Functional Medicine Meeting
Dr. Weitz

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Medical Director/CEO, Holtorf Medical Group
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Topics

- CFS/Fibromyalgia
- Thyroid dysfunction
  - Deidoninase
  - Thyroid transport
  - Alternative testing
    - SHBG
    - Thyroflex
    - Medical-legal
- Adrenal dysfunction
- Peptide therapy
- Stem cells
- Scheduled time: 6 hours

Cycle of Dysfunction

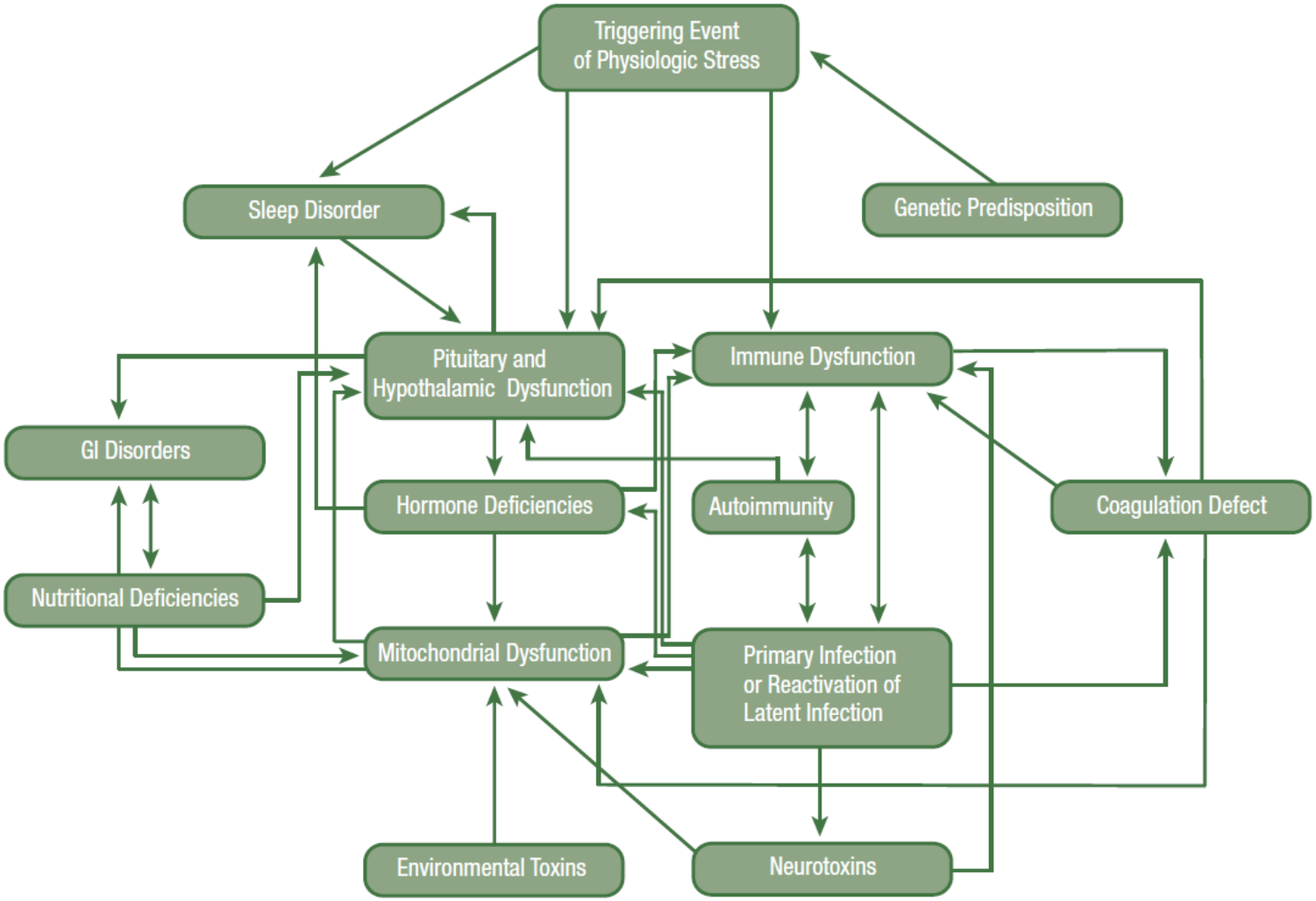
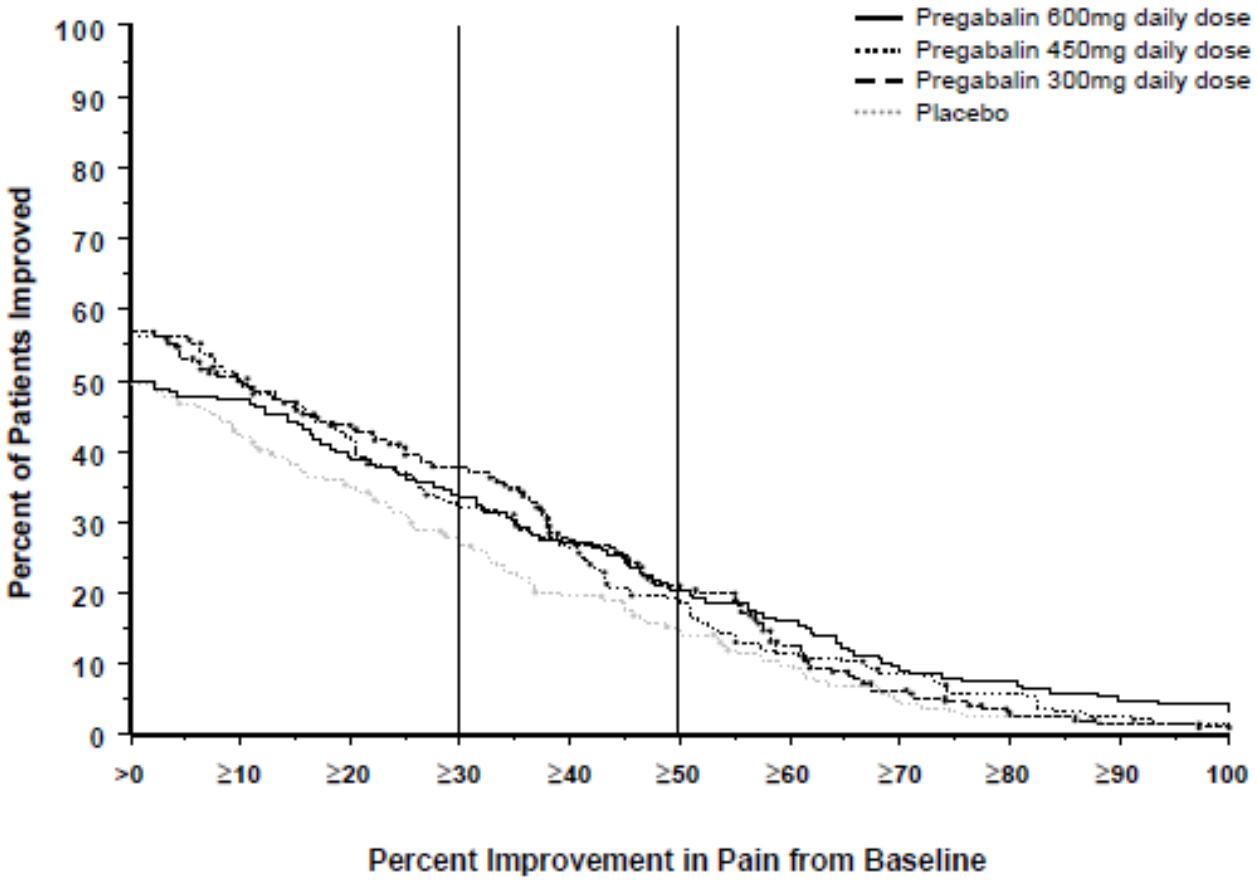


Figure 8: Patients Achieving Various Levels of Pain Relief – Fibromyalgia Study F1

