

Issues in measuring Low Thyroid Function
Comments to a Colleague

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The management of Low Thyroid disease is not so straight-forward in clinical practice, particularly in pregnancy.

The following outline was prompted by discussions with several colleagues, and I thank them for their time and comments. It covers an extensive dissection of some of the literature about Thyroid assays, particularly Free T4 Analog Ria assays.

Some Endocrinologists have been pushing for tighter control of Hypothyroid Disease, but the studies are just not there yet. I contrast the approach we take to Diabetes Mellitus with Hypothyroidism. If a person is acutely sick with high blood glucose, we give Insulin. If a person is sick with low Free Thyroxine levels, we do nothing?

Please accept that this is not as well organized as I would like. There are so many issues. I thank you for your indulgence.

Tim Bilash MD

October 2008

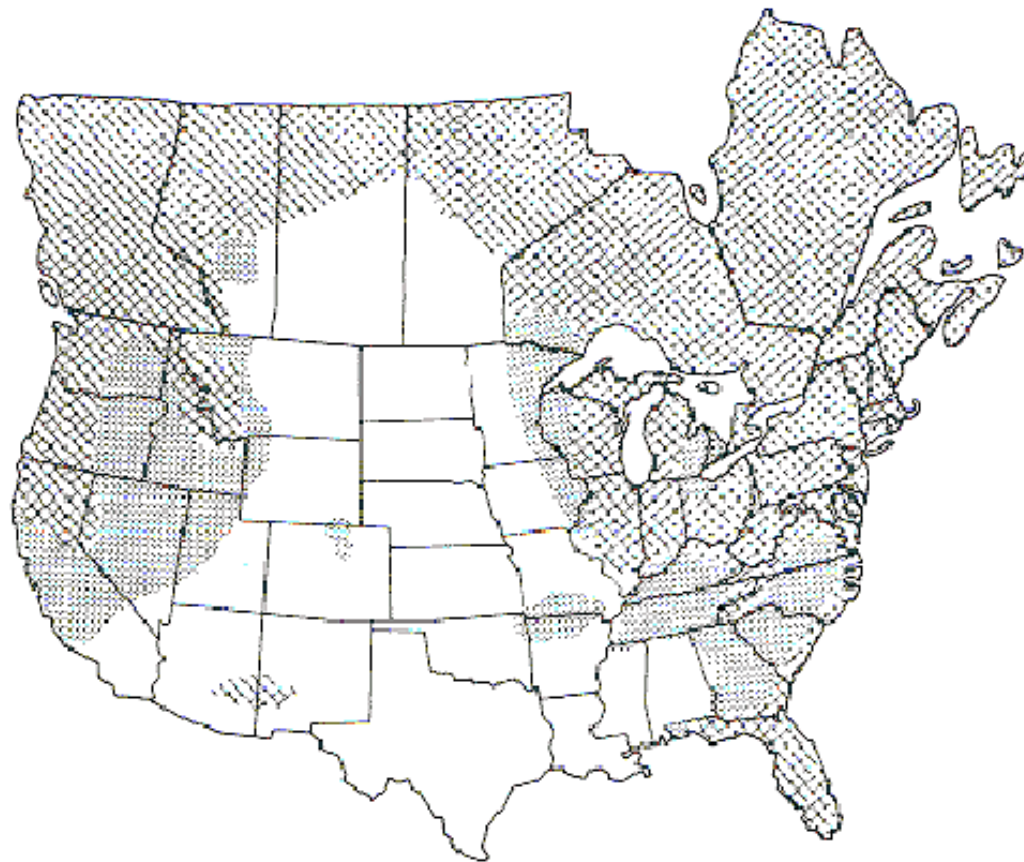
I. Some of the issues in measuring Low Thyroid function




- A. **Se** - As you suggested the role of Selenium in Thyroid disease, I looked for more information on this in the weeks since our meeting. Selenium and Iodide surprisingly appear to both require an optimal range for normal Thyroid function, with too low or too high causing problems (I2~200mcg/day, Se~50mcg/day). I found a map of areas where Selenium is adequate in the soil. It appears adequate in the the mid-continent, but deficient in the coastal soils. There is also some mentions that Se and I2 interfere with each others metabolism. Maybe these minerals need to be considered together as to Thyroid dysfunction? Se increases the conversion of T4 to T3 (which would decrease FT4/FT3 ratios?), I2 increases the T4 production (which would increase FT4/FT3 ratios).
- B. **Extra-Thyroidal Dysfunction** - We talk of Low Thyroid disease as if its only the Thyroid gland that gets sick. I challenge this, and submit that "extra-thyroidal disease", the control of Thyroid function beyond the Thyroid gland (inadequate pituitary TSH, changes in conversion of T4-to-T3, or inappropriately high or defective TBG, for example), has been largely ignored. We claim this is "rare", but TSH screening alone is unable to diagnose these conditions. If we don't consider it, how can we find it? It seems we follow a maxim, "Don't ask, don't look"?
- C. **Thyroiditis** - So we commonly think of Low Thyroid disease as caused either by 1) Low Iodide or the presence of 2) Thyroid gland Antibodies. Both these causes affect the Thyroid gland itself, and increase TSH. Just because antibodies are present does not prove that the antibodies cause the disease. Are there any blocking antibodies that reverse this problem? Are not those antibodies the result of disease rather than its cause, in particular a generalized increase in Immunoglobulins?
- D. **Goiters** - Goiters are uncommon in the patients I see. Classic goiter is associated with Iodide deficiency or Thyroiditis which elevate TSH. "Extra-Thyroidal Hypothyroidism" wouldn't raise TSH if the pituitary or hypothalamus can't respond. It is true some of the first trimester patients I see do show relatively elevated TSH (>3), especially in early pregnancy. So their early pregnancy loss may be associated with this "Thyroid gland doesn't respond/Thyroiditis". But I am finding many with low Free T4 with both TSH<2 and TSH>2 that have disease and respond to Thyroid supplementation.
- E. **Sub-normal FT4 in pregnancy?** - I would be expected that the Thyroid stimulating properties of HCG should raise FreeT4 in Pregnancy compared to non-Pregnancy. Thus I favor changing the pregnancy reference ranges. It is possible that inadequate HCG in some patients fails to compensate for inadequate T4 production demands with increasing TBG in Pregnancy?
- F. **Pregnancy vs NTI** - Pregnancy is different from Non-Thyroidal Illness (NTI). TBG is high 2nd to Estrogen, not low, yet these seemed to be lumped together. The thinking seems to be that low Albumin or Protein (Weak Thyroid-binding Proteins) decreases FT4 levels artifically in these assays. It is true that FT4 decreases from its higher levels as Pregnancy progresses, but the evidence is that it should not drop below the non-Pregnant norms unless there is an Iodide Deficiency or some other factor, especially for a given patient. How do we know "normal" Pregnant patient reference groups do not include patients who are actually Free Thyroxine Deficient and skewing the means? The FreeT4 normogram is extremely non-gaussian, clustering at the low end of normal range in pregnancy. Sapin(03) comparisons show a FT4 decrease in all groups, including pregnant patients compared to non-pregnant references. Why can't this be real hypothyroidim?

Map of Selenium Status in US & Canada

This is a very old map (Kubota et al., 1967). While the areas showing deficient may still be correct I'm aware that there have been other areas deficient in selenium that have been identified in the last decade(s) that are not represented on this map.

"Selenium deficiency is a major problem for livestock or wildlife in at least 37 states and costs beef, dairy, and sheep producers an estimated \$545 million in losses every year." soil scientist Gary S. Bañuelos



-  LOW-APPROXIMATELY 80% OF ALL FORAGE AND GRAIN CONTAIN <math>< 10</math> PPM SELENIUM
-  VARIABLE-APPROXIMATELY 50% CONTAIN <math>< 10</math> PPM SELENIUM INCLUDES ALASKA
-  ADEQUATE-80% OF ALL FORAGES AND GRAIN CONTAIN <math>< 10</math> PPM SELENIUM INCLUDES HAWAII

