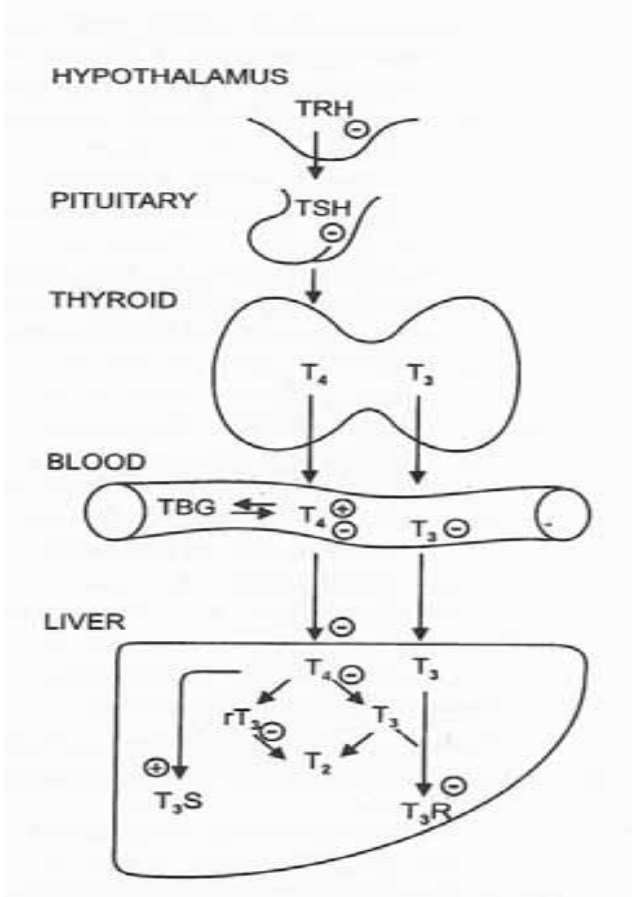
Tricky thyroid function tests



Pierre-Marc Bouloux

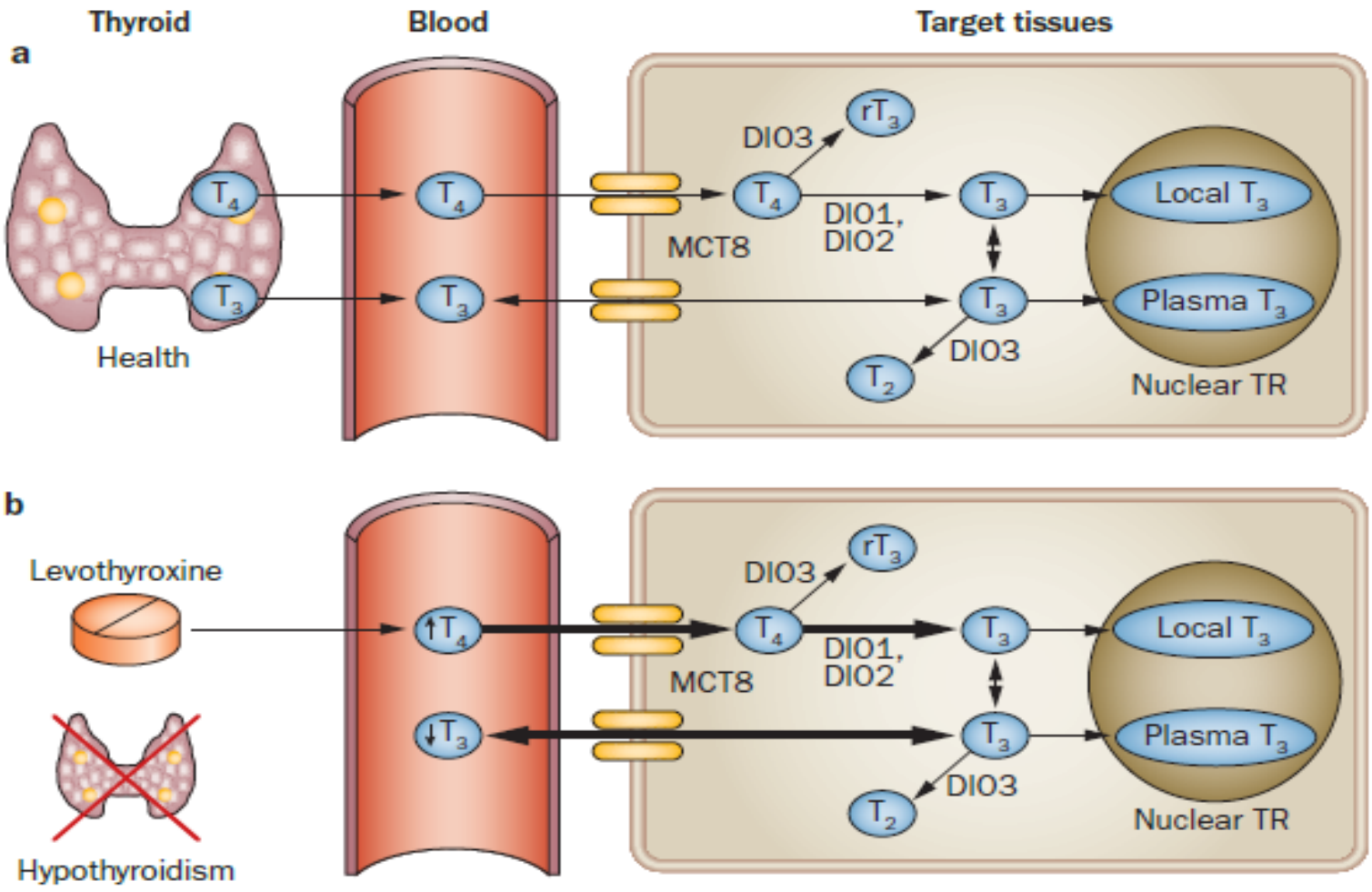
Mauritius

1st April 2015

Thyroid hormone transport

	TBG	TTR	ALB
Molecular weight (k daltons)	54*	55	6
Structure	Monomer	Tetramer	Monomer
Carbohydrate content (%)	20		
Number of binding sites for T_4 and T_3	1	2	Several
Association constant, Ka (M-1)			
For T_4	1×10^{10}	2×10^{8} **	1.5×10^{6} **
For T_3	1×10^{9}	1×10^{6}	2×10^{5}
Concentration in serum (mean normal, mg/liter)	16	250	40,000
Relative distribution of T_4 and T_3 in serum (%)			
T_4	75	20	5
T_3	75	<5	20
In-Vivo Survival			
Half-life (days)	5***	2	15
Degradation rate (mg/day)	15	650	17,000

Thyroid hormone metabolism



Wiersinga. Nature 2014.

Where things can make life difficult for the endocrinologist…..