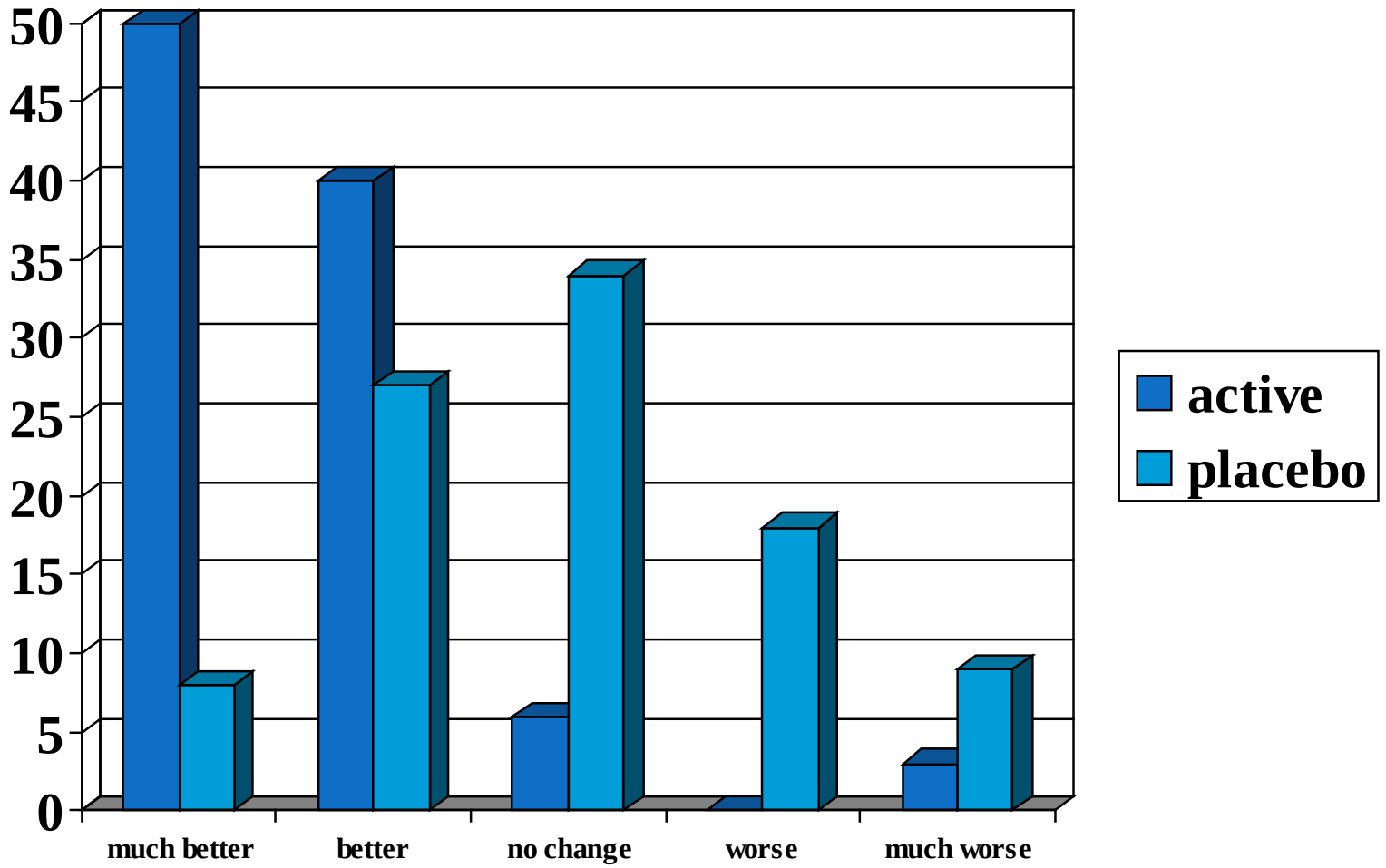


CFS/FM

Chronic Fatigue Syndrome and Fibromyalgia are very treatable Conditions!

When the multiple dysfunctions present are treated, significant improvement is seen, almost without exception.

Journal of Chronic Fatigue Syndrome 2001



Outcomes Published in JCFS

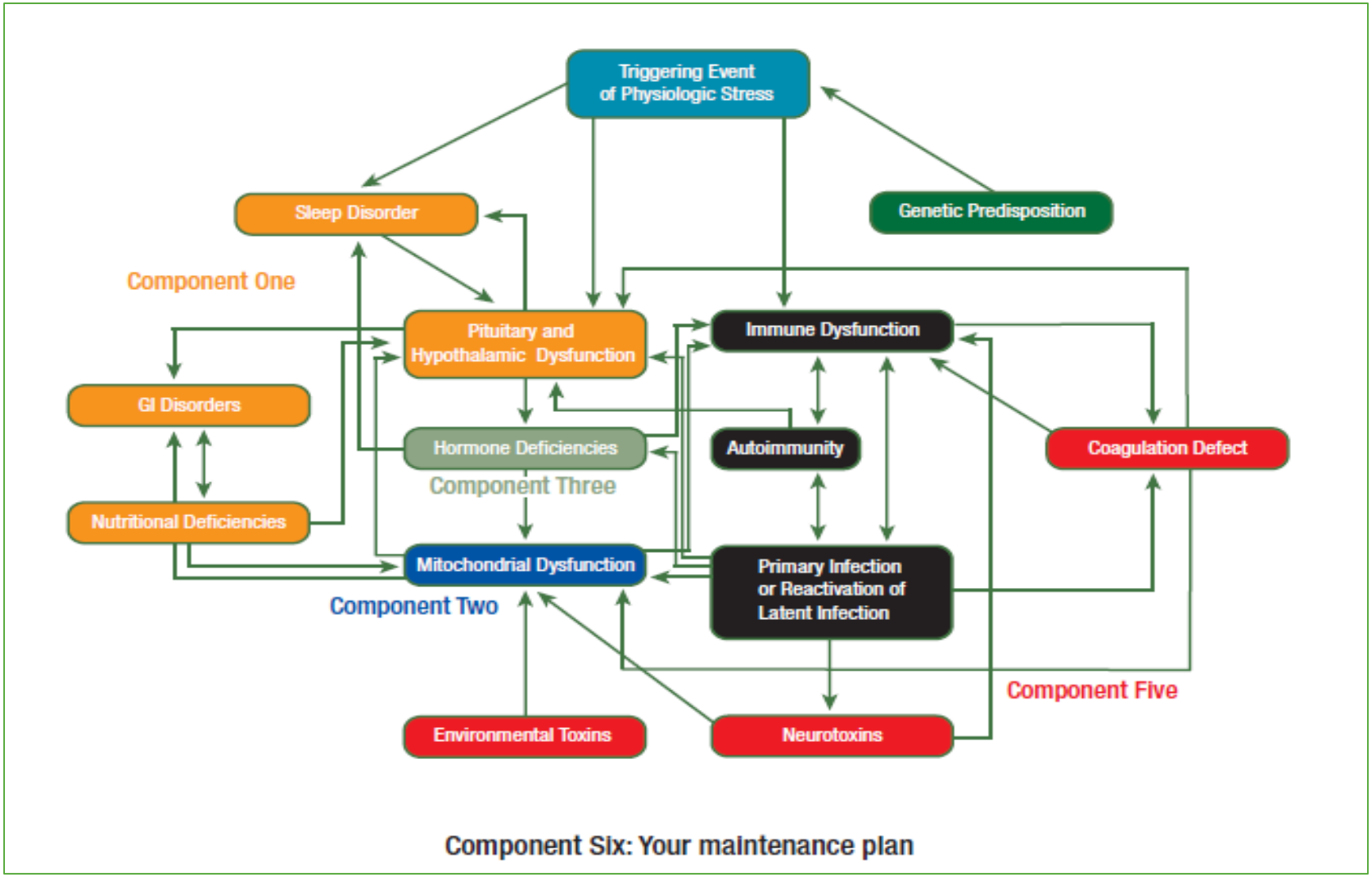
- 500 consecutive patients on computerized outcome assessment demonstrated that a multi-system treatment protocol that addresses the known physiologic abnormalities in CFS and fibromyalgia resulted in:
  - 94 percent of patients having overall improvement by the 4th visit
  - 75 percent noting significant overall improvement
  - 62 percent reported substantial overall improvement.
  - The average energy level and sense of well-being for patients doubled by the fourth visit
- The effectiveness of this multi-system treatment was further confirmed through the analysis of the cumulative findings of over 40 independent physicians and over 5,000 patients
- Prior to treatment at the center, the patients had seen an average of 7.2 different physicians for the treatment of CFS and/or FM without significant improvement.