- G. **True Thyroid Function** - I am puzzled by the arguments that the one step FreeT4 immunoassays are improper for Pregnancy. What determines the "true" Thyroid function? A problem is that as TBG concentration increases, the binding of T4 increases absolutely, and also increases as the ratio of T4-to-T3; FT4 goes down for a given Total T4 as TBG goes up. How do we know that equilibrium dialysis is the gold standard and reflects "true" Thyroid function? On close examination, both Equilibrium Dialysis and Ultracentrifugation may not correlate with clinical disease in this low range (see Fritz ahead). This is surely true of the patients I care for. We really have no good lab measure of thyroid function beyond these assays, and so clinical information becomes important.
- H. **Total T4 in Pregnancy** - Recently some are advocating that Total T4 as the most appropriate measure in Pregnancy (Fritz 2007, Nelson OBGYN News 2007). Perhaps it is because at high-T4 and high-FT4 levels, FT4 follows the TBG which is the Total T4. TBG is highly T4-bound with fewer available binding sites, and so they would pretty much measure the same thing. This does not appear to be the case for low-FT4 patients in late Pregnancy who have high TBG.
1. Fritz's paper appears inconsistent on the face of it, since different FT4 assays do not all give the same results with a given TT4. This is probably truer in the high-FT4 range of the assays, where FT4 approximates TT4, but not the lower hypothyroid range.
 2. They also looked at only one Labeled-Analog FT4 Assay, not representative of the diverse assays.
 3. None of the samples contained T3 as would be in normal serum, and certainly that would change the TBG-T4 saturation (see Maberly 1986 T4/T3).
 4. **Criticism of Fritz Total T4** - Midgley has written a reply to the latest Fritz claims (*Clinical Chemistry* 53: 1714, 2007), which went unanswered, that the conclusions in the Fritz paper are improper.
 - a. "I read with misgivings the recent communication by Fritz et al. (1) alleging that because under some circumstances an analog free thyroxine (FT4) immunoassay correlates total T4 and FT4 values, it does not measure FT4 but something akin to T4. I have shown repeatedly through mass action analyses that many experiments, including the one reported by Fritz et al. and others described in earlier papers, cannot meaningfully address the working of any of these assays. Allegations of shortcomings cannot be substantiated if they depend on experiments using artificial T4 solutions in which serum T4-binding proteins are either lacking or are present at concentrations insufficient to prevent overlarge T4 abstraction by the assay antibody (conditions strictly invalid for FT4 measurements)."
- I. **Sick-Pregnant patients** - I showed you some data on Sick-Pregnant patients with evidence of Thyroid disease. They displayed Medical (preterm labor, recurrent infections, PIH, DM) and Obstetric problems, and/or Low-Thyroid symptoms. They demonstrated Low FreeT4 (usually one-step non-analogue assay), with both TSH<2 and TSH>2. When treated with small doses of L-Thyroxine, they quickly improv . The plot of logTSH vs FT4 appears linear for these patients, supporting that the Thyroid gland on average is responding appropriately to its signals, but somewhere else there is a problem. It seems to me that it is the Pituitary Gland itself.
- J. **Sick-Neonates** - Studies using these one-step assays are showing Hypothyroid Clinical improvement in Children who test at Low FreeT4. Current opinions about these assays seem to ignore the clinical effects of supplementation in many patients. We concentrate on the fine points of assays, speculating and arguing back and forth, but prospective clinical studies to treat symptomatic Pregnant patients who test with Low FreeT4 to observe a possible response have not been done. These infants improve their respiratory symptoms as they improve their FT4 levels using these "inaccurate" assays. Something must be going on here. (TBG in infants?).
- K. **Opinion instead of observation** - The arguments about Albumin binding are an example of the dilemma. Serum Albumin is said to interfere with the accuracy of FT4 assays (a tradeoff to adjust for fatty acid effects, vs dilution at low albumin levels in the assays, vs low serum Albumin levels). These arguments are all over the map and don't stand up to closer scrutiny as outlined here. Another past claim that TSH is the measure of "true" Thyroid function. This is certainly now unsupportable, yet most physicians still use TSH only even to quantify replacement dosing. Opinion continues to abound masquerading as expertise.

- L. **Thyroxine toxicity** - I have heard Pediatricians and Family Practice Physicians express a concern that Maternal Thyroid supplements in Pregnancy will cause Fetal damage (Craniosyntosis). This is not shown to be the case.

First of all, the reverse is well evidenced across every Gestational Age, that infants born to Mothers with FreeT4 below the 10%-of-normal range (by current assays not ED) show neurological damage and developmental delay.

Second, there is strong evidence that TypeIII-Deiodinase in the Placenta prevents the pasage of excessive FreeT4 or FreeT3 to the Fetus. When Hyperthyroid mothers are normalized with Propylthiouracil, the FreeT4 by these one-step assays are kept in the high-to-above-normal ranges to avoid Fetal damage from Low Thyroid levels. Using small doses to raise Mom's FT4 levels to mid-normal has no expected risk in comparison.

II. Assay Effects

A. Some Types of FT4 Assays

1. Labeled-Equilibrium Dialysis/Ultrafiltration Assay (indirect - direct no longer used)
2. One-step Labeled FT4 Assay
 - a. Fluorescence Assay
 - b. Chemoluminescence/Magnetic Assay
 - (1) Amerlite (Horseradish-peroxidase-labeled T4 with enhanced Chemoluminescence)
 - (2) magic-Lite (Acridinium-ester-labeled T4 with enhanced Chemoluminescence)
3. One-step Labeled Analogue Assay
 - a. FT4 assays vary depending on the binding of the FT4-analogue to Albumin and TBG. This is a major effect for all analogues, an effect that would depend on the relative binding of analogue to TBG, Albumin and other Weak-Protein-Binders
 - b. Direct modified analogue assays supposedly are not affected by albumin addition
 - c. Specific Assays
 - (1) Amerlex-M (I125-labeled analog of T4)
 - (2) Coat-A-Count (I125-labeled analog of T4)
 - (3) Seria (I125-labeled analog of T4)
4. One-Step Labeled Antibody Assay
 - a. Amerlex-MAB
5. 2-step Labeled-Back-Titration Assay
6. 2-step Chromatography-FT3 RIA Assay
 - a. Lisophase

B. Serum Albumin effects

1. Close examination of the lo-Albumin/FT4 correlation demonstrates it is the high-Albumin/ high Free T4 patients that are skewing the means and normal ranges higher.
2. Low Serum Albumin appears to lower the FreeT4 assay, but it demonstrates a threshold effect, not correlated the group with serum albumin either below an albumin threshold of 4 g/L, or the other group for serum albumin above 4 g/L (see ahead). The FT4 at Albumin of 3.5 g/L appears to be lower than FT4 at Albumin of 4.5 g/L.
3. This certainly could alter the assay because of assay calibration differences.
4. Another possibility is that in patients who demonstrate low FT4, hypothyroidism causes the decreased Albumin. ED/UF would be insensitive to this.

C. Assay Albumin effects

1. Increasing Albumin concentration in the assay does not alter the measured FT4 level
2. Increasing Albumin concentration in the assay does raise some measured FT3 assays (+10% at 1g/L, +30% at 3g/L for Amerlex-M)

D. Serum TBG

1. FT4 assays appear to be correlated with TBG concentration. A 20x-dilution serum is estimated to raise the FT4 by 8% because of changes in FT4/FT3 binding. A doubling of the TBG concentration is estimated to lower FT4 by 1%. This would affect the measures by ED or UF to a bias higher, not lower (see ahead).

E. Low blood volume

1. Osmotic concentration artificially raises the FT4 level (see chiroweb.com/archives/18/07/03).
2. Not discussed as to effects in Sick patients or Pregnancy.

III. Specific Issues

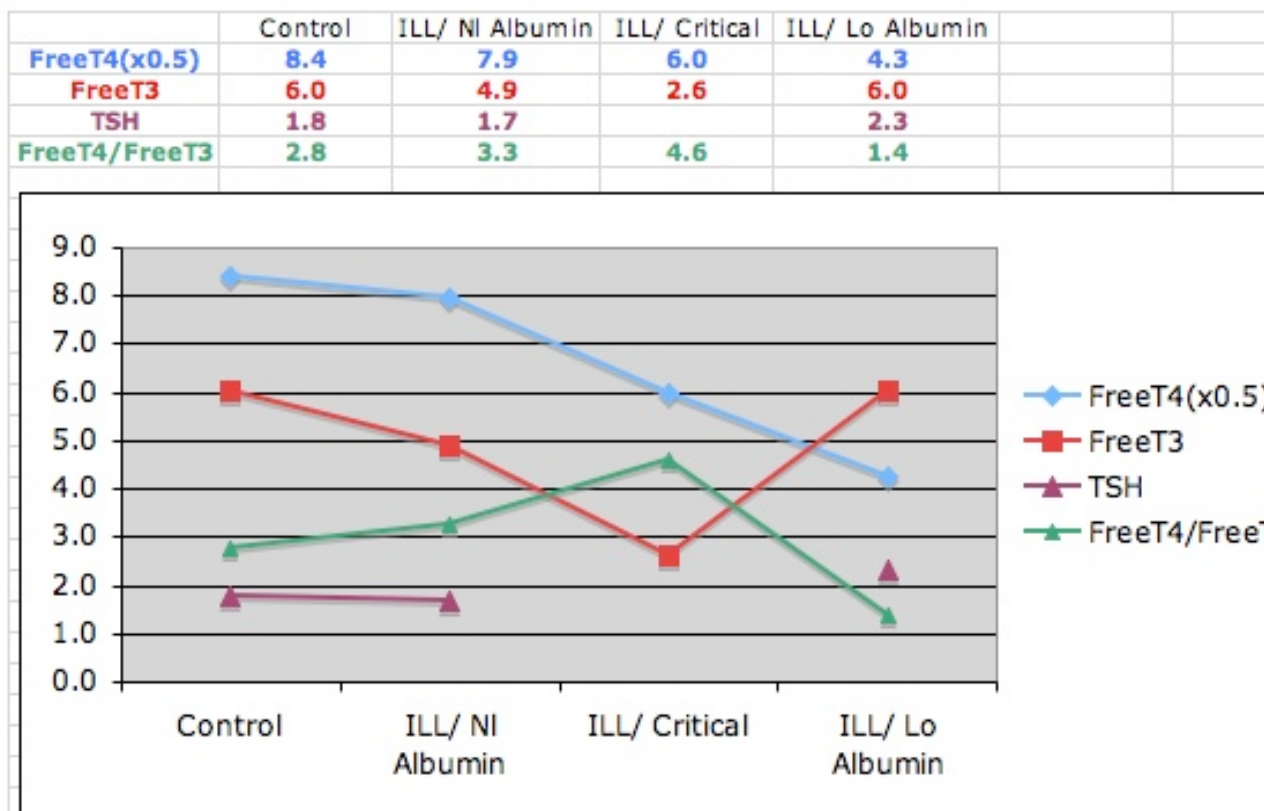
A. TBG/Thyroxine levels & Pregnancy

1. TBG concentration change is not a factor for measuring FT4 in Pregnancy. Maberly(86) looked at TBG-binding of T4&T3 at serum dilutions, and although "the distribution of thyroxin and triiodothyronine among the binding sites on TBG changes with [extreme] variations in TBG concentration" (see ahead), the TBG concentration only doubles in Pregnancy with negligible effect.
 - a. In Pregnancy, T4 increases 15%, T3 increases 50%, while TBG increases 150%
 - b. In Pregnancy there are thus fewer unoccupied T4 binding sites on TBG due to more T3 bound to them, but there is less bound FT4 to TBG.
2. The T4 to T3 binding-to-TBG ratios (TBG:T4/TBG:T3), are different in Sick(=5 ratio), Normal(=2.4 ratio), and Pregnant(=1.6 ratio) patients (using TDX and Immuophase-RIA). These are due mainly to the to FreeT3 level changes, not changes in the TBG binding constants.
3. Pregnancy increases FreeT4/FreeT3, and Sickness decreases FreeT4/FreeT3 compared to Normals. This does not seem to be considered in the discussions of FT4 assays in Pregnancy.