Pre-laboratory….paying insufficient attention to the clinical context

- Age (paediatric, elderly patients)
- Pregnancy changes
- Thyroxine therapy
- Confounding medications (heparin, furosemide, amiodarone)
- Non-thyroidal illness

Laboratory

Failing to recognise limitations of commonly used FT4, FT3, and TSH assays

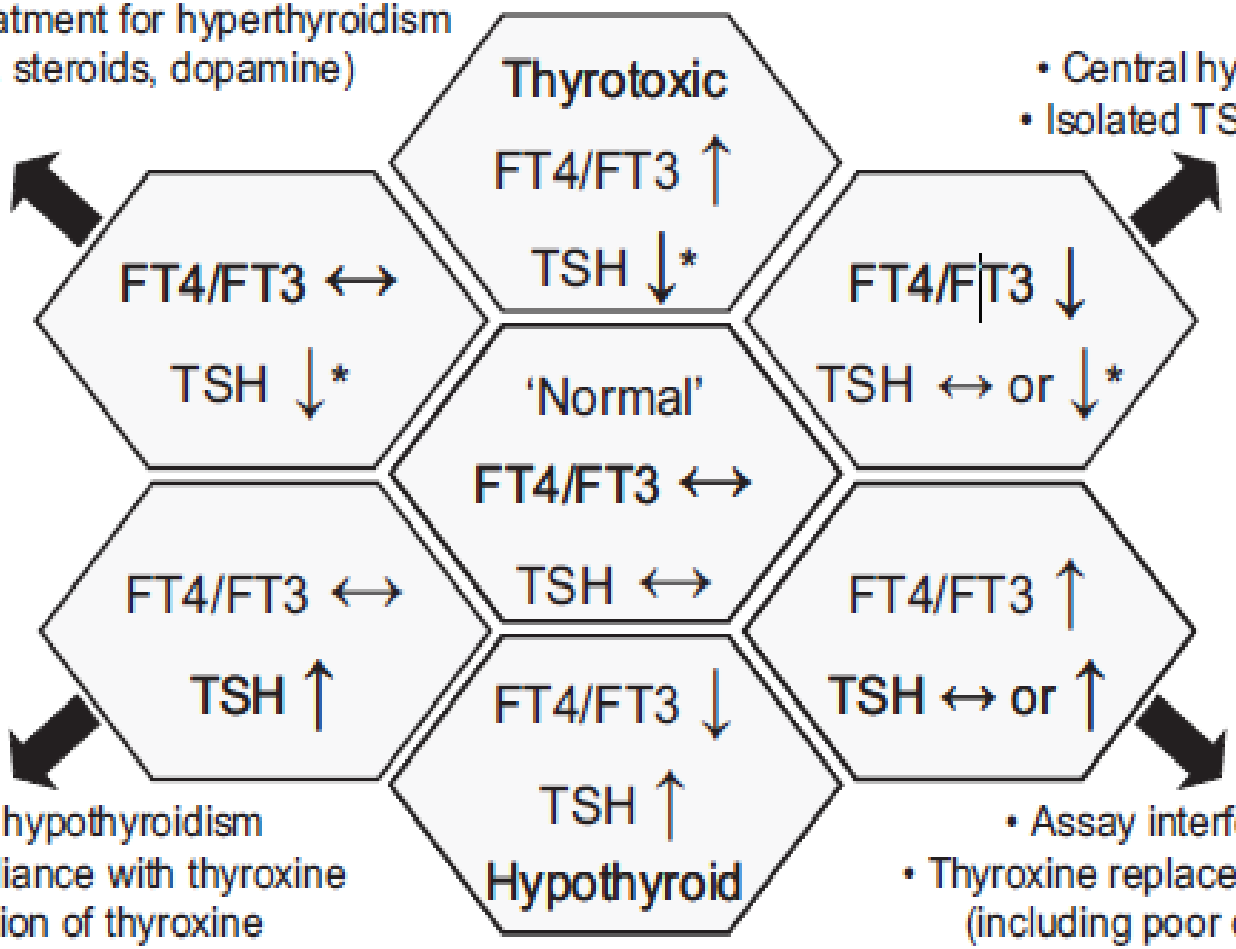
ASSAY interference:

- Heterophile Abs
- Human Anti-animal Igs (HAA)
- Anti-iodothyronine Abs
- High affinity ransporter protein (familial dysalbuminaemic hyperthyroxinaemia (FDH), and high affinity Transthyretin variants

Post-laboratory

Limited experience of dealing with rarer genetic or acquired HPT disorders

- Resistance to thyroid hormones (THRβ)
- Disorders of iodide transport (NIS)
- Disorders of TH transport (FDH)
- Disorders of TH metabolism (deiodinase)
- TSHomas



- Subclinical hyperthyroidism
- Recent treatment for hyperthyroidism
- Drugs (e.g. steroids, dopamine)
- NTI

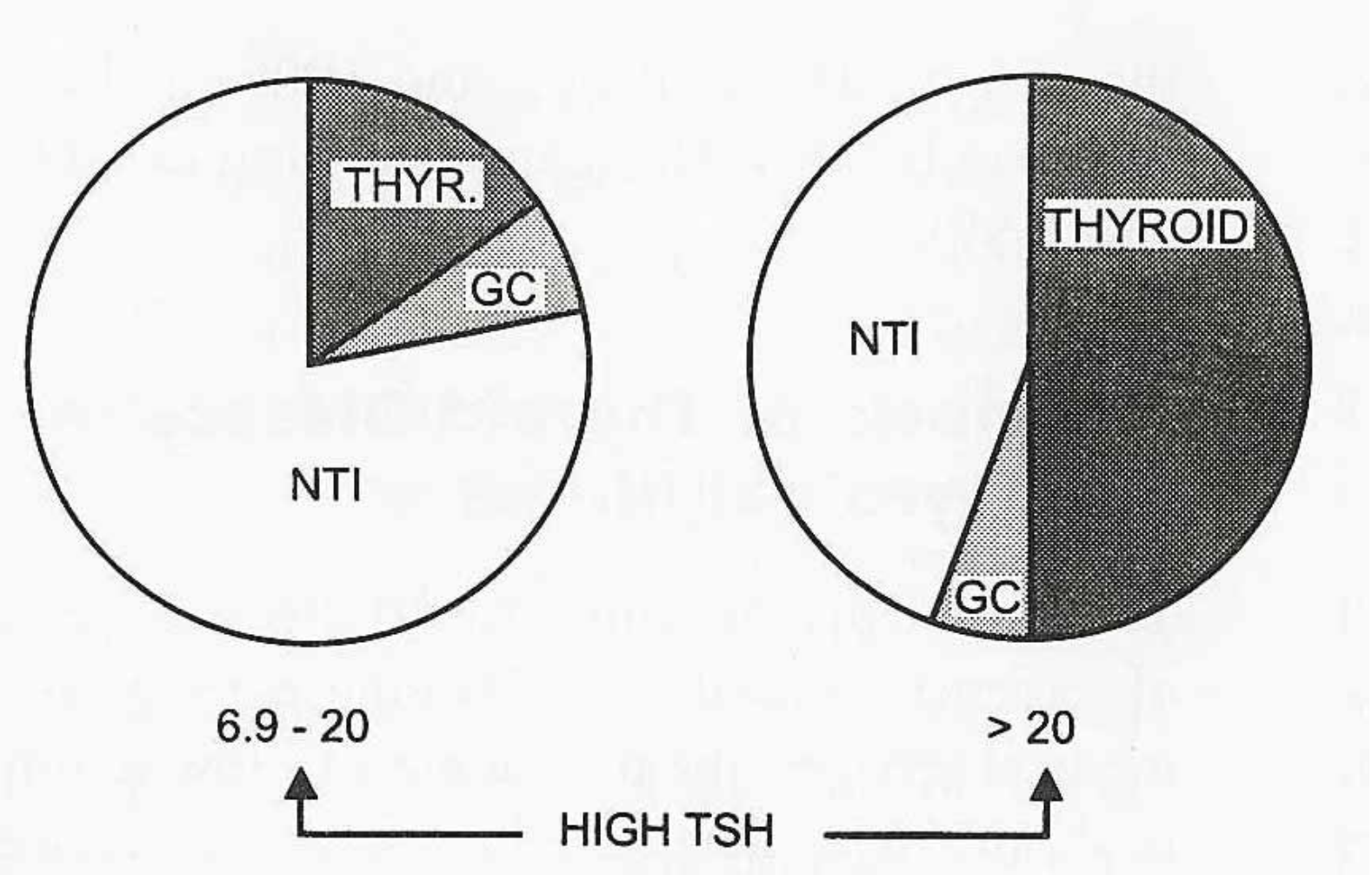
- NTI
- Central hypothyroidism
- Isolated TSH deficiency