Holtorf, K. Diagnosis and Treatment of Hypothalamic-Pituitary-Adrenal (HPA) Axis Dysfunction in Patients with Chronic Fatigue Syndrome (CFS) and Fibromyalgia (FM). J of CFS 2008;14(3):1-14

Six Component Approach

- Component One — Stabilize the Patient
- Component Two — Mitochondrial Enhancement
- Component Three — Balance the Hormones
- Component Four — Treat the Infectious/Immune Components
- Component Five — Addressing Unique Etiologies
- Component Six — Maintenance

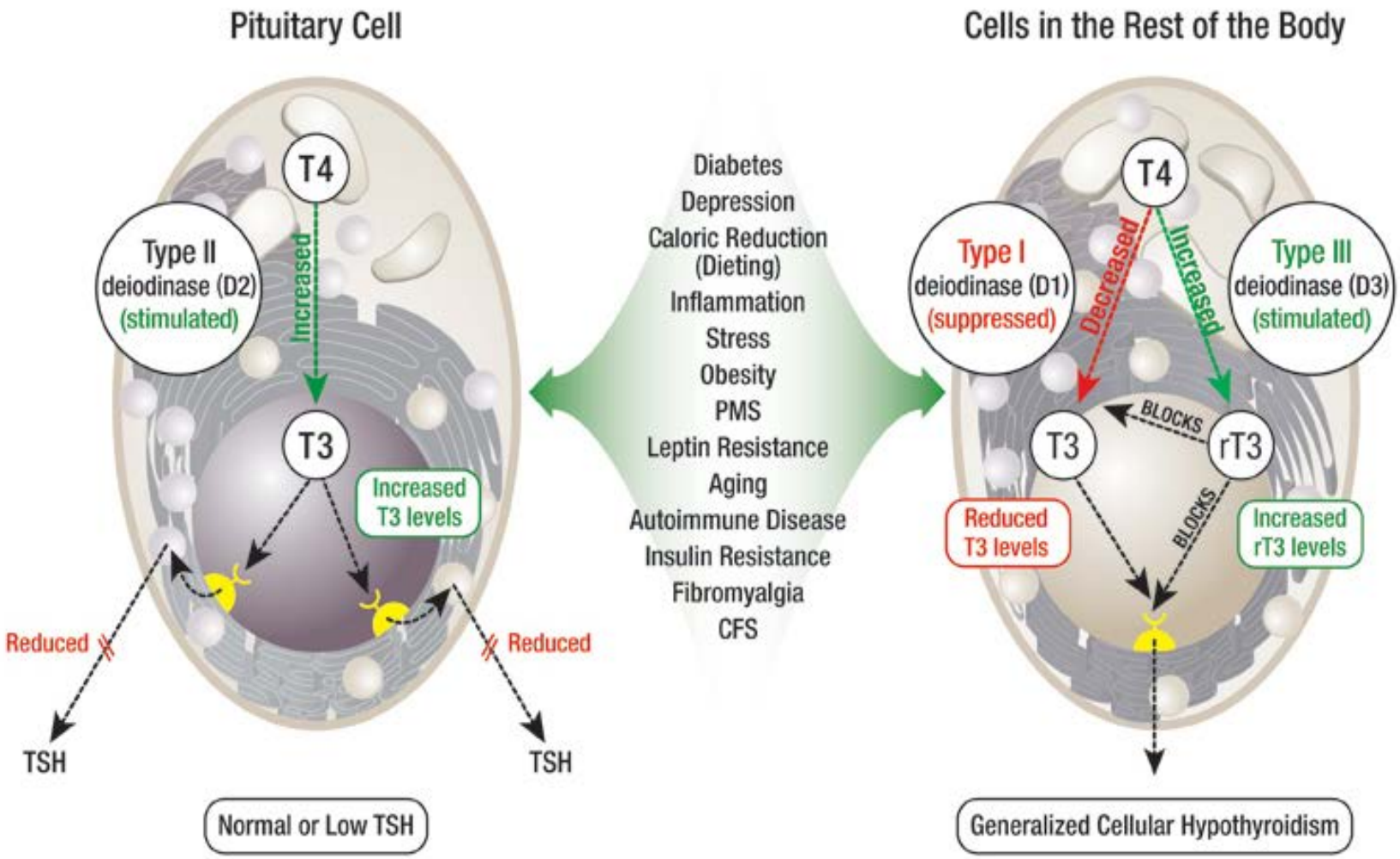
Integrated Approach to the Cycle of Dysfunction



Thyroid

- Almost all CF/FM are low thyroid
- Discussed in previous lectures
- Usually normal or low TSH because of pituitary dysfunction and inflammatory suppression of the TSH
- Thyroid resistance is very common with CFS/FM
- Blood tests indicate how much thyroid is in the blood, **not the effect**

Why the TSH is Unreliable?



Accuracy of TSH in Chronic Fatigue

- A study published in ***The Lancet*** **performed thyroid biopsies in patients with chronic fatigue and found that 40% of these patients had lymphocytic thyroiditis**
- **Only 40% of those with lymphocytic thyroiditis were positive for TPO or antithyroglobulin antibodies or had an abnormal TSH**
- Thus, the thyroid dysfunction would have gone undetected in the majority of patients if the biopsy had not been done
- This study also demonstrated that because the TSH is a poor indicator of thyroid function, it also does not predict whose symptoms will respond to thyroid replacement
- The authors state, **"After treatment with thyroxine, clinical response was favorable, irrespective of baseline TSH concentration."**

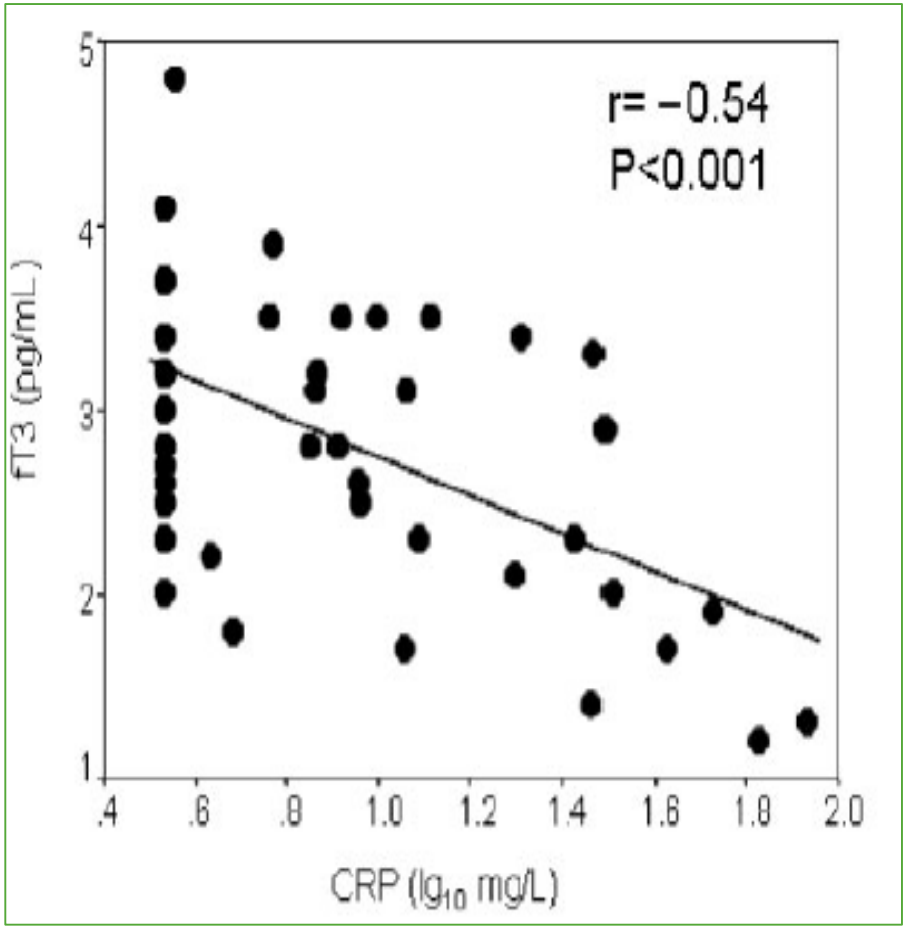
Wikland B. Fine needle aspiration cytology of the thyroid in chronic fatigue. *Lancet* 2001:357:956-57.
Wikland B, *et al*. Subchemical hypothyroidism. *Lancet* 2003;361:1305.

