The role of assay specificity in improving the diagnosis and treatment of endocrine disorders.

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Disclosures: Steroid, 3 thyroid patents issued. Free Vitamin D3 and 1:25 dihydroxy Vitamin D3 patents pending

NIH Intramural research support 2011-2015

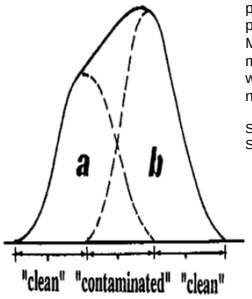
NIH CTSA support 2000-2011 (Children's National and Georgetown University)

To prescribe the right treatment, it is essential to have the correct diagnosis.

Correct diagnosis is dependent on reliable and accurate quantitation of disease markers

Disease markers need to correlate with the clinical picture.

From colorimetric to HPLC amperometric detection for catecholamines / metabolites. Gil Hill, Bill Purdy McGill University



These assays markedly improved diagnosis and management of pheochromocytoma and neuroblastoma patients.
Message: Lack of specificity in measurement changes both the means and widens the Gaussian distributions for the normal and diseased populations

Soldin SJ,Hill JG. Clin Chem 1980;26:291-4.
Soldin SJ, Hill JG. Clin Chem. 1981;27:502-3.

Marks the beginning of lessons in specificity dating back to 1980s

THYROID ISSUES

Why should we be interested in thyroid disorders ?

Total prevalence of hypothyroidism is 5.7% (***19 million in USA***).
Total prevalence of hyperthyroidism is 3.1%.(***10 million in USA***).
[European Journal of Endocrinology (2000) 143 639-647]

Endocrinologists constantly complaining about lack of correlation of free thyroid tests with TSH. CAP PT program shows major differences between different immunoassays.

Evidence is mounting that a substantial percentage of patients with hypothyroidism require T4 plus T3 replacement therapy. “Many hypothyroid patients are not tolerating and dissatisfied with LT4 treatment(palpitation, anxiety, insomnia, hypothyroid symptoms)”. Correct diagnosis depends on reliable measurement of T4, T3, FT4, FT3 and TSH.

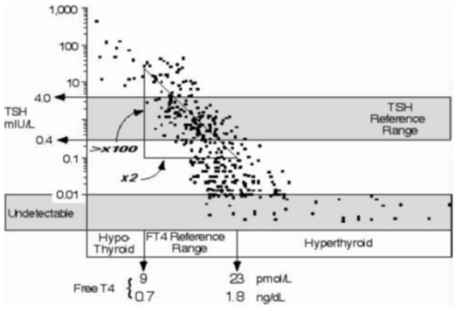
Suggested mechanisms of action

T4 pre-hormone…..D1 (plasma membrane)
D2 (Endoplasmic reticulum)

T3 active combines with TRs in nucleus triggering biological effects

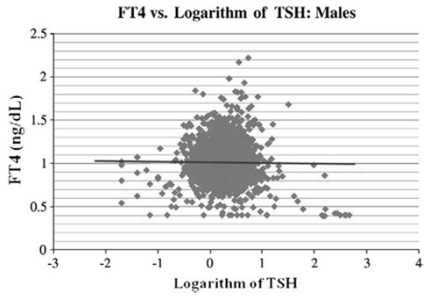
D3 plasma and nuclear membrane deactivates T3 to T2 and T4 to rT3

FT4 vs Log TSH



Spencer at. al. J Clin Endocrinol Metab 1990

ABBOTT ARCHITECT ci8200: Correlation of FT4 vs. log TSH: Males y=−0.0066x+1.013, r=0.010, n=2654.