- Subclinical hypothyroidism
- Poor compliance with thyroxine
- Malabsorption of thyroxine
- Drugs (e.g. amiodarone)
- Assay interference
- NTI recovery phase
- TSH resistance

- Assay interference; FDH
- Thyroxine replacement therapy (including poor compliance)
- Drugs (e.g. amiodarone, heparin)
- NTI (including acute psychiatric disorders)
- neonatal period
- TSH-secreting pituitary adenoma
- Resistance to thyroid hormone
- Disorders of thyroid hormone transport or metabolism

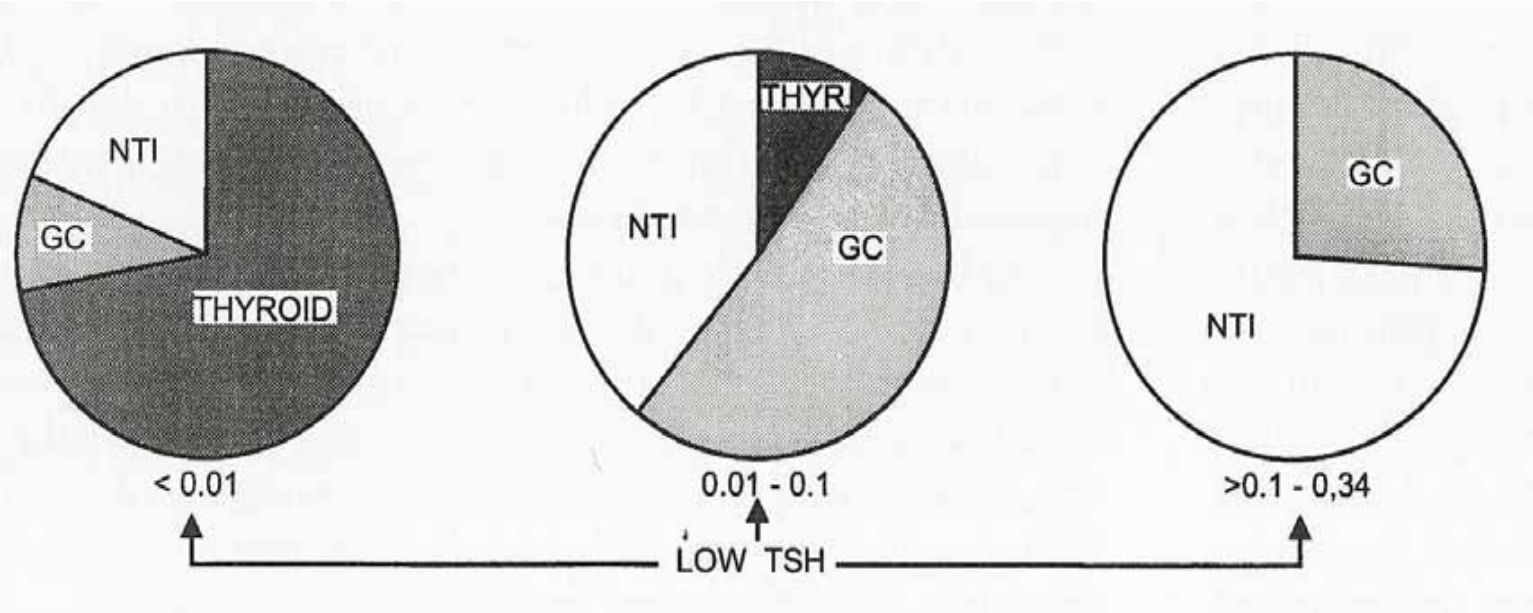
Gurnell et al, Clin Endocrinol 2011 74(6):673-8

Non thyroidal illness (NTI) 1



(Spencer et al, 1988)

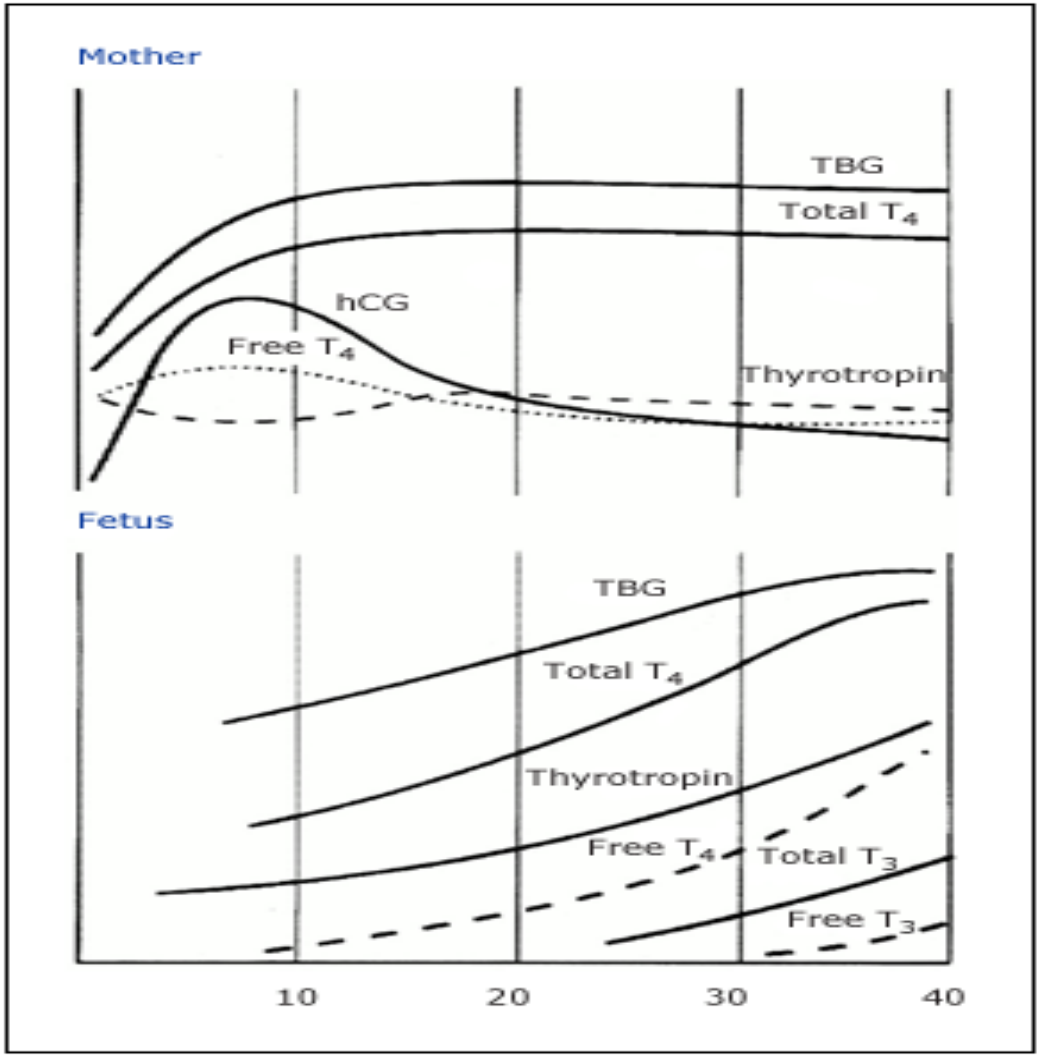
Outcome in acute admissions
Low serum TSH
(Spencer et al, 1988)