Accuracy of TSH in Fibromyalgia

- TRH testing of FM patients
- Found that all of the patients with fibromyalgia were hypothyroid despite the fact that standard thyroid function tests, including TSH, T4 and T3, were in the normal range.
- They found that these patients tended to have low normal TSH levels that averaged 0.86 vs 1.42 in normals with high normal free T4 and low normal T3 levels so doctors erroneously feel these patients are on the high side of normal because of the low normal TSH and high normal T4.

Neeck G, Riedel W. Thyroid function in patients with fibromyalgia syndrome. J Rheum 1992;19(7):1120-1122

Inflammation



Enia G et al. Subclinical hypothyroidism is linked to micro-inflammation and predicts death in continuous ambulatory peritoneal dialysis. Nephrol Dial Transplant (2007) 22: 538–544

Thyroid Levels with Chronic Dieting

- 25 days of calorie restriction (dieting) significantly reduced D1, resulting in reduced T4 to T3 conversion with a 50% reduction in T3.
- Associated with an increase in D2, so there was no increase in TSH but rather a decrease from an average of 1.20 ng/ml to 0.7 ng/ml.

Araujo RL, Andrade BM, da Silva ML, et al. Tissue-specific deiodinase regulation during food restriction and low replacement dose of leptin in rats. Am J Physiol Endocinol Metab 2009;296:E1157-E1163.

Thyroid Levels with Chronic Dieting

- Study by Leibel, et found that individuals who significantly dieted in the past had, on average, 25% lower metabolism (equal to someone who weighed 60% less).
- Additionally, the reduction was shown to be present years later.
- This 25% percent reduction in metabolism equates to an approximate deficit of 500-600 kcal per day.

Leibel RL, Jirsch J. Diminished energy requirements in reduced-obese patients. Metabolism 1984;33(2):164-170.

Thyroid Levels with Chronic Dieting

- Obese rats fed a high-fat diet with one group going through caloric restriction and refeeding
- After two cycles of caloric restriction, weight loss occurred at half the rate and weight gain occurred at three times the rate compared to the control group.
- *"The data suggests that frequent dieting may make subsequent weight loss more difficult."*

Brownell KD, Greenwood E, Stellar E, Shrager E. The Effects of Repeated Cycles of Weight Loss and Regain in Rats. Physiol Behav 1986;38:459-64.

Variable Bioactivity of TSH

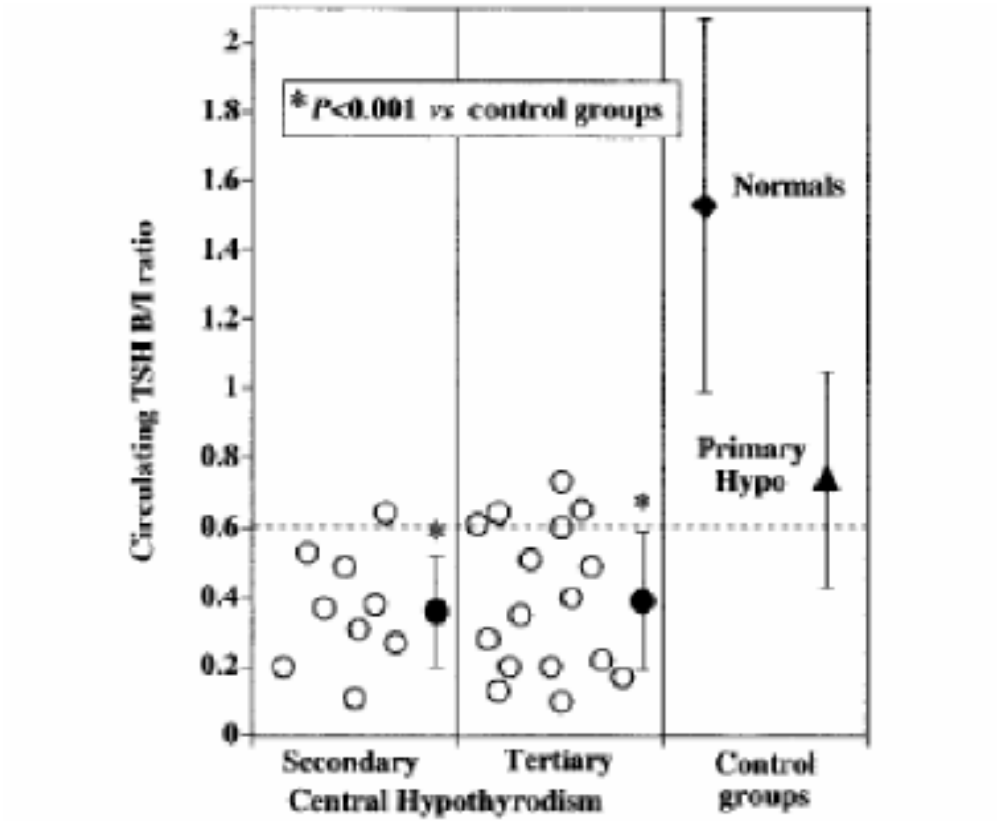


FIG. 3. The TSH B/I in CH patients with secondary or tertiary defect (○, single cases; ●, mean ± SD) and in control subjects (mean ± SD; ◆, normal subjects; ▲, primary hypothyroid patients). The *dotted line* indicates the lower limit of the normal range. Both groups of CH patients have B/I ratios significantly lower than those observed in normal or primary hypothyroid controls.

Endocrinology & Diabetes. 6(1):55, February 1999.
J Clin Endocrinol Metab 1973 37: 190-196.
J Clin Endocrinol Metab 1973 37: 595-601.
Clin Endocrinol (Oxrf)1972; 1:115-25.
J Clin Endocrinol Metab 1973; 37;595-601.
Am J Med Sci 1973. 265:315-8.
J Clin Endocrinol Me.lab 1974; 38:964-75.
J Clin Endocrinol Metab 1975; 41:722-8.
Clin Endocrinol (Oxf) 1975: 4;585-90.
Endocrinology 113:2145-2145-2154
Rec Prog Horm Res 41:577-606
Biochem J 287:665-679
Endocrinology, Vol 133, 1490-1503,
J Clin Endocrinol Metab 1989 69: 985-995.
Eur J Endocrinol. 131:331-340.
Thyroid. 8:941-946.
J Clin Endocrinol Metab. 48:989 -998

Thyroid Hormone Transport

- Because TT is energy dependent, any condition associated with a reduced production of the cellular energy (**mitochondrial dysfunction**) will also be associated with **reduced transport of thyroid** into the cell.
- Results in cellular hypothyroidism despite having standard blood tests in the “normal” range.

Holtorf K. Thyroid Hormone Transport into Cellular Tissue. J AARM 2014;3:53-68

Thyroid Hormone Transport

- Conditions associated with reduced mitochondrial function and impaired thyroid transport include: insulin resistance, diabetes and obesity;[68,69,70,71,106] chronic and acute dieting;[4,51,66,72,112,113,114,115,116,117,118] diabetes;[69,73,74,75,76] depression; [73,77,78,79] anxiety;[73,80] bipolar depression; [73,77,81,82] neurodegenerative diseases;[73,83,84,85,86,87)] aging;[73,74,88-100] chronic fatigue syndrome; [73,101,102] fibromyalgia; [73,103,104] migraines; [73] chronic infections; [73] physiologic stress and anxiety; [73,79] cardiovascular disease; [73,99,104,105,108] inflammation and chronic illness; [73,109,110,111] and those with high cholesterol and triglyceride levels. [58,60,72,106,107]

- Thus, standard blood tests can be very unreliable if any of these commonly occurring conditions are present.[1-107]

Holtorf, K. Thyroid Hormone Transport into Cellular Tissue J Restor Med 2014;3(1):53-68

Thyroid Hormone Transport

- Specific and separate transporters for T4 and T3
- The transporter for T4 is much more energy dependent than the transporter for T3[5,40,41,49,52,53,66] (see figure 1)
- Even slight reductions in cellular energy (mitochondrial function) results in dramatic declines in the uptake of T4 while the uptake of T3 is much less affected.[5,41,62,67]
- Pituitary has completely different transporters that are not energy dependent.