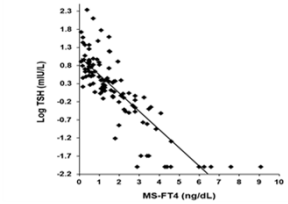


S.J. Soldin et al. Clinica Chimica Acta 411 (2010) 250–252 ? Role of FDA

NIH Study 2010 n=109 UF MSMS

Inverse log-linear relationship: LC-MS/MS-FT$_4$ and Log TSH

R = 0.84 (95 % CI 0.77 to 0.88)



Deventer HE ,Remaley A,Mendu DR,Soldin SJ Clin Chem 2011;57:122-7.

FT4 vs Log TSH

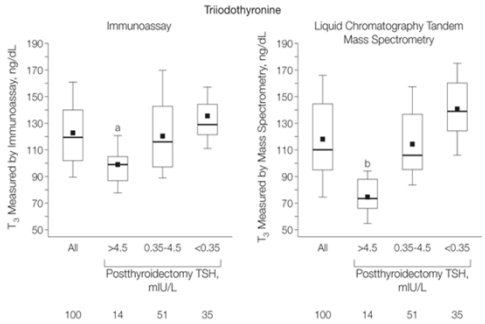
IA Studies

Platform	r	Reference	
Siemens RxL Dimension	0.48	Clin Chem. 55 (2009) 1380-1388.	Studies by
	0.58	Thyroid. 18 (2008) 1303-1311.	Soldin lab
	0.08	Thyroid. 18 (2008) 1303-1311.	
Abbott Architect Ci8200	0.05	Clin Chim Acta. 411 (2010) 250-252.	
Siemens Immulite 2500	0.45	Clin Chem. 57 (2011) 122-127	
Beckman Coulter Access DXI 800 Unicel	0.75	CCLM 2012;50(10):1849–1852	
Roche Modular E170	0.76	CCLM 2012;50(10):1849–1852	
Siemens ADVIA Centaur	0.72	CCLM 2012;50(10):1849–1852	

Mass Spectrometry Studies

Studies by	0.90	Clin Chem. 55 (2009) 1380-1388.
Soldin lab	0.77	Clin Chem. 55 (2009) 1380-1388.
	0.84	Clin Chem. 57 (2011) 122-127
	0.86	Thyroid. 18 (2008) 1303-1311.

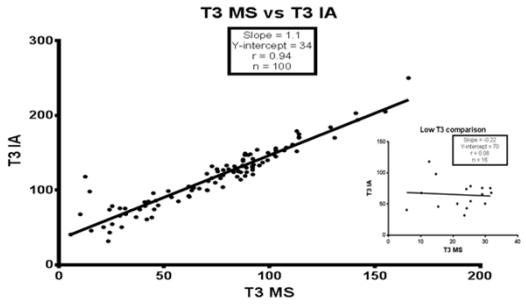
POST THYROIDECTOMY TSH vs T_3



Jonklaas J,Davidson B,Bhagat S,Soldin SJ. JAMA;2008:769-777.

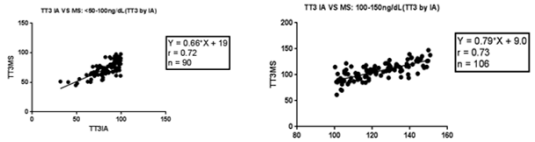
Georgetown University in and out-patient study

IA Siemens Immulite vs MSMS [9 vs 48 below 2.5th percentile. BIAS !! Intercept is 34 !!]

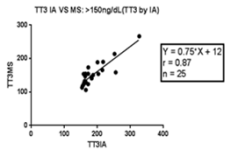


Soukhova N, Soldin OP, Soldin SJ. CCA 2004; 343:185-90
Nguyen LT, Gu J, Soldin OP, Soldin SJ. Clin Chem 2011;57:Abstract B99,pA82

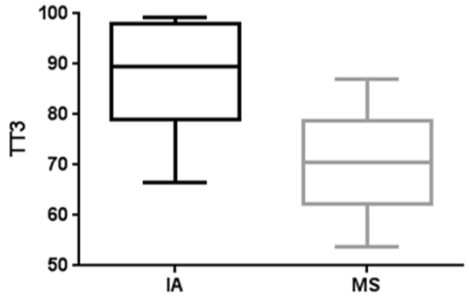
Roche 6000 T3 IA/MS comparisons at various concentration ranges



MS classifies 69 % as below 2.5th percentile.
IA classifies 35% as below the 2.5th percentile



TT3 (IA and MS) vs TSH >3.7uIU/mL



Georgetown in-patient study in non-thyroidal diseases.