Effect of pregnancy on thyroid gland physiology

- Increase in free thyroid hormone concentrations and reduction in TSH concentrations in first trimester through the action of human chorionic gonadotrophin (hCG)
- Increased renal I^- clearance (increasing dietary requirements in deficient areas).
- Increased serum TBG increasing total T4 and T3 concentrations
- Increased plasma volume that increases T4 and T3 pool size.
- Increased inner-ring deiodination of T4 and T3 by placenta increasing hormone degradation

Changes in maternal and foetal thyroid function during pregnancy



Drug interference in FT4 assays

- Heparin
- Furosemide (IV >80mg)
- Phenytoin
- Aspirin
- Non steroidal anti-inflammatory drugs

These drugs are capable of displacing T4 and T3 from their binding sites and alter hormone delivery and clearance and distort diagnostic tests for FT4 and FT3

Heparin Interference in FT4/3 measurements

- Both fractionated and unfractionated heparin can cause an artefactual elevation in measured concentrations of FT4/FT3 by displacement of T4 and T3 from their carrier proteins.

- The mechanism appears to involve generation of free fatty acids (FFAs) via heparin-mediated activation of endothelial lipoprotein lipase (LPL), with FFAs displacing T4 from albumin. The extent to which FFAs rise is variable and, as displacement continues *in vitro*, pre-analytical delay can compound the situation.

Case vignette - 1

68 year old male

GP referral: 'progressively rising TSH on long term L-T4 therapy' – what is going on?

HPC : Primary hypothyroidism 1992

- Euthyroid on L-T4 125-150 mcg/day
- Annual TSH monitoring

PMH : Hypertension, Aortic valve replacement, Antiphospholipid syndrome - PE

Case Vignette 1

- DH - LT4 125mcg/day
  - Lisinopril 10mg/day
  - Bisoprolol 2.5mg/day
  - Furosemide 40mg/day
  - Simvastatin 20mg/day
  - Warfarin 3-4mg/day
- DQ: Right hallux pain for several months

Case vignette 1

- O/E BMI 28.5
- Euthyroid
- No palpable thyroid gland
- P 68/min
- BP 125/60 mmHg
- **TSH 25.6mU/l (0.35-22.7)**
- **fT4 19.8 pmol/l (11.5-22.7)**
- Repeated: **TSH 28.5 fT4 16.8**
- Urate 0.43 µmol/l (0.2-0.4)
- Rheumatoid Factor 183 (0-30)

Diagnoses

- ? Poor compliance with L-T4 : perhaps took it a few days before and on the morning of testing
- ? Malabsorption of L-T4
- Rheumatoid arthritis

Serial TFTs

Date	TSH	FT4	L-T4 mcg/day
9.03.04	0.04	17.9	150
1.04.05	0.54	-	125
10.04.06	1.6	-	125
10.05.07	2.4	-	125
10.06.08	2.5	-	125
09.07.09	4.9	-	125
09.08.10	25.5	19.8	125
11.08.10	28.5	16.8	125
01.09.12	37.9	20.3	150
05.09.12	50.2	23.8	175

Further investigations

Assay Platform 1		Assay Platform 2 (DELFIA)	
TSH (0.35-5.5)	50.8	TSH (0.4-4.0)	0.06
FT4 (11.5-22.7)	23.8	FT4 (9-20)	26.0
		Rheumatoid Factor (0-30)	<10
		(human IgG only)	

TSH dilution studies

Control subject

Dilution	TSH	TSH x dil
1:1	27.50	27.50
1:2	14.00	28.00
1:4	6.88	27.52
1:8	3.35	26.80
1:16	1.64	26.24

Patient

Dilution	TSH	TSH x dil
1:1	13.30	13.30
1:2	3.75	7.50
1:4	0.95	3.84
1:8	0.26	2.08
1:16	0.07	1.12