Holtorf, K. Thyroid Hormone Transport into Cellular Tissue
J Restor Med 2014;3(1):53-68

Thyroid Hormone Transport (pituitary)

- The pituitary is different than every cell in the body [1,17,43,50,52,55,59,60,61]
  - Different deiodinases
  - Different high affinity thyroid receptors
  - Different thyroid transporters that are not energy dependent
  - The pituitary does not make RT3 (No D3)
- **Pituitary will maintain or increase the uptake of T4 and T3 in low energy states, while the rest of the body will have significantly reduced transport of T4 and T3 causing intracellular hypothyroidism[1,17,22,43,50,52,55,59,60,61]**

Holtorf, K. Thyroid Hormone Transport into Cellular Tissue
J Restor Med 2014;3(1):53-68

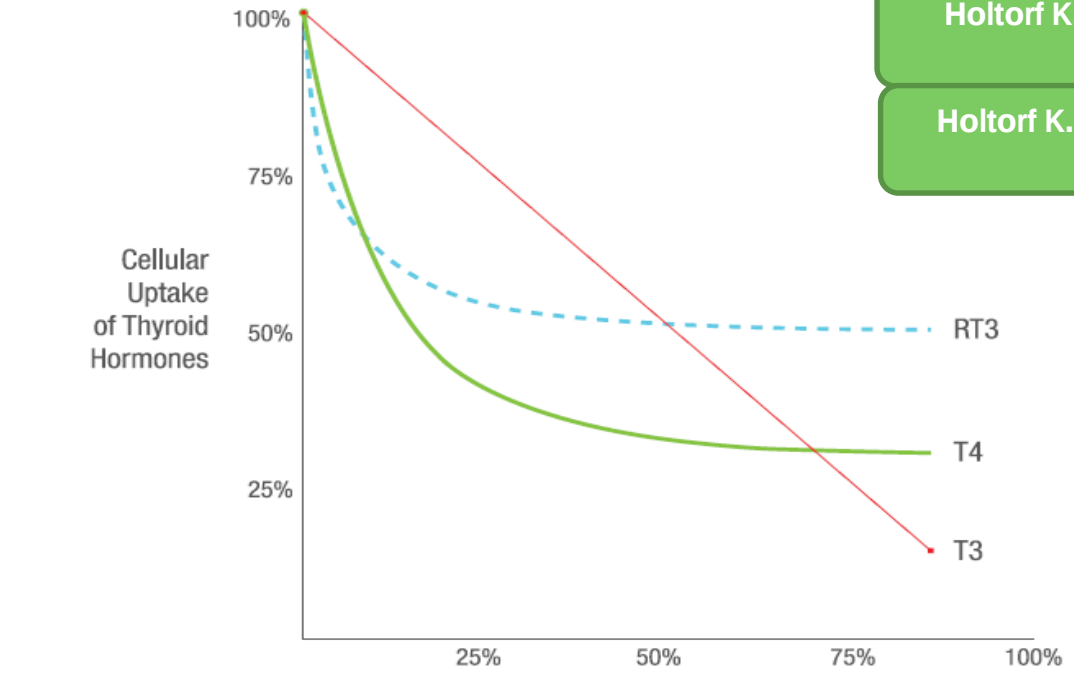
Thyroid Hormone Transport (reverse T3)

- How do you tell if you have reduced thyroid transport?
- RT3—The transporter for reverse T3 (rT3) has the same pharmacodynamics and kinetics as the T4.[6,41,45,62,66,67]
- Main reason that rT3 goes up with stress, etc. is reduced transport.
- This property makes rT3 (and SHBG) a useful indicator of diminished transport of T4 into the cell.[45]

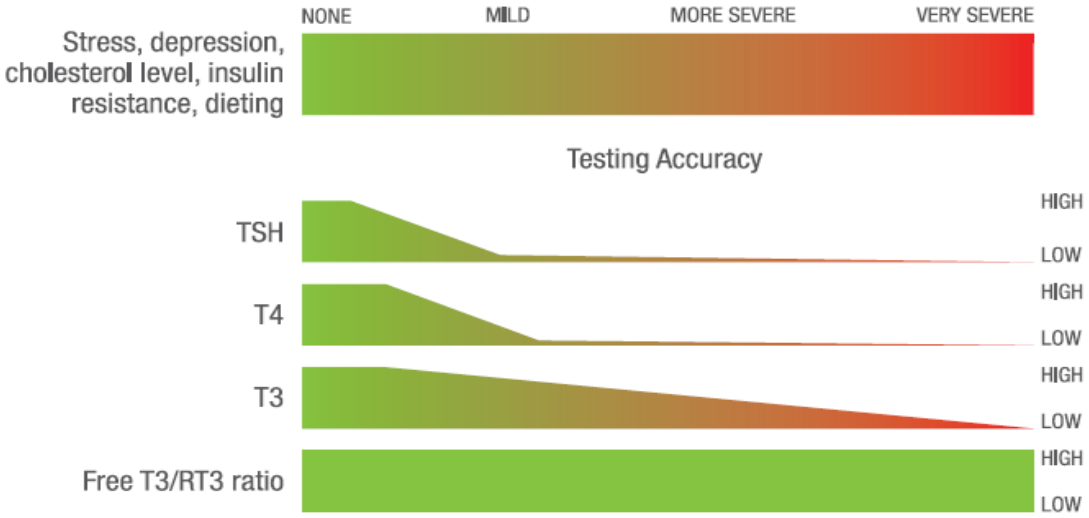
Holtorf K. Thyroid Hormone Transport into Cellular Tissue. J AARM 2014;3:53-68

Holtorf K. National Academy of Hypothyroidism (NAHypothyroidism.org)

Holtorf K. Thyroid Hormone Transport into Cellular Tissue. J AARM 2014;3:53-68



Percent drop in cellular energy (above) and severity of stress, depression, cholesterol level, insulin resistance and dieting (below)



Thyroid Hormone Transport (reverse T3)

- Thus, a high reverse T3 demonstrates that there is either increased T4 to reverse T3 formation or an inhibition of reverse T3 uptake into the cell (larger effect)
- These two effects occur together in a wide range of physiologic conditions .
- Hi RT3 also indicates that T4 would not be optimal or appropriate therapy.

Holtorf, K. Thyroid Hormone Transport into Cellular Tissue
J Restor Med 2014;3(1):53-68

Thyroid Hormone Transport (stress)

- Serum from non-stressed individuals had no effect on T4 cellular uptake, while those with significant physiologic stress had up to a 44% reduction in T4 uptake into the cell. [41,42,50]
- Chronic and yo-yo dieting, frequently done by a large percentage of the population, is shown to be associated with reduced cellular T4 uptake of 25%-50%.[3,49,112,114,115,116]

Vos RA et al. Impaired thyroxine and 3,5,3'-triiodothyronine handling by rat hepatocytes in the presence of serum of patients with nonthyroidal illness. J Clin Endocrinol Metab 1995;80:2364-2370.

Thyroid Hormone Transport (depression)

- The dysfunction present with depression and bipolar depression includes reduced uptake of T4 into the cell.[24-26,30,31,35,39-45]
- Thought to have "high normal" levels of thyroid—usually low normal TSH and high normal T4.
- Lack of benefit from T4 used as proof that they are not hypothyroid.
- T3 stimulates production of serotonin.

Holtorf, K. Thyroid Hormone Transport into Cellular Tissue
J Restor Med 2014;3(1):53-68

Depression

- Treated 159 bipolar patients with T3 who had failed to adequately respond to an average of 14 medications (average dose 90.4 mcg (range 13 mcg-188 mcg)).
- The medication was found to be well tolerated and 84% experienced significant improvement and 33% had a full remission.
- One patient who was switched to T4 for cost reasons experienced a return of symptoms, which resolved with the reintroduction of T3.
- The authors concluded, *"Augmentation with supraphysiologic doses of T3 should be considered in cases of treatment resistant bipolar depression..."*
- The authors thanked several doctors who encouraged them to go beyond the traditional 50 mcg of T3 because it has helped so many of their patients.

Kelly T et al. The use of triiodothyronine as an augmentation agent in treatment resistant bipolar II and bipolar disorder NOS. Journal of Affective Disord 2009;116:222-226.