Summary:

Patients did not have underlying /overt thyroid disease

48% of the T3's were below the 2.5th percentile when measured by MSMS

11% of them were below 2.5th percentile when measured by IA (Siemens Vista)

In-patients are on approximately 6-8 drugs.

Georgetown in-patient study in non-thyroidal diseases.

In-patients are on approximately 6-8 drugs.

QUESTION:

Are these multiple drug regimens affecting the deiodinases resulting in low T3"s

See Abdalla SM and Bianco AC, "Defending plasma T3 is a biological priority" Clinical Endocrinology 2014;0:1-9

Also, Rhee et al "Thyroid functional disease: an under-recognized cardiovascular risk factor in kidney disease patients" Nephrol Dial Transplant 2014;0:1-15

70 y old woman with Hashimoto's thyroiditis and problems converting T4 to T3:

IA results for T4,T3,FT4,FT3,TSH normal. Physicians refuse to change dose regimen as all tests normal. *All FDA approved tests-poor correlation with TSH or log TSH.* Patient complains of feeling sluggish and unwell.
Mass Spectrometry results – *good correlations with TSH*
3-Iodothyronamine <5pg/mL (<5 pg/mL)
T2 4.6 pg/mL (7.8-22.2 pg/mL)
T3 74 ng/dL (80-166 ng/dL) L
rT3 9.4 ng/dL (9.2-22.8 ng/dL) LN
T4 10.0 ug/dL (4.9-10.6 ug/dL)
FT4 1.9 ng/dL (1.3-2.4 ng/dL)
FT3 1.8 pg/mL (1.5-6.2 pg/mL) LN
T3 added to dosing regimen
Her FT3 is now fine at 3.3 pg/mL (1-6) and patient better

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50 y female with hypothyroidism

Complains of tiredness and feeling sluggish.
IA: FT4 1.0 (0.8-1.5); T3 91 (90-215); TSH 1.28 (0.4-4.0)

MSMS: FT4 2.1 ng/dL(1.3-2.4) ; FT3 1.9 pg/mL(1.5-6.0) ;
T3 79 ng/dL (80-187); T4 7.1 ug/dL(4.9-10.5)

Added 12.5 ug T3 bid.

Patient reports feeling well.

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68 YEAR OLD WOMAN WITH HYPOTHYROIDISM FEELING LETHARGIC

MASS SPEC RESULTS	IA RESULTS
FT4 1.2 ng/dL (0.8-2.0)	1.2 ng/dL (0.8-1.5)
FT3 1.5 pg/mL (1.5-6.0)	2.5 pg/mL (1.8-4.2)
TT3 64 ng/dL (80-187)	99 ng/dL (90-215)
rT3 8.7 ng/dL (9-21)	
T4 7.2 ug/dL (4.9-10.5)	8.0 ug/dL (4.5-12.5)

Heterezygous for human D2 Thr92Ala polymorphism (33-60% of population)
Treatment with T3 normalized MSMS measurements and improved clinical condition. Cholesterol normalized, 220 to 160 mg/dL.
JCEM 2011;96:E1527-E1533. Clinical Developmental Immunology 2012;Article ID 340542.