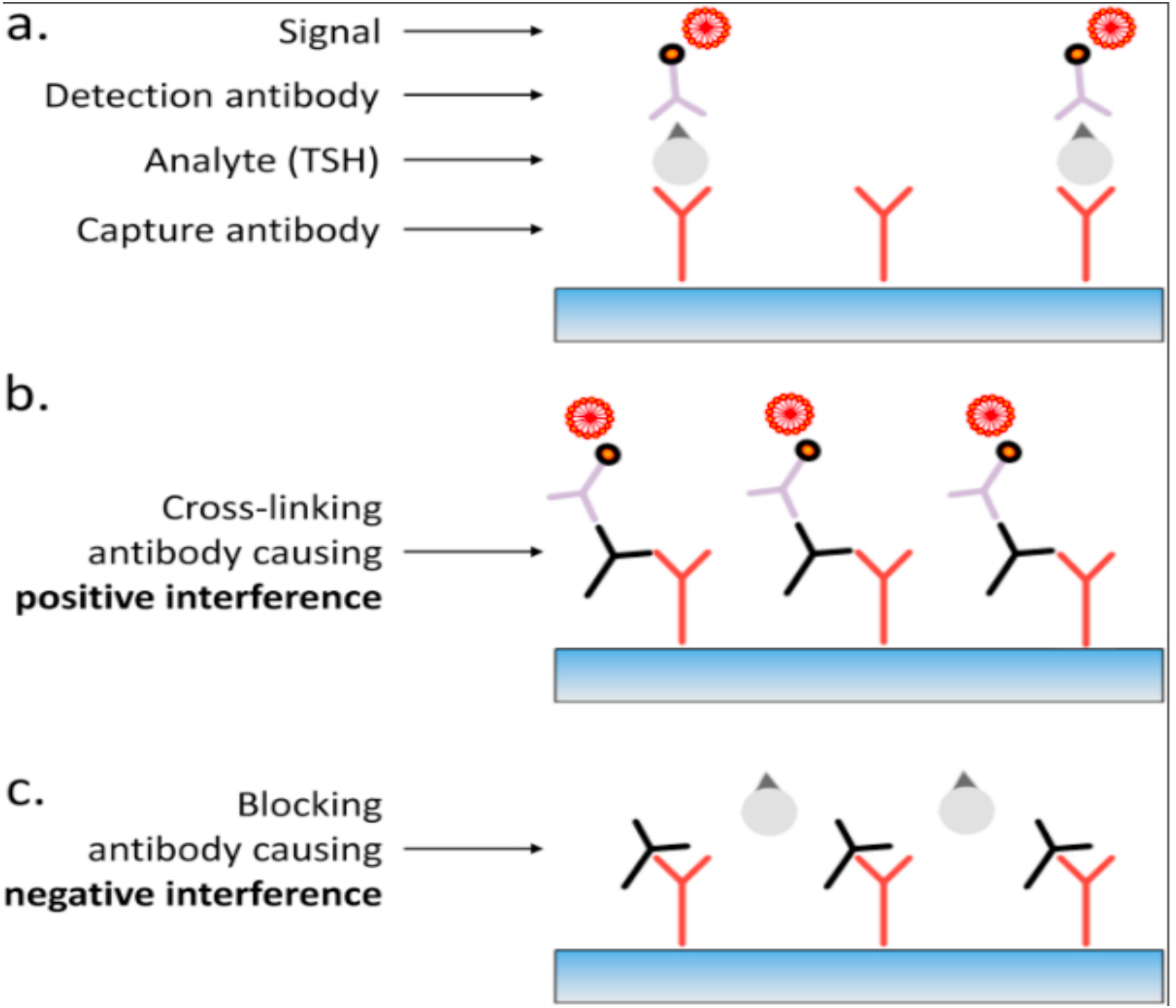
Subsequent management

- Reduce L-T4 dose to 125mcg/day
- Use of DELFIA platform for future TSH measurements

07.09.14 TSH 0.59 fT4 17.3 on L-T4 125mcg/d

Final Diagnoses:

1. Stable 1^{ary} Hypothyroidism
2. TSH and RhF assay interference
3. Gout


a.
Signal
Detection antibody
Analyte (TSH)
Capture antibody
b.
Cross-linking antibody causing positive interference
c.
Blocking antibody causing negative interference

Case 2- clinical vignette

7 day old baby

PC Blood spot TSH = 213

HPC: Normal pregnancy and labour

Neonatal period - dry skin

- mild jaundice

- no goitre

Rx. L-T4 therapy commenced Day 11

Case 2- clinical vignette

	TSH (0.4-4.0)	FT4 (9.0-20.0)	FT3 (3.0-7.5)
Day 7	213	-	-
Day 11	826	17.0	-
Day 18 (on L-T4)	308	33.0	7.9

Case 2- clinical vignette

Mother

PMH: Asymptomatic

No FH of thyroid disease

O/E Euthyroid

No goitre

TSH 287 mU/l

FT4 13.5 pmol/l

FT3 4.2 pmol/l

TPO Negative

TRAb Negative

Detection of macro TSH

- Discordant TSH results in an assay that utilizes different antibody pairs;
- Altered TSH result following immunosubtraction (using polyethylene glycol (PEG) or protein G/A)
- Nonlinear TSH measurement following sample dilution: if either TSH or the assay reagents are weakly bound by interfering antibodies, this interaction may be disrupted by dilution and a nonlinear dilution series will ensue.

Gel Filtration Chromatography of serum TSH *(Halsall et al 2006)*

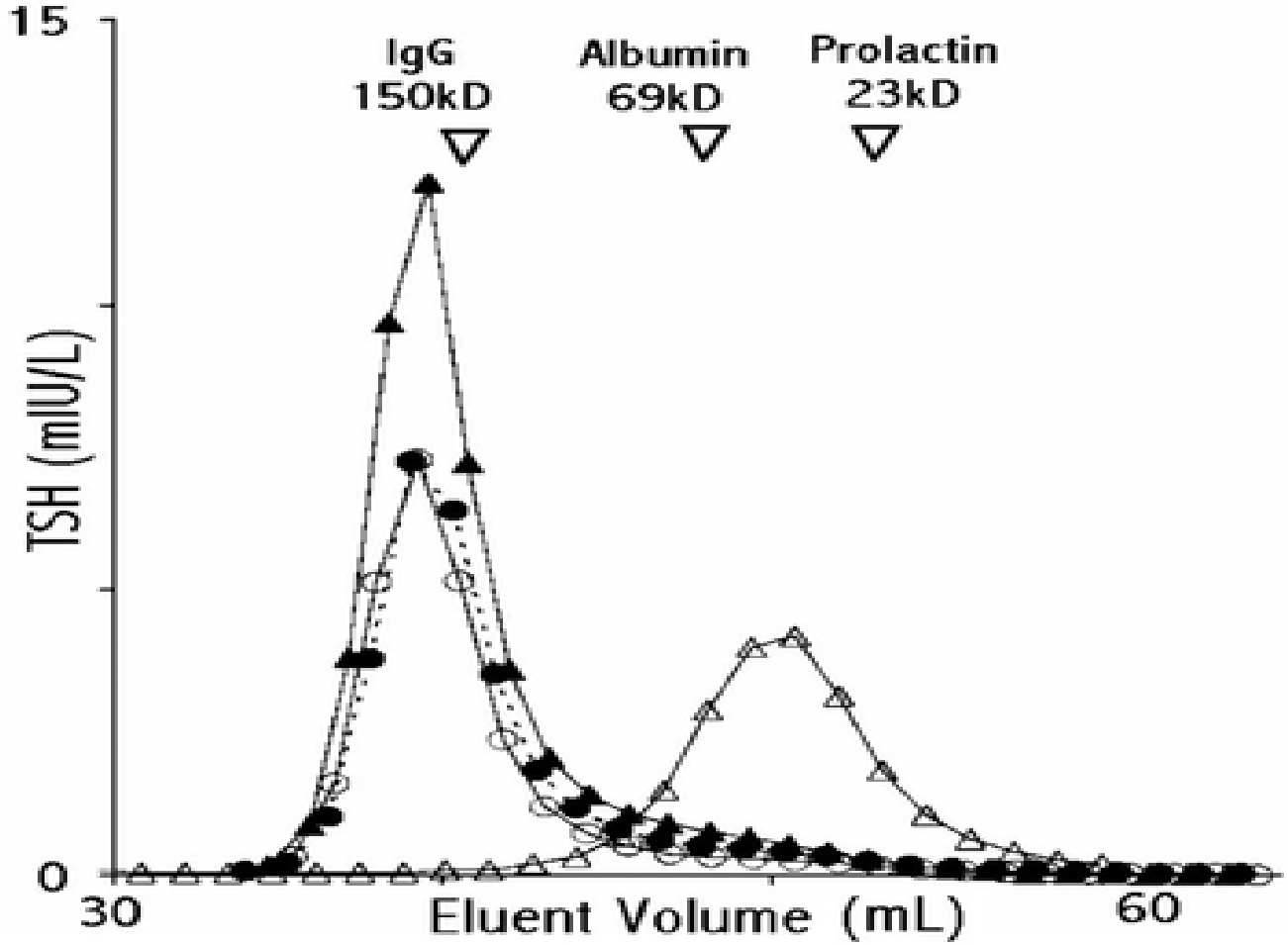


Fig. 1.Gel filtration chromatography of serum TSH.

Gel filtration chromatography of serum TSH in a control patient with increased TSH caused by hypothyroidism, Δ—Δ; in the neonate, ○—○; and in the mother before, ●—● and after incubation with serum from a control with increased TSH, ▲—▲. The peak elution volumes for endogenous serum prolactin, albumin, and IgG are shown as markers of molecular mass.