B. Ill patients and Low Albumin effects

1. Ill patients who are critically ill (nl albumin) or are severely ill and have low albumin measure low FT4 were compared to controls (using the Amerlix-M assay, from Desal 87).
 - a. Assays:
 - (1) **Amerlex-M, a direct-labeled-analogue** assay. The Amerlex-MAB uses a modified analogue that is supposed to block added-albumin effects.
 - (2) **DPC, a direct labeled-analogue** assay is not supposed to be affect by the addition of albumin. []
 - (3) The addition of Albumin to these assays did not affect FT4 levels, but increased FT3 for Amerlex-M and not DPC. The addition of Albumin to these assay should not affect the FT4 levels for Ill-lo-albumin patients.
 - (4) It is possible that the Albumin-bound T4 is an important component of Throid function at the local level (see TBG release ahead), maintaining FT4 available for uptake in tissues like the liver at times when TBG increases its hold on to T4 (Pregnancy).
 - (5) It appears that the severity of the illness, not albumin concentration, determines the low Thyroid function results in these patients. Many severely ill patients with high albumin show marked lowering of Thyroid hormones, and patients with low Albumin have normal Thyroid hormones.

2. Free levels vs Albumin, Illness Status

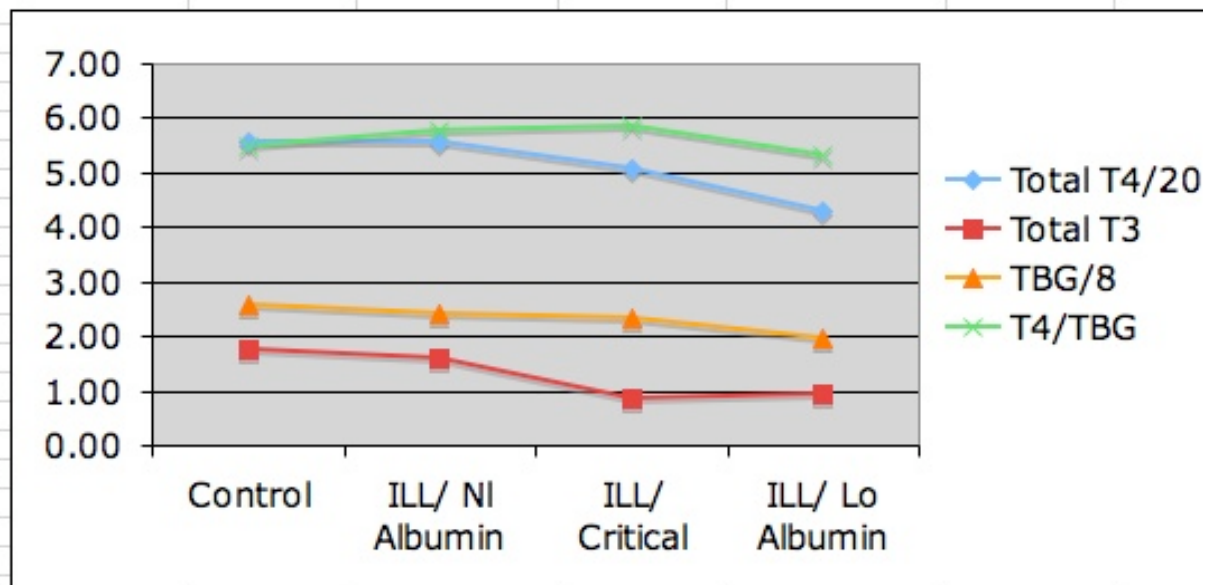


3. I propose that hypothyroid ought to be classified by the FT4, FT3 and FT4/FT3 (FT3/FT4) Ratio status. It seems to me that adding FreeT4/FreeT3 ratios to FT4 would be useful in evaluating whether hypothyroidism needs to be supplemented with Thyroxine. I interpret the Free Hormone Levels and Ratios in the above Desal patients in the following manner:

- a. **"Sick Euthyroid"** (inhibited peripheral conversion T4>T3)
 - (1) Mildly Ill patients (nl-albumin)
 - (2) **nl-FT4**, slight-lo-FT3
 - (3) nl-FT4/FT3 Ratio
 - (4) **decreased conversion T4 to T3**
 - (5) nl-TSH
- b. **"T3 Hypothyroid"** (lo peripheral conversion T4>T3)
 - (1) Critically Ill patients (nl-albumin)
 - (2) slight-lo-FT4, **Lo-FT3**
 - (3) hi-FT4/FT3 Ratio
 - (4) **lo conversion T4 to T3**
 - (5) (undocumented-TSH)
- c. **"T4 Hypothyroid"** (enhanced peripheral conversion T4>T3)
 - (1) Severely Ill patients (lo-albumin)
 - (2) **lo-FT4**, nl FT3
 - (3) lo-FT4/FT3 Ratio (increased T3/T4 ratio)
 - (4) **hi conversion T4 to T3**
 - (5) nl to increased-TSH

- (6) Why couldn't the increased FT3 prevent a rise in TSH in these patients, so they wouldn't measure as hypothyroid? Is this like iodine restriction?, NTI?
- d. **"T3,T4 Hypothyroid"** (normal peripheral conversion T4>T3)
- (1) **lo-FT4, lo-FT3**
 - (2) **nl-FT4/FT3 ratio**
 - (3) **nl conversion T4 to T3**
 - (4) Why couldn't these patients have low albumin caused by hypothyroidism, not low Thyroid tests caused by low albumin? See ahead for the discussion on Albumin effects.
4. The **Total T4 and T3 results** from this paper also support the above ideas (Desal 87).
- a. TT3 decreases more than TT4 in the Critically-ill-nl-albumin patient (low T4-to-T3 conversion).
 - b. TT4 and T4/TBG Ratio are lower in Severely-ill-lo-albumin. The TSH is also higher in these patients. These are consistent with these patients having true low Thyroxine and being Hypothyroid.

	Control	ILL/ NI Albumin	ILL/ Critical	ILL/ Lo Albumin
Total T4/20	5.57	5.56	5.07	4.28
Total T3	1.75	1.58	0.88	0.94
TBG/8	2.58	2.43	2.32	1.98
T4/TBG	5.50	5.77	5.85	5.31



C. TBG Binding/ Assay Dilutions -

1. FT4/FT3 ratios from TBG binding percentages changed with extreme serum dilution, which is approximately linear at low dilutions (Maberly 86, Amerlex-RIA).

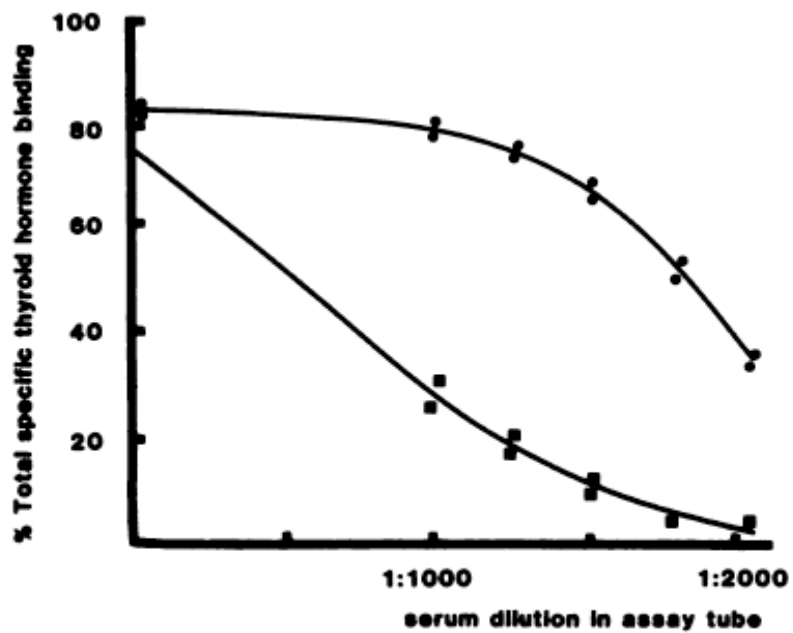
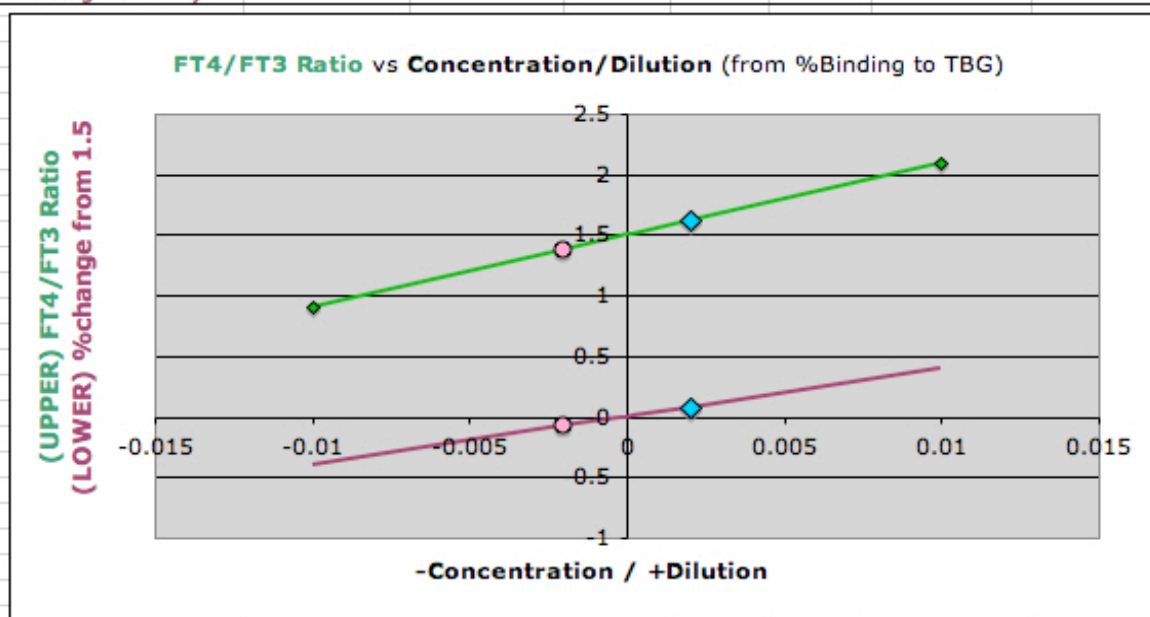