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Binding protein effects on FT4/FT3 by IA: 30 year old male **AIDS-related Kaposi sarcoma**

- Immunoassay results

FT4 0.66 ng/dL (0.8-1.5)	L	Euthyroid patient, IA results do not correlate with clinical condition.
FT3 1.41 pg/mL (1.80-4.20)	L	
TSH is 3.52 uIU/mL (0.36-4.0)	***N***	
T3 is 42 ng/dL (90-215)	L	
T4 is 3.1 ug/dL (4.5-12.5)	L	
TBG is 4.3 ug/mL (13-39)	L	
TG is 32 ng/mL (1.6-60)	N	

- Mass spec results

FT4 1.8 ng/dL (1.4-3.2)	N	Results correlate with clin symptoms
FT3 1.7 pg/mL (1.5-6.0)	N	
T4 2.4 ug/dL (4.9-10.2)	L	
T3 25.2 ng/dL (83-168)	L	
rT3 10.7 ng/dL (9-23)	N	

56 YEAR OLD CAUCASIAN FEMALE WITH 3 YEAR HISTORY OF HYPOTHYROIDISM

She presented with lethargy, loss of energy and struggling to loose weight. BMI 29.9 kg/m^2 . Patient was treated with Synthroid only and still feels lethargic, is also constipated.

Analyte	MSMS	Immunoassay
T4 ug/dL	13.4(5.1-11.3)	11.8 (4.5-11.7)
FT4 ng/dL	1.9(1.3-2.4)	1.6 (0.9-1.7)
TSH uIU/mL		2.65 (0.27-4.2) Normal
T3 ng/dL	82 (80-187) [3rd %]	114 (80-200)[25th%]
FT3 pg/mL	2.1 (1.5-6.2)[4th%]	2.6 (2.0-4.4)[20th%]
Cholesterol mg/dL		219(<200)

TBG normal, ATA and ATG below detection limit

Now started on additional dosing BID with 12.5 ug T3.

Case report continued

The low MS FT3 and TT3 may explain the unresolved symptoms of hypothyroidism even with the now normalized FT4 and TSH. Patient was started on 12.5 ug bid T3. After 10 days of treatment all her symptoms of hypothyroidism were alleviated.

Also cholesterol dropped from 219 to 190 mg/dL.

Her 10 day post T3 treatment LC-MSMS results for FT3 and TT3 at 8am (pre-dose) were 3.8pg/mL[1.5-6.2] and 129 ng/dL[80-187] and 2h post dose were 4.6 pg/mL and 145 ng/dL respectively.

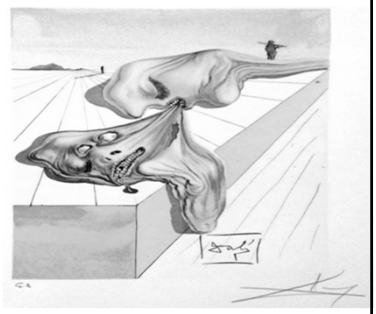
Wartofsky Curr Opin Endocrinol Diabetes Obes 2013;20:460-466

"Perhaps 20% of hypothyroid patients treated with T4 alone continue to complain of symptoms suggesting thyroid hormone deficiency ? deiodinase polymorphisms. These patients may benefit from T3/T4 combination therapy !!"

When patients complain and results do not correlate with the clinical picture there is usually a good reason.

What (wo)men do to (wo)men

"Do no harm", Thomas Dunne



FDA's contribution to diagnostic errors

- FDA has approved all the "problem" tests (IA's for FT4,FT3,T3) without assessing whether any of them are "clinically useful" (good correlation to: 1. log TSH and 2. clinical condition).
- *One could argue that the current system provides clinicians with the wrong result thereby contributing actively to both incorrect diagnoses and subsequent treatment.*
- In the low range, IA's give false normal results in 65% of FT4's and 50% of FT3's and T3's.This affects 4-6 million hypothyroid people in the USA alone. These tests therefore will account for many wrong diagnoses resulting in incorrect or no treatment with T3 when T3 treatment is warranted.

Summary and conclusions for thyroid hormone testing

T4 testing by immunoassay appears to be reliable.

