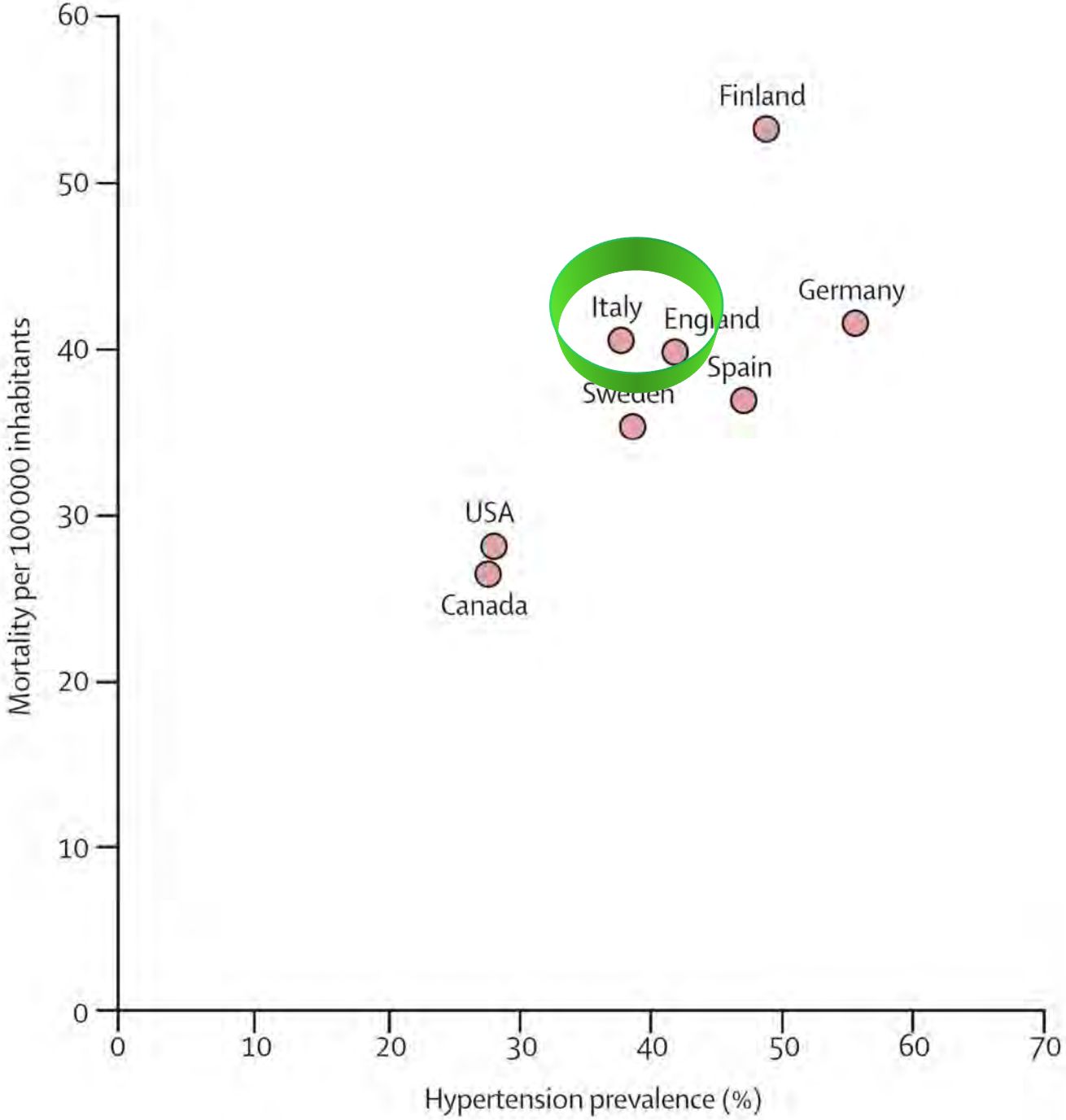
Lancet 2006; 367: 168–76



Lancet 2006; 367: 168–76

Controversies in hypertension

Norman M Kaplan, Lionel H Opie



Laboratorio OCHI Verona

RMD

J. Lab. & Clin. Med.
January, 1955

PRESIDENTIAL ADDRESS

PART I. PAINTING BACKGROUND

PART II. PRIMARY ALDOSTERONISM, A NEW CLINICAL SYNDROME*

JEROME W. CONN, M.D.
ANN ARBOR, MICH.

PART I. PAINTING BACKGROUND

Presented at the Twenty-seventh Annual Meeting of the Central Society for Clinical Research, Chicago, Ill., Oct. 29, 1954.

Persistence . . . and Prayer: From the Artificial Kidney to the AutoAnalyzer

Clinical Chemistry 46:9
1425–1436 (2000)

LEONARD T. SKEGGS, JR.

History

Renin Substrate

Asp Arg Val Tyr Ile His Pro Phe His Leu Leu Val Tyr Ser R -----

↓ ← **Renin**

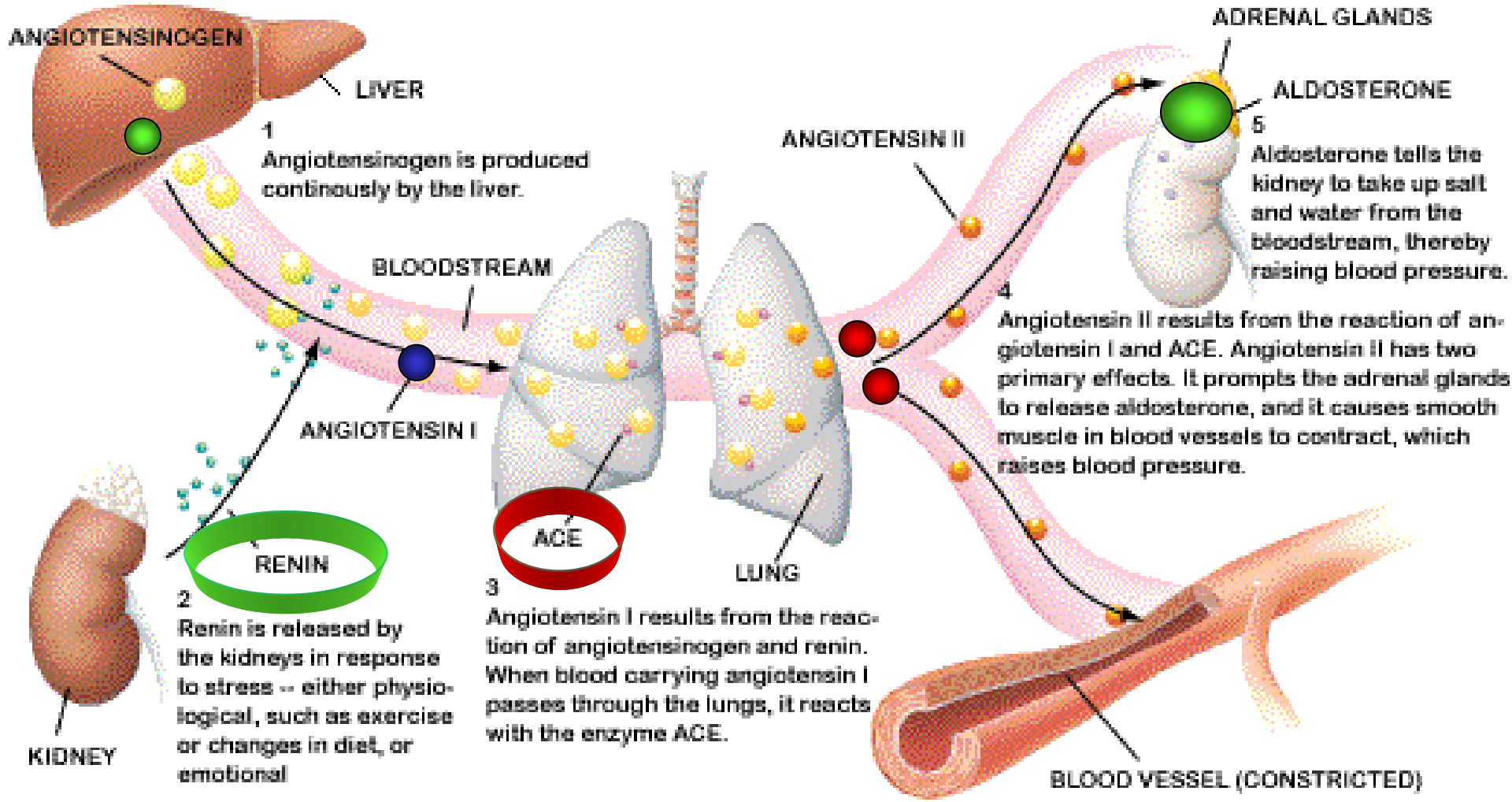
Angiotensin I

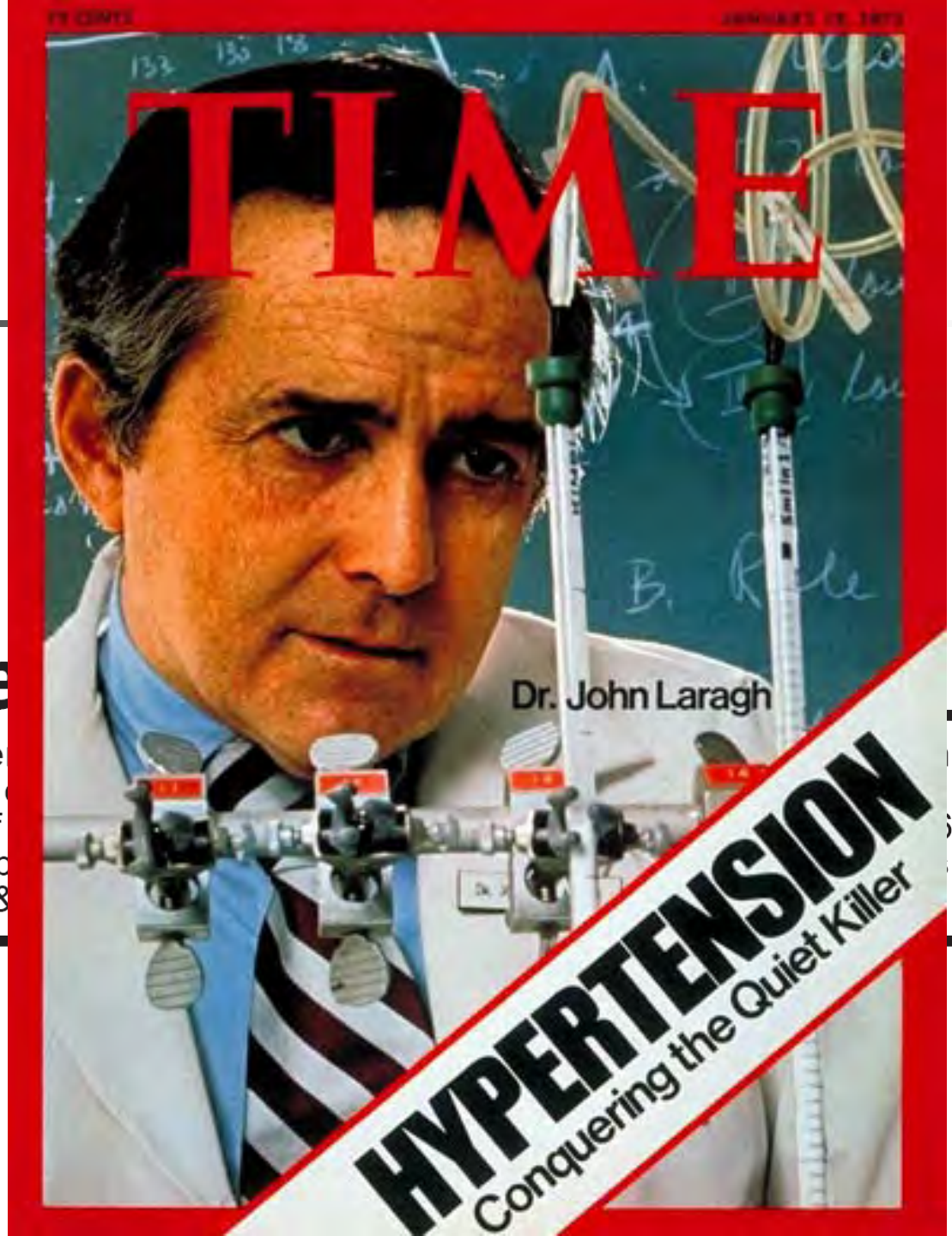
Asp Arg Val Tyr Ile His Pro Phe His Leu + L

Converting Enzyme → ↓

Angiotensin II

Asp Arg Val Tyr Ile His Pro Phe + His Leu


ANGIOTENSINOGEN
LIVER
1
Angiotensinogen is produced continously by the liver.
BLOODSTREAM
ANGIOTENSIN I
ANGIOTENSIN II
ADRENAL GLANDS
ALDOSTERONE
5
Aldosterone tells the kidney to take up salt and water from the bloodstream, thereby raising blood pressure.
4
Angiotensin II results from the reaction of angiotensin I and ACE. Angiotensin II has two primary effects. It prompts the adrenal glands to release aldosterone, and it causes smooth muscle in blood vessels to contract, which raises blood pressure.
RENIN
2
Renin is released by the kidneys in response to stress -- either physiological, such as exercise or changes in diet, or emotional
KIDNEY
ACE
3
Angiotensin I results from the reaction of angiotensinogen and renin. When blood carrying angiotensin I passes through the lungs, it reacts with the enzyme ACE.
LUNG
BLOOD VESSEL (CONSTRICTED)



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CC/NUMBER 35
AUGUST 27, 1979

Laragh J H, Ange
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secretory rate of
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Columbia Univ. &

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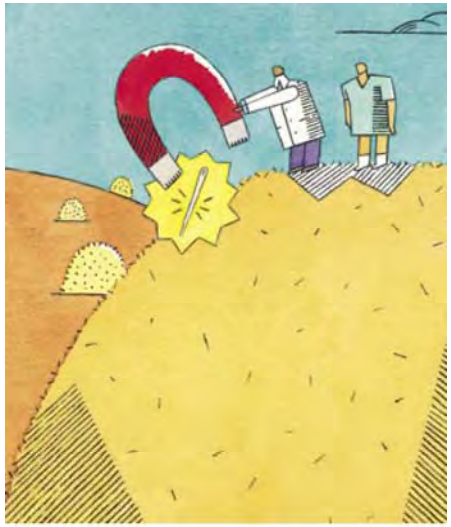
others on the
60.
and Surgeons,

Primary Aldosteronism: A Needle in a Haystack or a Yellow Cab on Fifth Avenue?

Gian Paolo Rossi, MD, FACC, FAHA



Current Hypertension Reports 2004, **6**:1–4

IMAJ • Vol 4 • January 2002

In conclusion, normokalemia does not exclude the diagnosis of hyperaldosteronism. Patients with resistant hypertension should be systematically evaluated for this condition, using the PAC/PRA ratio and the suppression-stimulation tests.

Normokalemic Hyperaldosteronism in Patients with Resistant Hypertension

Sydney Benchetrit MD, Jacques Bernheim MD and Eduardo Podjarny MD

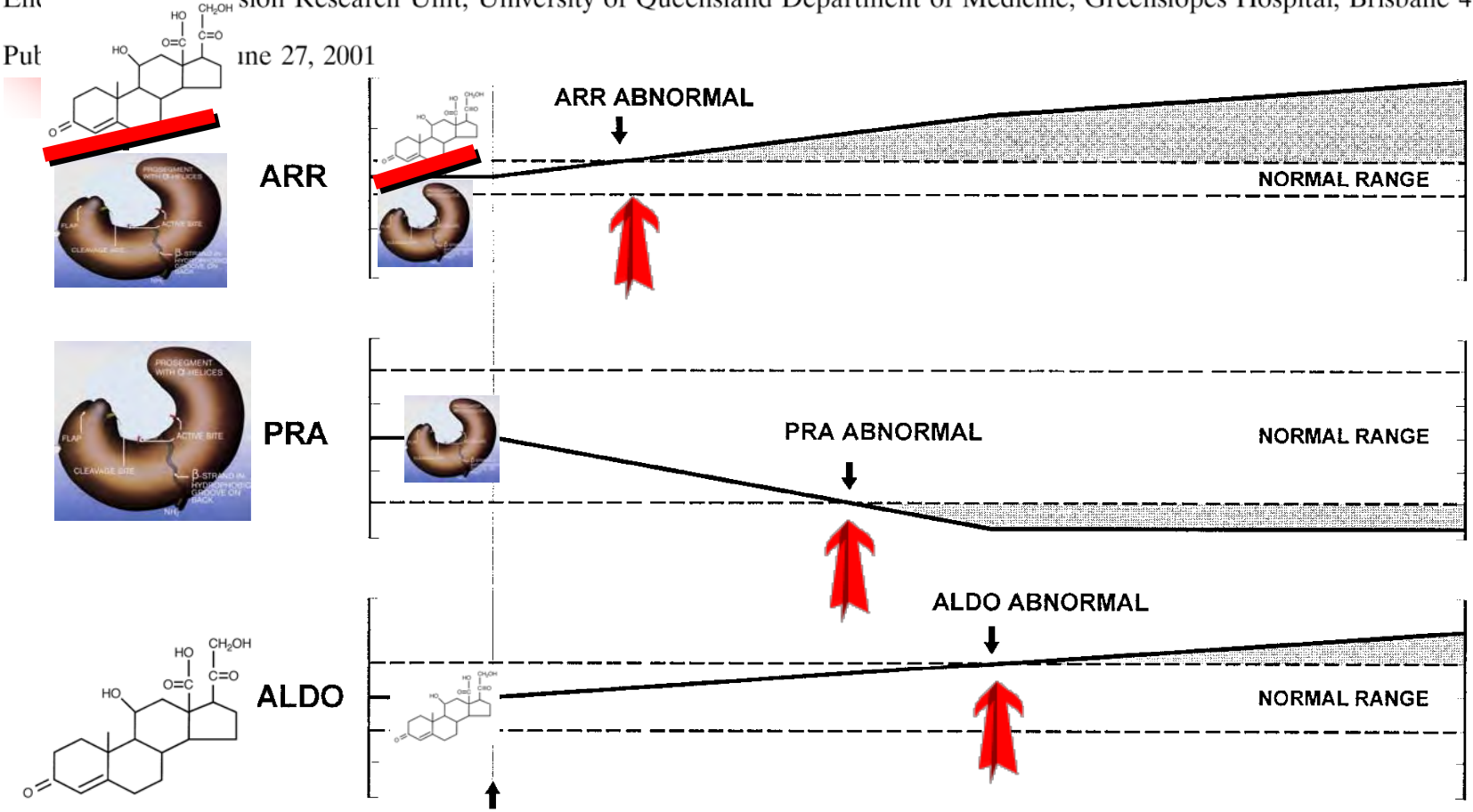
Department of Nephrology, Meir Hospital, Sapir Medical Center, Kfar Saba, and Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

Primary Aldosteronism: Are We Diagnosing and Operating on Too Few Patients?

Richard D. Gordon, M.D., Ph.D., Michael Stowasser, M.B.B.S., Ph.D., John C. Rutherford, M.b.B.S.

Endocrine Hypertension Research Unit, University of Queensland Department of Medicine, Greenslopes Hospital, Brisbane 4120, Australia

Published online: June 27, 2001



World J. Surg. 25, 941–947, 2001
DOI: 10.1007/s00268-001-0033-4

WORLD Journal of SURGERY

Viewpoint

Cautions over the current epidemic of primary aldosteronism

Lancet 2000; **357:** 953–54

Norman M Kaplan

ARR should not be done as a routine procedure on all patients with hypertension and the diagnosis of hyperaldosteronism should not be based solely on the finding of a raised ARR.

Clinical Endocrinology (2003) **59**, 427–430

Commentary

Primary aldosteronism, diagnosed by the aldosterone to renin ratio, is a common cause of hypertension

Pitt O. Lim* and Thomas M. MacDonald†
**Department of Cardiology, Wales Heart Research Institute, University of Wales College of Medicine,*

($n = 56$, 161/98 mmHg to below 140/90 mmHg) of patients with mild hypertension with the 'best' monotherapy but only 39% when a random approach was adopted. Furthermore, it appeared

We believe that primary aldosteronism is probably a common form of hypertension. Such hypertension appears aldosterone-driven, is identified quite easily by the ARR and is effectively managed with appropriate treatment.

0021-972X/04/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 89(9):4221–4226

doi: 10.1210/jc.2003-032179

Aldosterone to Renin Ratio in a Primary Care Setting: The Bussolengo Study

OLIVIERO OLIVIERI, ALBERTO CIACCIARELLI, DENISE SIGNORELLI, FRANCESCA PIZZOLO, PATRIZIA GUARINI, CHIARA PAVAN, ANGELA CORGNATI, SALVATORE FALCONE, ROBERTO CORROCHER, ALESSIO MICCHI, CHIARA CRESSONI, AND GIANSTEFANO BLENGIO

An aldosterone to active renin ratio (AaRR) of 32 pg/ml was taken as the cut-off value, equivalent to an ARR of 50 ng/dl/ng/ml/h. As an elevated AaRR is frequent in the general hypertensive population.