Fig. 1. Total specific T4 (●) and T3 (■) binding at increasing dilutions of sera in assay buffer, each dilution assayed in duplicate

- FT4/FT3 Ratio thus increases an estimated +8% for a 20x dilution, +1% for a 2x dilution. Assays that dilute the sample would increase the measured FT4/FT3 artificially, mostly from an increased measured FreeT4 (FT3 changes are negligibly over this range) when TBG is high (as in PRegnancy).
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Serum Dilution increases FT4 assay values artificially							
	T4TBG (est)	T3TBG (est)	FT4	FT3	FT4/FT3 ratio	%cng(FT4/FT3)	
Concentr(-)/Dilut(+)			1-(T4TBG)	1-(T3TBG)		(from no dil)	
-0.01 (100:1)	0.85	0.83	0.15	0.17	0.9	-40%	
0.00 (1:1)	0.75	0.83	0.25	0.17	1.5	0%	
0.01 (1:100)	0.65	0.83	0.35	0.17	2.1	40%	

	Serum Concentration			Normal	Serum Dilution		
	100x	20x	2x		1x	1/2x	1/20x
Concentration/Dilution	-0.01	-0.002	-0.0002	0	0.0002	0.002	0.01
FT4/FT3 Ratio	0.9	1.38	1.49	1.5	1.51	1.62	2.1
%cng(FT4/FT3) from no dil (~ = %cng from FT4)	-40%	-8%	-1%	0%	1%	8%	40%



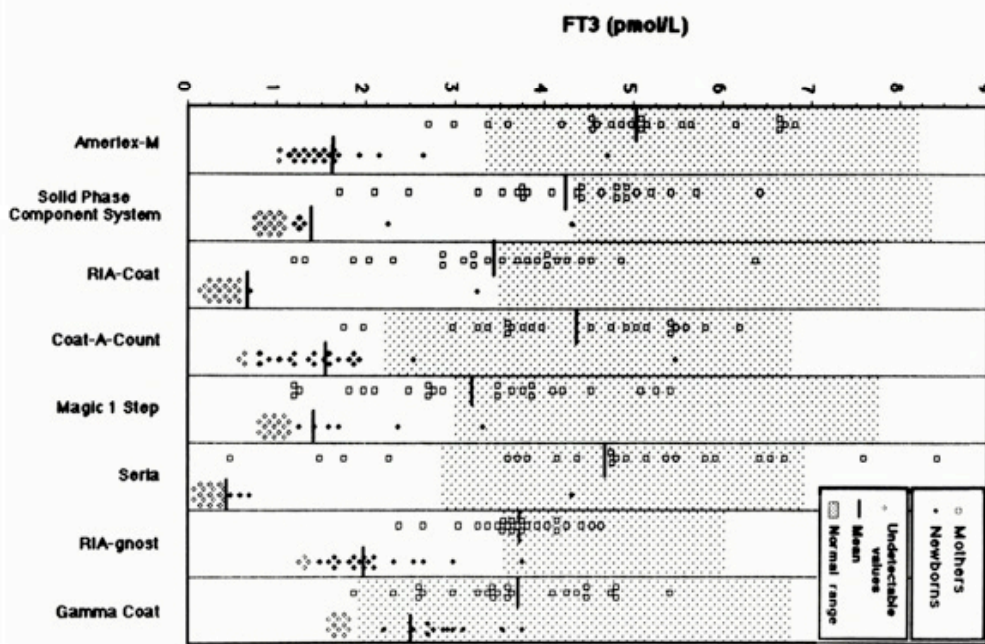
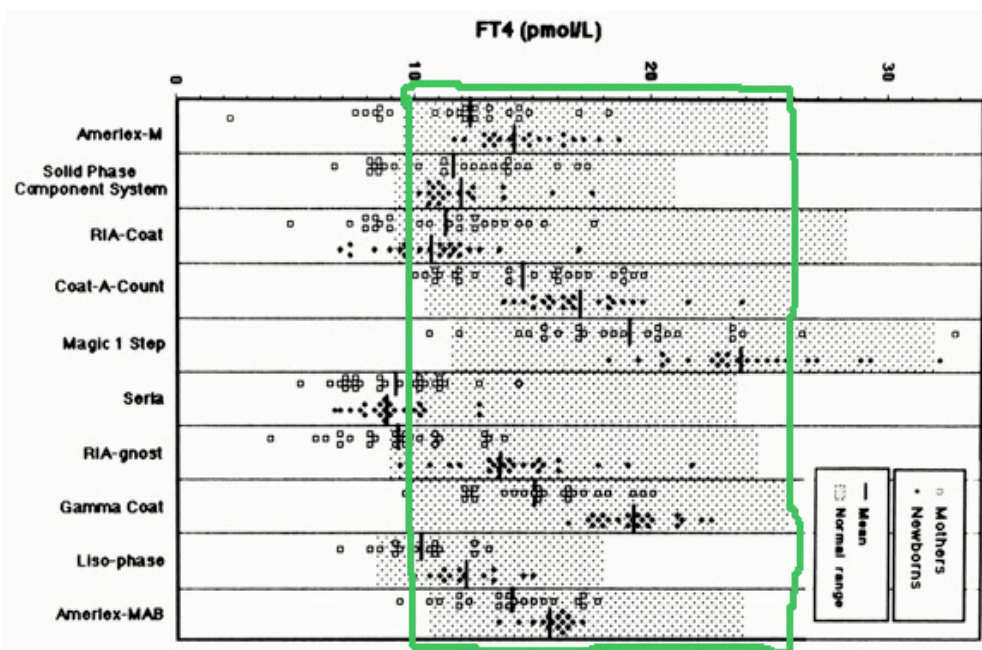
- A doubling of the TBG concentration, as in Pregnancy, would cause a -1% decrease in T4/T3 Ratio by this, a negligible difference.
- What could be different in pregnancy for non-diluted serum vs dialyzed filtrate could be the amount of T3 bound to TBG. The Bound-T4/T3 ratio increases with dilution, so that serum FT4 assays that dilute samples like Ultrafiltrates or Equilibrium Dialysis will measure *higher* FT4. "If the [FT4] method relies simply on estimating the TBG concentration and expressing this as the product of the concentration of total hormone, the method will not account for this redistribution of T4 and T3 among sites." Assays are affected differently by this. TDX estimated lower FT4 in pregnancy, while FTI, Corning and Amerlex estimated higher FT4 in pregnancy.
- It does not look to me that ED would be such a gold-standard in Pregnancy.

D. Assay comparisons in Pregnant patients (Roti et al 1991, in Glinoeer 1997).

1. The Coat-A-Count, Magic, Gamma Coat and particularly the Amerlix-MAB assays all show the pregnant patients within the assay norms. These assays appear less sensitive to decreased FT4 at low levels.
2. Factors that can affect assays
 - a. Assays require external calibration because they don't determine concentrations directly
 - b. Influence of serum levels or binding properties of TBG, albumin and FFA associated with pregnancy
 - c. Excessive dilution of serum stripping T4 from TBG producing an increased FT4 artifact (as noted above)
 - (1) TDX estimated lower FT4 in pregnancy, while FTI, Corning and Amerlex estimated higher FT4 in pregnancy. (Maberly 86).
 - d. Iodine deficiency can lower FT4 by 10-15% with increase in TSH as pregnancy progresses

3. Graph of FT4 Assay comparison

Glinner, D. Endocr Rev 1997;18:404-433



ENDOCRINE
REVIEWS

4. These results do not support Fritz's(2007) and Nelson's (OBGYN News 2007) statements that FT4 measures Total T4. See above.