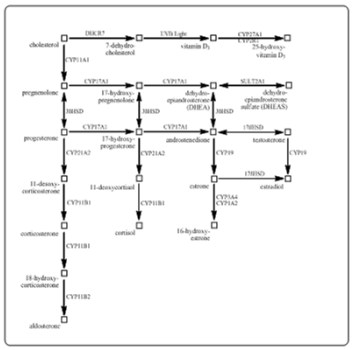
T3 testing by immunoassay is suboptimal at low concentrations ,during pregnancy and in many in-patients.

FT4/FT3 and TT3 tests need to correlate with log TSH. The FDA has a role to play when they license IA for FT4, FT3 and TT3 .

We recommend that UF-MSMS be employed to measure FT4/FT3 for all specimens in which the TSH >95%-tile or <5%-tile. We also recommend that IA T3's below the 10th percentile be repeated by LCMSMS.

T4,T3,FT4,FT3 tests by LCMSMS are available at commercial laboratories.

STEROID METABOLIC PATHWAY



Lack of Specificity of FDA Approved Immunoassays. Data from CAP PT Program Y-A Survey 2010

Analytes	Low	High	H/L
Testosterone (ng/dL)	83	213	(2.6)
	715	1309	(1.8)
Estradiol (pg/mL)	38	366	(9.6)
	274	1140	(4.2)
Progesterone (ng/mL)	4.6	6.3	(1.4)
	7.4	12.5	(1.7)
17OH P (ng/dL)	765	2121	(2.8)

Tandem Mass Spectrometry. Data from CAP PT Program Y-A Survey 2010

Analytes	Low	High	H/L
Testosterone (ng/dL) n=13	71	125	(1.7)
	635	959	(1.5)
17OH P (ng/dL) n=11	821	1289	(1.5)
Estradiol (pg/mL)	142	184	(1.3)
n=4 NYS PT	44	152	(3.4)
Progesterone (ng/mL)	3.9	4.8	(1.2)
n=4 NYS PT	47	67	(1.4)

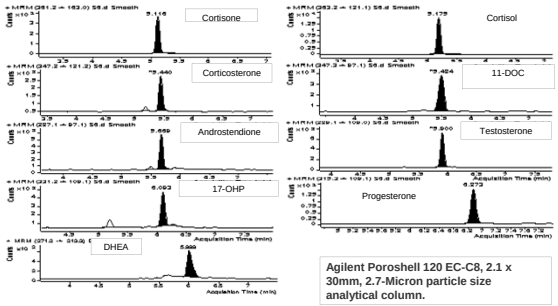
SINGLE ANALYTE vs PROFILE

- The case of large commercial reference laboratories
- The majority of teaching hospitals have less than 1,000 beds and a single tandem MSMS performing steroid profiles would provide physicians to access >1 steroid in the panel.
- Panel testing provides the clinician with more information

Soldin SJ,Soldin OP Clin Chem. 2009;55:1061-6.

3rd Generation Steroid Profile Assay by LCMSMS

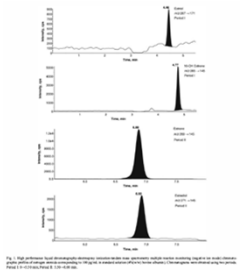
Stolze BR, Gounden V, Gu J and Soldin SJ A408. Development and Validation of a High Performance Liquid Chromatography Tandem Mass Spectrometry 9 Steroid Panel using Minimal Sample Volume. Clinical Chemistry, Abstracts 2014;S133



Agilent Poroshell 120 EC-C8, 2.1 x 30mm, 2.7-Micron particle size analytical column.

ESTROGEN PROFILE ASSAY

Guo T, Gu J, Soldin OP, Singh RJ, **Soldin SJ** Clin Biochem, 2008;41:736-41.