ADALTIS

Nichols Institute Diagnostics

PRA pitfalls-1

Two assays

Prorenin

37°C

4°C

Renin

1-3 ore
pH 5.5-6.0

1-3 ore
pH 5.5-6.0

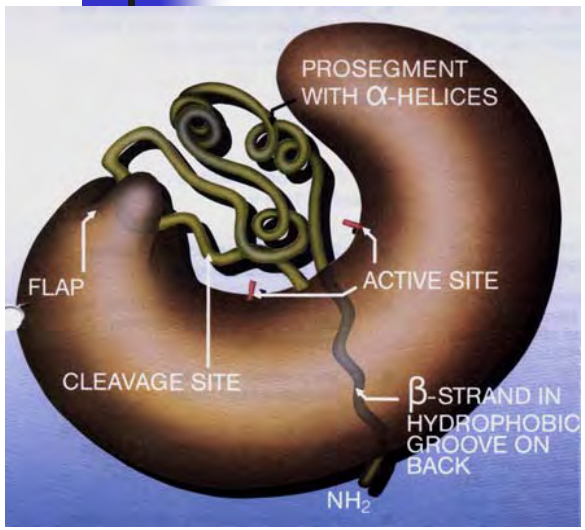
Angiotensin I

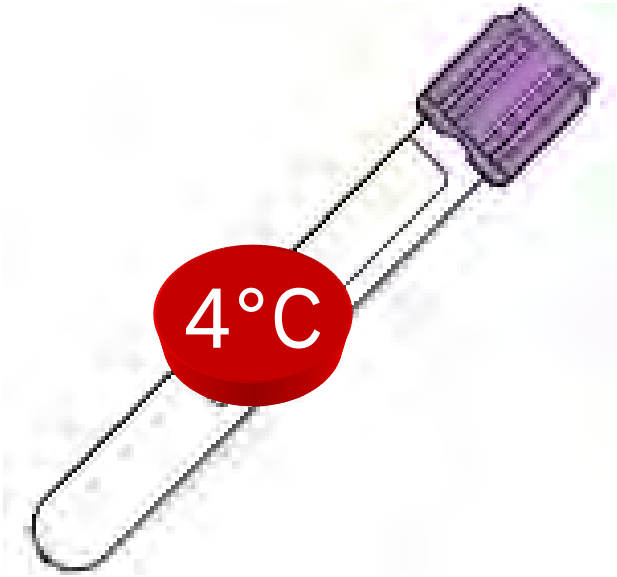
24 h

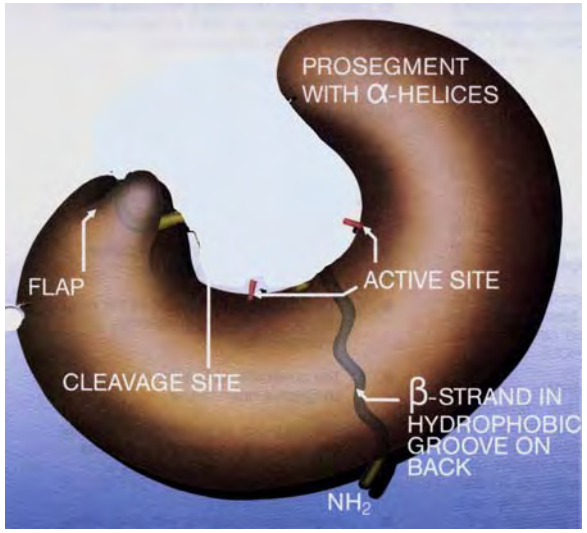
Ria 1

Ria 2

PRA pitfalls-2







Sample handling

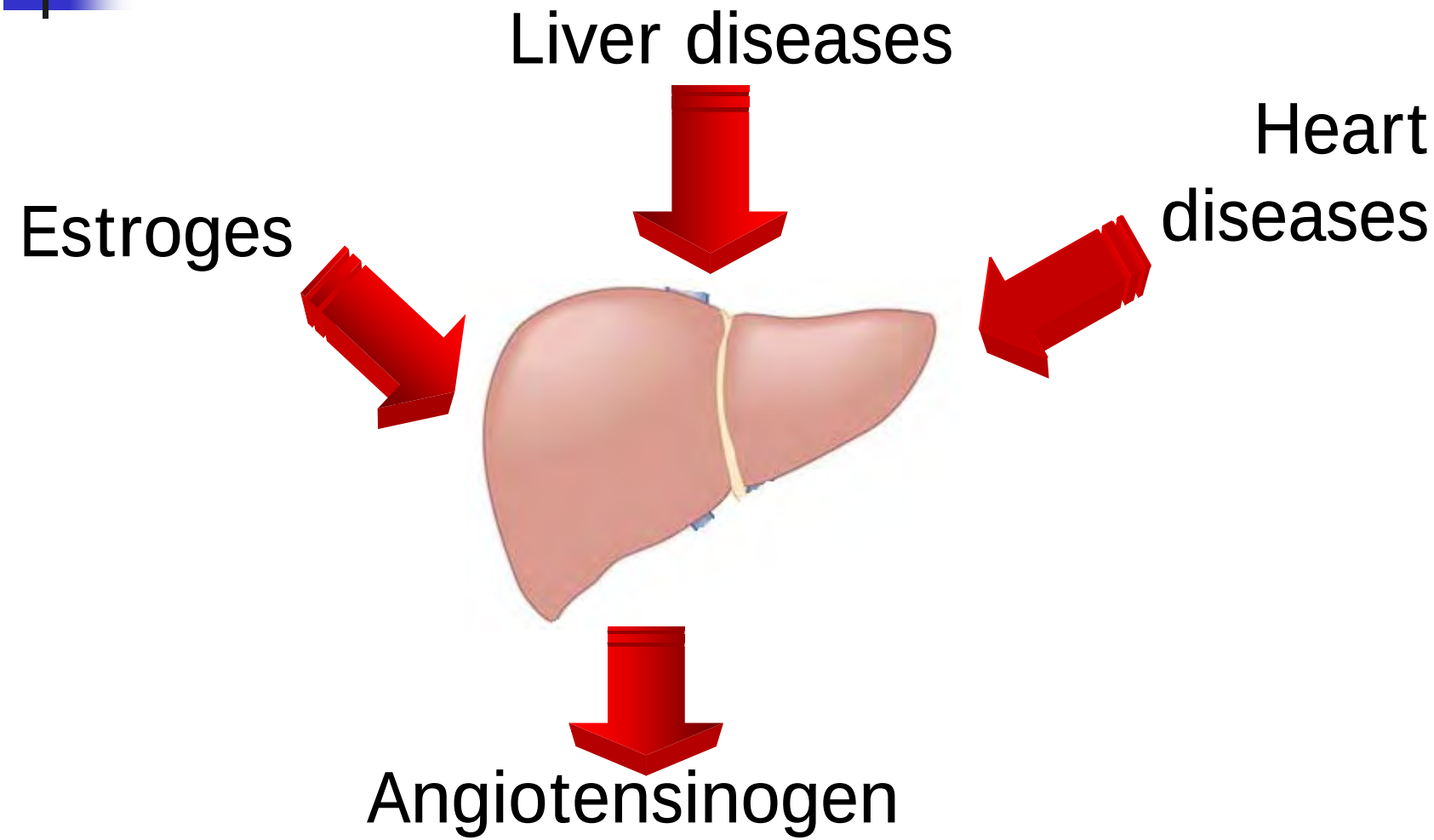
Collection in cold tubes

Stored in ice before centrifugation

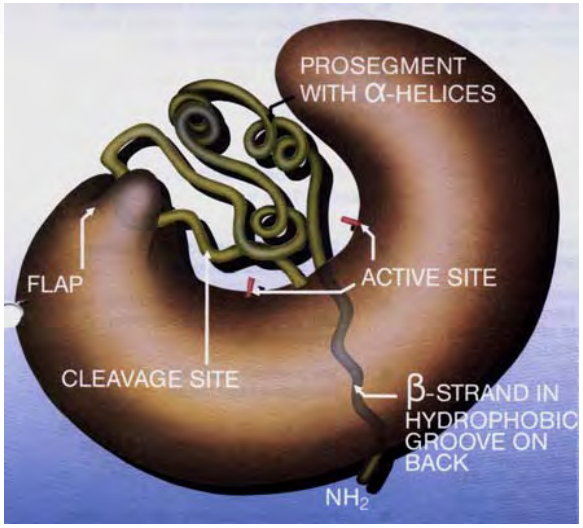
Prorenin-Renin cryoactivation

- Active Renin derives from enzymatic cleavage of a 43 amino acid prosegment
- Prorenin concentration in circulation is usually 10 times higher than active renin
- Cryoactivation significantly increases Renin concentration

PRA pitfalls-3

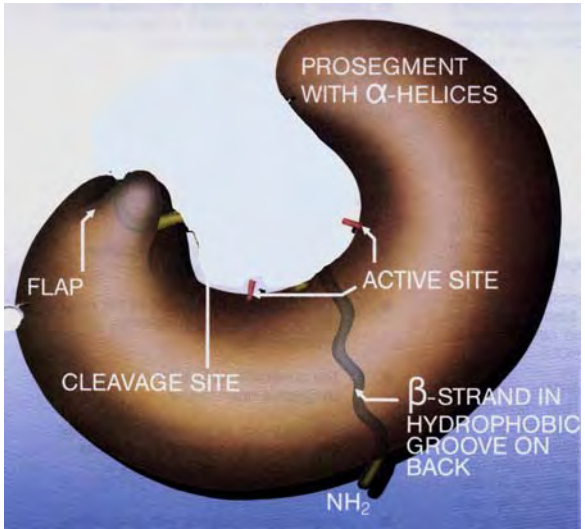


Prorenin vs Active Renin



Prorenin

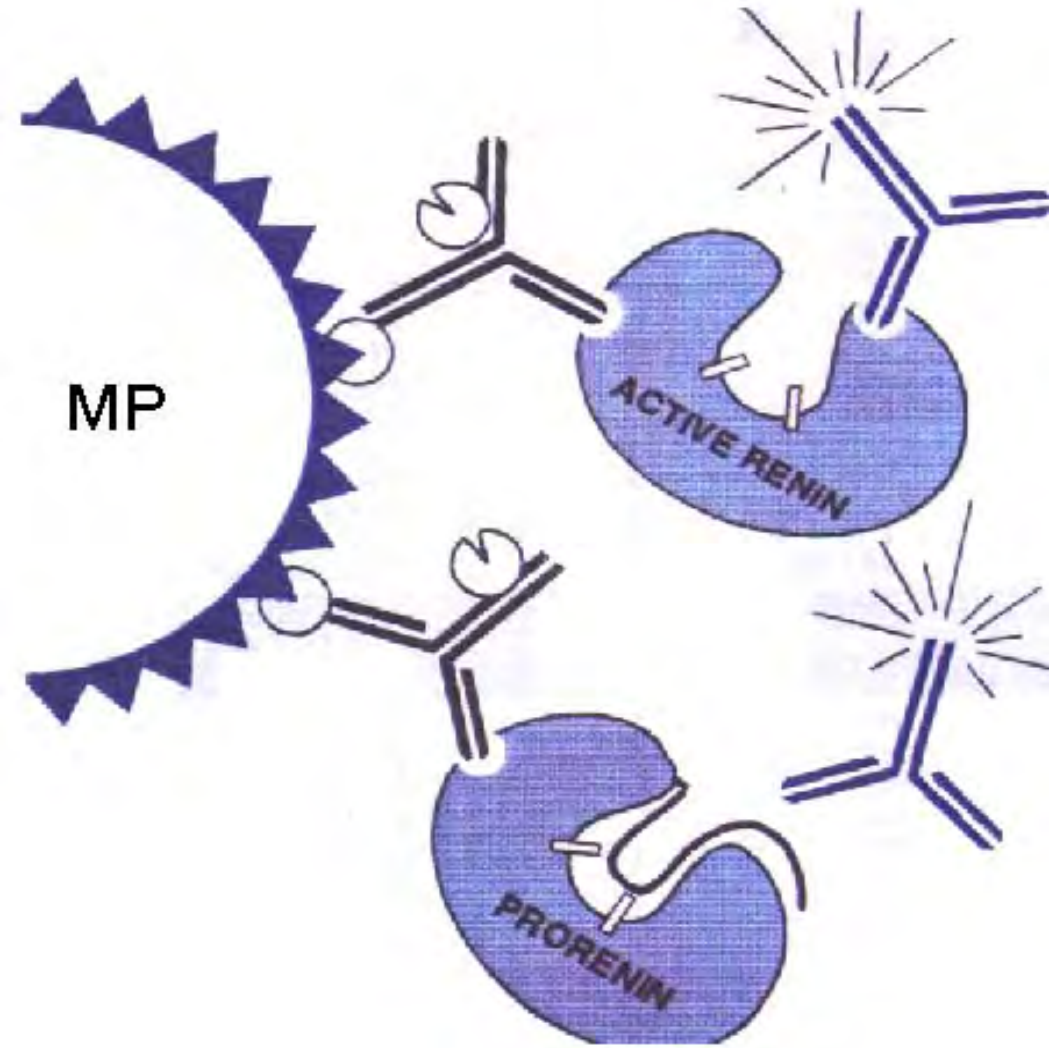
Prosegment covering the active site and the epitope Not measured by the Direct renin assays



Active Renin

Prosegment cleaved
Active site exposed
Epitope exposed measured by the Direct renin assays

Direct Renin: principle of the assay



A sandwich is formed only in presence of renin molecules that bridge both antibodies

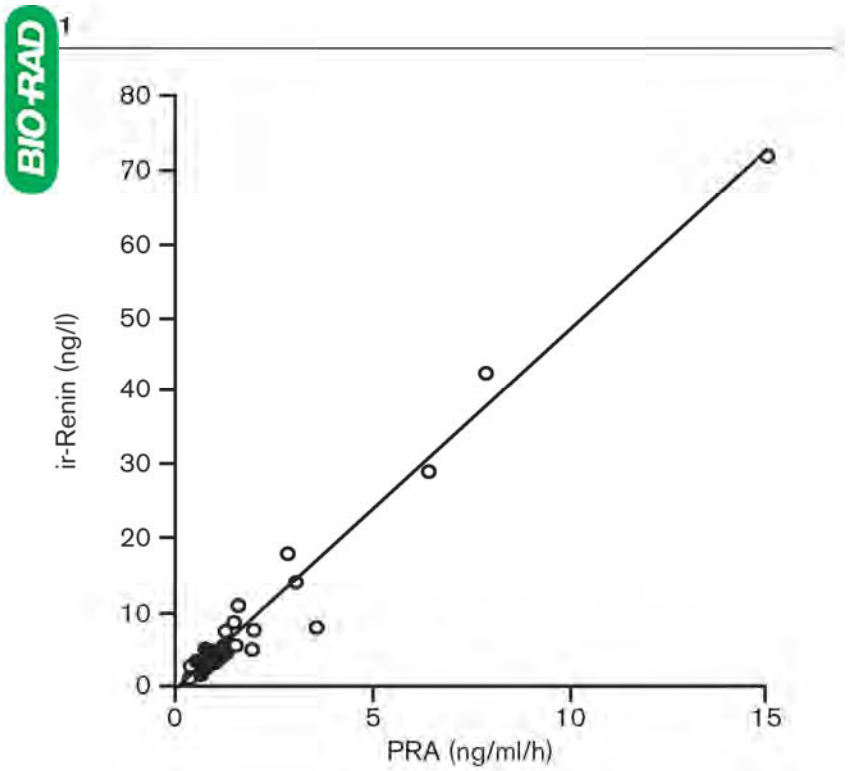
Direct Renin - Advantages

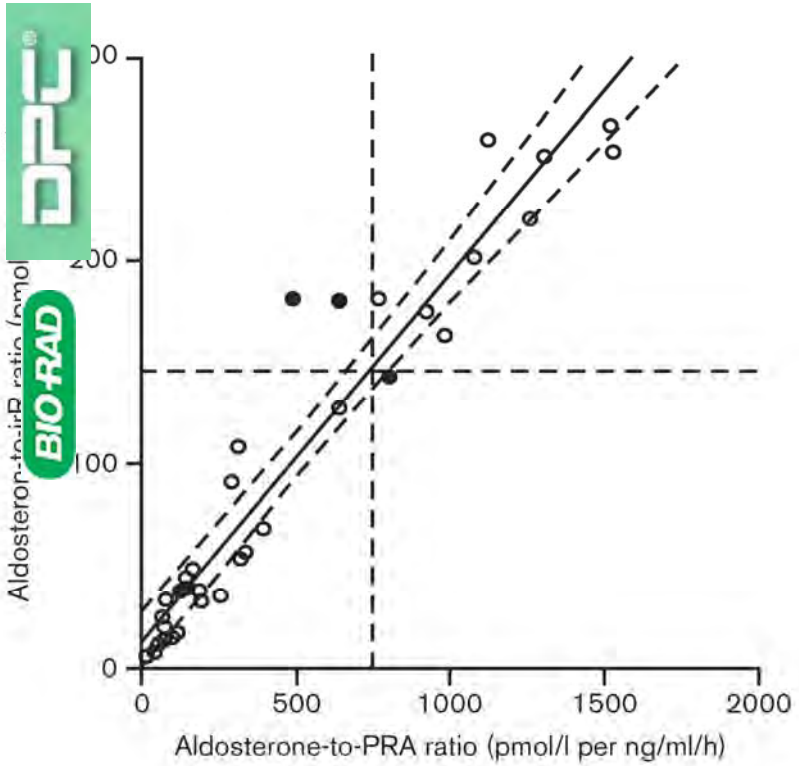
- Completely automated
- Non-isotopic
- Reduced workload
- Accurate and sensitive
- Standardized assay (WHO 68/356)
- More consistent (inter-assays and inter-laboratories)

Active renin versus plasma renin activity to define aldosterone-to-renin ratio for primary aldosteronism

Paolo Ferrari[a], Sidney G. Shaw[c], Jérôme Nicod[b], Esther Saner[b] and Jürg Nussberger[d]

Journal of Hypertension 2004, 22:377–381





DPC

Active renin versus plasma renin activity to define aldosterone-to-renin ratio for primary aldosteronism

Paolo Ferrari[a], Sidney G. Shaw[c], Jérôme Nicod[b], Esther Saner[b] and Jürg Nussberger[d]

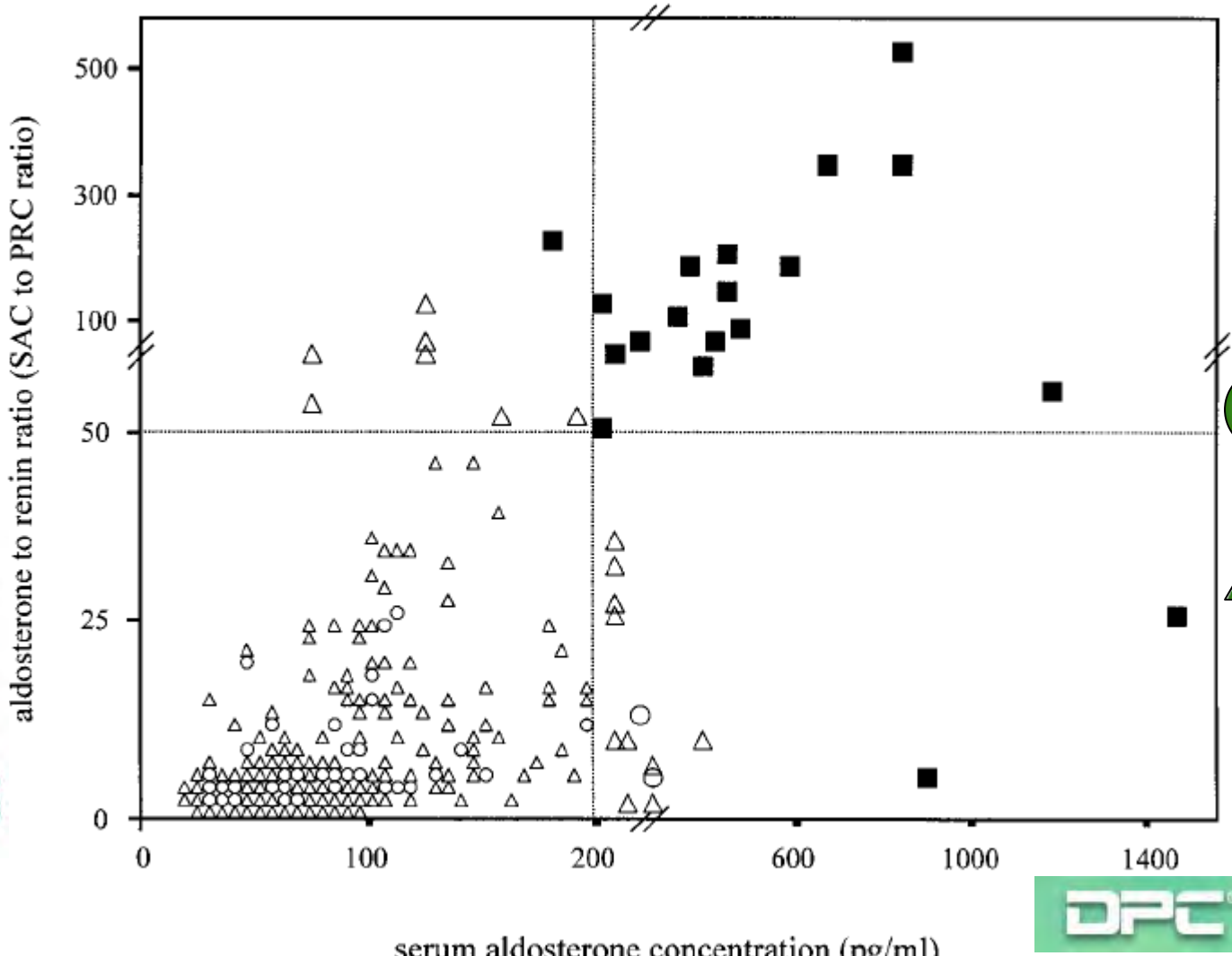
Measurements of irR were highly correlated with PRA in hypertensive subjects, and the correlation between ARR derived by measuring PRA or irR is also excellent.

Journal of Hypertension 2004, **22**:377–381

Ratio of serum aldosterone to plasma renin concentration in essential hypertension and primary aldosteronism

S. Trenkel [1]
C. Seifarth [2]
H. Schobel [3]
E. G. Hahn [2]
J. Hensen [4]

Trenkel S et al. Ratio of ... Exp Clin Endocrinol Diabetes 2002; 110: 80–85



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J. Hensen [4]

Trenkel S et al. Ratio of ... Exp Clin Endocrinol Diabetes 2002; 110: 80–85

In summary, measuring plasma renin concentration without the necessity of ice-cooling and the aldosterone to renin-concentration ratio are useful in the screening for primary aldosteronism and very efficient to identify patients with the potentially curable form of an aldosterone producing adenoma.

HYPERTENSION Canada

Published by the Canadian Hypertension Society

Canadian Cardiovascular Congress 2002

Usefulness of the Aldosterone-to-Renin Ratio for Screening Primary Aldosteronism

September 2003
Bulletin No. 76

Table of contents

Table 2

Aldosterone/renin Ratio

False (+)

- Beta-blockers
- Chronic renal failure
- Low renin hypertension
- Elderly patients

False (-)

- Spironolactone
- Severe hypokalemia

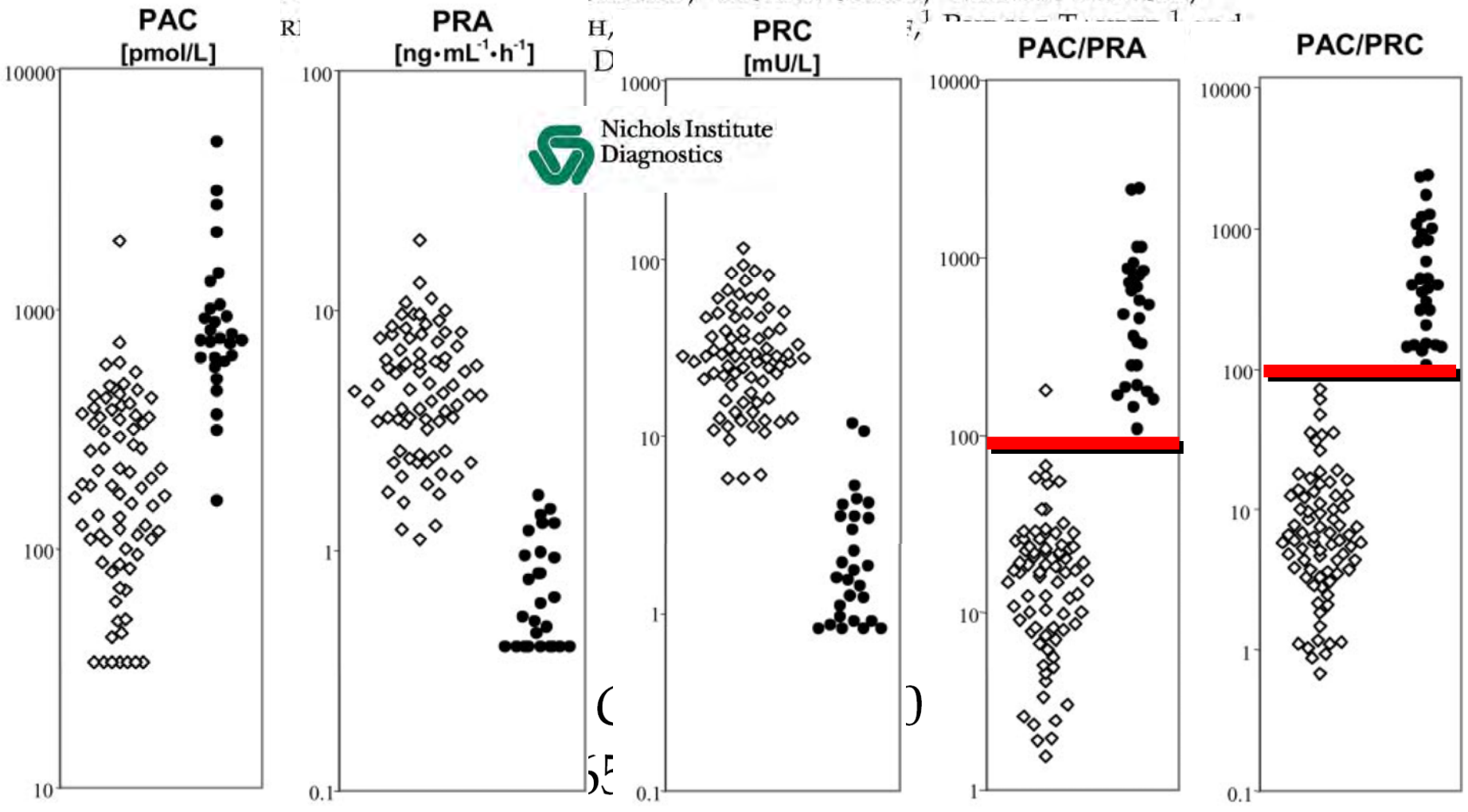
Table 3

Aldosterone/renin Ratio (pmol/L/ng/L)

• ≤ 100	Essential hypertension
• 100-140	Grey zone (repeat)
• ≥ 140	Primary aldosteronism (combined with plasma aldosterone > 400 pmol/L)

Rapid Screening Test for Primary Hyperaldosteronism: Ratio of Plasma Aldosterone to Renin Concentration Determined by Fully Automated Chemiluminescence Immunoassays

PERSCHEL,[1*] RUDOLF SCHEMER,[3] LYSANN SEILER,[4] MARTIN REINCKE,[4]



Rapid Screening Test for Primary Hyperaldosteronism: Ratio of Plasma Aldosterone to Renin Concentration Determined by Fully Automated Chemiluminescence Immunoassays

Table 3. Calculated cutoff values and resulting specificity with respect to 100% sensitivity to differentiate patients with PHA from healthy volunteers.