E. FT4, Albumin and Non-Thyroidal Illness (Midgley 1990)

1. I find that this paper clarifies the effects of serum Albumin and NTI on FT4 assay measurements.
2. Two FT4 assay types were used
 - a. The first assay was a Labeled antibody (Amerlex-MAB RIA/Amerlite Luminescence), thought to be resistant to serum Albumin levels
 - b. The second was an Analog-RIA (Amerlix-M), thought to be affected by serum Albumin levels.
3. 2 groups were compared
 - a. First group was "**Euthyroid Reference**" (Euth). These were non-pregnant, ambulatory "with suspected thyroid disease..., found to be euthyroid by clinical and biochemical diagnosis". This pre-presumes that the group with ranges <4.5 were euthyroid. There is currently no definitive laboratory way to demonstrate this. The best is to presume that they were more euthyroid than the other group. Patients who are hypothyroid with low FT4 and normal TSH would be included in the Euthyroid group.
 - b. Second group was "**Severe Nonthyroidal Illness**" (sNTI). These were non-pregnant, older, with illnesses ranging from heart disease to severe malnutrition. Most Amerlix-M sNTI values in this group were subnormal, but this was the assay partially used to identify them as sNTI patients in the first place.
 - c. The sNTI group had serum albumin levels <40g/L, whereas the Euth group had albumin levels >40g/L.

4. Lab scatter plots, Euthyroid vs sNTI

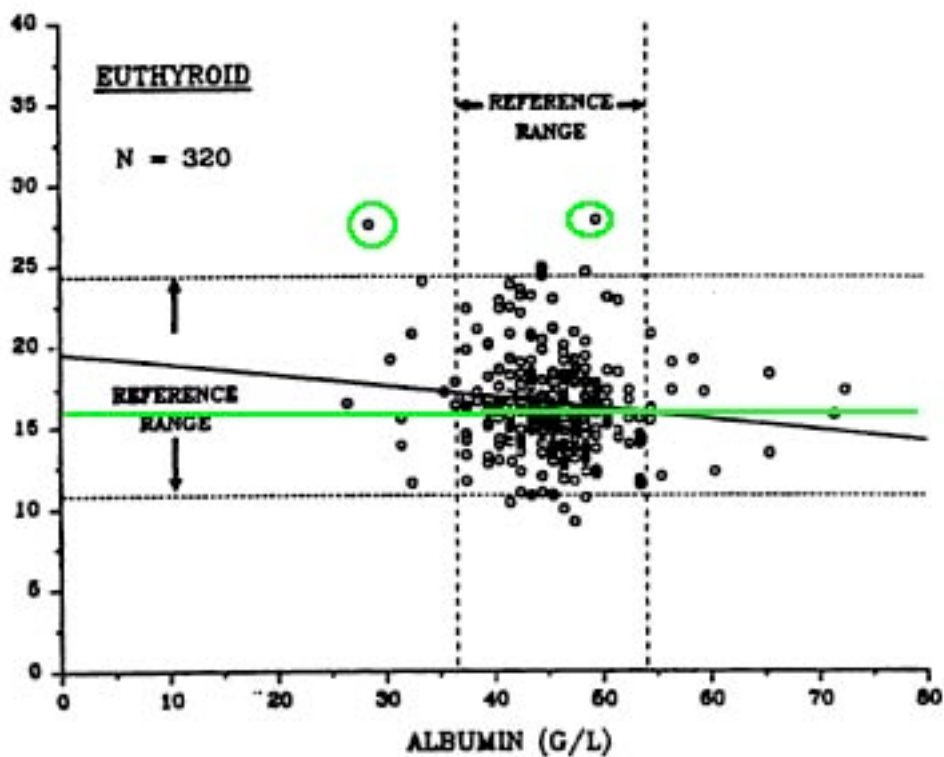
a. Lab normograms

- (1) I was puzzled that the distribution for both assay results appeared gaussian in shape. This is in contrast to the distributions in Pregnancy, where the FT4 values bunch in the low 20% of the range. This would be significant when comparing pregnant to non-pregnant patients, because the different shapes drastically undermine any statistical comparison.

b. Euthyroid Group (Euth)

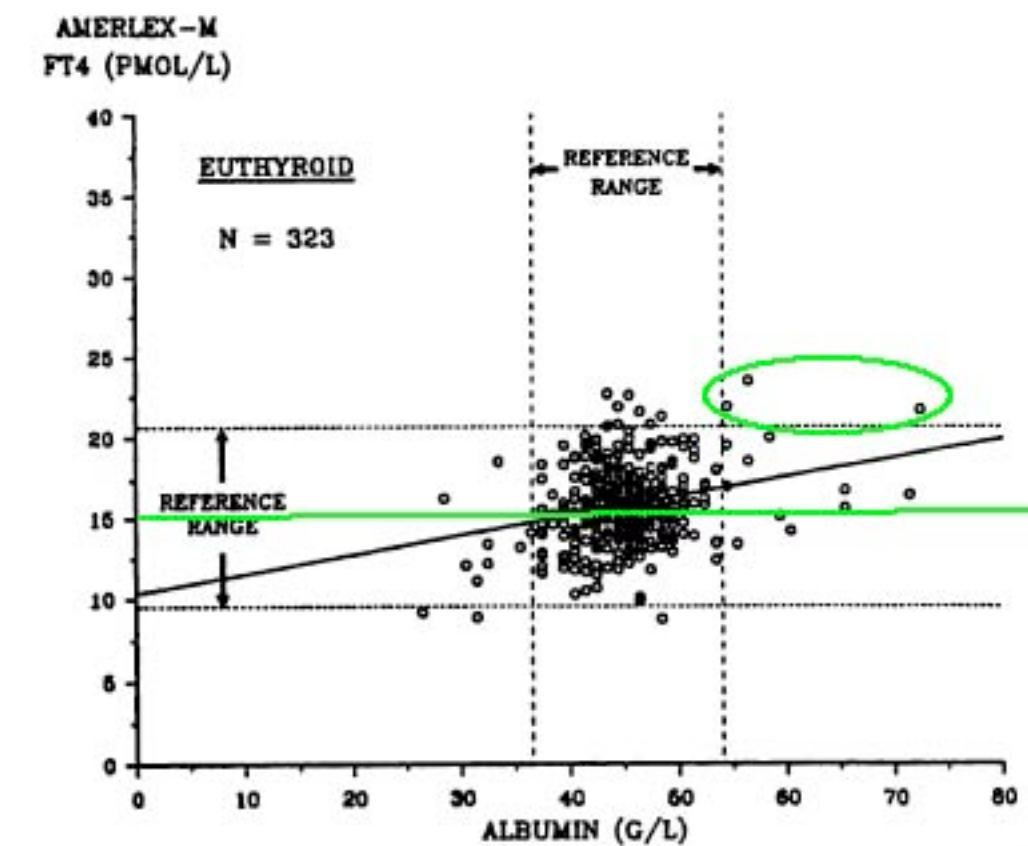
- (1) Most Euth patients had serum Albumin > 40g/L.
- (2) **Euth group/MAB assay (E-MAB)**
 - (a) The mean FT4 was 16.5 pmol/L, with essentially all in the FT4 nl-ref range.
 - (b) Most MAB assay measurements are in the euthyroid range, essentially **independent of serum albumin levels above 40 g/L**.
 - i) The slight negative trend with albumin disappears when one patient of the 320 with an outlying hi-FT4 value is removed from the analysis (28pmol/L; albumin 28g/L, lo-albumin range). This demonstrates how tenuous these correlations are.
 - ii) The mean FT4 also drops slightly, estimated here as 16 pmol/L, if the 2 outlier hi-FT4 patients are omitted. It appears that the higher-FT4 patients may skew the MAB assay towards higher mean FT4 and higher normal ranges.
- (3) The MAB assay identifies the "euthyroid" patients as euthyroid.

AMERLEX-MAB
FT4 (PMOL/L)