Recent new steroid profile applications

CAH

PCOS (most common gynecological endocrinopathy 7-14% of women)

Marathon running

Adrenal insufficiency (0.6%)

Diurnal effects on steroid concentrations

Neurosteroids

Case Study CAH: Steroid Panel

- 2 day infant,normal penis, no palpable testes
- 17 OH Prog >20,000 ng/dL(<100 ng/dL)
- 11 Deoxycortisol 1.2 ug/dL(<0.15)
- Testosterone 441 ng/dL(<10 ng/dL)
- Androstenedione >11,900 ng/dL(<50 ng/dL)
- Progesterone 3.31 ng/mL(<0.33 ng/mL)
- DHEAS 15 ug/dL(<1.25 ug/dL)
- CAH with 21 OH-ase deficiency (1 in 15 thousand births) Low cortisol (3.8 ug/dL)
- Karyotype on peripheral blood 46 XX
- Early detection leads to early treatment (glucocorticoid replacement) and avoids salt wasting crises.

DRUG TREATMENT OF CAH

DRUG	POTENCY	SIDE EFFECTS
• Hydrocortisone/cortisone	equipotent	few
• Prednisolone	5X	long acting,impair growth
• Dexamethasone	40-80X	long acting,impair growth

Hydrocortisone preferred medication for children with CAH
If a salt-retaining hormone is needed, use fludrocortisone

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Hormonal Circadian Rhythms in Patients with Congenital Adrenal Hyperplasia:

The treatment goal in congenital adrenal hyperplasia (CAH) is to replace glucocorticoids while avoiding androgen excess and iatrogenic Cushing's syndrome.

Hormonal Circadian Rhythms in Patients with Congenital Adrenal Hyperplasia: Identifying Optimal Monitoring Times and Novel Disease Biomarkers. Debono M, Mallappa A, Gounden V, Nella AA, Harrison RF, Crutchfield CA, Backlund PS, Soldin SJ, Ross RJ, Merke DP.Eur J Endocrinol. 2015 Sep 4. pii: EJE-15-0064.

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Continuous Subcutaneous Hydrocortisone Infusion (CSHI)

Background: CAH treatment focuses on replacement of cortisol and aldosterone and prevention of ACTH – driven androgen excess. This approach is frequently inadequate.

5 patients with difficult to treat (7am 17-OHP>1200 ng/dL and androstenedione >210 ng/dL) classic CAH due to 21-hydroxylase deficiency.

Results: At 6 months all but 1 patient had 7am 17-OHPs 2-6 fold lower than baseline values.

Conclusion: CSHI is a safe and well-tolerated therapy.

Paper submitted to JCEM

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PCOS STUDY

ANALYTE	PCOS mean (SD)	CONTROLS Mean (SD)	p value 2 sample t-test	p value non-parametric
HbA1c %	5.6 (0.4)	5.3 (0.4)	<0.003	<0.008
ALDOSTERONE pg/mL	321 (178)	182 (110)	0.0006	0.0005
ANDROSTENEDIONE ng/dL	110 (57)	73 (45)	0.007	0.005
TESTOSTERONE ng/dL	46 (24)	26 (14)	0.0003	0.0001
PROGESTERONE ng/mL	1.3 (2.9)	2.1 (3.3)	0.32	0.024
ESTRONE pg/mL	80 (48)	53 (25)	0.008	0.05
ESTRIOL pg/mL	62 (30)	123 (49)	0.001	0.001

DHEAS,DHEA,11-DOC,CORTICOSTERONE,17-OHP,VIT D3,ESTRADIOL p>0.1

Summary PCOS Study

30 PCOS , 30 age matched controls

DHEA,DHEAS,Androstenedione,

Testosterone and Aldosterone are higher in patients with PCOS.

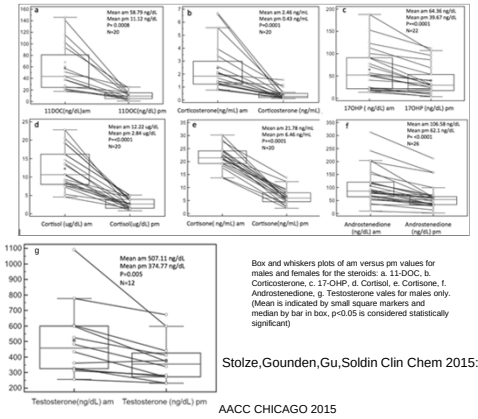
5/30 PCOS patients had very elevated aldosterones (>550 pg/mL) some of which were due to an adrenal tumor.