Method	Units	Optimum value for cutoff	Sensitivity, %	Specificity, %
PAC	pmol/L	155	100	43.4
PRA	$ng \cdot mL^{-1} \cdot h^{-1}$	1.7	100	94.7
PRC	mU/L	5.2	100	89.5
PAC/PRA ratio	(pmol/L)/ ($ng \cdot mL^{-1} \cdot h^{-1}$)	185	100	98.7
PAC/PRC ratio	pmol/mU	71	100	100

Nichols Institute Diagnostics

Clinical Chemistry 50:9 1650–1655 (2004)

Nichols Institute Diagnostics

1311 Calle Batido
San Clemente, California 92673
949.940.7200
800.286.4NID

Nichols Institute
Diagnostics

Laboratorio OCHI Verona
RMD

nols Institute
gnostics

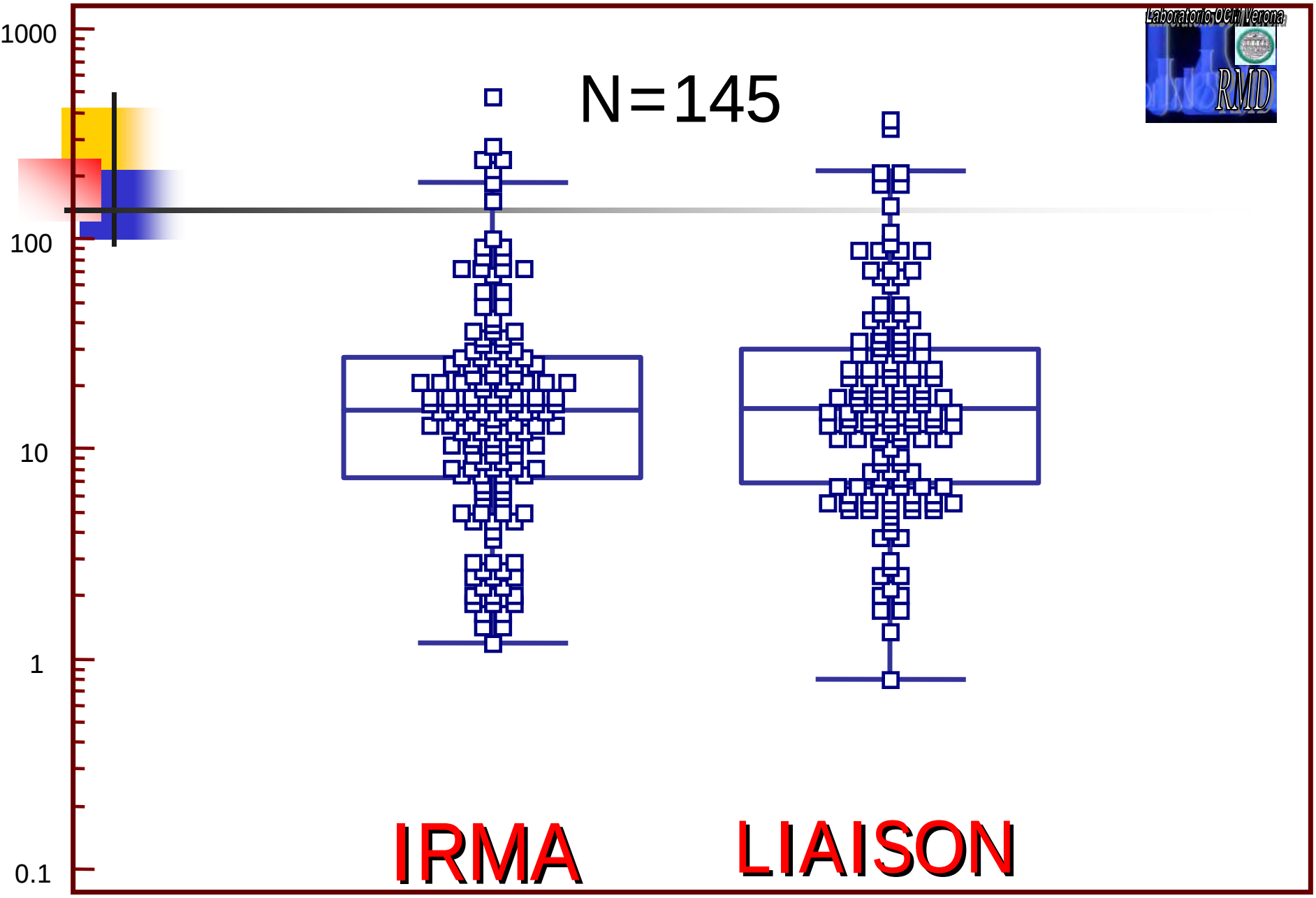
April 25, 2006

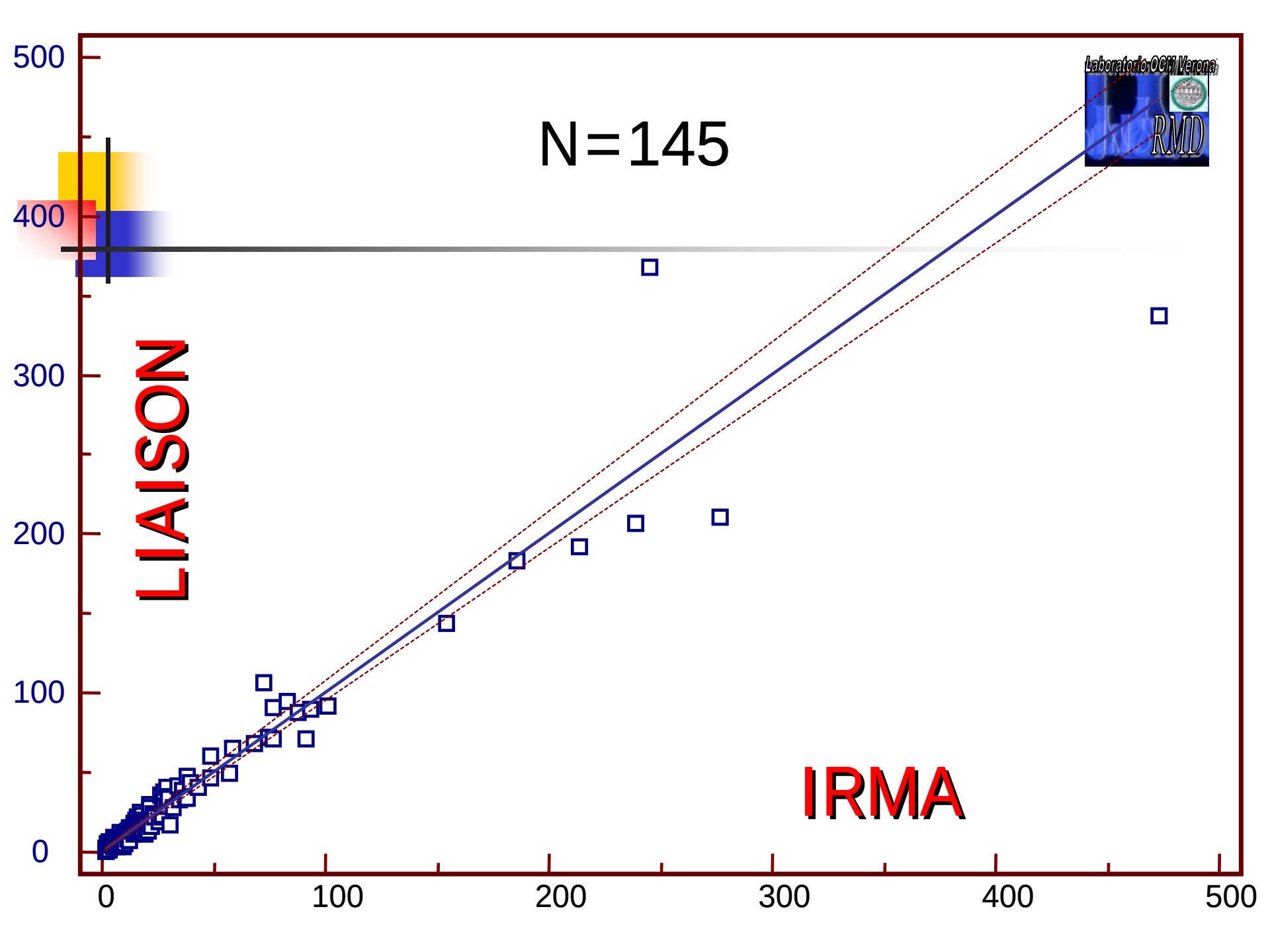
Dear Valued Customer:

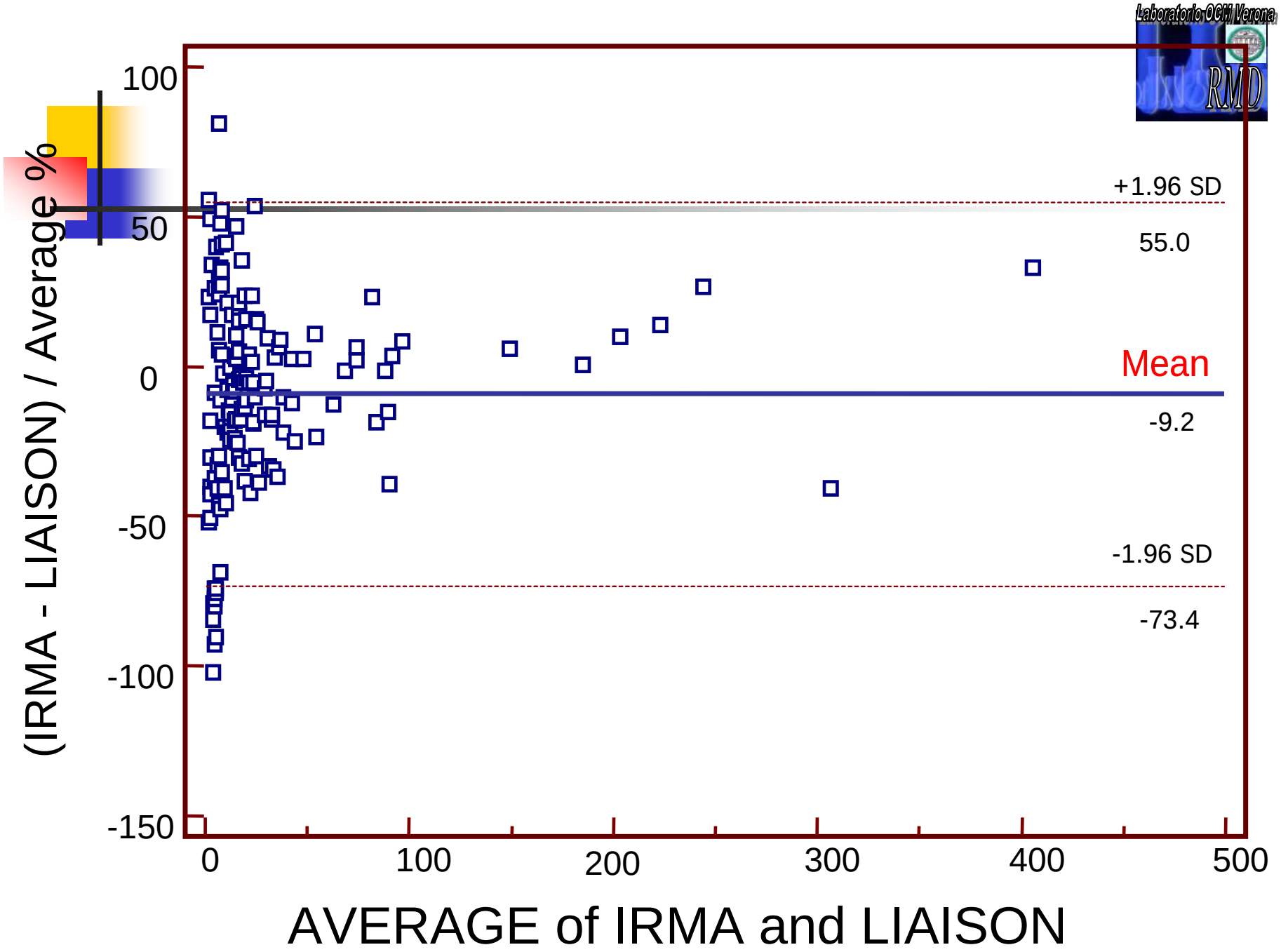
It is with great regret that I must inform you on April 20, 2006, after evaluating a number of alternatives, we have decided to discontinue product shipments and discontinue the operations o Nichols Institute Diagnostics (NID). This was a difficult decision and was made only after muc study and deliberation. We have been infusing new resources and focusing on Quality System

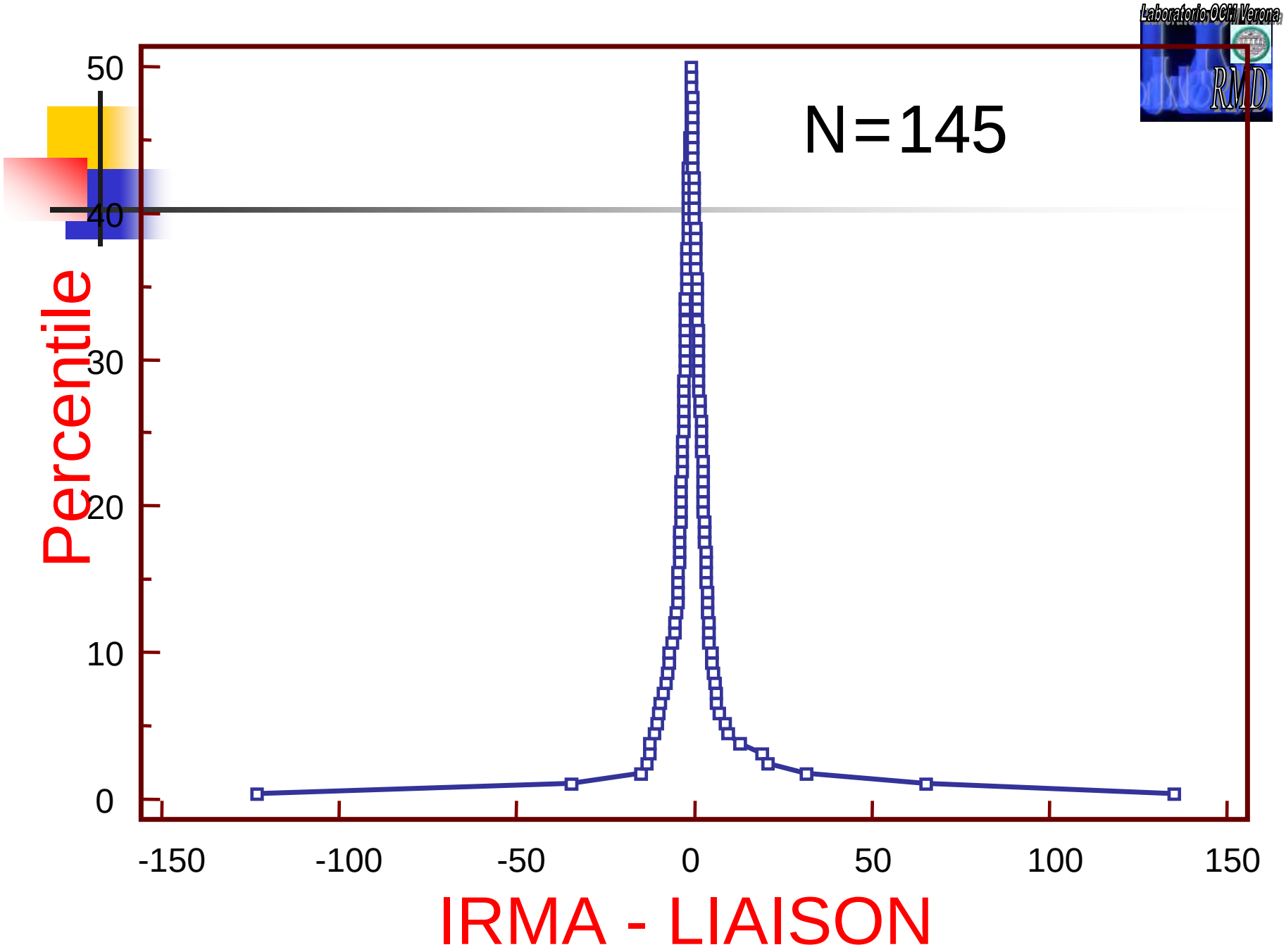
LIAISON – Dia-Sorin

CEA	TPA
PSA	fPSA
NSE	S-100
CA 15-3	AFP
CA 125	hCG/ß-hCG
CA 19-9	Ferritin
Tg	PCT


1000
100
10
1
0.1
N=145
IRMA
LIAISON
Laboratorio OCM Verona
RMD

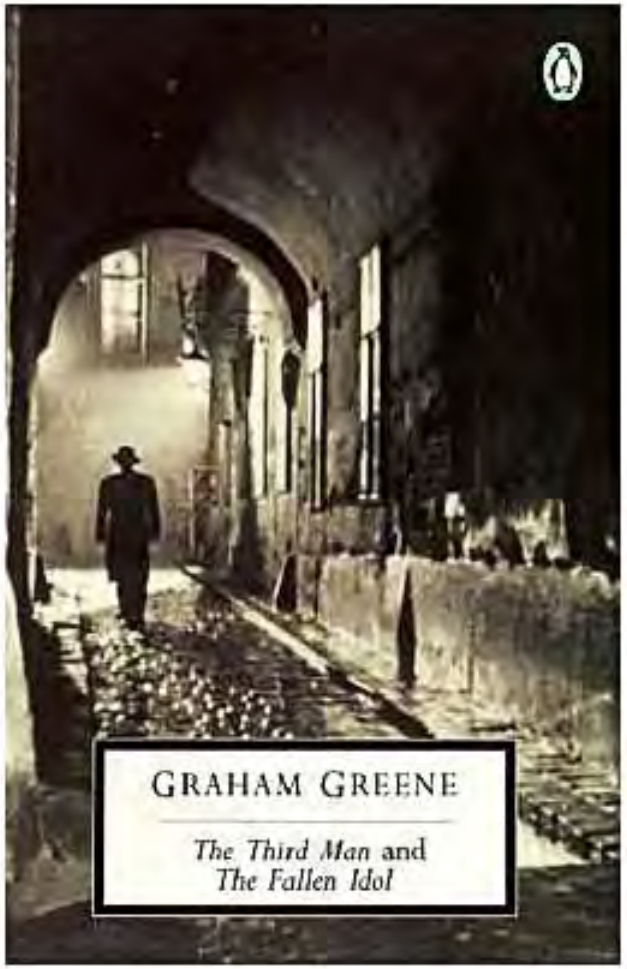

N=145
LIAISON
IRMA
Laboratorio OCRI Verona
RMD
0
100
200
300
400
500


Laboratorio OCHI Verona
RMD
100
50
0
-50
-100
-150
+1.96 SD
55.0
Mean
-9.2
-1.96 SD
-73.4
0
100
200
300
400
500
(IRMA - LIAISON) / Average %
AVERAGE of IRMA and LIAISON


Laboratorio OCRI Verona
RMD
N=145
50
40
30
20
10
0
Percentile
-150
-100
-50
0
50
100
150
IRMA - LIAISON

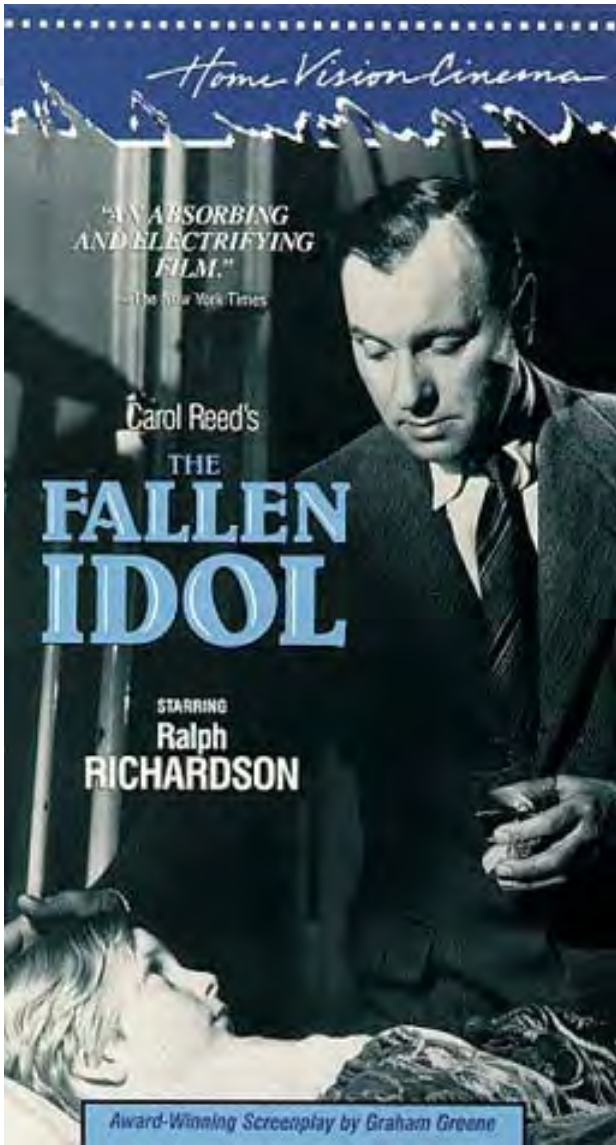
First fallen idol

Low prevalence



Second fallen idol

Hypokalemia



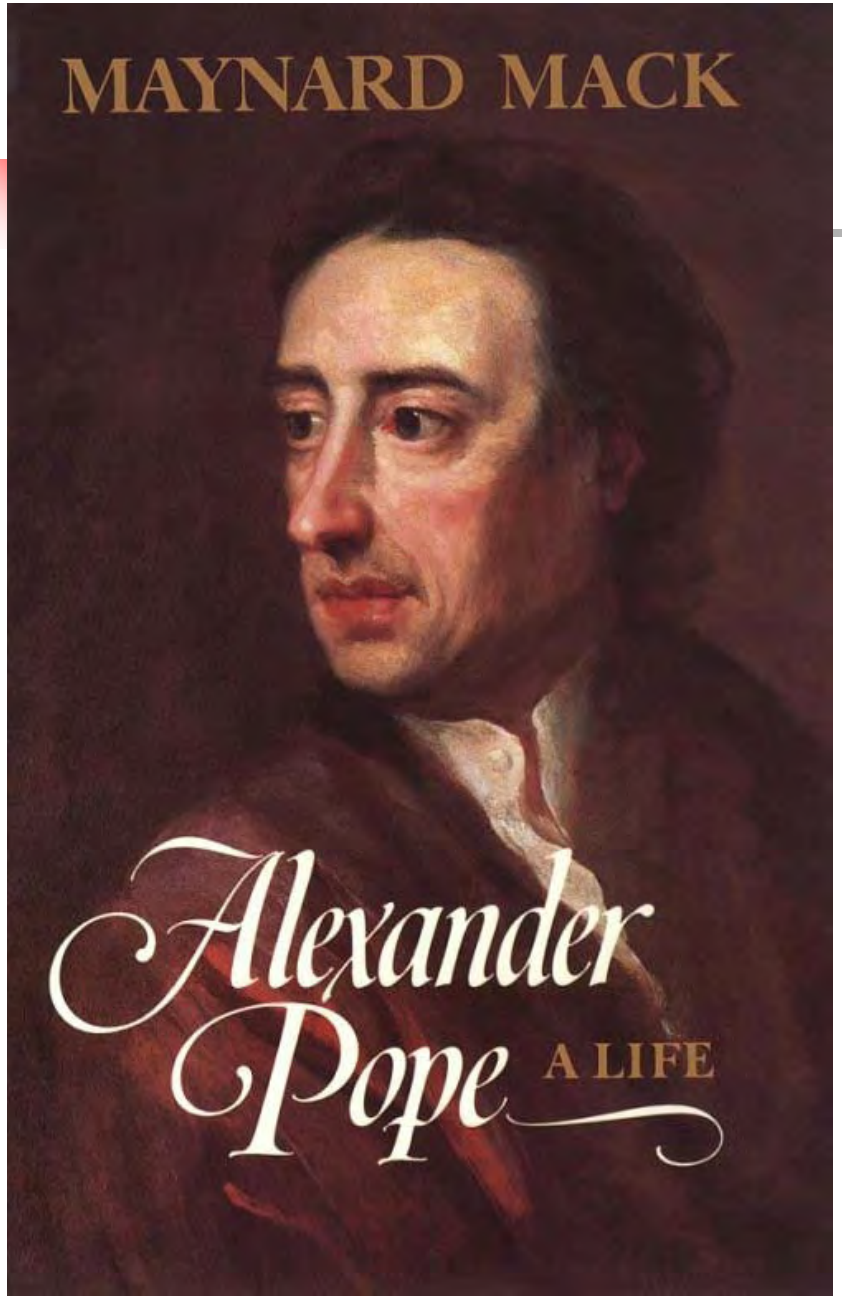
Third fallen idol

Direct renin

not

acceptable

assay



Be not the first by whom the new are tried,

Nor yet the last to lay the old aside.