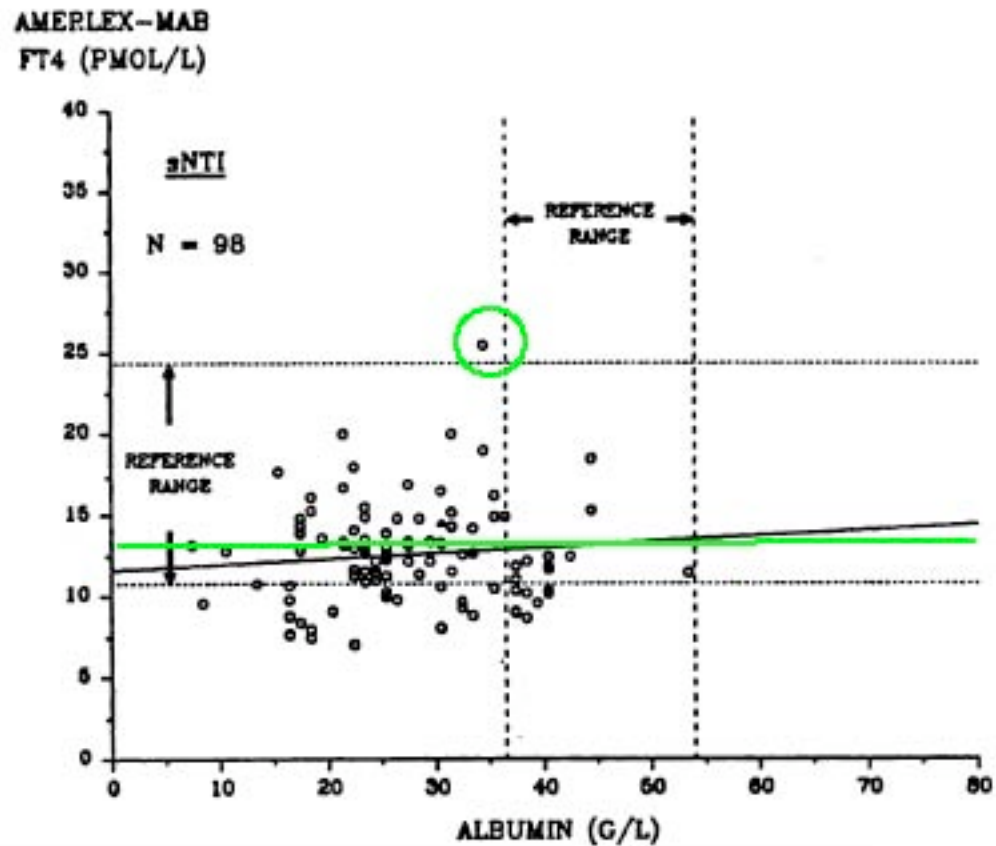
(4) **Euth group/M assay (E-M)**

- (a) The mean FT4 was 15.9 pmol/L, essentially the same as the MAB assay, in the FT4 nl-ref range.
- (b) A correlation of increasing FT4 with increasing serum albumin is claimed in the paper. However, I contend that removing the 3 *hi-FT4* values (>24pmol/L normal) with hi-albumin (> 54g/L) collapses this correlation. Further removing all hi-out-of-range FT4 patients would further worsen any correlation.
- (c) Most M assay measurements are in the euthyroid range, as with the MAB assay, essentially **independent of serum albumin levels above 40 g/L.**
- (d) The patients who measured *hi-FT4* at low albumin with MAB did not correspondingly do so with the M assay. This indicates the possibility that the **MAB assay has a constant bias to higher FT4** when serum albumin is < 40 g/L, rather than the **M assay having a linear bias to lower FT4** with albumin.
- (5) The M assay identifies the "euthyroid" patients as euthyroid.



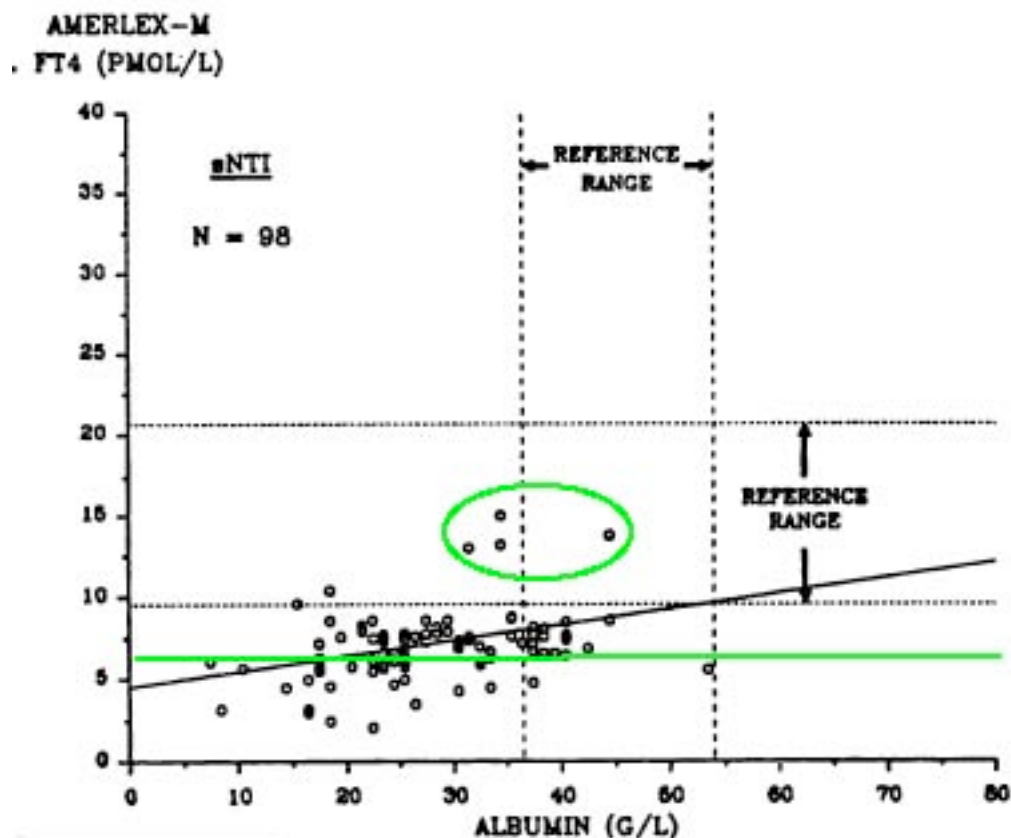
c. sNTI Group, Sick Nonthyroidal Illness (sNTI)

- (1) Most sNTI patients had serum albumin < 40g/L.
- (2) **sNTI group/MAB assay (sNTI-MAB)**
 - (a) The mean FT4 was 12.7 (lower than Euth/MAB), most were below the mid-normal range value, with 22% sub-normal.
 - (b) FT4 levels were independent of serum albumin below 40g/L
- (3) The MAB assay measures a small constant shifted lower FT4 than the euthyroid group (-3.8 pmol/L).



(4) **sNTI group/M assay (sNTI-M)**

- (a) The mean FT4 was 7.0 (lower than Euth/M), and all were below the mid-normal value, with most sub-normal using the M assay.
- (b) A correlation of increasing FT4 with increasing serum albumin is claimed in the paper. However, if the 4 relatively *hi-FT4* values are again removed (>12pmol/L), then there appears to be **essentially independent of serum albumin below albumin 40g/L**.
- (5) The M assay measures a constant lower FT4 than the euthyroid group (-9.5 pmol/L) in the range < 40g/L.

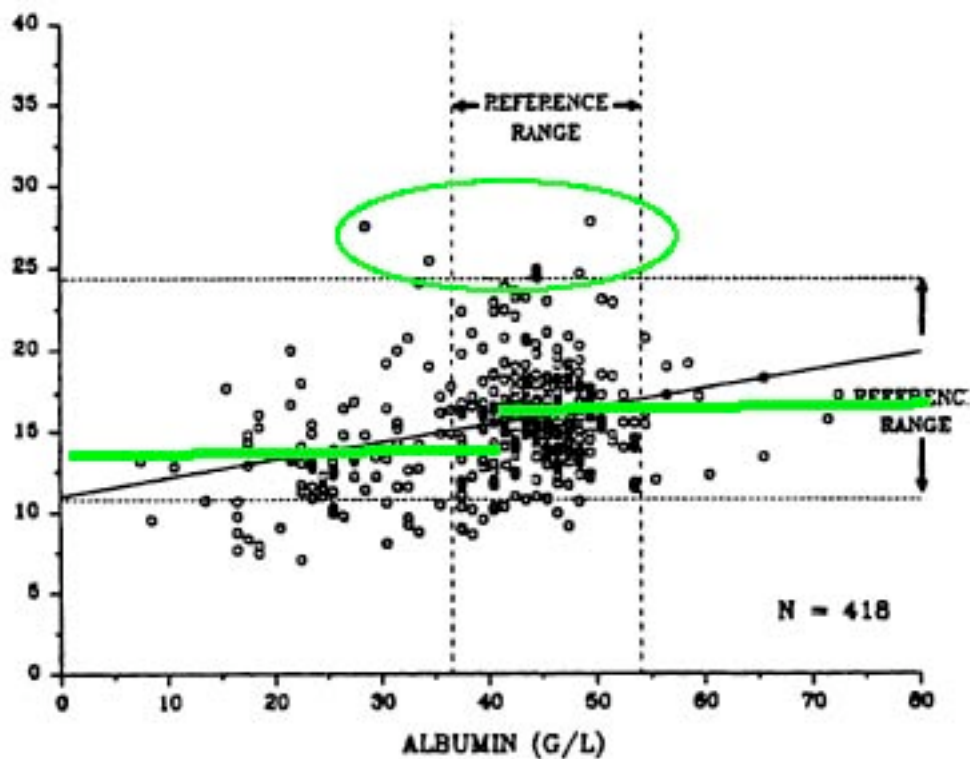


d. **Combined Euthyroid+sNTI groups**

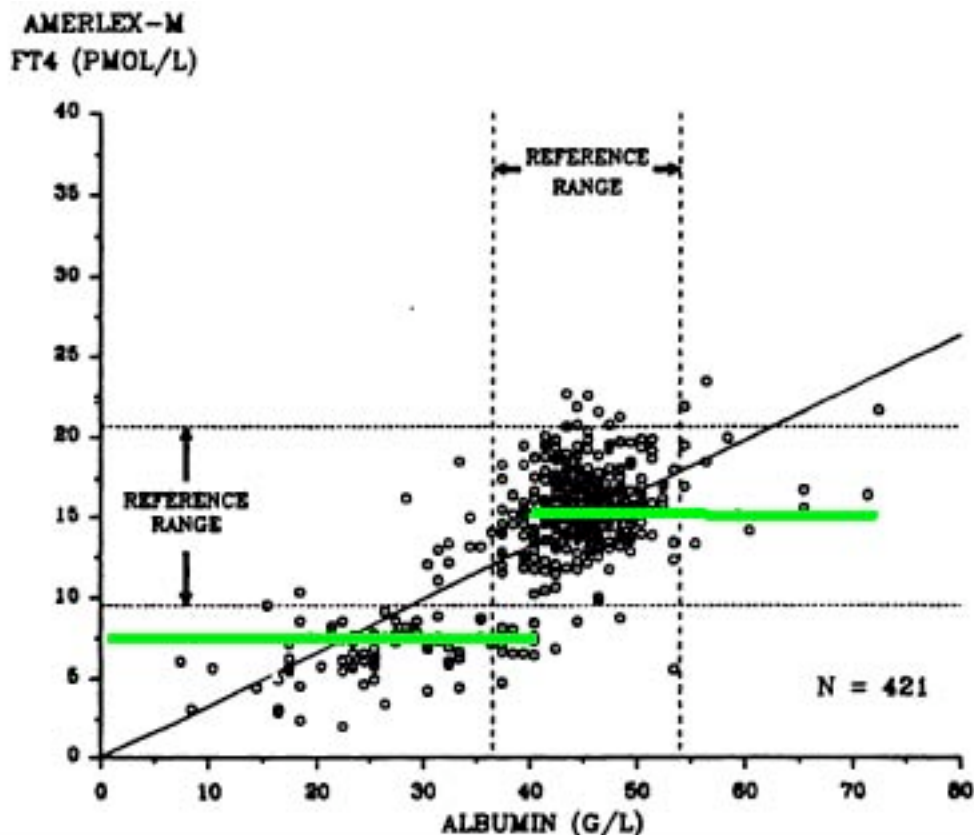
- (1) When the groups are combined, the claim is for a stronger FT4 correlation with serum Albumin. However, I suggest once again both the MAB and M assays are independent of albumin for albumin ranges either < 40 g/L and > 40 g/L. The difference by Albumin groups $< / > 4$ g/L is 3.5 pmol/L for MAB and 8 pmol/L for M, but constant within each Albumin range. It is of note that the MAB measures 3.9pmol/L higher than ED at normal Albumin (ED is independent of serum albumin, Christofides 1992).