Macro TSH artefact

Thus, in the 'macro hormone' artefact, a specific anti-TSH immunoglobulin binds TSH and neutralizes its biological activity, but leaves epitopes exposed for interaction with the assay antibodies. This is analogous to macro CK, macroamylase and macroprolactin

Case Vignette 3

- 52 year old man
- HPC : 6 months tiredness
  Cold intolerance
  Dry skin
- PMH – Prostatic carcinoma aet 50
- DH : Bicalutamide
- OE: P 60 SR. No goitre
- **FT4 51.0 (44.7)**
- **TSH 16.3 (24.5)**
- TPO (0-100) 2550 TRAb 0.4

Case 3 - clinical vignette

- FT4 19.6 pmol/l (9-22)
- TSH 30.0mU/l (0.4-4.4)
- FT3 2.9 pmol/l (3.0-7.5)

TRH Test

Time (min)	TSH (mU/l)
0	16.3
20	32.2
60	58.9

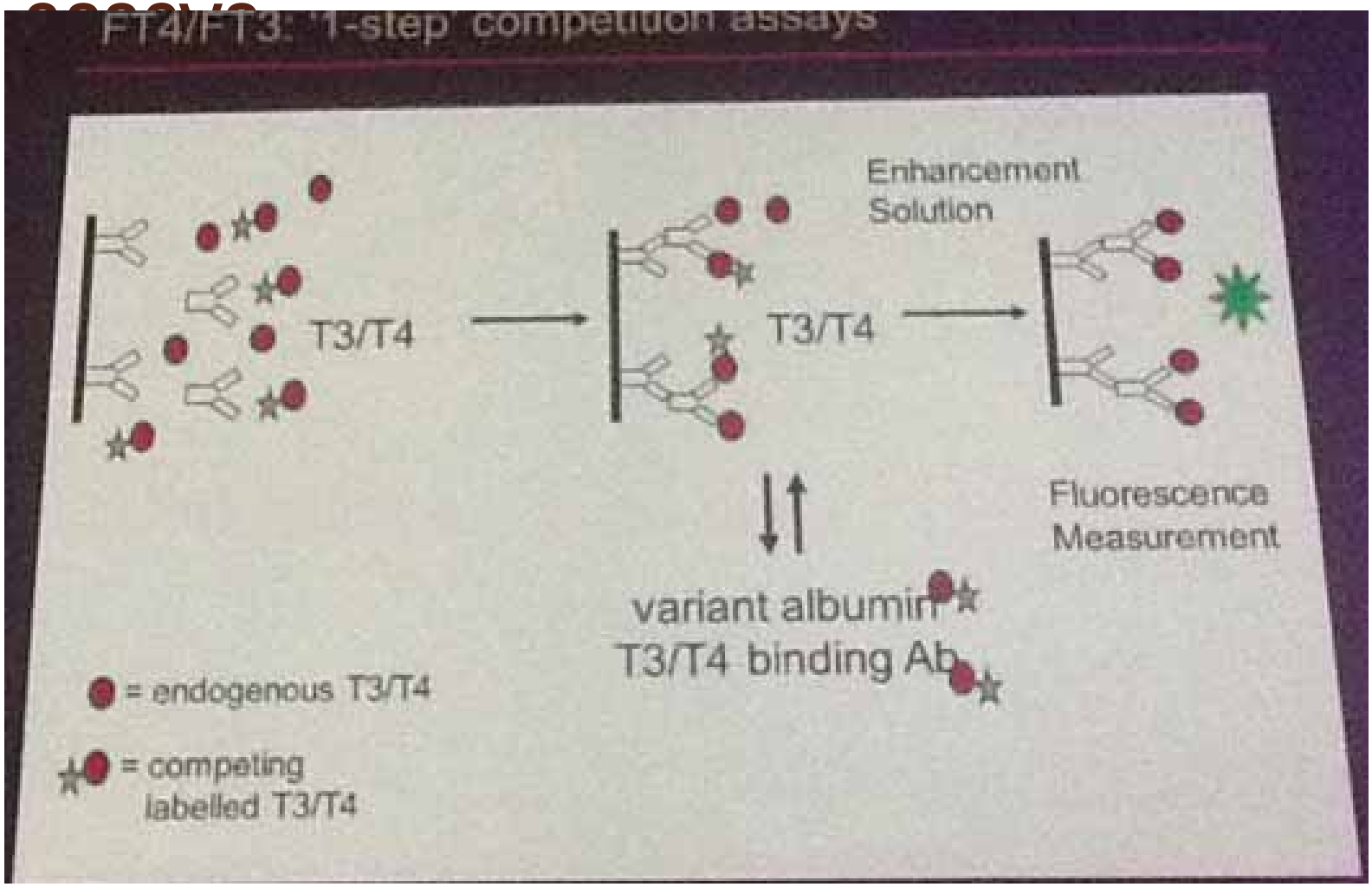
Further Investigations

	Beckman Access	Abbott AxSYM	Delfia P/Elmer DELFIA	Roche E170	Immulite 2500	Bayer Centaur
FT4	2.0	5.0	3.3	19.5	21.6	44.7
TSH	24.5	25.0	22.0	25.0	30.0	-