Alexander Pope

An Essay on Criticism, 1711

English poet & satirist (1688 - 1744)

TOTAL AND FREE TESTOSTERONE, REALLY BETTER THAN A GUESS ?

Giagulli V.A. MD, PhD

O.U. Internal Medicine,Subunit Endocrinology PP.OO. Putignano-Noci-Gioia del Colle ASL Ba/5 Noci (Ba); O.U. Pathophysiology of Reproduction IRCCS Castellana Grotte (Ba), Italy.

6th AME National Meeting & 3rd Joint Meeting with AACE
Verona, October 27-29,2006

ANDROGENS

The modern definition includes:

1. **Sexual effects:**
    - *Differentation and development of male internal and external genitalia as well as differentation of secondary characteristics*
    - *Maintenance of reproduction function*
    - *Muscle development*
    - *Bone density*
2. **Metabolic effects:**
    - *Proteins*
    - *Carbohydrates*
    - *Fat metabolism*

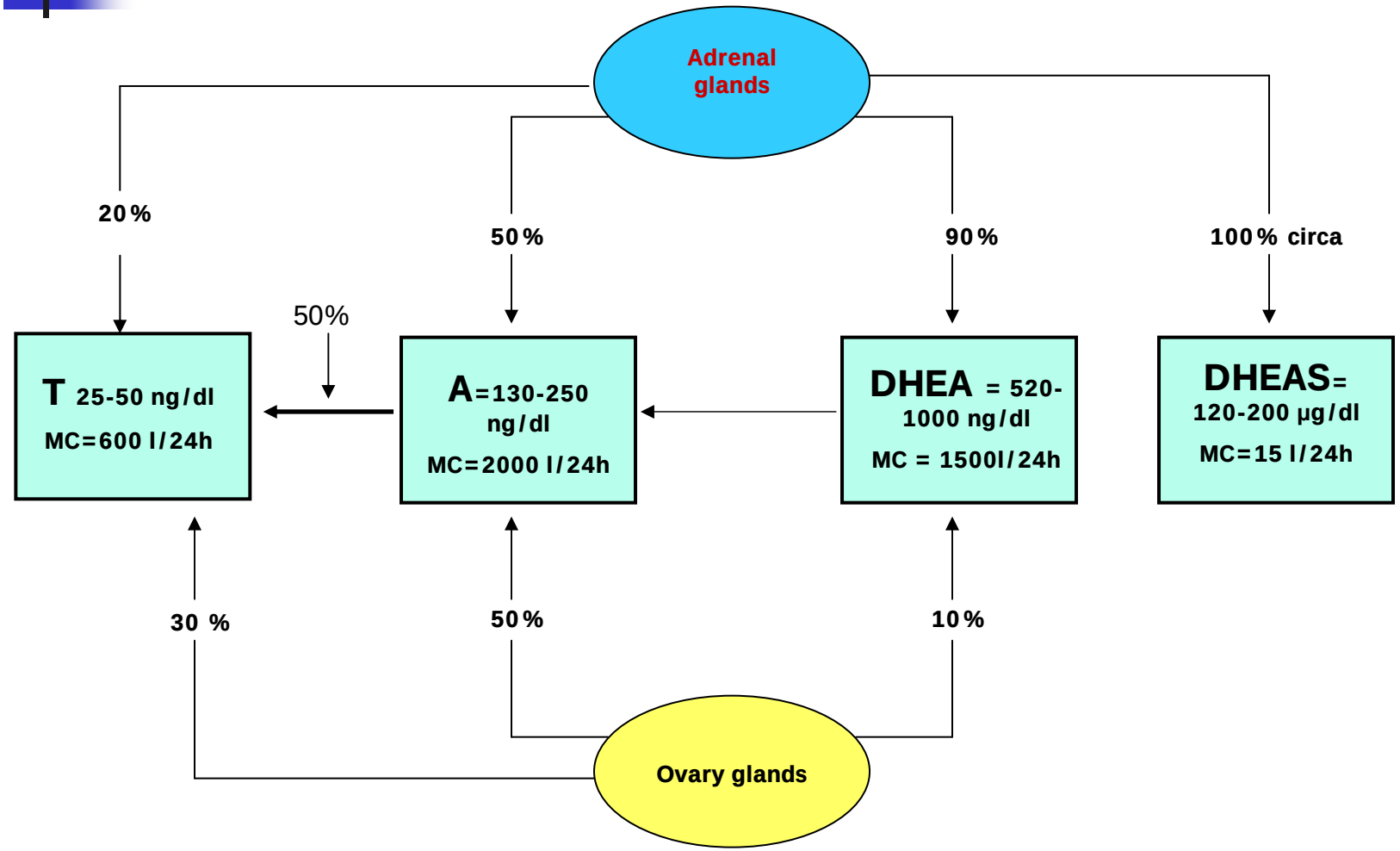
PLASMA ANDROGENS

1. Active ones: **T** and **DHT**

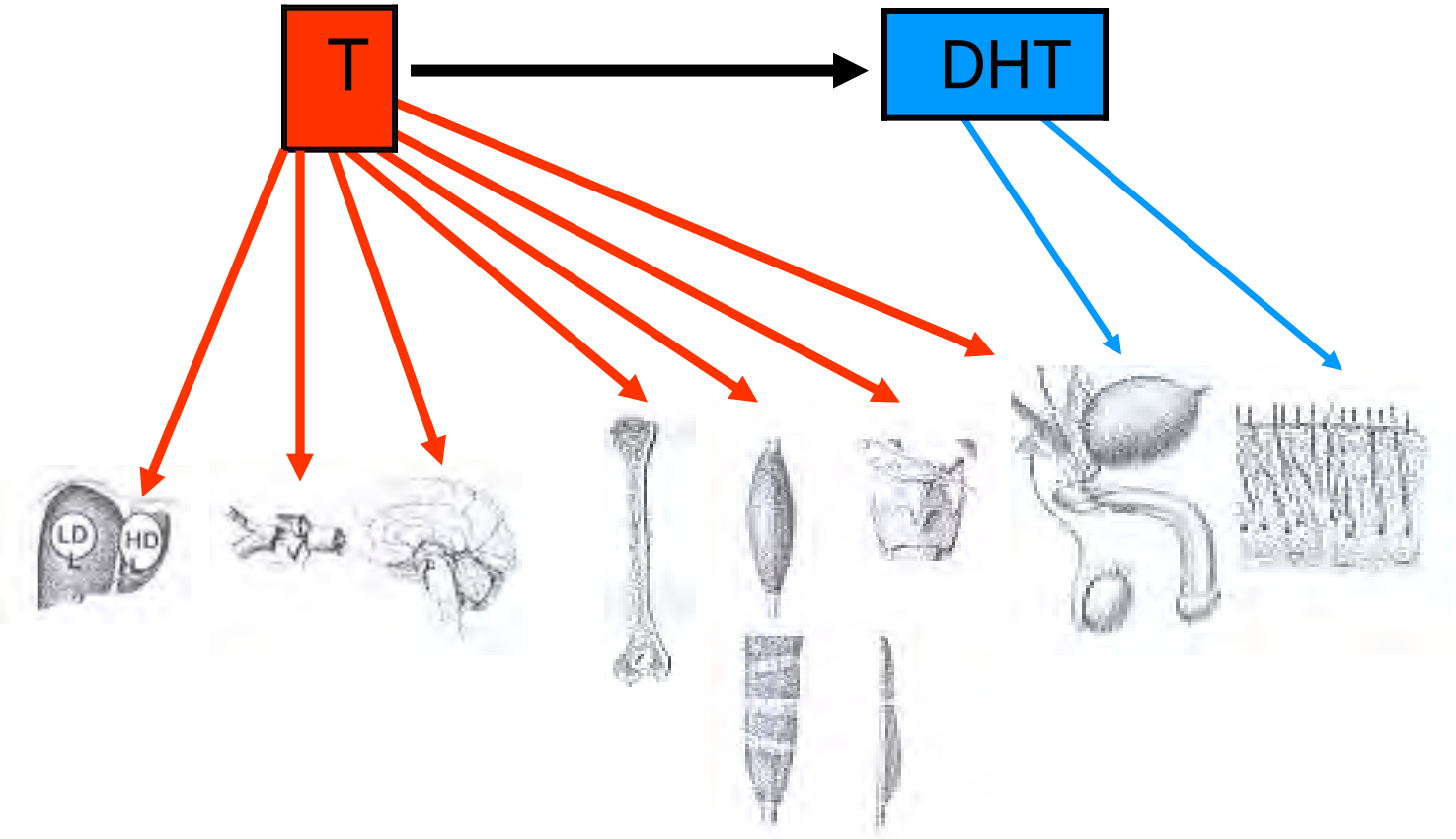
2. Active ones after being metabolized into **T** and/or **DHT**:
   - Androstenedione
   - DHEA(S)

(Important precursor of both active and less active androgens: 17α OH P)

Origin of plasma androgens in normal women



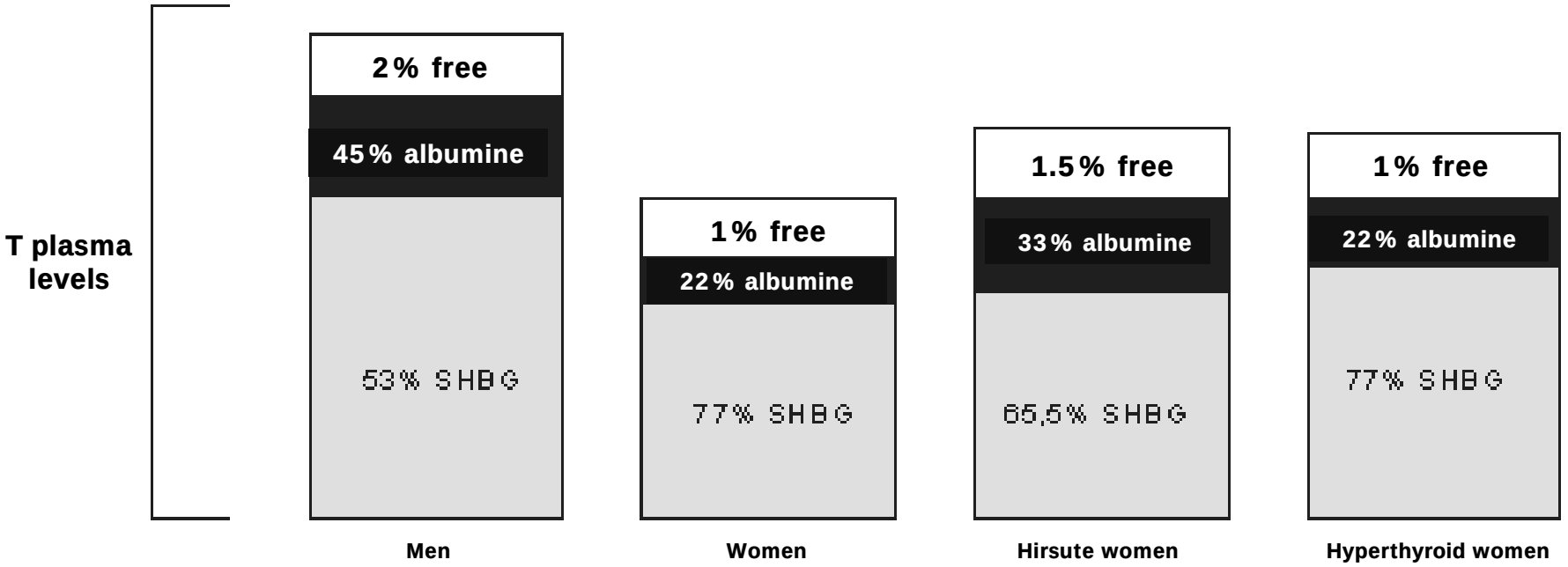
TARGET ORGANS FOR TESTOSTERONE AND DIHYDROTESTOSTERONE



Neischlag E. & Behre HM, 2004

ANDROGENS PRODUCTION AND THEIR METABOLISM

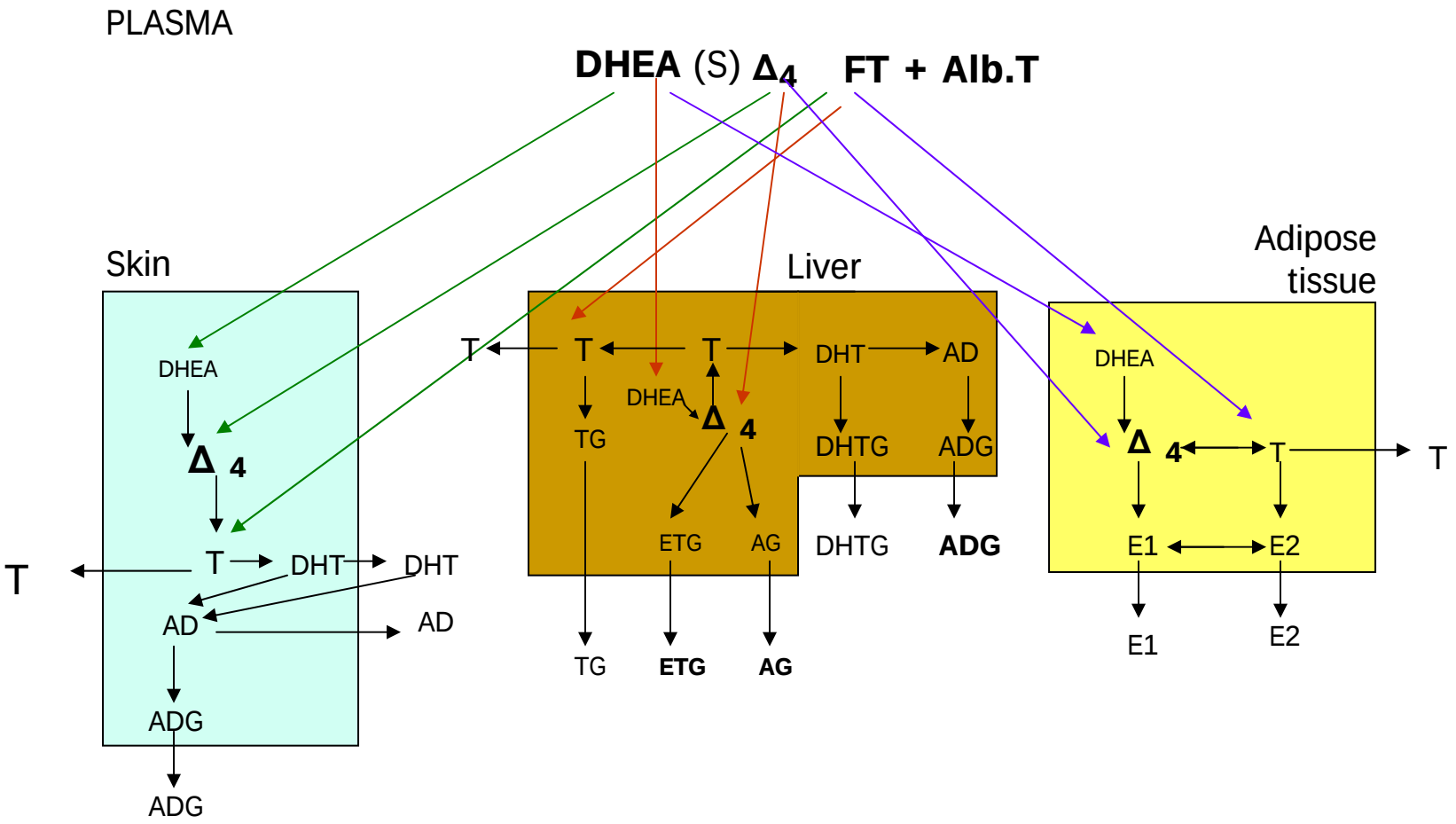
PLASMA TESTOSTERONE LEVELS
PHYSICAL STATE



Vermeulen A. et al JCEM, 1969, 1999.

Androgens metabolism

Deslypère et al, 1985;Toscano & Horton,1987; Giagulli et al, 1989; Quinkler et al, 2004



WHEN DO WE HAVE TO MEASURE ANDROGENS LEVELS IN MEN AND WOMEN ?

ANDROGEN EXCESS IN WOMEN

(AACE Hyperandrogenism guidelines Endocrine Pract,2001)

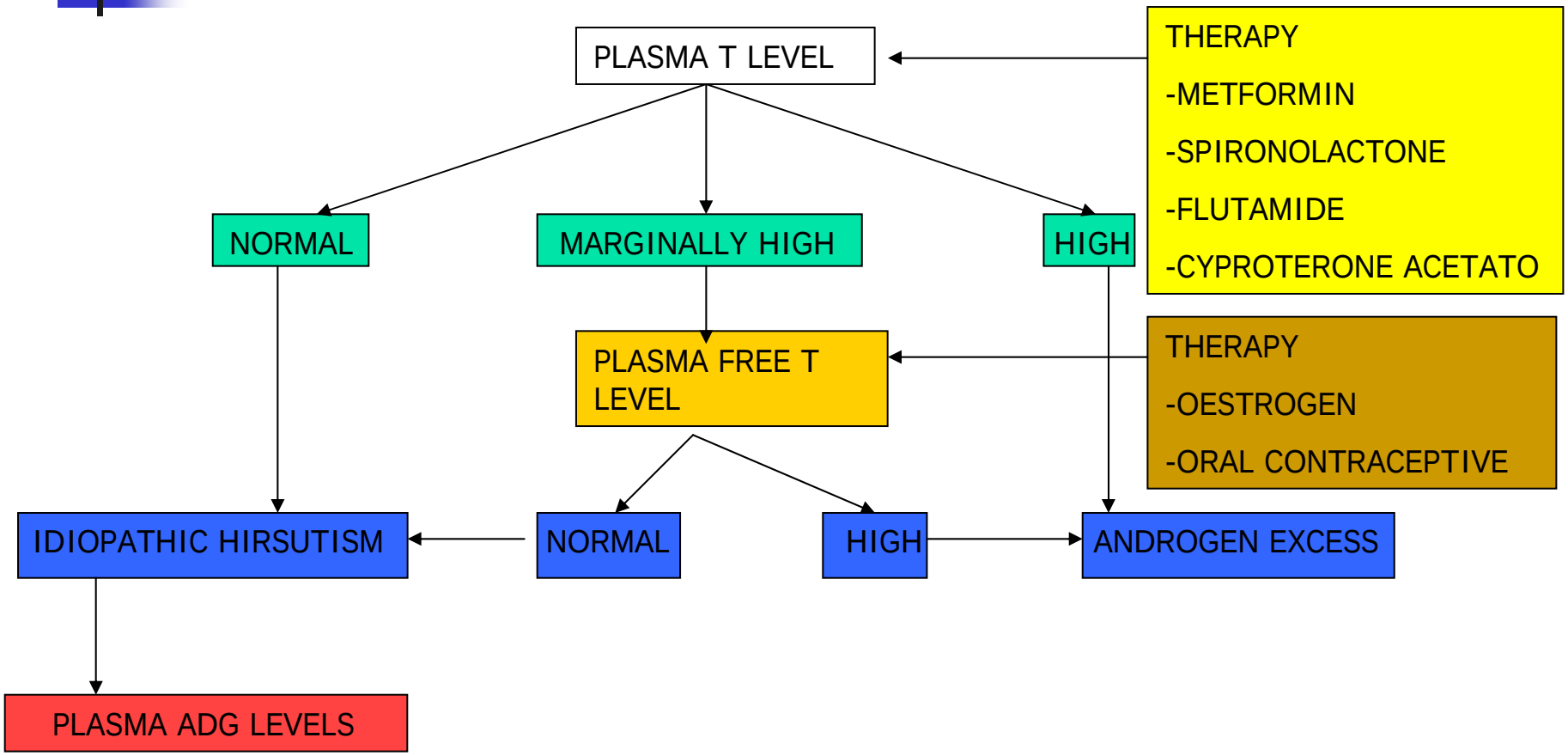
Hirsutism, androgenic alopecia, acne, ovulatory dysfunction and, in extreme and prolonged cases, even *virilization* and *masculinization,* are the androgenic features shown by women affected by ANDROGEN EXCESS.

HIRSUTISM is defined as the presence of terminal hairs in women's body areas where we do not normally expect to find them: cheeks, chin, above upper lip, midline chest etc.

VIRILIZATION is characterized by deep voice, increased muscle mass, temporal balding, and clitoral enlargement.

HIRSUTISM AND VIRILISM

(PROGESSION OF HIRSUTISM, OBESITY, MENSTRUAL IRREGULARITY, GLUCOSE ABNORMALITIS, DISLIPIDEMIA ETC.)