Depression

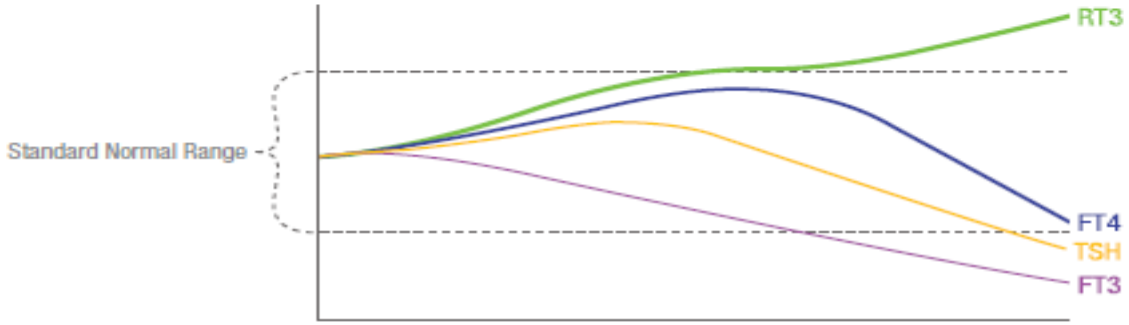
- With over 4000 patients, The Star*D Report is the largest trial comparing antidepressant effectiveness for depression.
- It found that 66% of patients fail to respond to antidepressants or have side-effects severe enough to discontinue use.
- Of those who do respond, over half will relapse within one year.
- The trial found that T3 was effective even when other medications -- such as citalopram (Celexa), bupropion (Wellbutrin), sertraline (Zoloft), venlafaxine (Effexor), or cognitive therapy – were not.
- It was shown to be 50% more effective, even with the less than optimal dose of 50 mcg, under direct comparison with significantly less side effects than commonly used therapeutic approaches with standard antidepressants.

Nierenberg AA, et al. A comparison of lithium and T3 augmentation following two failed medication treatments for depression: A STAR*D Report. Am J Psychiatry 2006;163:1519-1530.

Thyroid Hormone Transport

Associated serum thyroid levels with progressively decreasing tissue thyroid levels due to stress, illness, depression, calorie reduction or aging (Why standard blood tests lack sensitivity to detect low thyroid in the presence of such conditions)

Demonstrates why TSH levels lack the accuracy to detect cellular levels and the free T3/reverse T3 ratio is the most accurate method to determine cellular thyroid levels in the presence of physiologic stress, illness, depression or obesity.



Severity of illness/depression stress/calorie reduction	none	mild	moderate	severe
Normal aging	young	middle	older	elderly
Tissue hypothyroidism (diminished tissue T3 level)	none/mild	mild/moderate	moderate/severe	severe
Inaccuracy of TSH and T4 levels	none	potentially significant	significant	substantial
Diminished utilization of T4	none/mild	mild/moderate	moderate/severe	severe

Holtorf K. National Academy of Hypothyroidism (NAHypothyroidism.org)

Holtorf K. Peripheral Thyroid Hormone Conversion and Its Impact on TSH and Metabolic Activity. J AARM 2014;3:30-52

ESTIMATION OF TISSUE HYPOTHYROIDISM

TABLE 1. Sensitivity and specificity of the 14 symptoms and signs of hypothyroidism and analysis of their positive and negative predictive values

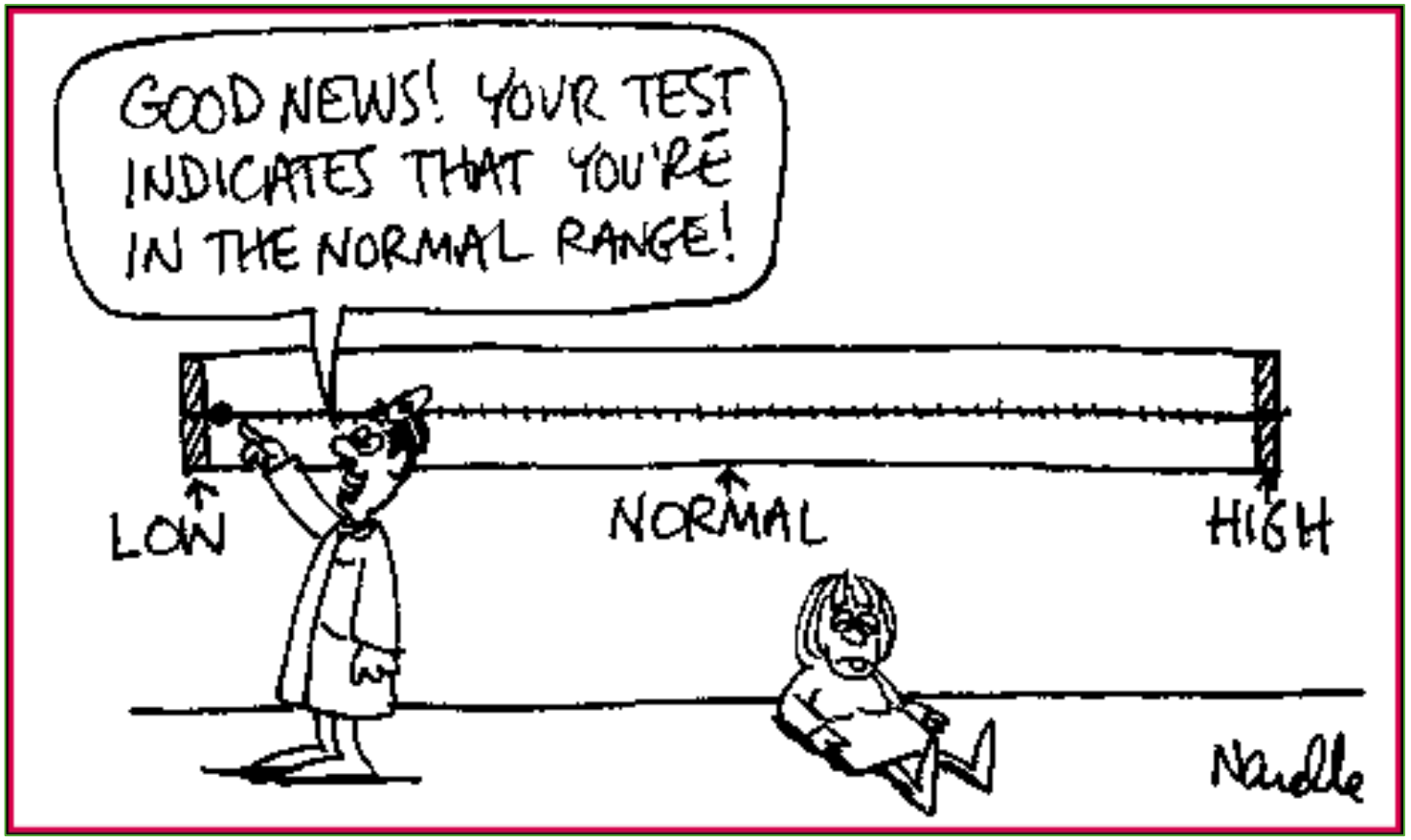
Symptoms and signs	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Ankle reflex	77	93.5	92.2	80.3
Dry skin	76	63.8	67.7	72.7
Cold intolerance[a]	64	65	64.6	64.4
Coarse skin	60	81.2	76.1	67
Puffiness	60	96.3	94.2	70.7
Pulse rate[a]	58	42.5	50.2	50.3
Sweating	54	86.2	79.6	65.2
Wt increase	54	77.5	70.6	62.8
Paraesthesia	52	82.5	74.8	63.2
Cold skin	50	80	71.4	61.5
Constipation	48	85	76.2	62
Slow movements	36	98.7	96.5	60.7
Hoarseness	34	87.5	73.1	57
Hearing	22	97.5	89.8	52.6

Zulewski H, et al. Estimation of Tissue Hypothyroidism by a New Clinical Score: Evaluation of Patients with Various Grades of Hypothyroidism and Controls JCEM 1997;82(3):3 771-776