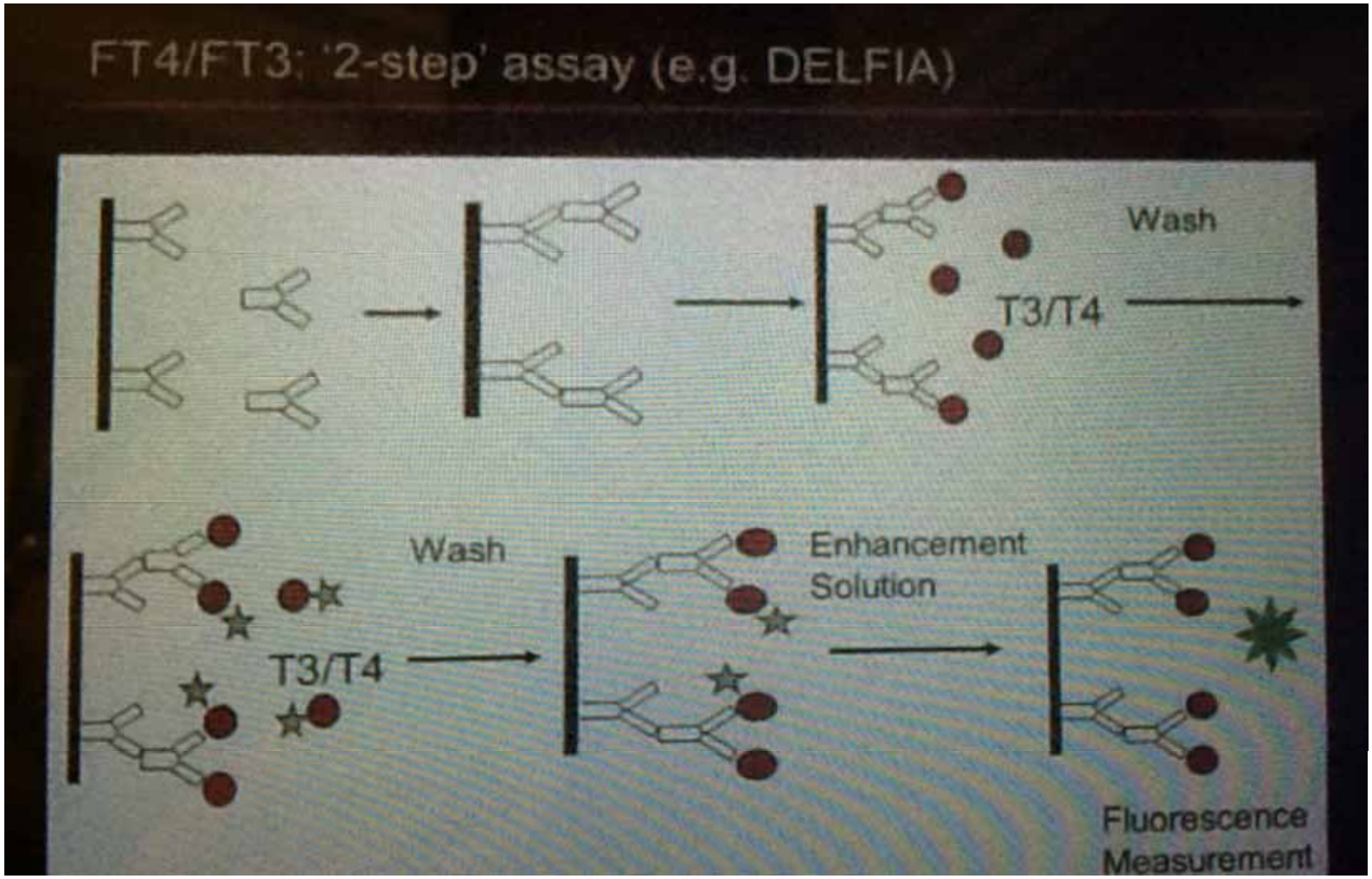
TWO STEP ASSAY

ONE STEP ASSAY

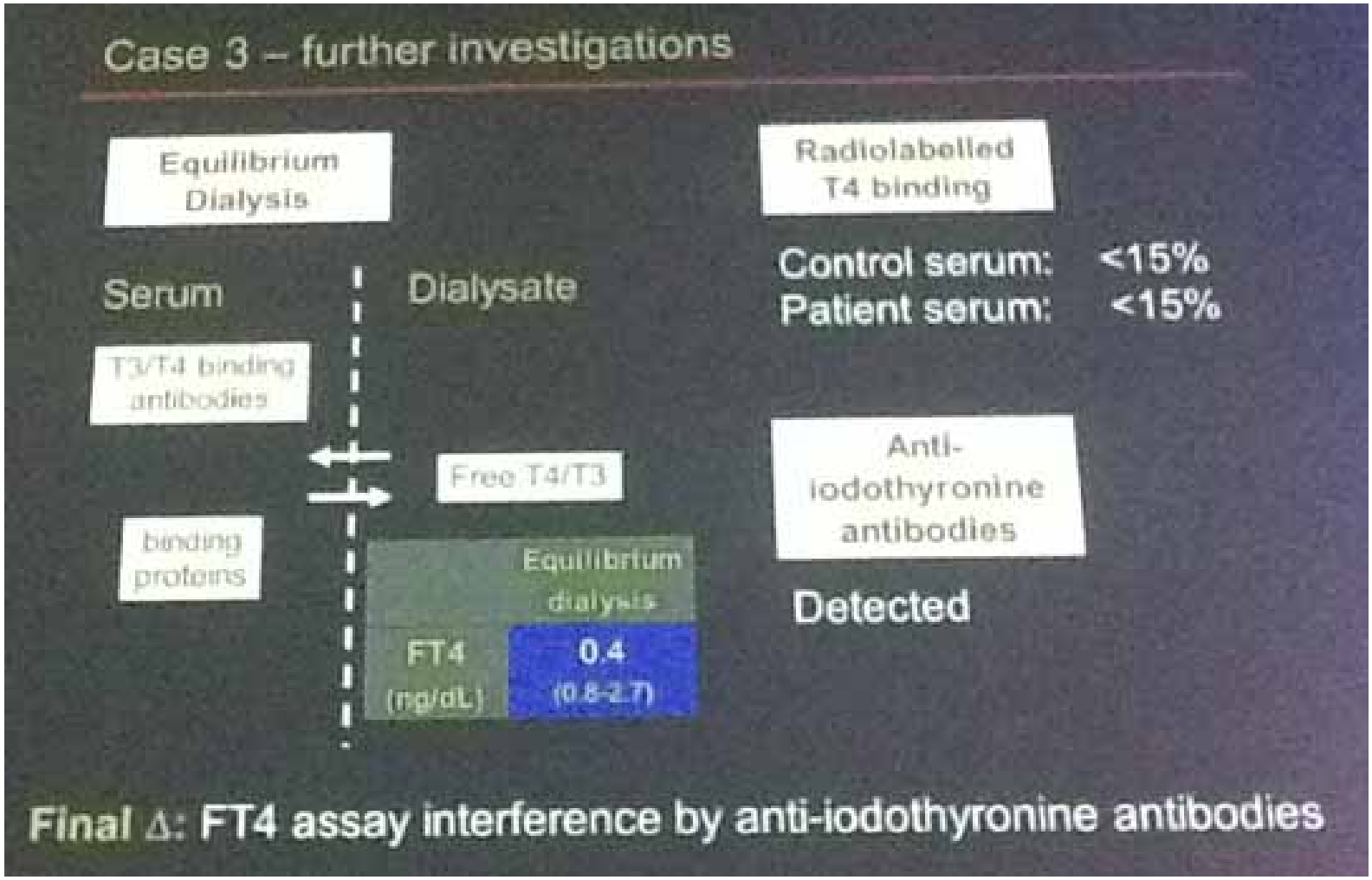
FT4/FT3: '1-step' competition



From a lecture by M Gurnell 2014



Further Investigation



Familial Dysalbuminaemic Hyperthyroxinaemia (FDH)

- T4 binding to subdomain IIA (Tr1) of mutant albumin (leading up to 30% T4 being bound to albumin rather than the normal 10%)
- 2 step assays **may** reduce likelihood of abnormal binding of labelled tracer analogue T4 binding to mutant albumin