Rosenfield RL New Engl. J Med., 2005

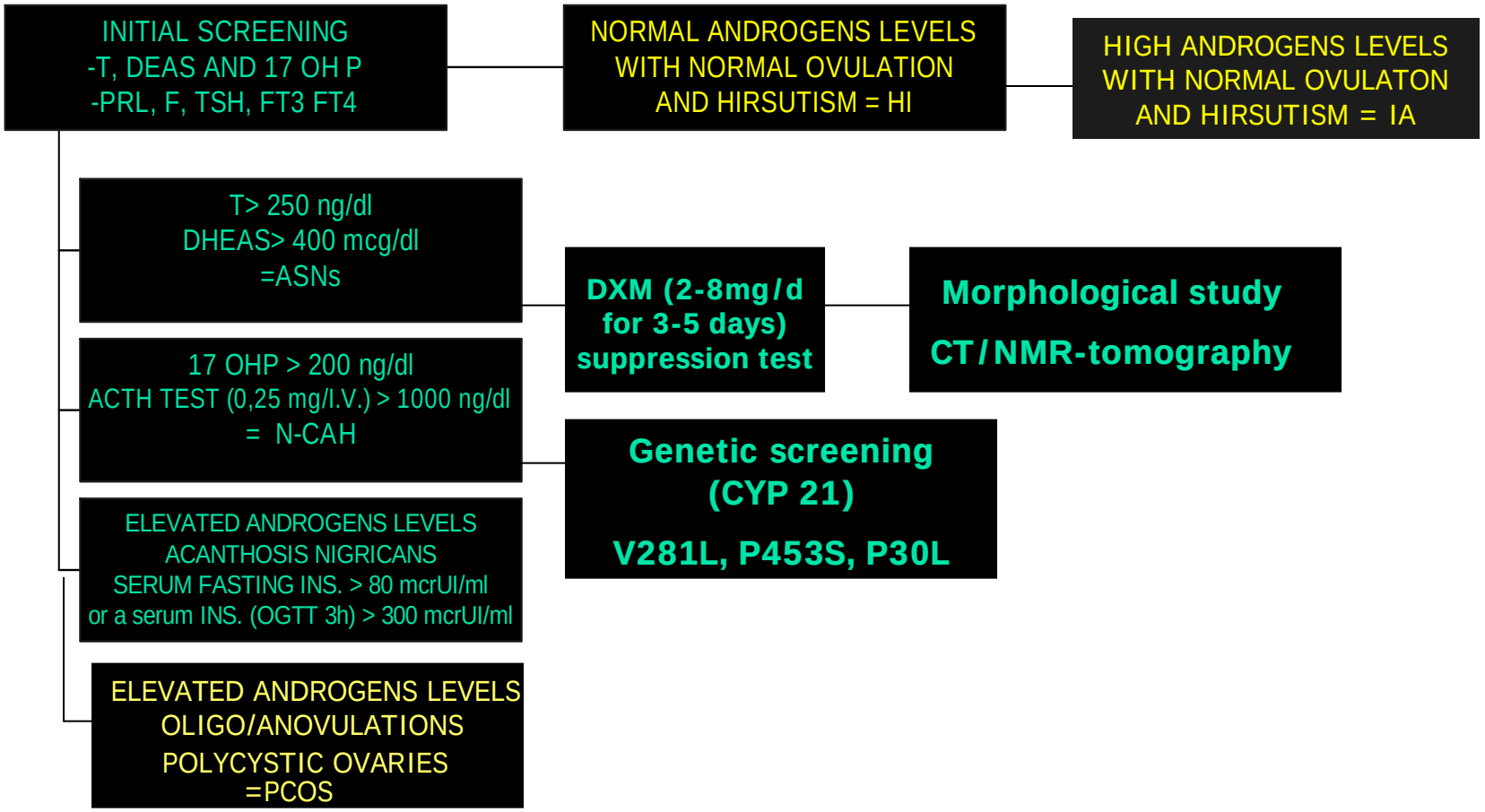
PHYSICAL CHARACTERISTICS AND PLASMA ANDROGEN LEVELS (X ± SD) IN PREMENOPAUSAL WOMEN WITH SIMPLE OBESITY (O) (N.18), IDIOPATHIC HIRSUTISM (IH) (N.16), PCOS (N.25), N-CAH (N.5) AND WITHOUT HIRSUTISM (CG) (N.40)

	CG	O	IH	PCOS	NCAH
BMI	22±2,5	39,5±8,7***	22±3.0	28±3,5**	26±3.0*
Cycle	R	R & O	R	O & A	O
17OHP (ng/ml)	0,9±06	n.d.	1,0±0,9	1,1±06	8,8±2,2***
T (ng/dl)	30,3±9,3	30,6±8,2	40,6±19,2	60,6±25,2**	105,5±89,2***
FT (ng/dl)	0,40±0,11	0,53±0,10*	0,46±0,11	1,15±0,29***	n.d.
SHBG (nM/l)	70±29	58±26**	68±30	52±37**	n.d.
A (ng/dl)	170±100	196±74	201±160	265±136**	560±450***
DHEA (ng/dl)	640±210	589±240	622±257	890±322*	690±310
DHEAS (µg/dl)	130±50	128±71	147±65	186±45**	120±106
ADG (ng/dl)	130±30	127±75	168±20*	221±85 **	350±160***
*= P<005; **=P<001; ***=P<0001 O, IH, PCOS and NCAH vs CG					
R=regular cycle; O= oligomenorrhoea; A= amenorrhoea					

Giagulli VA et al,1991,1992; Vermenlen A. & Giagulli VA. 1991

HOW TO DIAGNOSE HIRSUTISM IN WOMEN

(Rotterdam ESHRE/ASRM consensus, 2003; CARMINA et al. JCEM, 2006)



ANDROGEN EXCESS IN WOMEN

(AACE Hyperandrogenism guidelines,2001; Azziz R. et al JCEM, 2004; Carmina E. et al JCEM, 2006)

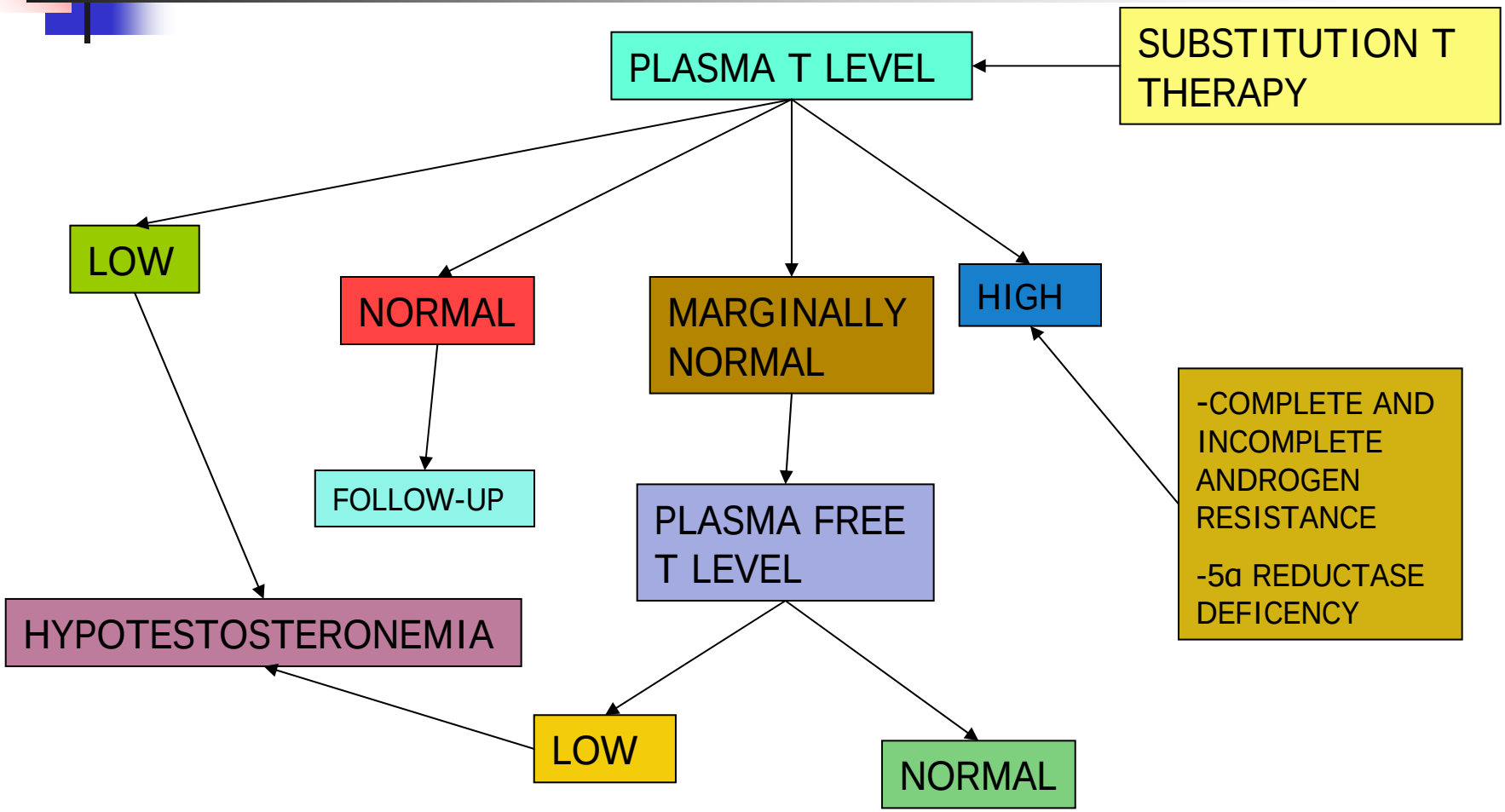
Disorders include

1. **Specific identifiable disorders (< 10%):**
   - *Classical or non classical adrenal hyperplasia (CAH or NCAH)*
   - *Androgen secreting neoplas (ASNs)*
   - *Hyperandrogenic insulin acanthosis nigrigans (HAIRAN)*
2. **Functional androngen excess (>85%):**
   - *Polycystic ovary syndrome (PCOS)*
   - *Idiopathic hirsutism (IH)*
   - *Idiopathic Hyperandrogemia (IA)*

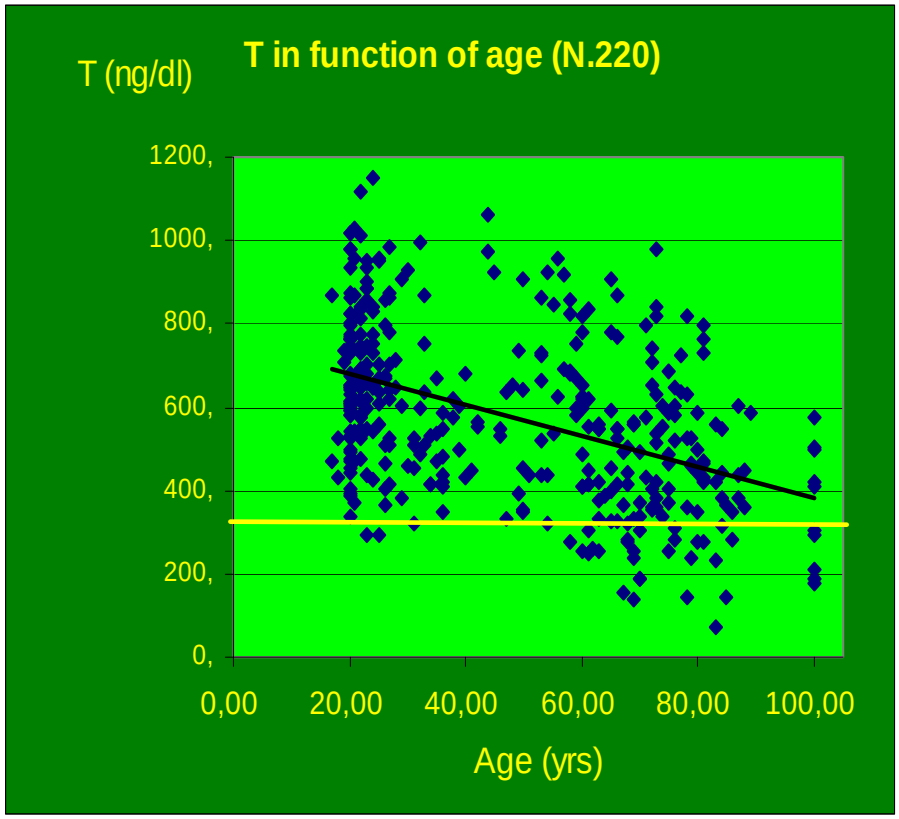
MALE HYPOGONADISM

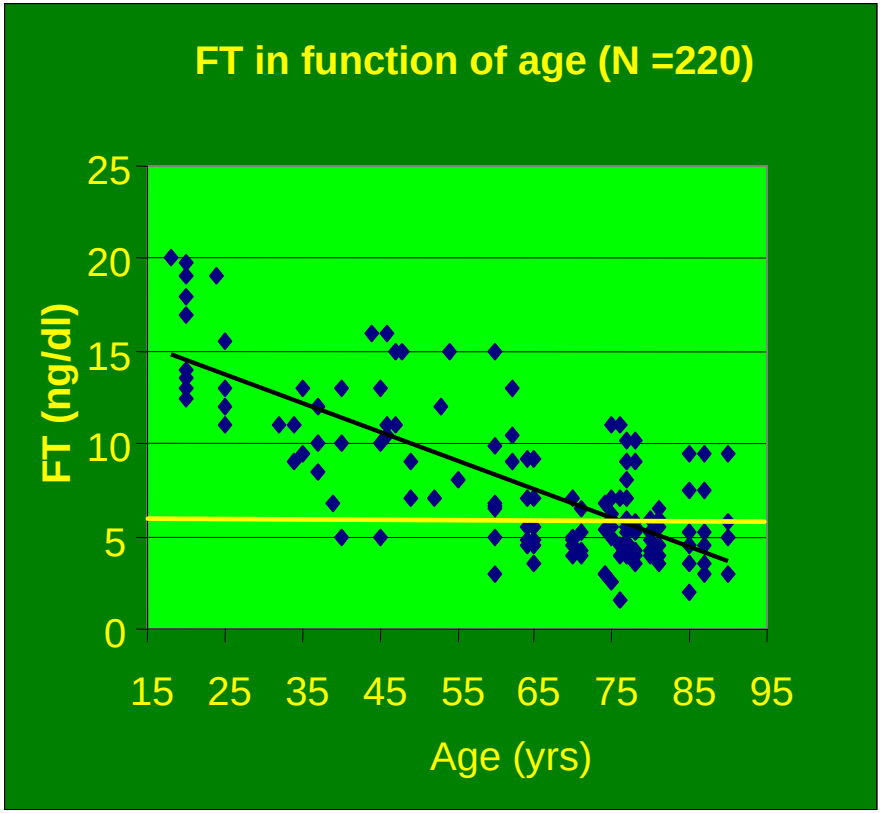
(Small testis and phallus, scant pubic and axillary hair, diminishing body hair, gynecomastia, eunuchoid tall stature, reduce male musculatur, loss libido, impotentia, obesity etc)

(AACE Hypogonadism Guidelines, 2002; BHASIN S. et al JECM, 2006)



(F)T PLASMA LEVELS IN NORMAL MEN IN FUNCTION OF AGE





Veurmeulen A., 1996, Kaufman JM & Vermeulen A Endocr. Rev.,2005

LABORATORY CRITERIA FOR AGE ASSOCIATED HYPOGONADISM

(Kaufman JM & Vermenlen A, 2005; Neischlag E et al , 2005; Bhasin S. et al, 2006)

L.n. tranformed T levels in healthy young men 20-40 years old:

Mean: 673 ng / dl

M - 2.5 S.D.: 319 ng / dl = 11 nMol/l

L.n. transformed FT (dialysis or calculated from T and SHBG capac.)

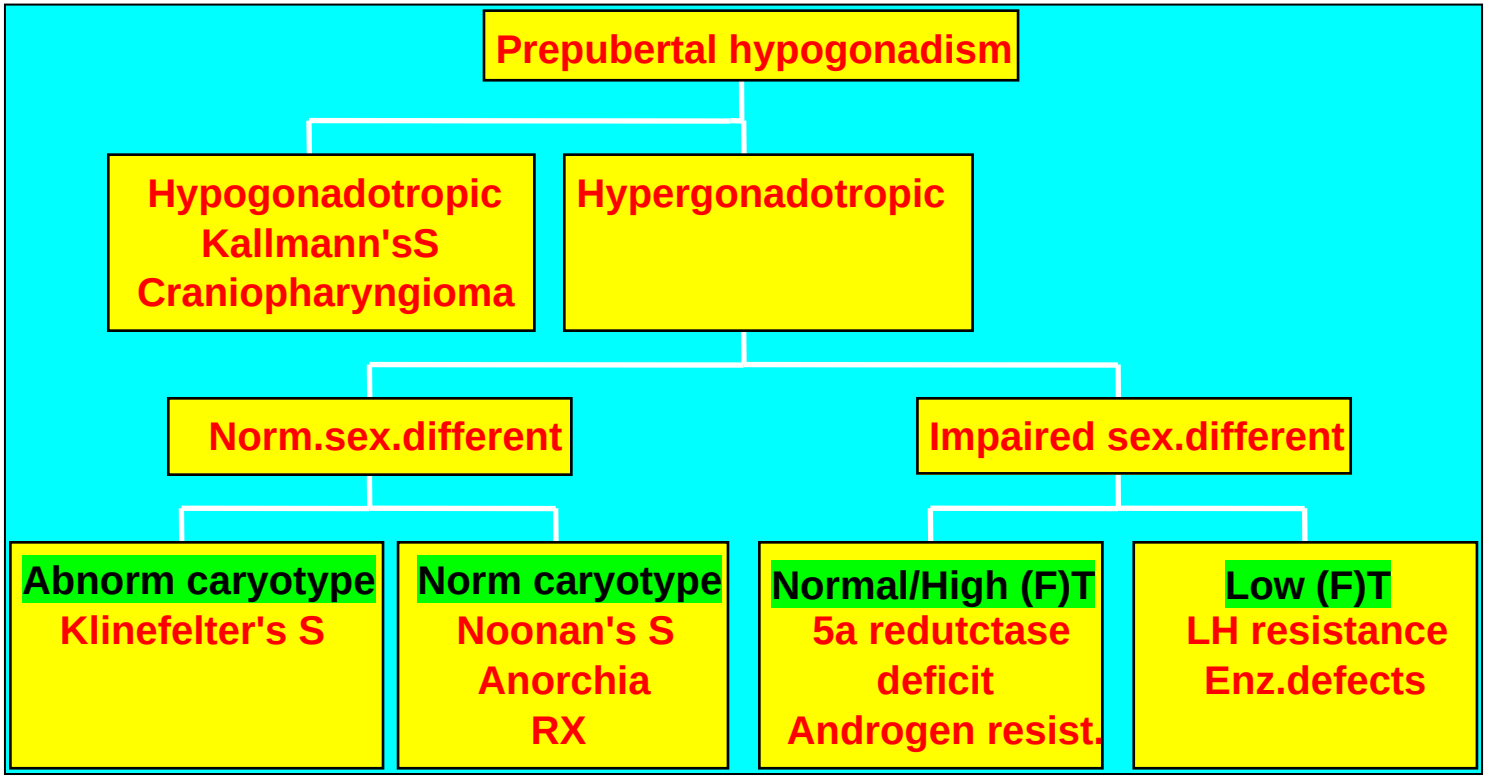
Mean : 14 ng/dl

M - 2.5 S.D.: 6.5 ng/dl= 0.225 nMol/l

P.S. Increased LH levels not required !

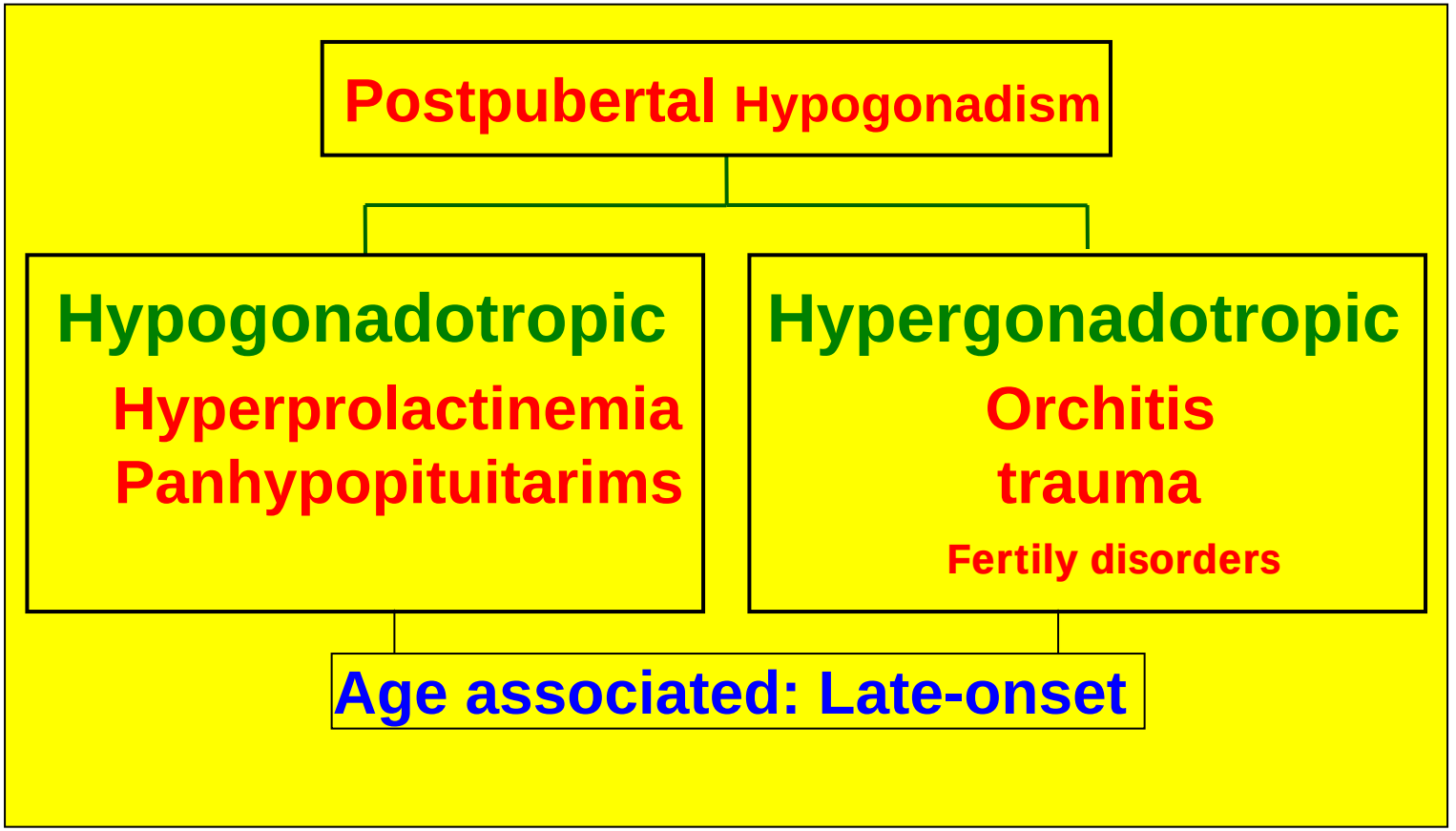
PREPUBERTAL HYPOGONADISM

AACE Hypogonadism Guidelines, 2002



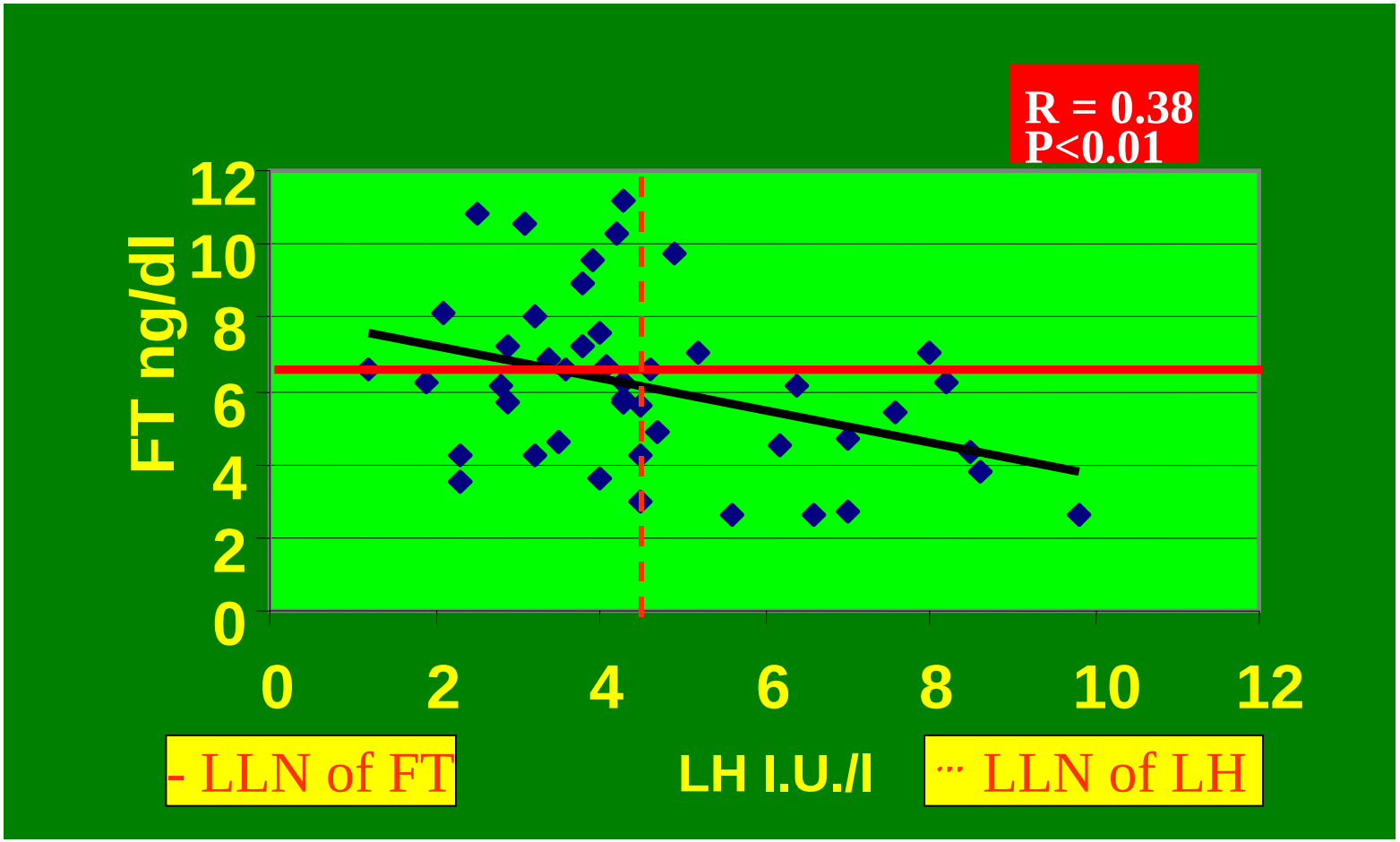
POSTPUBERTAL HYPOGONADISM

AACE Hypogonadism Guidelines, 2002; Neischlag E et al , 2005



FT VERSUS LH IN ELDERLY MEN

(Kaufman JM., & Vermeulen A.,2005)



Total and free testosterone, better than a guess?