Conclusion: results emphasize the power of profile monitoring

Marathon runner study (n=51, distance 52 Km)

Hew-Butler T ,Soldin SJ et al. JCEM 2008 Jun;93(6):2072-2078.

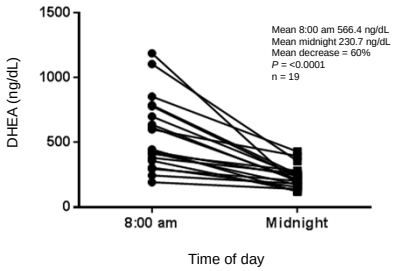
- Pre vs post race steroid profiles
- Aldosterone 6.1 to 19.7 ng/dL p<0.001
- Corticosterone 226 to 3491 ng/dL p<0.001
- 11-Deoxycortisol 0.03 to 0.54 ug/dL p<0.001
- Cortisol 10 to 33 ug/dL p<0.001
- DHEA,DHEAS and Androstenedione all showed increases p<0.001



Box and whiskers plots of am versus pm values for males and females for the steroids: a. 11-DOC, b. Corticosterone, c. 17-OHP, d. Cortisol, e. Cortisone, f. Androstenedione, g. Testosterone vales for males only. (Mean is indicated by small square markers and median by bar in box, p<0.05 is considered statistically significant)

Stolze,Gounden,Gu,Soldin Clin Chem 2015:61;556-58

Diurnal Variation for DHEA Concentration



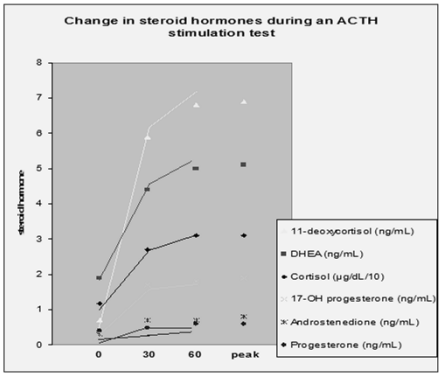
Summary of Diurnal rhythms for steroids

- Diurnal effects are very significant for most steroids studied
- This means that it is necessary to standardize collection times and have reference intervals for 8am,8pm and midnight

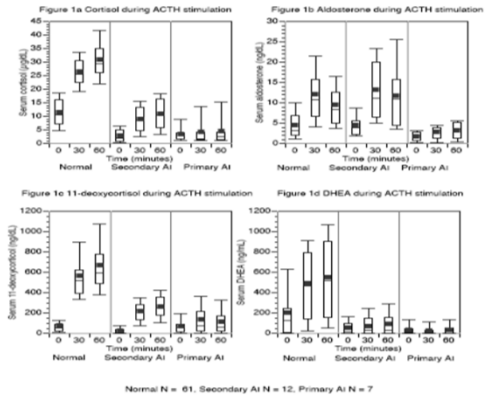
Steroids In Adrenal Insufficiency

STUDY SUBJECTS						
	Number of patients	Sex	Age (years)	Patient type	Outcome of cortisol measurements	Designation
Group 1	21	57% F	33 ± 9	No suspicion AI	≥ 20 µg/dL by MS/MS	Normal
Group 2	40	64% F	46 ± 15	AI suspected	≥ 20 µg/dL by MS/MS	Normal
Group 3	19	50% F	46 ± 13	AI suspected (4 patients with known AI)	< 20 µg/dL by MS/MS	AI