Do you have or feel the following symptoms?	No Symptom Never	Few or Sometimes	Moderate or regularly	Much or Often	Always or Extreme
Sensitive to cold	☐	☐	☐	☐	☐
Cold hands or feet	☐	☐	☐	☐	☐
Generalized fatigue	☐	☐	☐	☐	☐
Morning fatigue	☐	☐	☐	☐	☐
Fatigue unless exercising	☐	☐	☐	☐	☐
Sleepy during day	☐	☐	☐	☐	☐
Distracted easily	☐	☐	☐	☐	☐
Poor motivation for required tasks	☐	☐	☐	☐	☐
Depression	☐	☐	☐	☐	☐
Headaches	☐	☐	☐	☐	☐
Water retention	☐	☐	☐	☐	☐
Constant swollen eyelids	☐	☐	☐	☐	☐
Swollen eyes in morning	☐	☐	☐	☐	☐
Swollen calves/feet	☐	☐	☐	☐	☐
Difficulty losing weight despite dieting	☐	☐	☐	☐	☐
Constipation	☐	☐	☐	☐	☐
Bedwetting as child	☐	☐	☐	☐	☐
Slow heart palpitations	☐	☐	☐	☐	☐
Muscle cramps	☐	☐	☐	☐	☐
Carpal tunnel syndrome	☐	☐	☐	☐	☐
Stiff joints in morning	☐	☐	☐	☐	☐
Joint pain worsens with cold	☐	☐	☐	☐	☐
Hoarse voice in morning	☐	☐	☐	☐	☐
Dry skin (general/feet or elbows)	☐	☐	☐	☐	☐
Slow growing or brittle nails	☐	☐	☐	☐	☐
Diffuse hair loss	☐	☐	☐	☐	☐
Muscle achiness or soreness	☐	☐	☐	☐	☐
Low body temperature	☐	☐	☐	☐	☐
Diminished sweating	☐	☐	☐	☐	☐
Tingling or numbness in extremities	☐	☐	☐	☐	☐
Hoarse voice (constant or in morning)	☐	☐	☐	☐	☐
Decreased hearing	☐	☐	☐	☐	☐
Course skin (rough skin)	☐	☐	☐	☐	☐

Available at HoltorfMed.com

Diagnosis (laboratory)



Brian Narelle

Diagnosis (laboratory)

Best Method of Diagnosis

- Look at T3 to reverse T3 (T3/rT3)ratio (should be >2)
- Free T3 should be above 3.5
- Reverse T3 less than 16 LC/MS/MS or 150 on RIA
- TSH greater than 2.0 is significant
- Usually low normal free T3 and above average reverse T3
- Signs and symptoms (body temp, pulse, cold ext, etc.)
- SHBG < 70 for women; < 30 for men
- Relaxation phase ankle reflexes
- Basal metabolic rate
- Leptin > 12
- Any positives on TMP
- High C4a, ECP

Treatment with T4

T4 preparations are not optimal or adequate for patients who have CFS/FM/Lyme dz, depression, chronic illness, diabetes, weight gain, etc. because they have a high incidence of reduced T4 to T3 conversion, reduced uptake of T4 and thyroid resistance.

Treatment with T4 (tissue levels of T3)

- Measure tissue thyroid levels in hypothyroid animals receiving T4 replacement.
- Plasma TSH, T4 and T3 levels and 10 different tissue levels of T4 and T3 were measured.
- Demonstrated that all tissues, except the brain (pituitary), required supraphysiologic levels of plasma T4 to provide normal tissue levels of T3.
- The pituitary was able to maintain normal levels of T3 despite the rest of the body being hypothyroid.
- Consequently, the pituitary levels of T3 and subsequent level of TSH is a poor measurement of tissue thyroid levels.
- Demonstrated that T4 only preparations such as Synthroid or Levoxyl, are unable to achieve normal levels of T3 in all tissues, except the brain and pituitary, unless supraphysiologic plasma levels of T4 and T3 are obtained and the TSH is suppressed.

Escobar-Morreale HF et al. Replacement therapy for hypothyroidism with thyroxine alone does not ensure euthyroidism in all tissues, as studied in thyroidectomized rats, J Clin Invest 96:2828-2838

Treatment with T4

- Normal T4 or T3 levels did not ensure tissue euthryoidism.
- Supraphysiologic plasma T4 and T3 levels were required to obtain normal levels in some tissues.
- At some amounts of T4 infused, the level of thyroid hormones in the pituitary was over twice that of other tissues, again demonstrating that pituitary levels and thus TSH secretion is not an accurate measure of tissue hypothyroidism.
- T4/T3 combinations are required to normalize tissue levels of T4 and T3 or T3 to normalize tissue T3 levels.

Escobar-Morreale HF et al. Replacement therapy for hypothyroidism with thyroxine alone does not ensure euthyroidism in all tissues, as studied in thyroidectomized rats, J Clin Invest 96:2828-2838

Optimal Thyroid Replacement
(monitoring TSH)

The false positive rate that a suppressed TSH indicates hyperthyroidism in someone on thyroid replacement is 80%.

The positive predictive value of a suppressed TSH is demonstrating hyperthyroidism is equal to:

$$\text{Positive predictive value (PPV)} = \frac{\text{sensitivity x prevalence}}{((100\text{-specificity}) \times (100\text{-prevalence})) + (\text{sensitivity x prevalence})}$$

$$\frac{59 \times 20}{(100-51) \times (100-20) + (59 \times 20)}$$

$$\frac{1180}{49 \times 80 + 1180} = 23\ \%$$

Meier C et al. Serum TSH in assessment of severity of tissue hypothyroidism in patients with overt primary thyroid failure: cross sectional survey. The British Medical Journal 2003;326:311-2.

Optimal Thyroid Replacement
(monitoring TSH)

0021-972X/94/7806-1368$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 78, No. 6
Printed in U.S.A.

Comparison of Second and Third Generation Methods for Measurement of Serum Thyrotropin in Patients with Overt Hyperthyroidism, Patients Receiving Thyroxine Therapy, and Those with Nonthyroidal Illness*

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ABSTRACT

We compared serum TSH results determined in second and third generation assays in patients with thyroid disease and nonthyroidal illnesses (NTIs) to evaluate the usefulness of the more sensitive assay. We studied 19 subjects with untreated hyperthyroidism, 12 hyperthyroid subjects sampled at 4-week intervals after beginning carbimazole, 153 subjects receiving T_4 replacement, and 300 hospital in-patients with a variety of NTIs. Serum TSH was measured, using a second generation immunometric method, together with free T_4 and free T_3. Samples with subnormal TSH (<0.5 mU/L) were reassayed, using a more sensitive chemiluminescent immunometric method.

Both assays revealed undetectable serum TSH levels in 18 of 19 overtly hyperthyroid patients. Undetectable TSH values (in both assays) were found in 30 of 33 patients with low serum TSH levels who were receiving treatment for hyperthyroidism, in association with normal thyroid hormone levels in 11. Undetectable TSH was evident in ($P < 0.001$) and in those receiving T_4 with low TSH ($r = -0.33$; $P < 0.05$); no significant correlation was evident in subjects with low serum TSH levels associated with NTI.

An improvement in assay sensitivity led to a reduction in the number of patients being treated with T_4 or with NTI in whom serum TSH was undetectable and, hence, an increase in those in whom overt hyperthyroidism could be excluded. Undetectable TSH results, even in a third generation assay, are not diagnostic of overt hyperthyroidism, but are also found in subjects with treated thyroid disease and NTI.' (*J Clin Endocrinol Metab* **78:** 1368–1371, 1994)

- **Suppressed TSH is not diagnostic of hyperthyroidism**
- **Can be due to NTI or seen with adequate replacement with NTI**

Medical-Legal

- Document pulse, body temp
- Need to use alternative means of diagnosis (thyroflex, BMR, SHBG)
- Document used an alternative means of diagnosis and patient understands that this is not standard therapy (can prewrite with a check box)
- Educate the patient
- Give studies to patient and put studies in chart
- Document more if suppress the TSH
- Use T3SR (cardiac side-effects are mainly not from nuclear receptor (slow on/slow off)(variable levels don't matter much) but rather from surface cell receptor-CA+ channels (variable levels matter)
- Significant respectable minority rule
- Symptom assessment each visit

HPA Axis Dysfunction in CFS/FM

- Analysis of the data in over 150 studies that assessed adrenal function in CFS and FM patients demonstrates that the majority of CFS and FM patients have abnormal adrenal function due to hypothalamic-pituitary dysfunction.
- The majority of patients should be treated for adrenal dysfunction (especially if AM cortisol <10-12)
- ACTH stimulation test is no better than flipping a coin in determining adrenal dysfunction in CFS/FM, as it is not a primary adrenal dysfunction
- The data shows that such treatment is safer and more effective than commonly used treatments such as antidepressants.
- While these conditions are similar, the abnormality in CFS is in the pituitary while the FM patients have abnormalities of the hypothalamus.