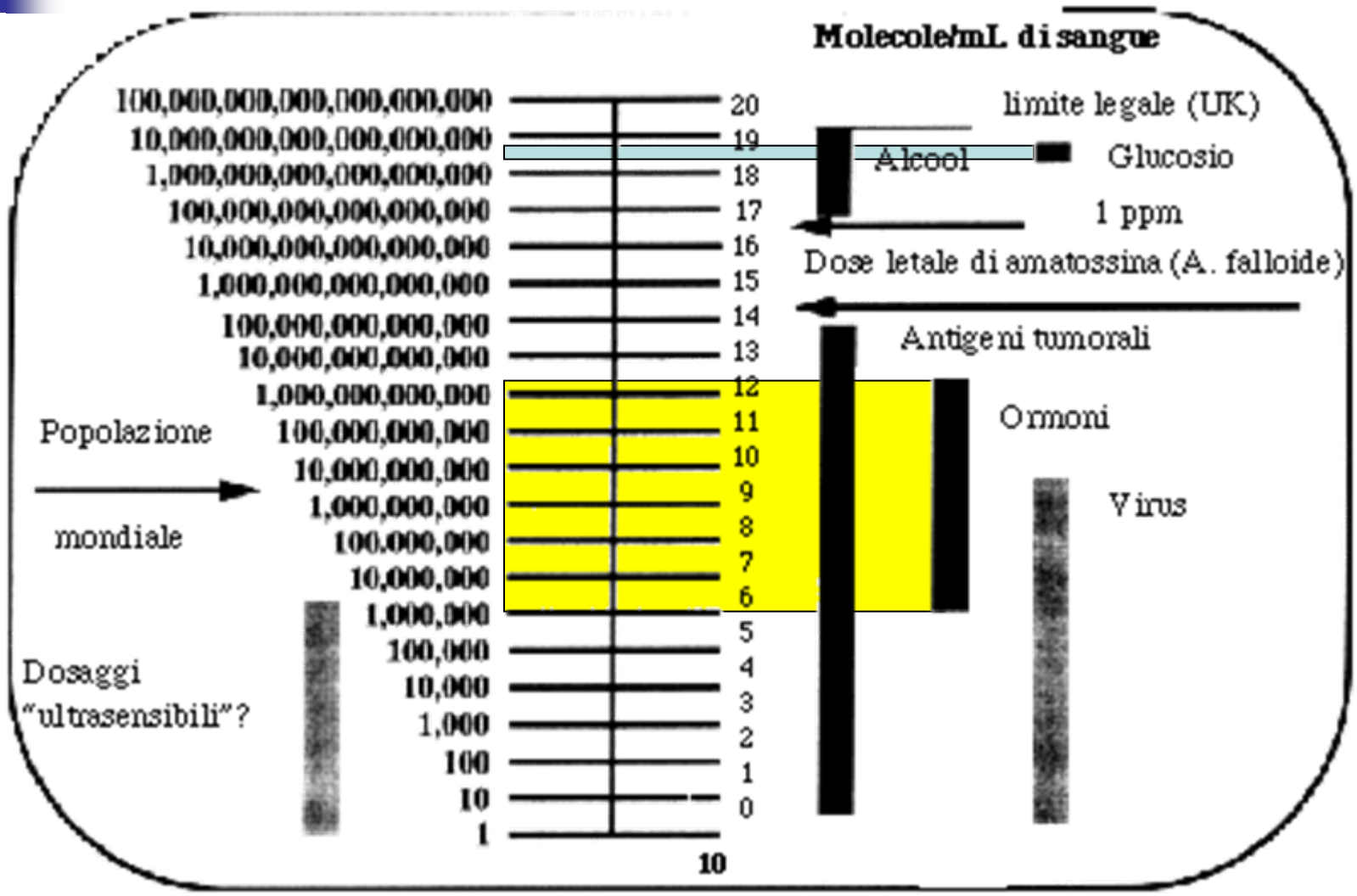
The laboratory point of view

A. Fortunato

Ospedale “San Bortolo” – Vicenza

antonio.fortunato@ulssvicenza.it

Sensitivity



Sensitivity

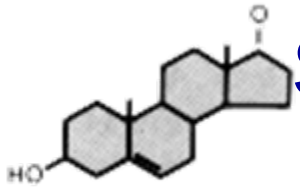
tT4 100 nmol/L

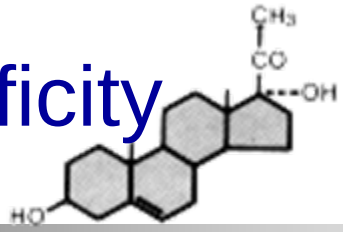
tTST 5 nmol/L

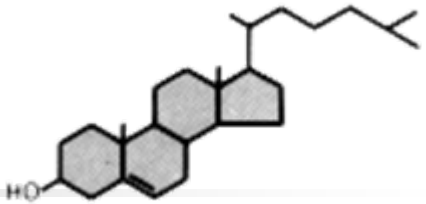
fTST 10 pmol/L

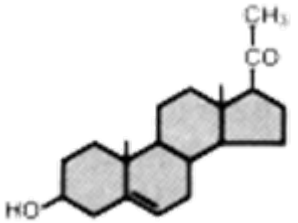
fTST tTST tT_4

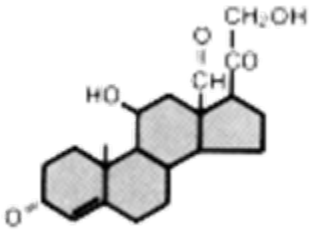
Specificity

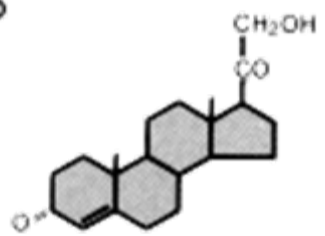

deidroepiandrosterone

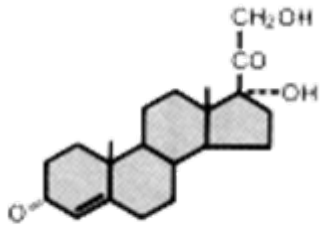

17-idrossipregnenolone

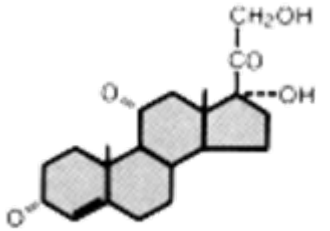

colesterolo

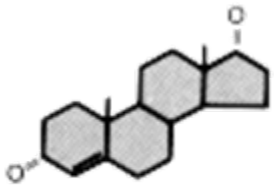

pregnenolone

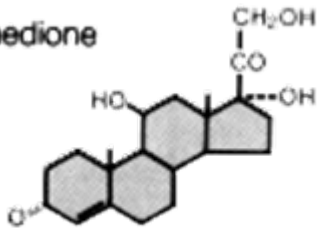

aldosterone

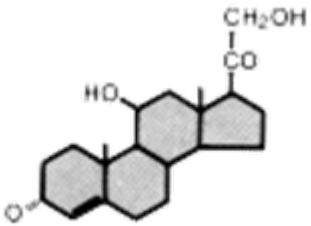

desossicorticosterone

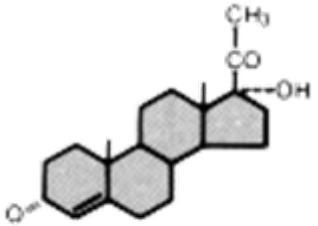

11-desossicortisolo (sostanza 'S')

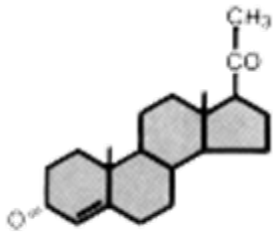

cortisone

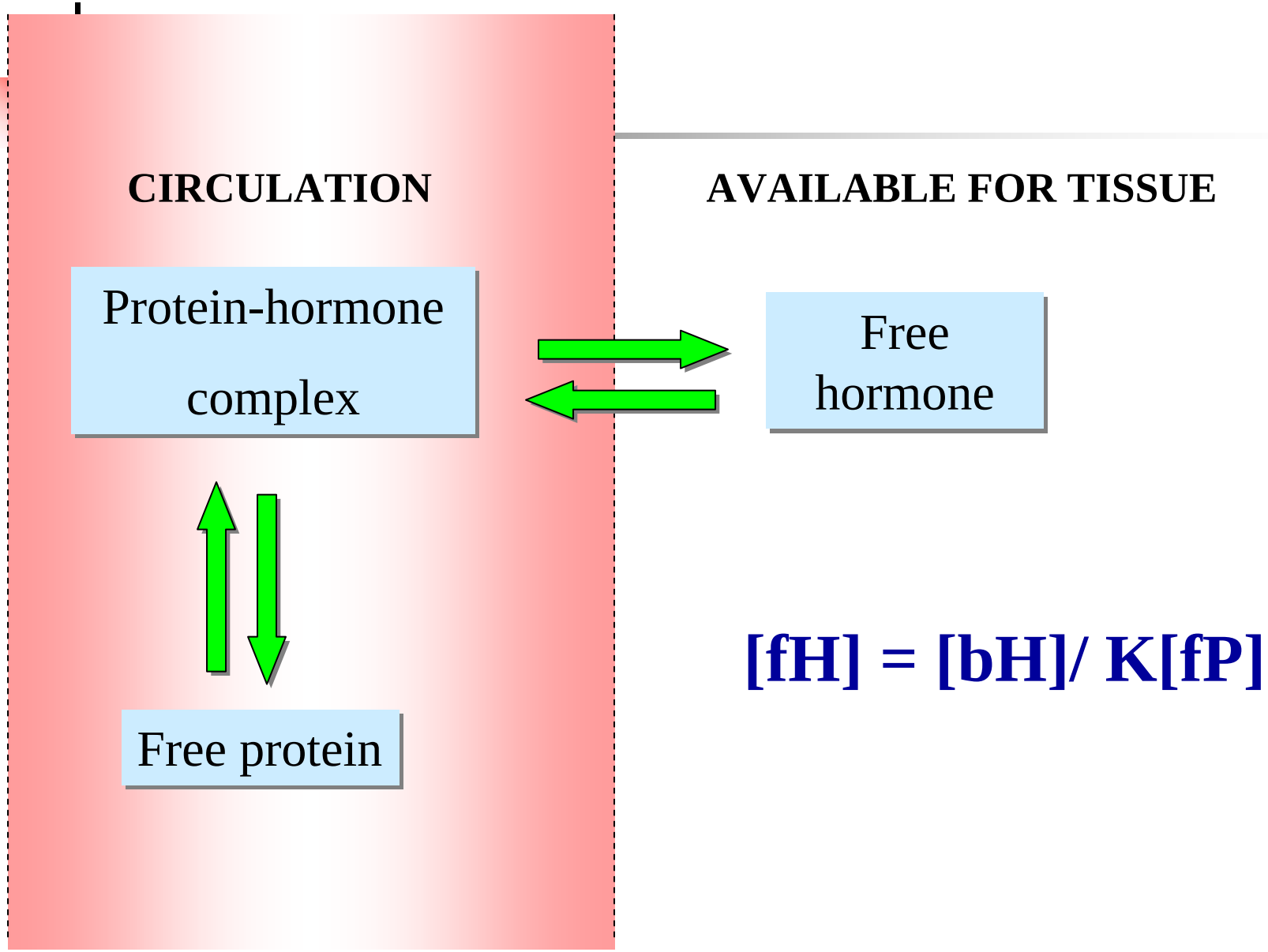

androstenedione


cortisolo


corticosterone


17-idrossiprogesterone


progesterone


CIRCULATION
AVAILABLE FOR TISSUE
Protein-hormone complex
Free hormone
Free protein

$$[fH] = [bH]/ K[fP]$$

Sex Hormone-Binding Globulin

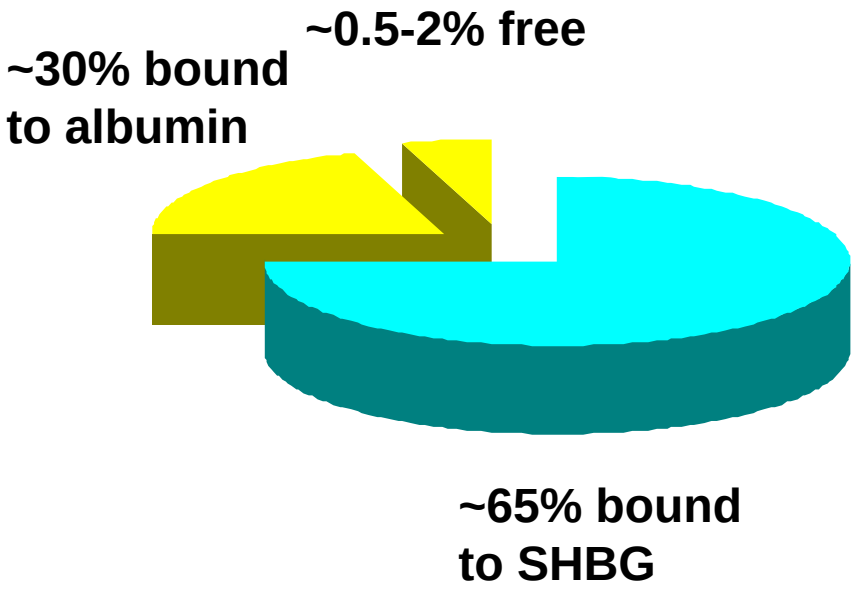
- SHBG is the carrier protein for estrogen and testosterone
  - SHBG-bound fraction is unavailable for biological activity
- Production regulated by estrogen-testosterone balance
  - Estrogen stimulates SHBG production
  - Testosterone decreases SHBG synthesis

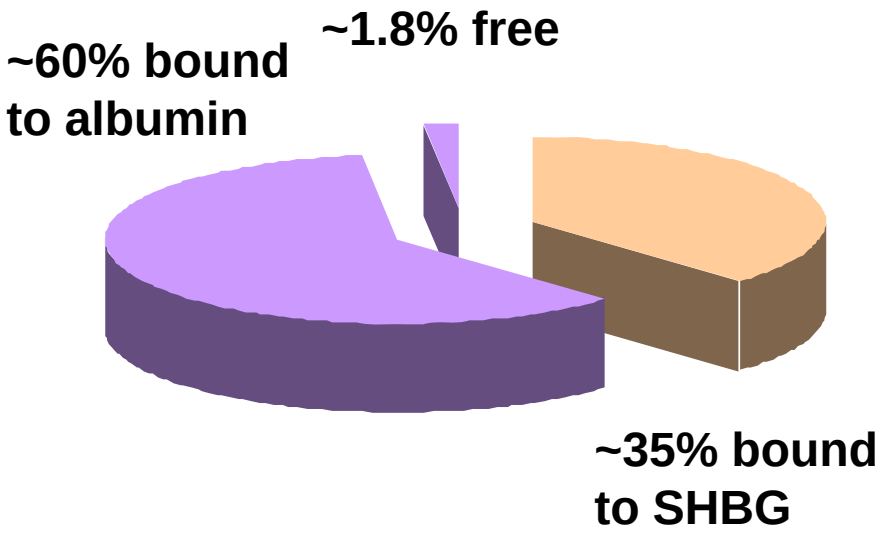
Selby C. *Ann Clin Biochem*. 1990;27:532-541.

Testosterone and Estrogen Circulation in the Body

Bioavailable Testosterone

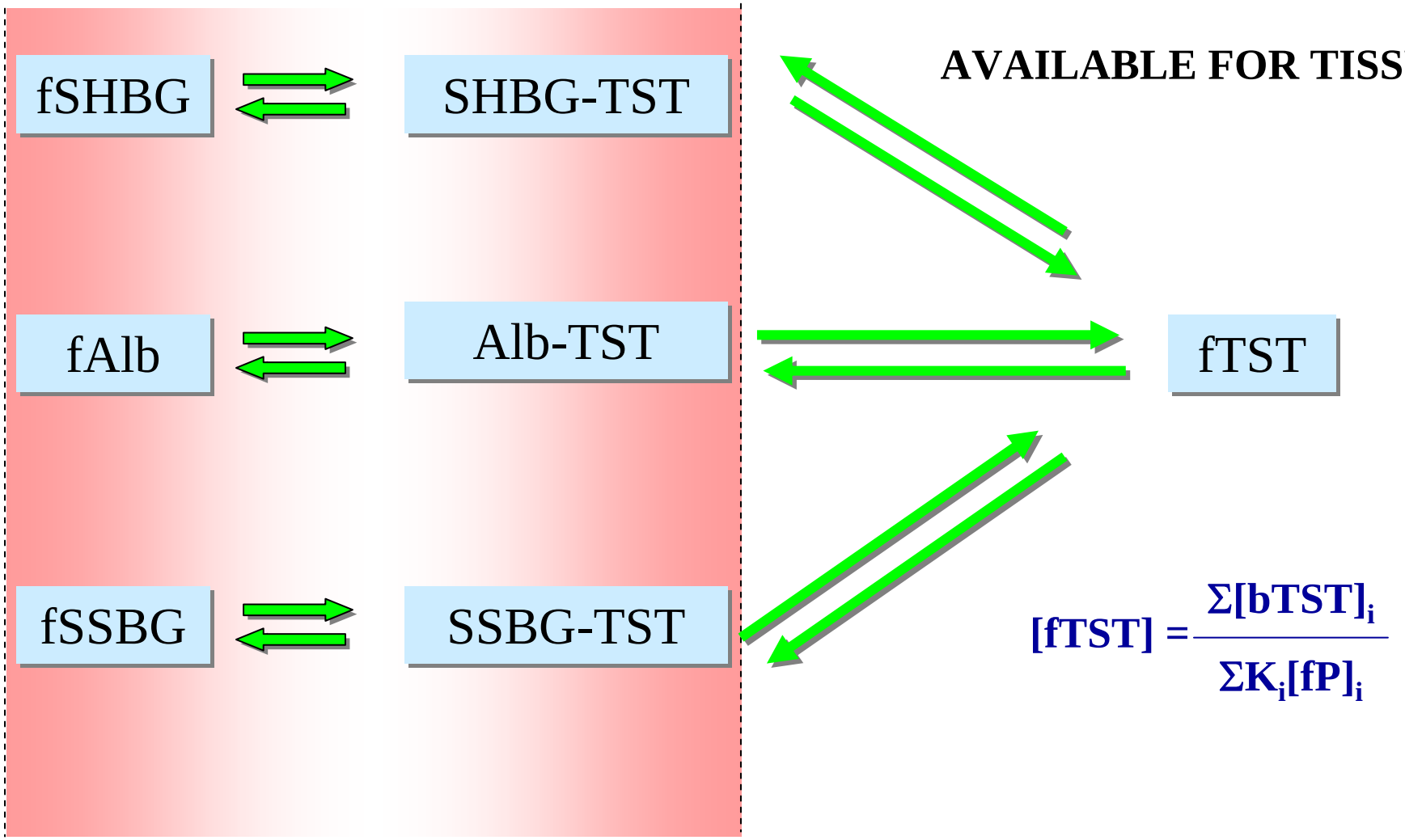
Bioavailable Estrogen





Simon JA. *Fert Steril*. 2002;77:S77-S82.
Demers LM. In: Redmond, G, ed. *Androgenic Disorders*. Raven Press, New York, NY; 1995:21-34.

CIRCULATION



$$[fTST] = \frac{\Sigma[bTST]_i}{\Sigma K_i[fP]_i}$$

Assays for Measuring Testosterone

- Commercial assays for testosterone lack sensitivity and reliability
  - Do not accurately measure low ranges found in women
- Total testosterone immunoassay
  - With or without purification
- Analog tracer free testosterone assay
  - Not labor intensive, reproducible
  - Level of accuracy is controversial
- Salivary measures
  - Highly variable

Guay AT. *Fertil Steril.* 2002(suppl 4);77:S83-S88.