Case 4 – clinical vignette

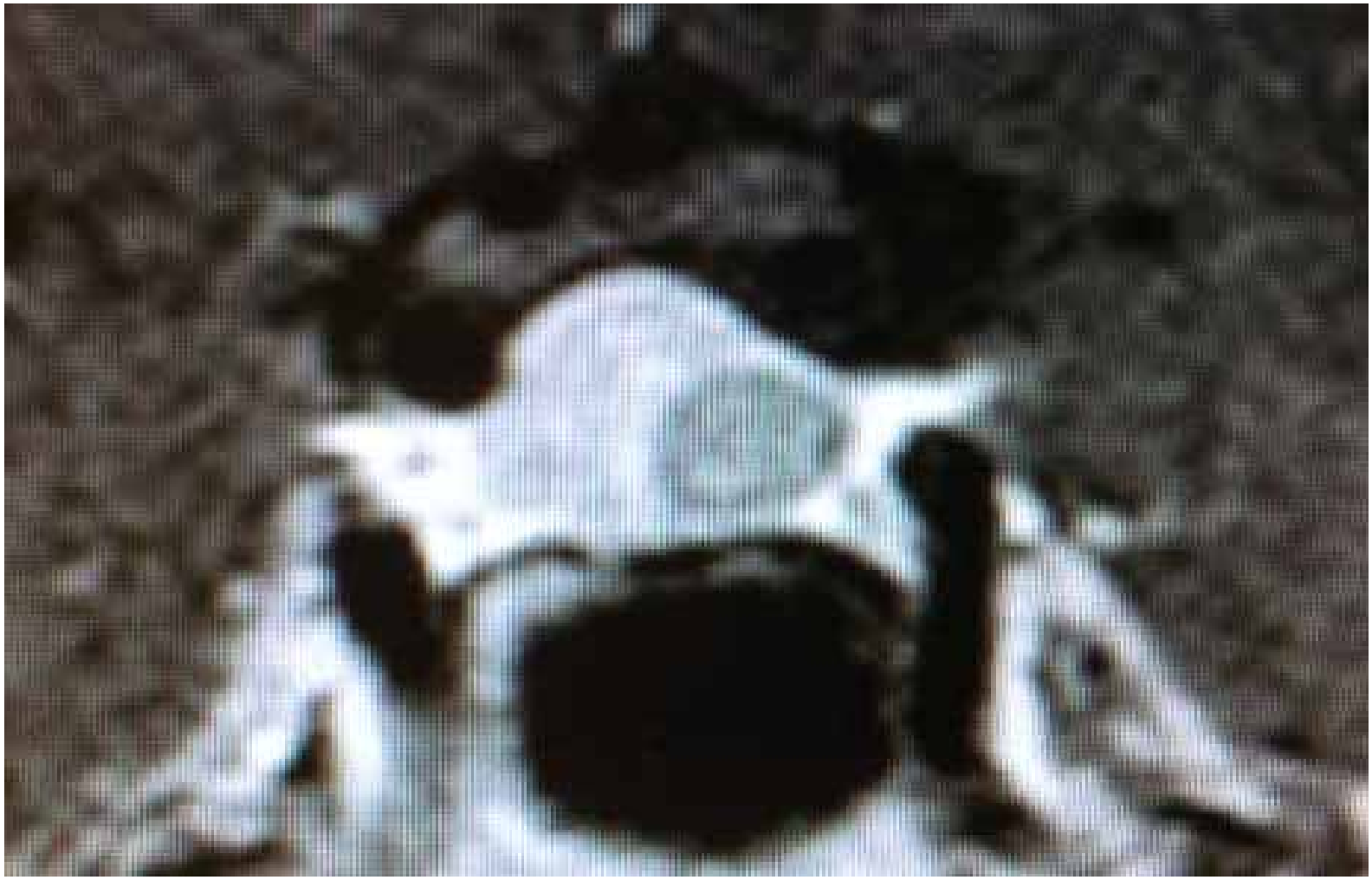
- 26 year old female
- PC Galactorrhoea
- HPC 12 month history of bilateral galactorrhoea, oligomenorrhoea, reduced libido
- No headaches/visual disturbances
- OE Euthyroid, small smooth goitre
- Normal secondary sexual characteristics
- Bilateral galactorrhoea, normal visual fields
- Clinical diagnosis: prolactinoma

Investigations

- Prolactin 5037 (<620)
- LH 5.0, FSH 4.3 E2 180pmol/l (100-750)
- IGF-1 35.2 nmol/l (9.5-45)
- Short synacthen test: F 367-657nmol/l
- **TSH 2.9 FT4 29.2 (9-20) FT3 14.6 (3-7.5)**

Revised diagnoses: Microprolactinoma, Free TH assay interference ??TSHoma

MRI of pituitary (post Gd)



Progress

- No assay interference
- No drug therapy
- No intercurrent illness
- Re-revised diagnoses:

  - TSHoma

  - PRL co-secretion or ‘disconnection syndrome?

Elevated T4/3 with detectable TSH

TSHoma		Resistance to thyroid hormone
No	?affected Relatives	Yes
Elevated	alpha SU/TSH ratio	Normal
Elevated	SHBG	Normal
Adenoma	Pit MRI	No adenoma
No	TRH response	Normal/exagerrated
No	T3 suppression	Yes
Yes	SRL response	No
Normal	THR beta gene analysis	Mutation in >90% cases

Further investigations

- TRH test:

	Patient	
Time	TSH (0.4-4.0)	TSH
0	2.4	3.4
20	23.8	4.0
60	17.0	4.7
SHBG	26	131
alpha subunit	0.45	1.44 (0.4-1)
	TH resistance	**TSHoma**

Elevated T4/T3 with detectable TSH

- Look at the patient – clinical thyroid status
- Confirm elevated fT4/fT3 by equilibrium dialysis / 'two step' method
- Confirm linearity of TSH assayed in dilution
- Exclude confounding drug therapy/intercurrent illness

Interpreting discordant TFTs

1. Re-evaluate clinical history

Be aware of the effects of age, pregnancy changes, LT4 therapy, medications & NTI

2.Re-assess the clinical thyroid status of patient

? Hypothyroid

? Euthyroid

? Hyperthyroid

3.Decide which TFT is most likely to be discordant

4.Exclude TH/and or TSH assay interference

5.Investigate for rare genetic/acquired disorders of HPT function

Discordant TFTs

Step 1: Re-evaluate clinical history

Age

Consider:
- neonatal period
- elderly

Pregnancy changes

Consider:
- ↓TSH (1st trimester; 2° to ↑hCG)
- ↑TT4 & ↑TT3 (from 1st trimester; 2° to ↑TBG)
- changes in FT4 & FT3
- pregnancy RR

Thyroxine therapy

Consider:
- confounding dietary factors or medications
- malabsorption syndromes
- altered TH metabolism
- non-compliance
- other factors (see Table 2)

Confounding medications

Consider:
- amiodarone
- furosemide
- heparin
- corticosteroids
- dopamine
- others (see Table 3)

Non-thyroidal illness (NTI)

Step 2: Re-assess thyroid status

? hypothyroid | ? euthyroid | ? hyperthyroid

Step 3: Decide which TFT is most likely to be discordant

Step 4: Exclude TH &/or TSH assay interference (consider specialist laboratory input)

Step 5: Investigate for rare genetic/acquired disorders of HPT function (consider referral to specialist centre)

NIS mutations

- Biallelic mutations in the *NIS* gene lead to:
- Congenital autosomal recessive iodide transport defect
- Characterized by hypothyroidism, goitre
- Low thyroid iodide / 99mTc uptake
- Low saliva/plasma iodide ratio
- **TSH raised, fT4 low, raised fT3**
- Exclude severe iodine deficiency

MCT8 Mutations

- These cause an X-linked disorder of childhood-onset, with psychomotor retardation including speech and developmental delay and spastic quadriplegia, caused by defects in the *MCT8 (SLC16A2)* gene, encoding a membrane transporter. In addition to neurological abnormalities, male patients exhibit a characteristic pattern of abnormal TFTs with elevated FT3, low FT4 and normal TSH levels

Functional Deiodinase Deficiency.

- The deiodinase enzymes are part of a larger family of 25 human proteins containing selenocysteine. Recently, a multisystem selenoprotein deficiency disorder, manifesting with growth retardation in childhood or other features (male infertility, skeletal myopathy, photosensitivity, hearing loss) in adults, has been associated with a thyroid signature – **raised FT4, normal/low FT3 and normal TSH levels**, because of functional DIO deficiencies.