Holst JP, Soldin SJ et al. Steroids 2007:72;71-84



ACTH STIMULATION: MSMS PROFILES

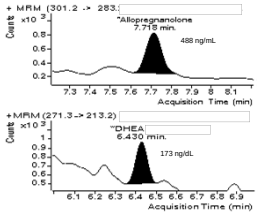


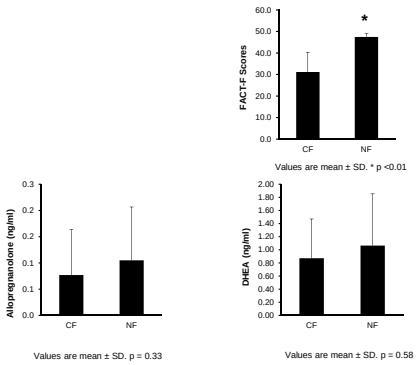
Summary of Adrenal Insufficiency Study

- Change the textbooks ! Cortisol is not the best marker to measure after ACTH stimulation
- The combination of 11 DOC, Cortisol, DHEA and Aldosterone optimizes correct classification of primary vs secondary adrenal insufficiency. Aldosterone concentrations <60 pg/mL at 30 min = primary AI

Holst JP,Soldin SJ et al. Steroids 2007:72;71-84

Neurosteroids





FACT-F: cancer fatigue scores. All subjects are men with prostate cancer who have received radiation therapy. Fatigue and neurosteroid levels were measured at one year after radiation therapy

Studies on free vitamin D3 in our laboratory

Suggested review: Groves et al Annual Rev. Nutr. 2014.34:117-41.

Why do we need to assess free vitamin D3 levels?

There is a poor correlation between total vitamin D3 and PTH

Some areas of clinical interest for free vitamin D3 include:

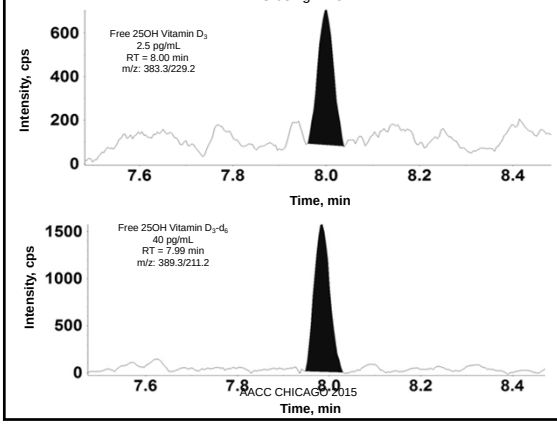
Depressive illness, Seizure disorders, Oncology, Bone diseases, Alzheimer's disease, Schizophrenia, Autism, Parkinson's disease, Multiple Sclerosis, Pregnancy

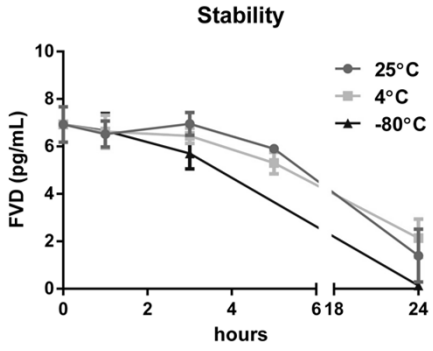
How to measure free vitamin D3

Measurement of many free steroid and thyroid concentrations is easy. Why did we fail for so long to develop a method for measurement of free Vitamin D3 ?

Vitamin D3 binds to its binding protein in serum/plasma. Ultrafiltration results in a protein free ultrafiltrate. Vitamin D3 in this ultra-filtrate sticks to both glass and plastic. However, if filtered into an organic solvent it remains in solution and can be quantified.

MS using APCI





I love talking about nothing. It is the only thing I know anything about .

Oscar Wilde

We are all amateurs…..we don't live long enough to be anything else

Charlie Chaplin, Limelight

Don't let schooling interfere with your education ***Grant and/or Mark Twain***

Final comment on fate of creative people:

"It is the finest blades that are most easily blunted, bent or broken"