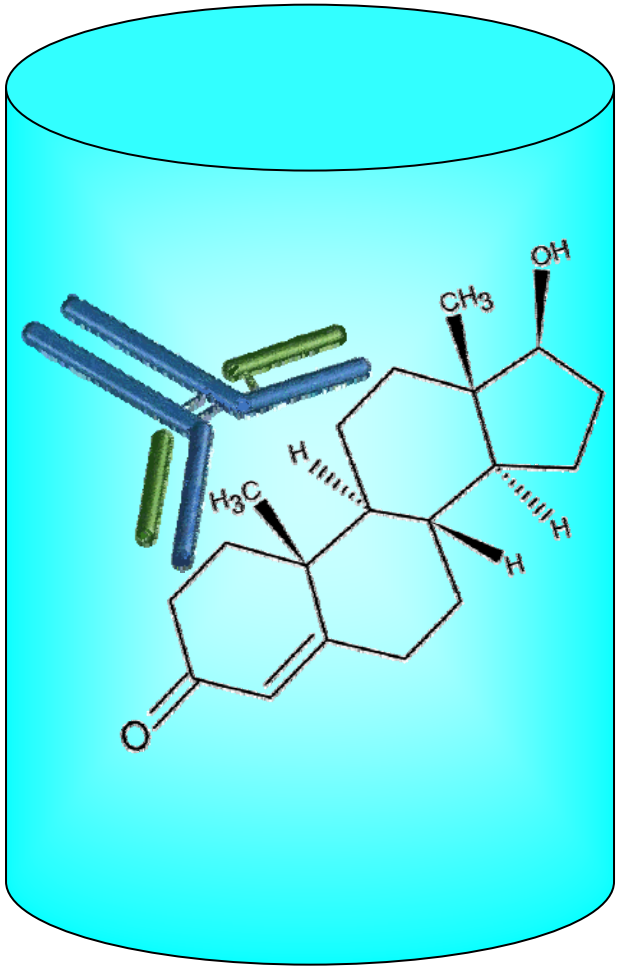
Assays for Measuring Testosterone (cont'd)

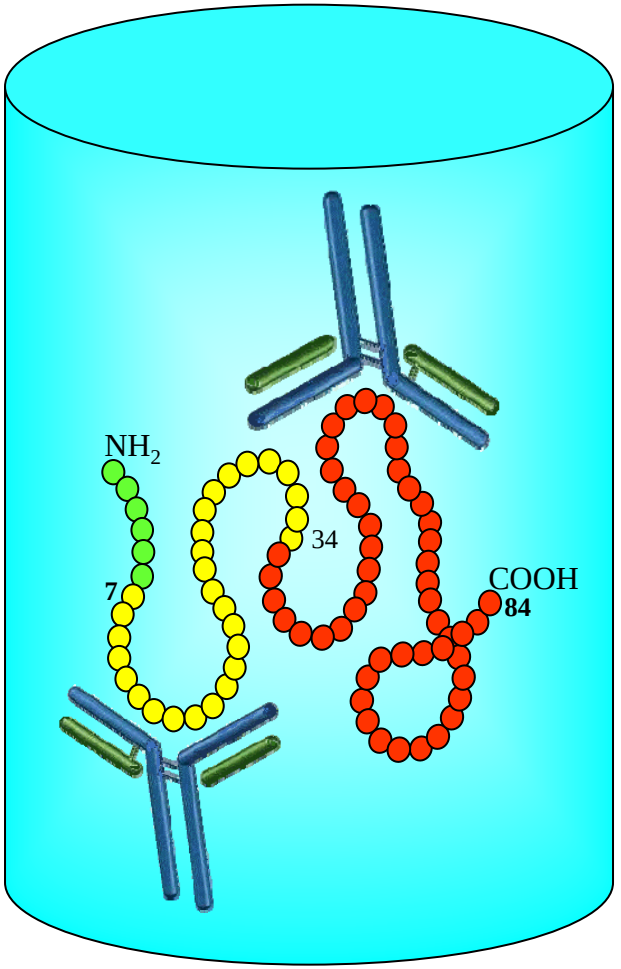
- Equilibrium dialysis or equilibrium ultrafiltration
  - "Gold standard" for measuring free testosterone
  - Difficult, time consuming

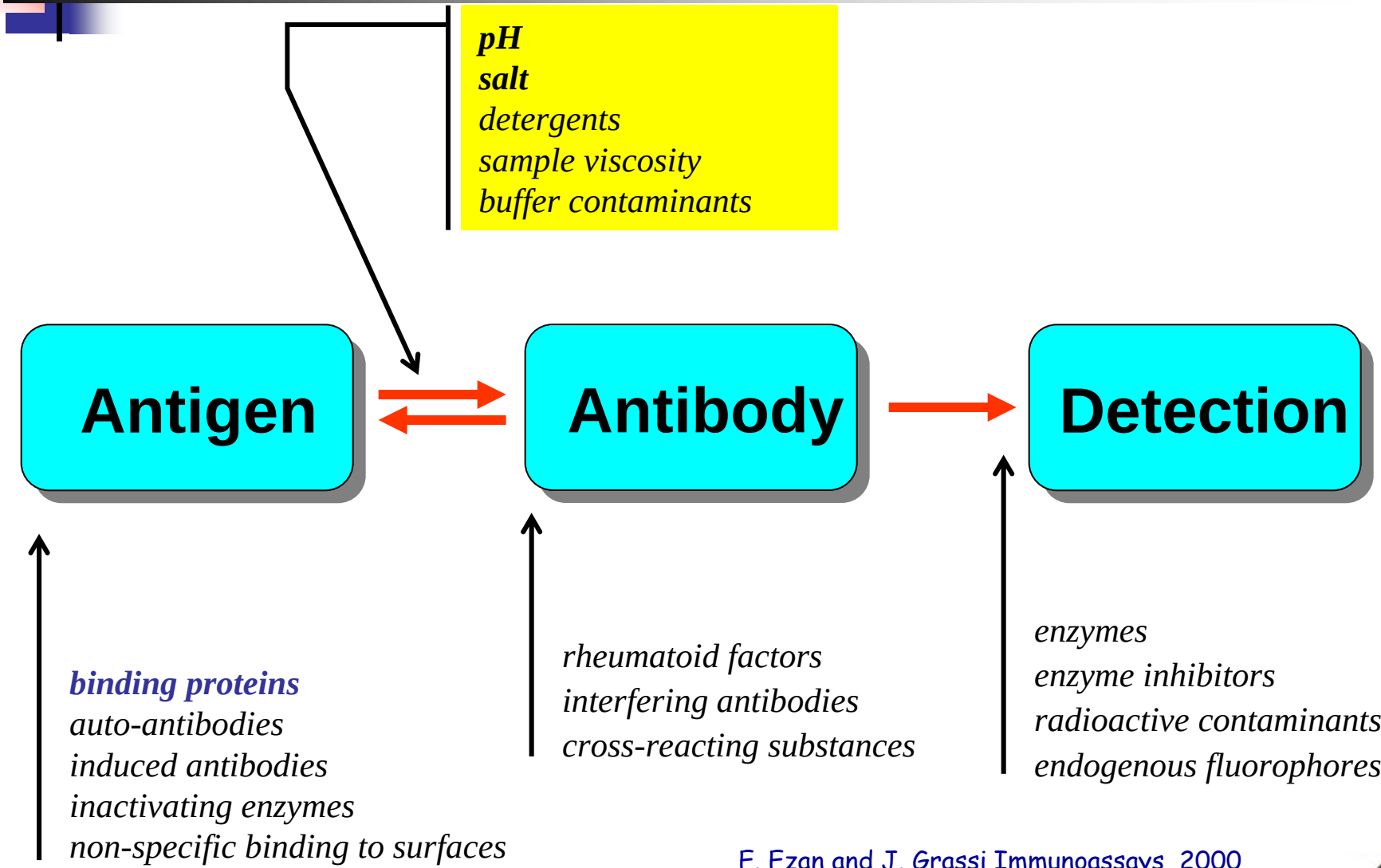
- Gas chromatography-mass spectrometry

Guay AT. *Fertil Steril.* 2002(suppl 4);77:S83-S88.

Ligand Assays

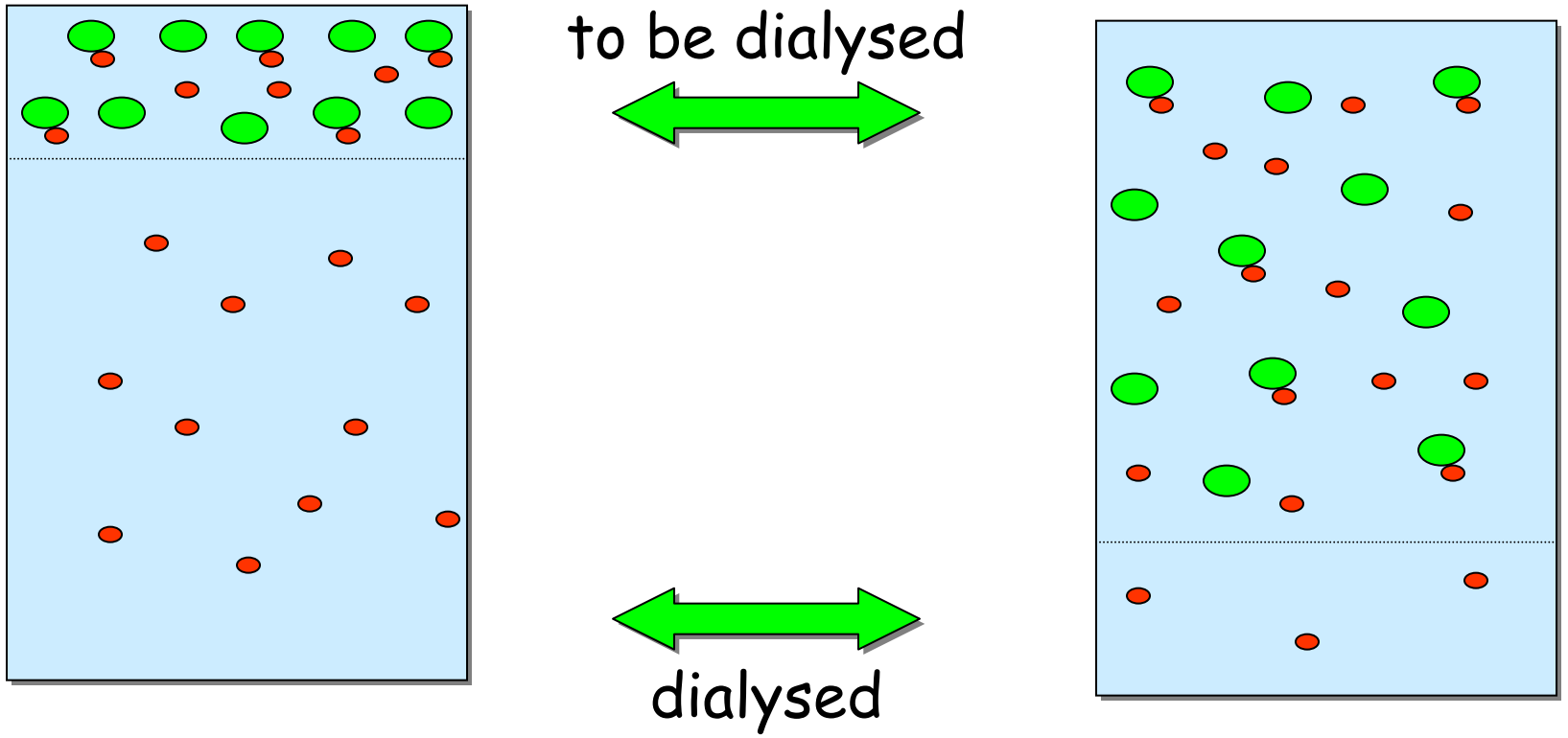




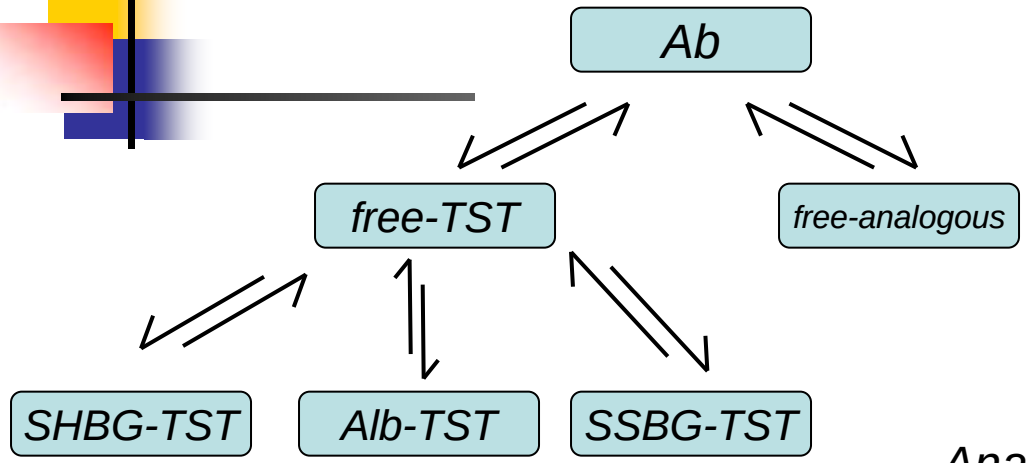


E. Ezan and J. Grassi,Immunoassays, 2000

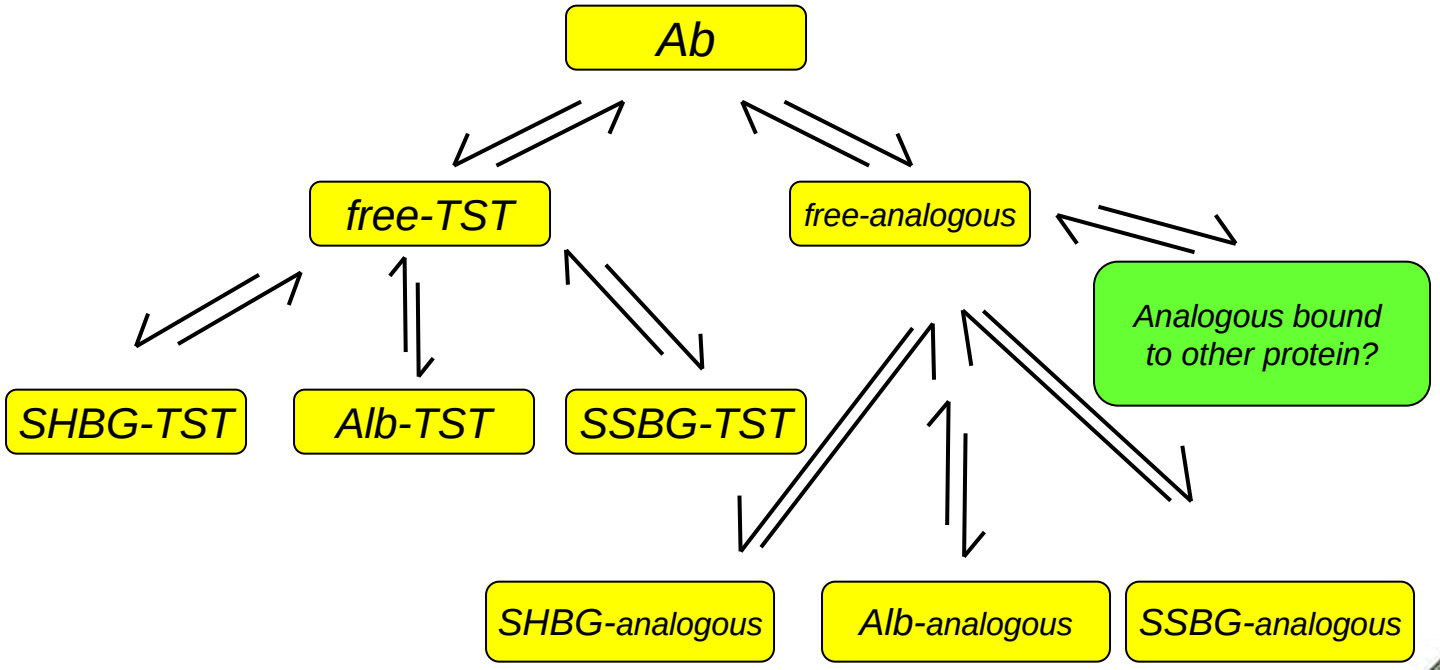
EQUILIBRIUM DIALYSIS



*Analog tracer **not bound** to protein*



*Analog tracer **bound** to protein*

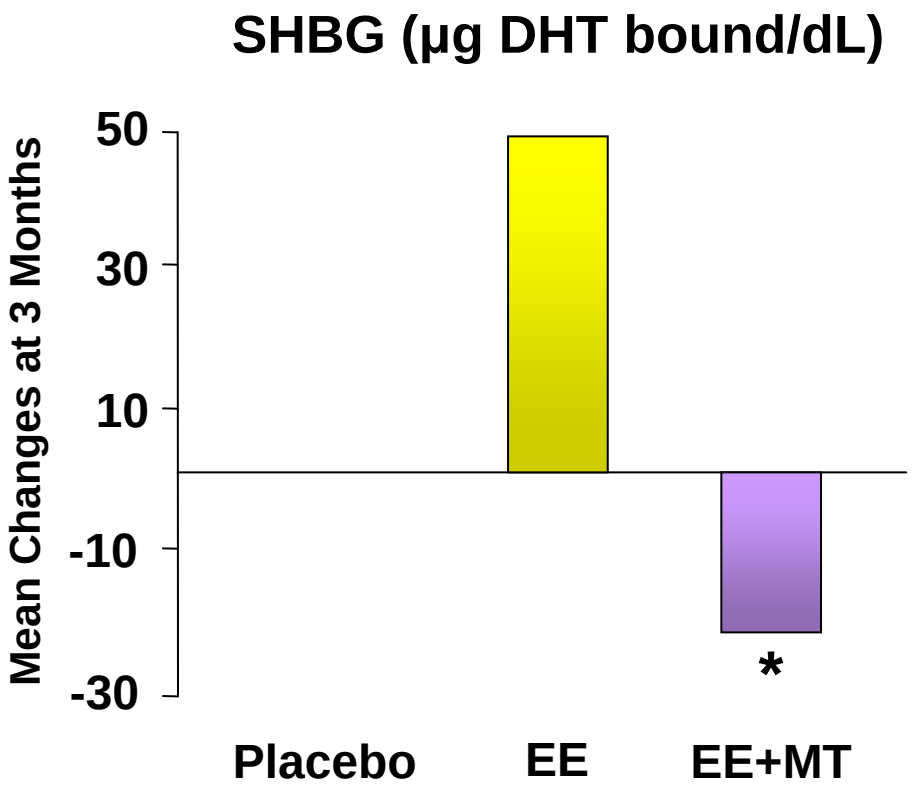


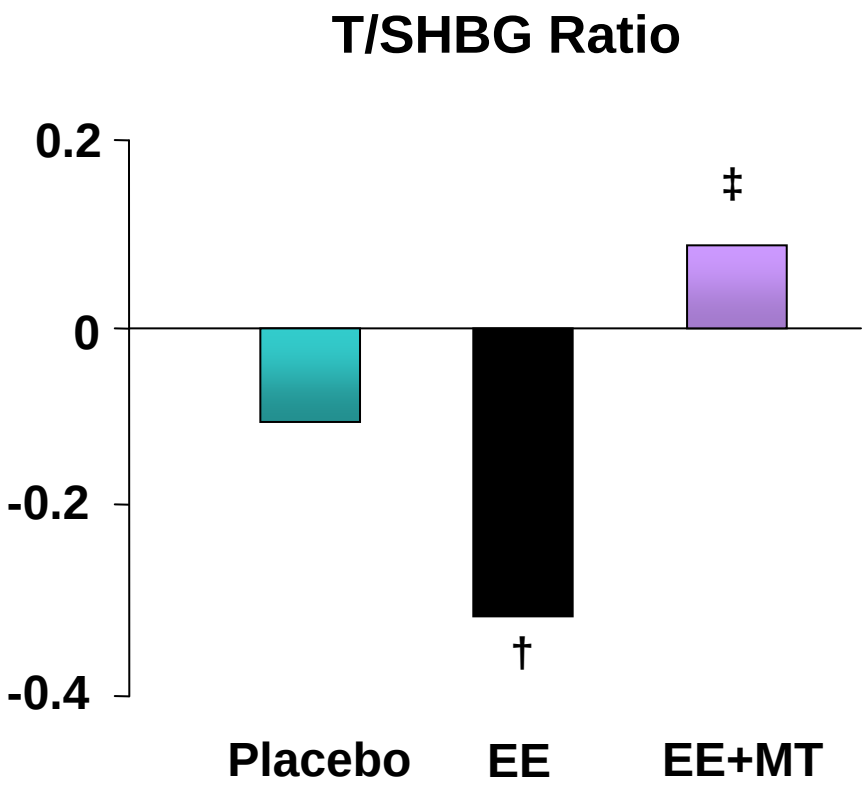
Analog tracer

It works ….

…. when sample is “normal”!

Effect of Estrogen and Estrogen-Androgen Therapy on SHBG and T/SHBG ratio





EE=esterified estrogens; MT=methyltestosterone

*$P \leq 0.01$; †$P \leq 0.001$; ‡$P < 0.05$ vs baseline

Simon JA, et al. *Menopause.* 1999;6:138-146.

0021-972X/04/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 89(2):520–524

doi: 10.1210/jc.2003-032175

February 2004

Editorial: Serum Testosterone Assays—Accuracy Matters

By its very nature, endocrinology is a specialty that relies on sharp and comprehensive clinical and investigative skills and particularly on accurate, precise, sensitive, and reliable measurements of circulating hormone concentrations. With the realization that manifestations of endocrine disease may be subtle and affected by comorbid illness, medications, aging, and other factors that cloud clinical diagnosis, rugged and robust hormone assays are especially important to the clinician. Even in the presence of obvious classical manifestations of endocrine disease, reliable laboratory studies are needed to confirm the diagnosis.

Serum testosterone (T) assays play an important role in the clinical evaluation of a number of very common endocrine disorders. In males, T assays are used primarily to confirm the diagnosis of hypogonadism, and also to evaluate boys with delayed or precocious puberty and monitor the adequacy of T therapy. Because the clinical manifestations of androgen deficiency are nonspecific, the presence of low

In this issue of *JCEM*, the papers by Wang *et al.* (2) and Miller *et al.* (3) both carefully evaluate the accuracy and reliability of assays for serum total T in men and free T in women, respectively.Wecomplement the authors for their well-conceived and well-designed studies examining the performance and validity of T assays. These reports serve as excellent models for the rigorous assessment of the accuracy of T assays, so important in clinical medicine and research.

(both measurement and statistical), but the results are very important for both clinical and research audiences.

0021-972X/97/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 82, No. 6
Printed in U.S.A.

June 1997

LETTERS TO THE EDITOR

Errors in the Measurement of Plasma Free Testosterone[a]

To the editor:

In their investigations on the effect of weight loss on serum androgens in obese women with and without polycystic ovary syndrome (PCOS), Jakubowicz and Nestler (**1**) measured the concentrations in plasma of testosterone, free testosterone, and sex hormone-binding globulin (SHBG). Among their observations was that, although testosterone and free testosterone fell significantly after weight loss in patients with PCOS, this was not true in the weight-

I believe that some of the conclusions need to be changed because the values for plasma free testosterone appear to be incorrect.

on internal inconsistencies in the data, disagreements with calculated values for free testosterone, and substantial discrepancies from the percentage of free testosterone in the literature obtained using a variety of other methods.

Lab Tests Online®

A public resource on clinical lab testing from the laboratory professionals who do the testing

Peer-reviewed
Non-commercial
Patient-centered

Use the search box and menus below to quickly navigate the Lab Tests Online site:

What are free and bioavailable testosterone? Testosterone is present in the blood as "free" testosterone (2-3%) or bound testosterone. The latter may be bound to either albumin (a serum protein) or to a specific binding protein called Sex Steroid Binding Globulin (SSBG) or **Sex Hormone Binding Globulin (SHBG)**. The binding of testosterone to albumin is not very tight and is easily reversed; so the term bioavailable testosterone (BAT) refers to the sum of free testosterone plus albumin-bound testosterone. Alternatively, it is the fraction of circulating testosterone that is not bound to SSBG. It is suggested that BAT represents the fraction of circulating testosterone that readily enters cells and better reflects the bioactivity of testosterone than does the simple measurement of serum total testosterone. Also, varying levels of SSBG can result in inaccurate measurements of BAT. Decreased SSBG levels can be seen in obesity, hypothyroidism, androgen use, and nephritic syndrome. Increased levels are seen in cirrhosis, hyperthyroidism, and estrogen use. In these situations, measurement of free testosterone may be more useful. However, technically, free testosterone is difficult to measure accurately.

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Non-commercial
Patient-centered

Use the search box and menus below to quickly navigate the Lab Tests Online site:

SHBG

Also known as: Testosterone-estrogen Binding Globulin (TeBG)
Formal name: Sex Hormone Binding Globulin
Related tests: **Testosterone**, Free Testosterone, Bioavailable Testosterone

Why get tested?

To help evaluate whether the concentration of SHBG is affecting the amount of testosterone available to the body's tissues

When to get tested?

If your **total testosterone** results seem inconsistent with clinical signs, suggesting a testosterone deficiency or excess production

Sample required?

A blood sample drawn from a vein in your arm

0021-972X/99/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 84, No. 10
Printed in U.S.A.

A Critical Evaluation of Simple Methods for the Estimation of Free Testosterone in Serum

ALEX VERMEULEN, LIEVE VERDONCK, AND JEAN M. KAUFMAN

Laboratory for Hormonology and Department of Endocrinology, University Hospital Ghent, 9000 Ghent, Belgium

1999

ELSEVIER

CLINICAL BIOCHEMISTRY

Clinical Biochemistry 36 (2003) 591–596

2003

Evaluation of an algorithm for calculation of serum "Bioavailable" Testosterone (BAT)

Pasha Emadi-Konjin[a,b], Jerald Bain[c], Irvin L. Bromberg[a,*]

[a]*Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Avenue, Toronto M5G 1X5, Canada*
[b]*Department of Laboratory Medicine, The Hospital For Sick Children, 555 University Avenue, Toronto, M5G 1X8, Canada*
[c]*Division of Endocrinology and Metabolism, Department of Medicine, Mount Sinai Hospital, 600 University Avenue, Toronto M5G 1X5, Canada*

Received 23 December 2002; accepted 9 May 2003

European Journal of Endocrinology (2005) 152 471–478 ISSN 0804-4643

EXPERIMENTAL STUDY

Empirical estimation of free testosterone from testosterone and sex hormone-binding globulin immunoassays

Lam P Ly and David J Handelsman

Department of Andrology, Concord Hospital and ANZAC Research Institute, University of Sydney, Sydney NSW 2139, Australia

(Correspondence should be addressed to D J Handelsman; Email: djh@anzac.edu.au)

2005

Clinical Chemistry 52:3
474–481 (2006)

Endocrinology and Metabolism

Serum Bioavailable Testosterone: Assayed or Calculated?