e. **COMBINED Euth+sNTI**

AMERLEX-MAB
FT4 (PMOL/L)



f. COMBINED Euth+sNTI

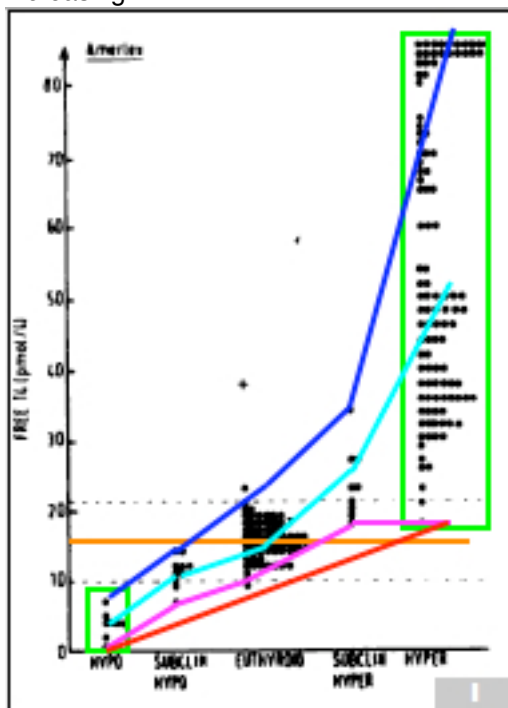


- g. The MAB assay has a larger spread in FT4 values (also higher mean) at a given Albumin than the M assay. This increases the spread for the MAB normal range (from 10-21 for M, to 11-24 for MAB). This can be interpreted as MAB being less precise for each patient, making MAB less sensitive at low or high FT4 values (see ahead).
- h. For both assays, the spread in FT4 measures at low Albumin is greater than at high Albumin. It may be that both MAB and M measures are less precise at low Albumin, so that the low FT4 at low albumin results have a larger spread about the mean than at high Albumin (see ahead). Since the M assay is more precise, it can more clearly distinguish the hypothyroid from euthyroid patients.
- i. It is very possible that either low albumin and hypothyroidism in Pregnancy co-exist, or that low Thyroid function actually causes the low serum Albumin. The often quoted idea that Albumin drops proportionally between 20 and 40 weeks of pregnancy which causes the lowering of FT4 measurements does not fit with the findings for these Analogue or Labeled-antibody assays.

5. In this study, **FT4 appears independent of serum Albumin within two respective albumin ranges <40 g/L, and >40 g/L**, using either the representative "albumin-insensitive" or "albumin-affected" assays. There is a constant 7pmol/L difference between these ranges.
- a. The correlation of FT4 with serum albumin appears to be enhanced by the inclusion of patients who test High for FT4 (not low). It is easy to conflate these clinical situations, using experience with Hyperthyroid/ Higher-FT4 patients to extrapolate to patients who test low for FT4, with the assumption that the assays perform the same at high and low FT4 measurements.
 - b. A FreeT4 below the mid-normal range with either assay seems to be suspicious for hypothyroidism. This would fit with the observation by Momotani et al that treatment for hyperthyroidism in Pregnancy requires maintaining FT4 in a mild hyperthyroid state (~1.9 ng/dl) to prevent adverse fetal effects. The upper range of normal FT4 may indicate a different physiologic state than the lower range.
 - c. It is curious that this study did not include any clearly hypothyroid patients (TSH>5) to assess whether albumin would affect their FT4 values. It is also curious that a clinical study treating these suspected low-thyroid patients testing was not done.
 - d. Hypothyroid patients do not demonstrate increased Albumin levels, Hyperthyroid patients do not demonstrate decreased Albumin levels.

F. FT4/FT4 range biased to higher values at high FT4 values

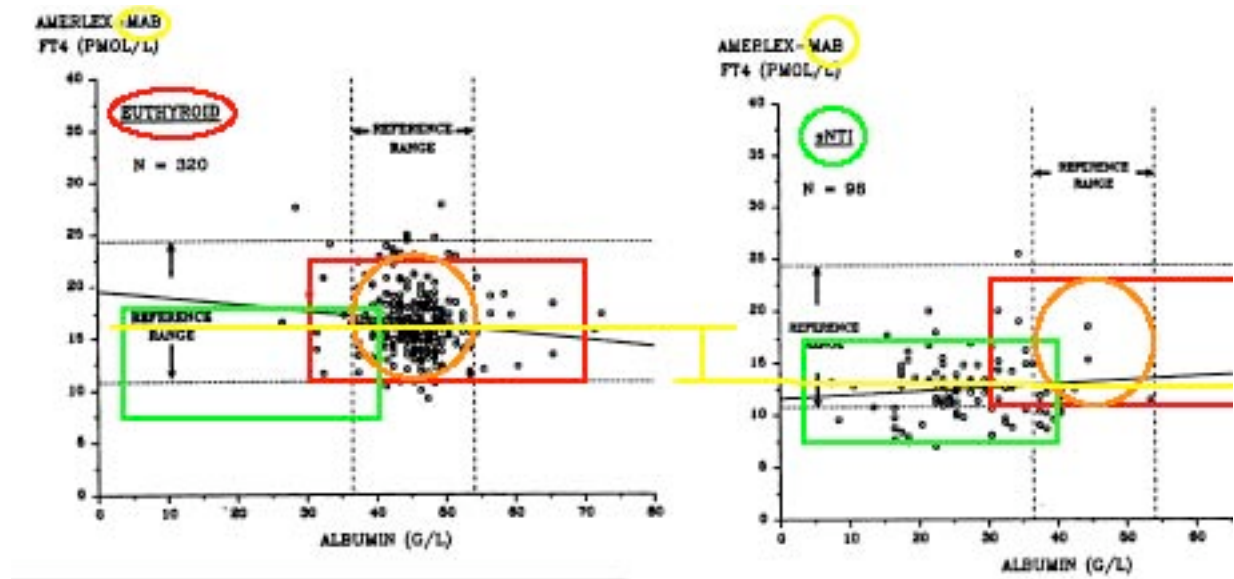
1. To support the idea that FT4 assays are skewed to higher FT4 at hi-FT4, rather than skewed to lower FT4 at low Albumin, look at the following measurements using the Amerlex-M assay (this is seen with the other assays used in the study in a similar manner). Gow(85)
2. There appears to be a differential bias to higher FT4 at hiFT4 values, with less hi-side bias at loFT4 values. The FT4 range of values increase in both in spread and mean as FT4 increases from hypothyroid values thru hyperthyroid values.
 - a. The **low-range** values (**Purple line**) for the different categories of patients identified as hypo-to-hyper thyroid follows a **more-or-less linear increase from 0 to 18 pmol/L** with increasing FT4 (**Red line approximation**).
 - b. The **mid-range** values (**Lt Blue line**) **increases in a parabolic manner from 5 to 50 pmol/L** with increasing FT4, different from the low-range value .
 - c. The **hi-range** values (**Dk Blue line**) **increases even faster from 8 to 80 pmol/L** with increasing FT4.



3. The interpretation of Albumin effects on FT4 measurements is complicated by this, because FT4 and Albumin levels are correlated. Lo-FT4 patients tend to have low Albumin, Hi-FT4 patients tend to have high Albumin. (Hypothyroidism lowers Albumin).
- a. This increasing bias to the high side would inflate FT4 values at hi-Albumin/hi-FT4, making it seem like there is an Albumin dependence of FT4.
 - b. It is thus not easy to distinguish if:
 - (1) Thyroid status changes Albumin levels
 - (2) Albumin levels change Thyroid status
 - (3) Thyroid status and Albumin level are correlated, caused equally by another unidentified condition
 - (4) Low Albumin artificially decreases the lab test that measures FT4
 - (5) High Albumin artificially increases the lab test that measures FT4
 - (6) Low FT4 artificially decreases the lab test that measures FT4
 - (7) High FT4 artificially increases the lab test that measures FT4
 - c. However, it does appear that these patients who test low for FT4 have low Albumin, and the patients who test high for FT4 have high Albumin. The Midgely separation by euthyroid/ sick segregates into hi/ lo Albumin.
 - d. Whichever of the above are valid, one cannot do a linear regression on a indendent quantity (FT4) that is correlated to the dependant variable (Albumin). It will always show a correlation that is due to increasing higher bias with the independent variable (Albumin).

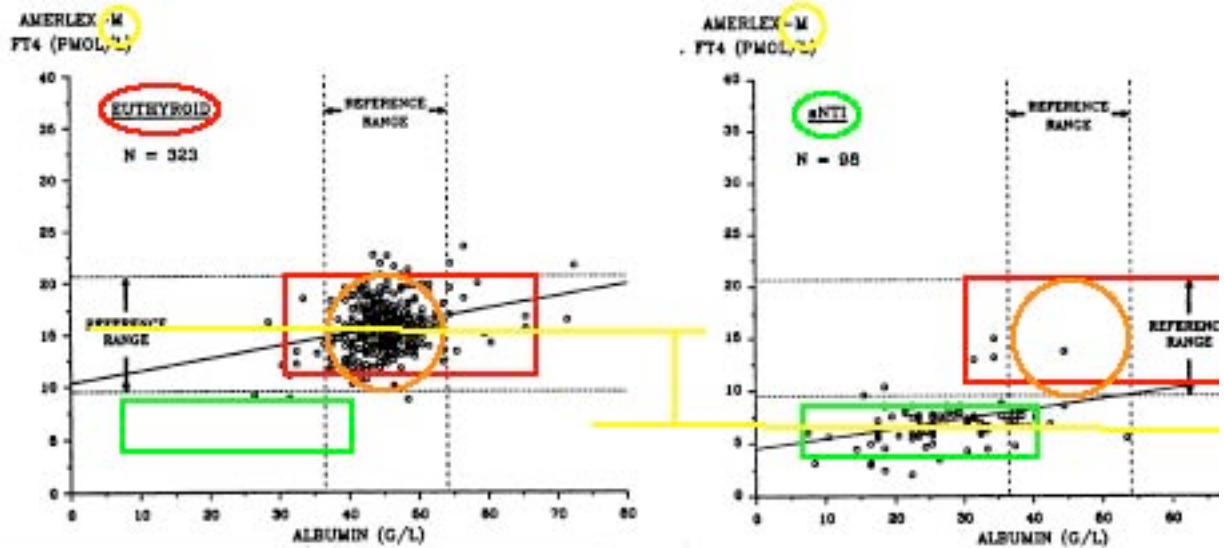
4. Assay Accuracy contribution to differences between MAB and M assays

- There appears to be larger sample errors in the MAB assay (increased SD inferred from the increased range for the MAB assay) compared to M assay. Using the graphs from Midgley 1990 again, an effort is made to ignore outliers and look at a trimmed distribution for the assays (boxes).
- The decrease in the FT4 trimmed range midpoint for sNTI compared to Euth with the MAB assay is $\sim 17 - 13 = 4$ pmol/L.
- The spread in the FT4 range for sNTI is $\sim 17.5 - 7.5 = 10$ pmol/L vs $\sim 23 - 10 = 13$ pmol/L for Euth with the MAB assay.
-



- e. The decrease in the FT4 trimmed range midpoint for sNTI compared to Euth with the M assay is $\sim 11 - 7 = 4$ pmol/L. This is the same (or greater if not trimmed) decrease as for the MAB assay.
- f. The spread in the FT4 range for sNTI is $\sim 9 - 4 = 5$ pmol/L vs $\sim 21 - 11 = 10$ pmol/L for Euth with the M assay

g.



- h. So the mean decrease in FT4 values for the MAB assay is the same for the M assay, 4 pmol/L.
- i. But the spreads in the MAB is greater than for the M. The spread in FT4 values for sNTI with the MAB assay divided by the spread for the M assay = $10/4 = 2.5x$. The spread in FT4 values for with the M assay is $5/4 = 1.3x$ the spread in FT4 for the sNTI patients with the M assay.
- j. This allows a better discrimination of the mean decrease with the M assay then the MAB, identifying the patients as truly T4-hypothyroid, and independent of the Albumin concentration between 10-40 g/L. Both assays show decreased FT4 for the sNTI patients, but the MAB assay errors, evidenced by the the larger assay spread of values, are too large to resolve it.

5. Additional comparison of these without any trim is below, showing the decrease in mean for both MAB and M assays in the sNTI group, but of smaller magnitude with MAB as if it is blunted. FT4 above 10 pmol/L is biased to higher values in both assays.

G.

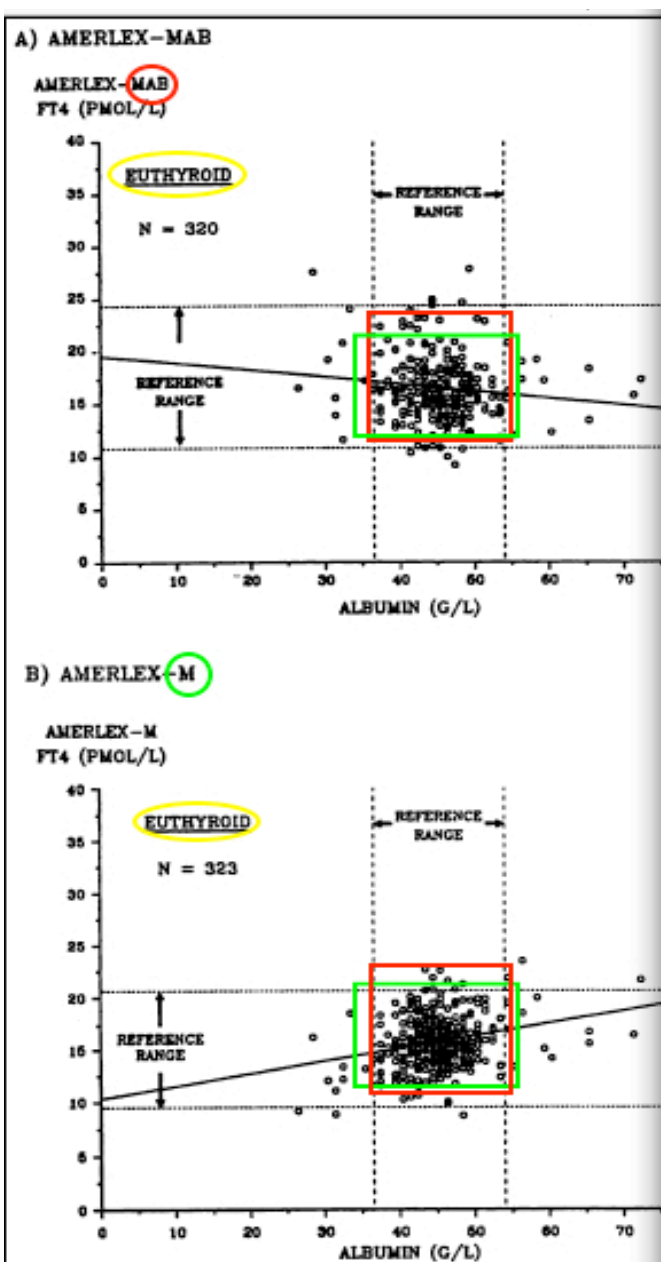


Fig. 2. Correlation of FT₄ and albumin concentrations in euthyroid subjects

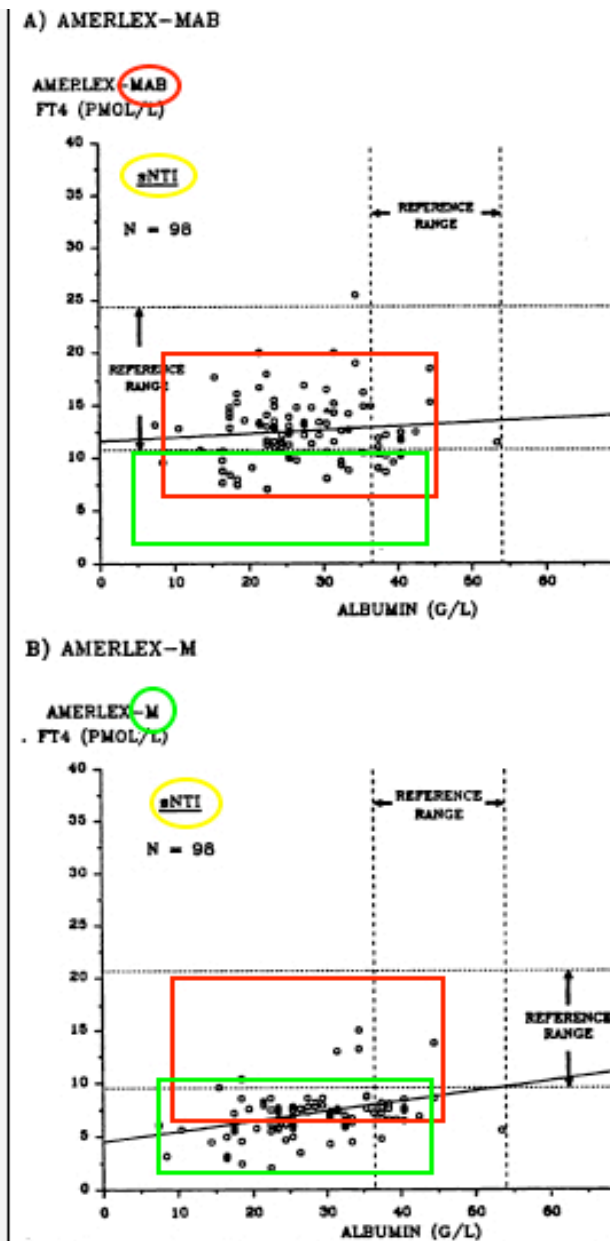


Fig. 3. Correlation of FT₄ and albumin concentrations in sub severe nonthyroidal illness