Frank Giton,[1] Jean Fiet,[1,2*] Jérôme Guéchot,[3] Fidaa Ibrahim,[4] Françoise Bronsard,[5] Dominique Chopin,[1] and Jean-Pierre Raynaud[6]

Clinical Chemistry 52:9
1777–1784 (2006)

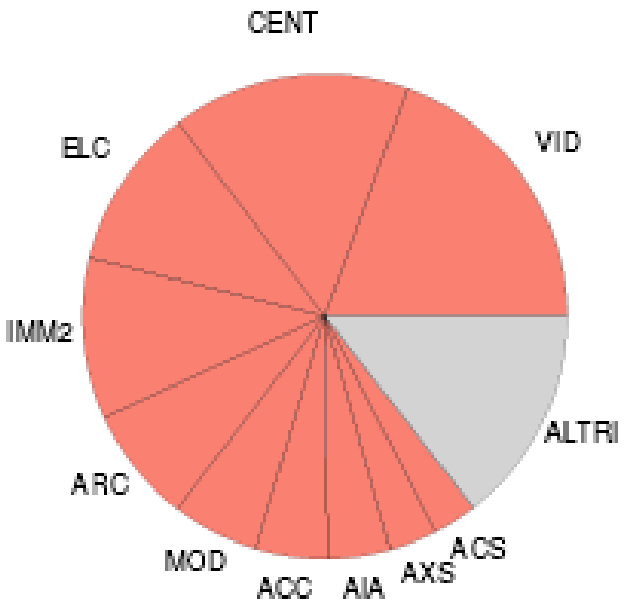
Endocrinology and Metabolism

Calculation of Bioavailable and Free Testosterone in Men: A Comparison of 5 Published Algorithms

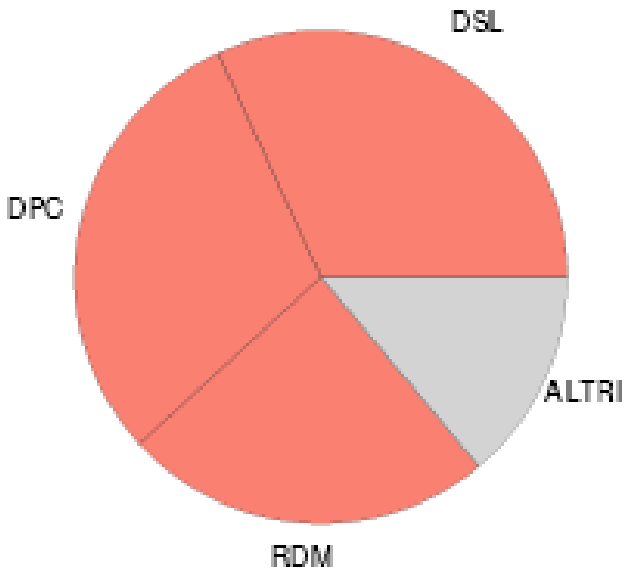
Willem de Ronde,[1,3*] Yvonne T. van der Schouw,[2] Huibert A.P. Pols,[3,4] Louis J.G. Gooren,[1] Majon Muller,[2] Diederick E. Grobbee,[2] and Frank H. de Jong[3]

METHODS

Total TESTOSTERONE

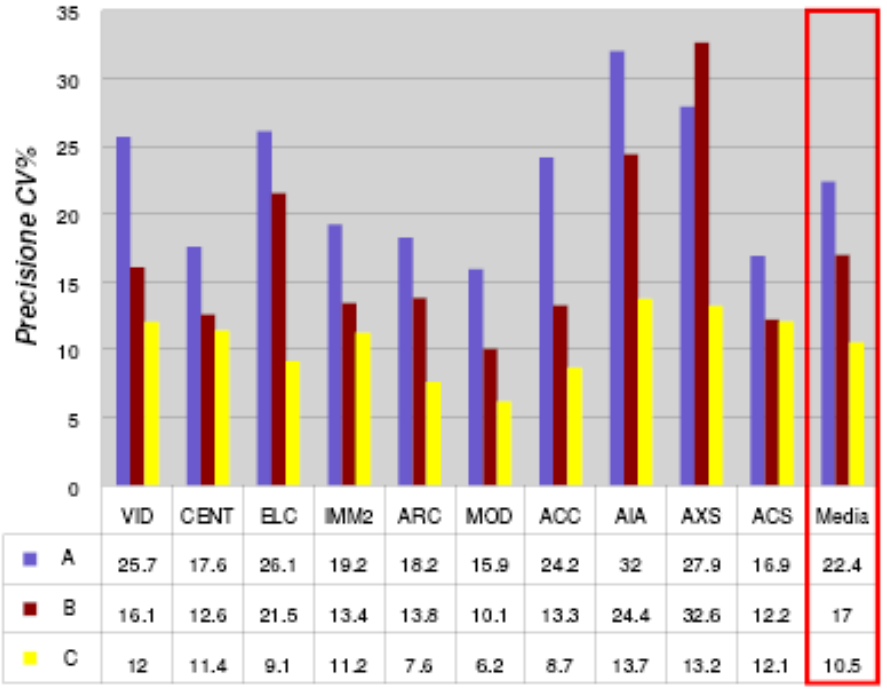


Free TESTOSTERONE

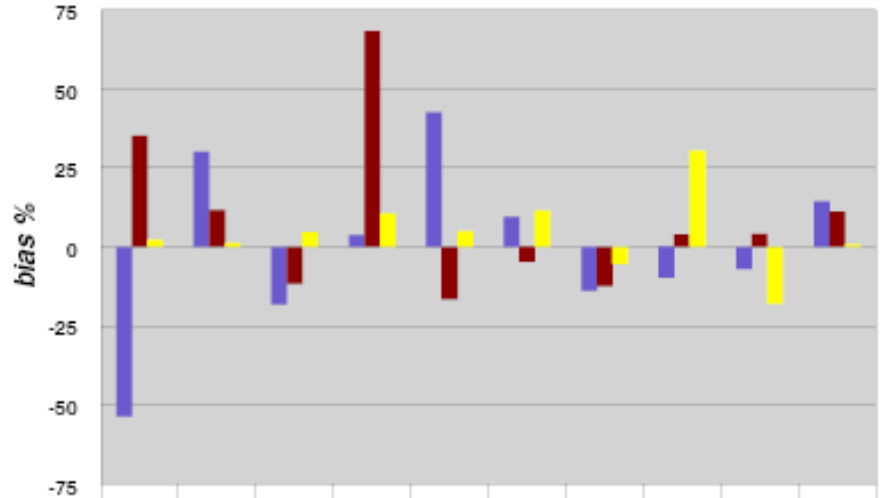


Total TESTOSTERONE

		CONS 6593 (100%) ng/mL	CV%
A	IM53b (P067)	0.46	41.4
	IM52b (P067)	0.48	39.7
	IM51a (P067)	0.49	41.4
B	IM51b (P071)	0.98	31.0
	IM54c (P071)	1.01	27.7
C	IM52c (P065)	2.29	30.9
	IM51c (P062)	2.34	25.7
	IM55b (P070)	2.35	20.7
	IM54a (P070)	2.37	21.5
	IM53c (P072)	2.75	18.6
	IM55c (P060)	3.1	13.1
	IM52a (P068)	3.25	16.4
	IM54b (P068)	3.34	15.2
	IM55a (P073)	5.17	13.9
	IM56c (P073)	5.29	14.7
	IM53a (P069)	8.49	14.3
	IM56a (P061)	8.81	11.7
	IM56b (P069)	8.9	11.9



	VID	CENT	ELC	IMM2	ARC	MOD	ACC	AIA	AXS	ACS	Media
A	25.7	17.6	26.1	19.2	18.2	15.9	24.2	32	27.9	16.9	22.4
B	16.1	12.6	21.5	13.4	13.8	10.1	13.3	24.4	32.6	12.2	17
C	12	11.4	9.1	11.2	7.6	6.2	8.7	13.7	13.2	12.1	10.5

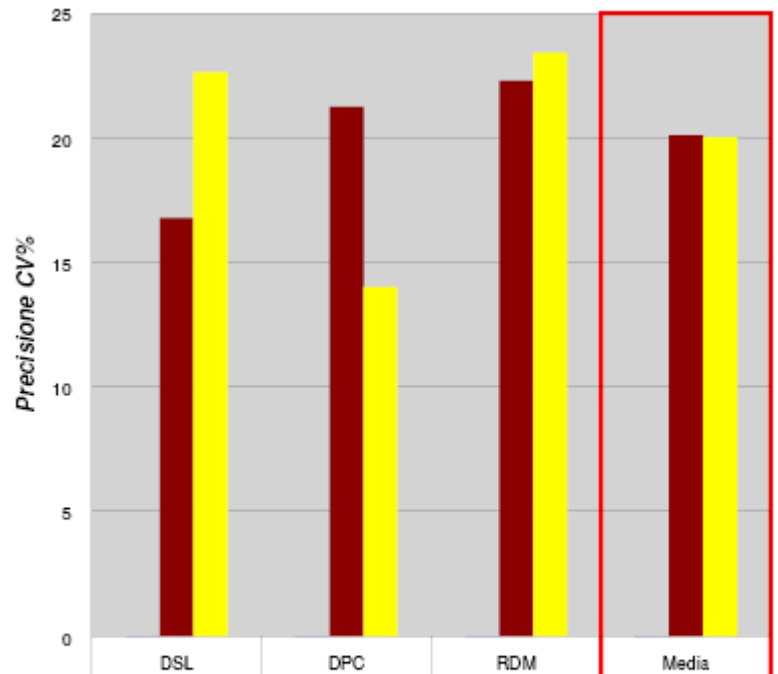


	VID	CENT	ELC	IMM2	ARC	MOD	ACC	AIA	AXS	ACS
A	-53.6	30.2	-18.2	3.6	42.6	9.5	-13.8	-9.7	-6.9	14.3
B	35.2	11.7	-11.7	68.2	-16.5	-4.7	-12.4	3.9	4	11.1
C	2.3	1.1	4.6	10.6	5	11.5	-5.3	30.4	-18.1	0.9

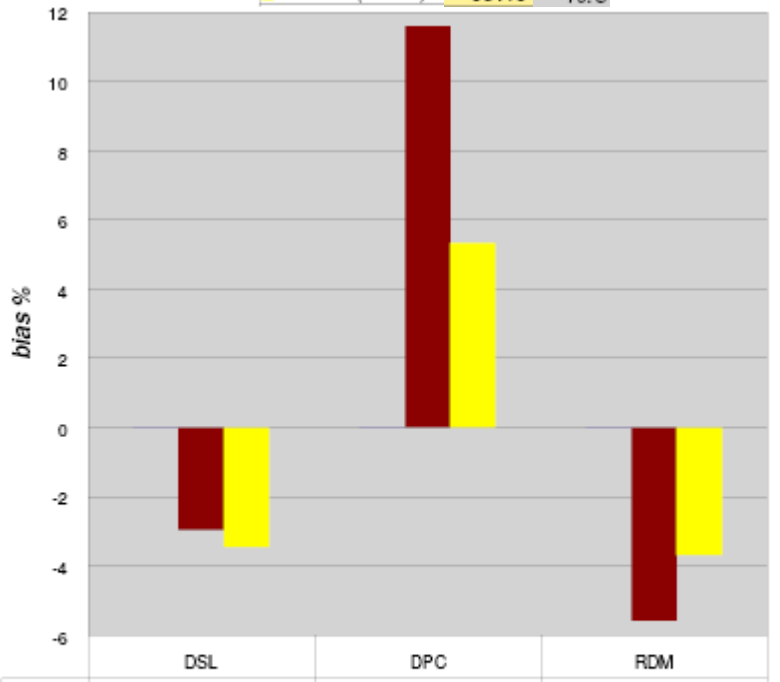
Free TESTOSTERONE

CONS
474 (100%)

		pg/mL	pg/mL
B	IH54a (H005)	2.32	36.2
	IH51a (H005)	2.55	24.1
	IH52b (H001)	2.63	22.9
	IH55b (H005)	2.99	23.0
C	IH56a (H004)	8.96	15.2
	IH53b (H006)	14.54	23.7
	IH52a (H006)	15.64	15.0
	IH56b (H006)	17.93	14.0
	IH54b (H007)	51.44	21.3
	IH53a (H007)	58.8	23.1
	IH51b (H007)	63.08	18.4
	IH55a (H003)	63.46	40.5



	DSL	DPC	RDM	Media
A	-	-	-	-
B	16.8	21.3	22.3	20.1
C	22.6	14	23.4	20



	DSL	DPC	RDM
A	-	-	-
B	-3	11.6	-5.6
C	-3.5	5.3	-3.7

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Original Evaluation: 6/6/2006

EVALUATION
ORIGINAL

Y-A 2006 Ligand-Special

Test / Unit of Measure / Peer Group	Specimen	Your Result	Mean	S.D.	No. of Labs	S.D.I	Limits of Acceptability Lower	Limits of Acceptability Upper	Your Grade	Plot of the Relative Distance of Your Results from Target as Percentages of allowed Deviation
Testosterone	**Y-01**	780	769.2	77.2	354	+0.1	537	1001	Acceptable	
ng/dL	**Y-02**	20	30.3	8.9	356	-1.2	3	57	Acceptable	
BAYER ADVIA CENTAUR										
SHBG nmol/L	**Y-01**	32.90	35.096	1.986	85	-1.1	29.13	41.06	Acceptable	
nmol/L	**Y-02**	<2.00			3				[28]	
DPC IMMULITE 2000										
Testosterone, free	**Y-01**	31.8	28.680	4.426	15	+0.7	15.40	42.00	Acceptable	
	Y-02	1.2	1.281	0.214	16	-0.4	0.60	2.00	Acceptable	
DIAGNOSTIC SYS SOLID										
pg/mL										

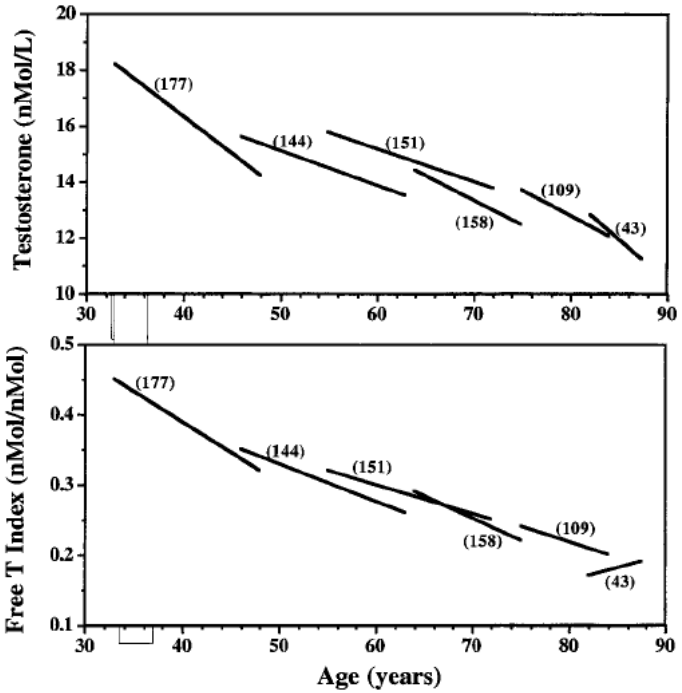
0021-972X/01/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

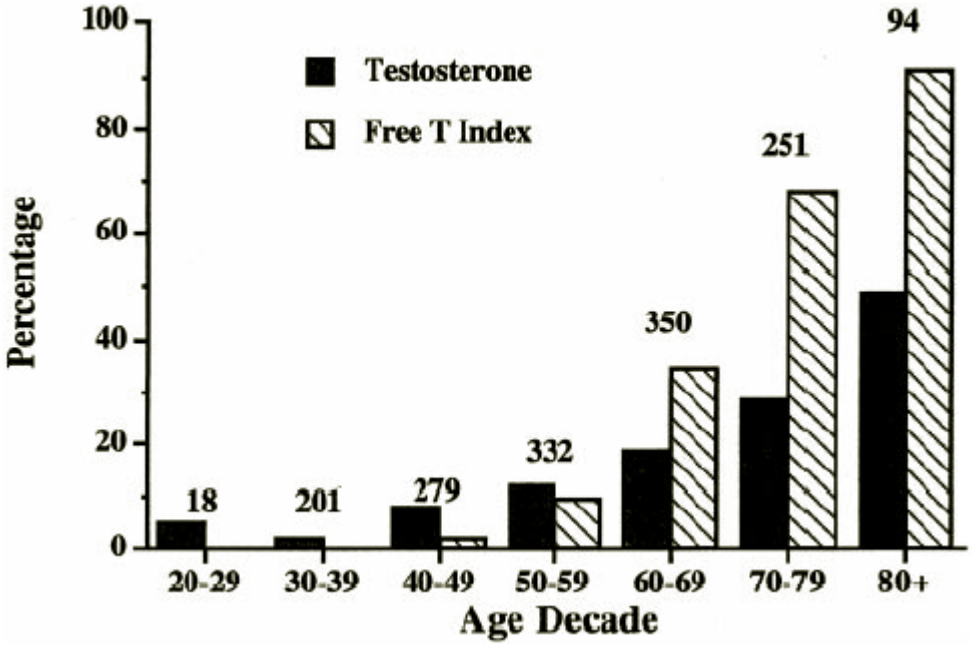
Vol. 86, No. 2
Printed in U.S.A.

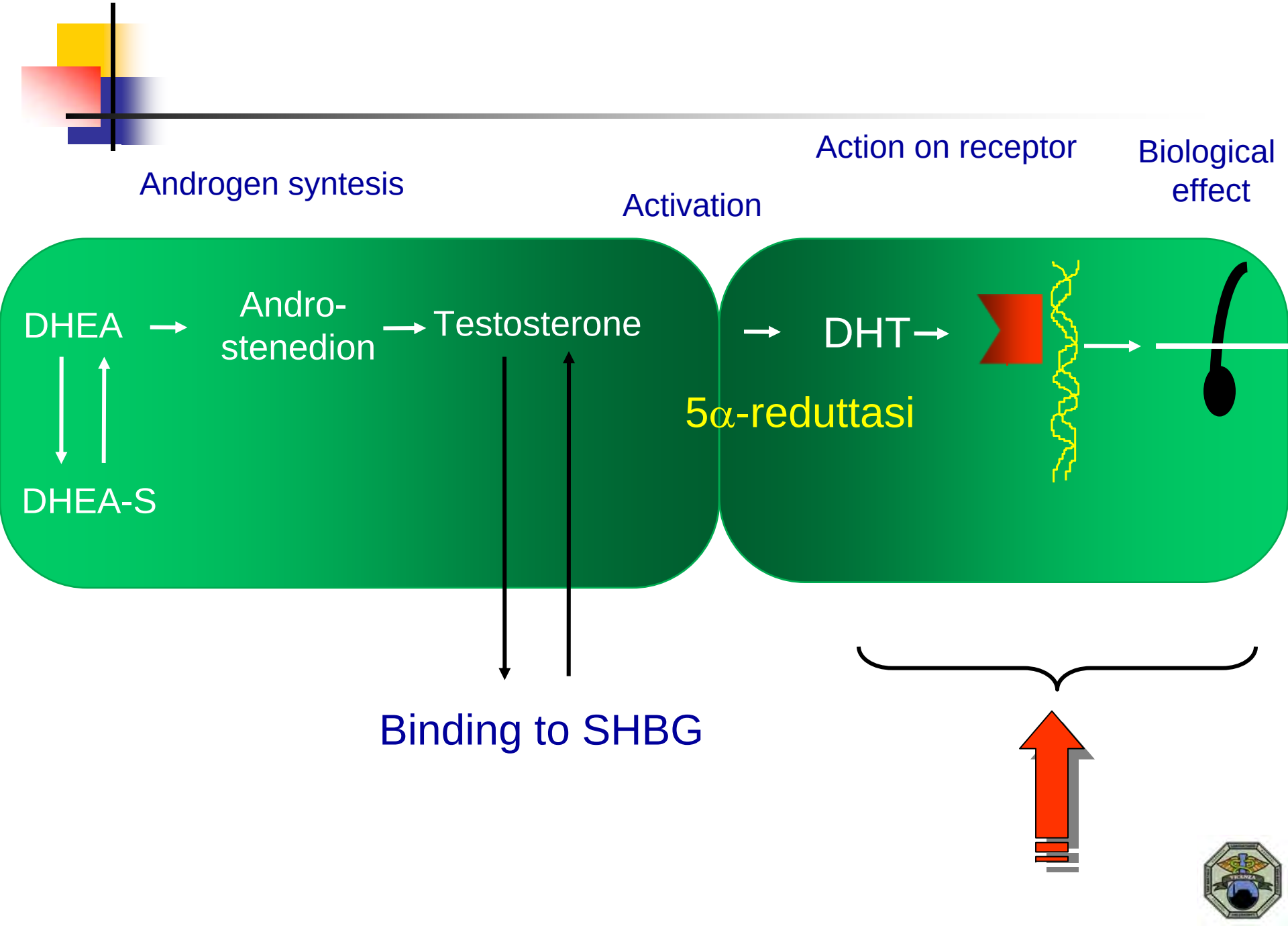
Longitudinal Effects of Aging on Serum Total and Free Testosterone Levels in Healthy Men

S. MITCHELL HARMAN, E. JEFFREY METTER, JORDAN D. TOBIN, JAY PEARSON, AND MARC R. BLACKMAN

The Intramural Research Program, National Institute on Aging, National Institutes of Health (S.M.H., E.J.M., J.D.T., J.P.) and Department of Medicine (M.R.B.), The Johns Hopkins University School of Medicine, Baltimore, Maryland 21224






Androgen syntesis
Activation
Action on receptor
Biological effect
DHEA
DHEA-S
Andro-stenedion
Testosterone
DHT
5α-reduttasi
Binding to SHBG

1: J Endocrinol Invest. 2005;28(3 Suppl):28-31.

Hormonal cut-offs of partial androgen deficiency: a survey of androgen assays.

Vermeulen A.

Section of Endocrinology, Department of Internal Medecine, University Hospital, Ghent, Belgium.
Alex.vermeulen2@telenet.be

As to the methodology, neither direct measurement of free testosterone by analog assay, nor the FT index (T/ SHB) can be recommended, only values obtained by dialysis, ammoniumsulfate precipitation or calculation yielding reliable estimates of androgen bio-activity. Dialysis and ammoniumsulfate precipitation are however work intensive and not widely used.

CONCLUSION
(n.1)

In Women:

• after clinical examination and evaluating medical history, there is no doubt that serum T is **really better than a guess** to evaluate the androgen status in **hyperandrogenism**;

•moreover, whenever serum T is marginally high or during a particular therapy (oestorgens,oral contraceptive etc.) or there are particular clinical symptons or signs (obesity, acne, midle hirsutism, hyperthyroidism etc), the measuremet of **serum SHBG to calculate the serum FT** or the determination of serum **feeble androgens (DHEA(S), A, 17 OHP)** or **serum ADG** might be required

•however, to evaluate androgen deficiency in women, the clinical significance of which is doubtful in any cases, due to the lack of accuracy of methods used in labs, **a guess** may be as good as unreliable T determinations

CONCLUSION
(N.2)

In men:

- after evaluating medical history and physical examination, there is no doubt that serum T levels are really better than a guess to evaluate the androgen status;

- however, whenever circulating T is stlightly normal and there are particular signs or symptons (obesity, impotence, decreasing muscle mass, loss of libido, osteoporosis etc) the measurement of plasma SHBG levels to calculate FT levels might be required.

WELCOME TO PUGLIA

THANKS FOR YOUR ATTENTION…