Holtorf K. Diagnosis and Treatment of Hypothalamic-Pituitary-Adrenal (HPA) Axis Dysfunction in Patients with Chronic Fatigue Syndrome (CFS) and Fibromyalgia (FM). J. Chron Fatigue Syndrome 2008;14(3):1-14

HPA Axis Dysfunction in CFS/FM

JOURNAL OF CHRONIC FATIGUE SYNDROME • VOLUME 14:3 (pub) 2008

REVIEW

Diagnosis and Treatment of Hypothalamic-Pituitary-Adrenal (HPA) Axis Dysfunction in Patients with Chronic Fatigue Syndrome (CFS) and Fibromyalgia (FM)

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Abstract

There is controversy regarding the incidence and significance of hypothalamic-pituitary-adrenal (HPA) axis dysfunction in chronic fatigue syndrome (CFS) and fibromyalgia (FM). Studies that utilize central acting stimulation tests, including corticotropin-releasing hormone (CRH), insulin stress testing (IST), d-fenfluramine, ipsapirone, interleukin-6 (IL-6) and metyrapone testing, have demonstrated that HPA axis dysfunction of central origin is present in a majority of these patients. However, ACTH stimulation tests and baseline cortisol testing lack the sensitivity to detect this central dysfunction and have resulted in controversy and confusion regarding the incidence of HPA axis dysfunction in these conditions and the appropriateness of treatment. While both CFS and FM patients are shown to have central HPA dysfunction, the dysfunction in CFS is at the pituitary-hypothalamic level while the dysfunction in FM is more related to dysfunction at the hypothalamic and supra-hypothalamic levels. Because treatment with low physiologic doses of cortisol (<15 mg) has been shown to be safe and effective and routine dynamic ACTH testing does not have adequate diagnostic sensitivity, it is reasonable to give a therapeutic trial of physiologic doses of cortisol to the majority of patients with CFS and FM, especially to those who have symptoms that are consistent with adrenal dysfunction, have low blood pressure or have baseline cortisol levels in the low or low-normal range.

Key words: HPA axis dysfunction; hypothalamic-pituitary-adrenal axis; chronic fatigue syndrome; fibromyalgia; CFIDS; cortisol, hydrocortisone

Introduction

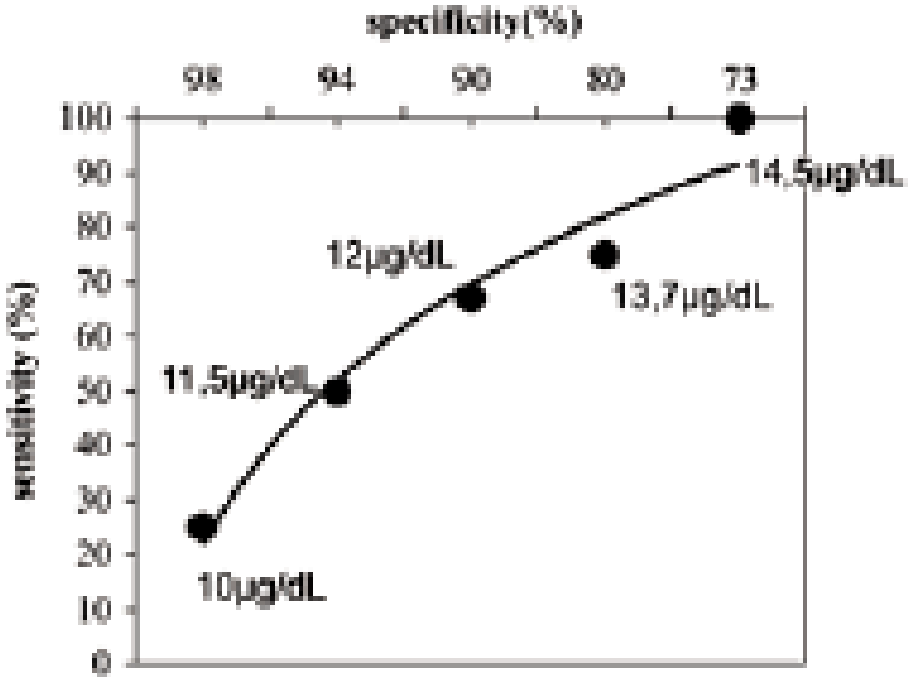
HPA Axis Dysfunction in CFS/FM

- A study published in the *Brazilian Journal of Infectious Disease* demonstrates that in patients with chronic infections, cortisol level of less than **12 has a specificity of greater than 90% for adrenocortical dysfunction** and a level less than 10 ug/dl has a specificity of 98% for adrenocortical dysfunction.
- **The most appropriate cutoff that optimizes specificity and sensitivity as found in this study as well as by others is 12 ug/dl.**
- In addition, a normal ACTH does not rule-out secondary hypoadrenalism, but an abnormally low or low normal ACTH level can be considered confirmatory.

Braz. J. Infect. Dis. Apr. 2001, vol.5, no.2
Journal of Chronic Fatigue Syndrome Volume 8(2) 2001APMIS 2005:113:269 77.

HPA Axis Dysfunction in CFS/FM

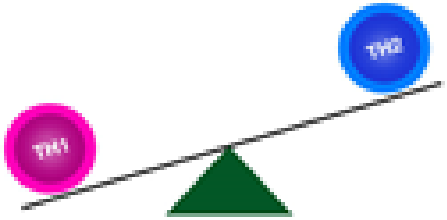
Figure 2. ROC curve showing the relation of sensitivity and specificity of the basal cortisol level to detect adrenocortical dysfunction



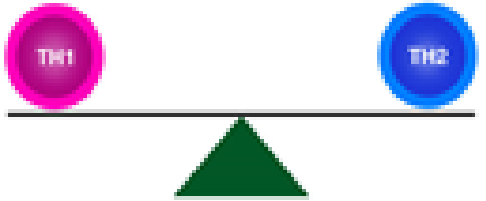
Laboratory "normal" range 4-21 ug/dl but if less than 12 ug/dl, there is a 90% chance of having adrenal insufficiency and with less than 10 ug/dl there is a 98% chance

Infections/immune dysfunction

Treat the immune dysfunction and Infectious component



FROM IMBALANCE TO BALANCE
FROM INEFFECTIVE IMMUNITY TO EFFECTIVE IMMUNITY



TH1-TH2 Shift in CFS

Clin Exp Immunol 2004; 135:294–302 doi:10.1046/j.1365-2249.2004.02354.x

High levels of type 2 cytokine-producing cells in chronic fatigue syndrome

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(Accepted for publication 17 November 2003)

SUMMARY

The aetiology of chronic fatigue syndrome (CFS) is not known. However, it has been suggested that CFS may be associated with underlying immune activation resulting in a Th2-type response. We measured intracellular production of interferon (IFN)-γ and interleukin (IL)-2; type 1 cytokines), IL-4 (type 2) and IL-10 (regulatory) by both polyclonally stimulated and non-stimulated CD4 and CD8 lymphocytes from patients with CFS and control subjects by flow cytometry. After polyclonal activation we found evidence of a significant bias towards Th2- and Tc2-type immune responses in CFS compared to controls. In contrast, levels of IFN-γ, IL-2 and IL-10-producing cells were similar in both study groups. Non-stimulated cultures revealed significantly higher levels of T cells producing IFN-γ or IL-4 in CFS patients. Concluding, we show evidence for an effector memory cell bias towards type 2 responsiveness in patients with CFS, as well as ongoing type 0 immune activation in unstimulated cultures of peripheral blood cells.

Keywords chronic fatigue syndrome cytokines immune activation Th1/ Th2 cytokines

Published in final edited form as:
Bull IACFS ME. 2008 ; 16(3): 19–33.

Evidence for T-helper 2 shift and association with illness parameters in chronic fatigue syndrome (CFS)

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DePaul University

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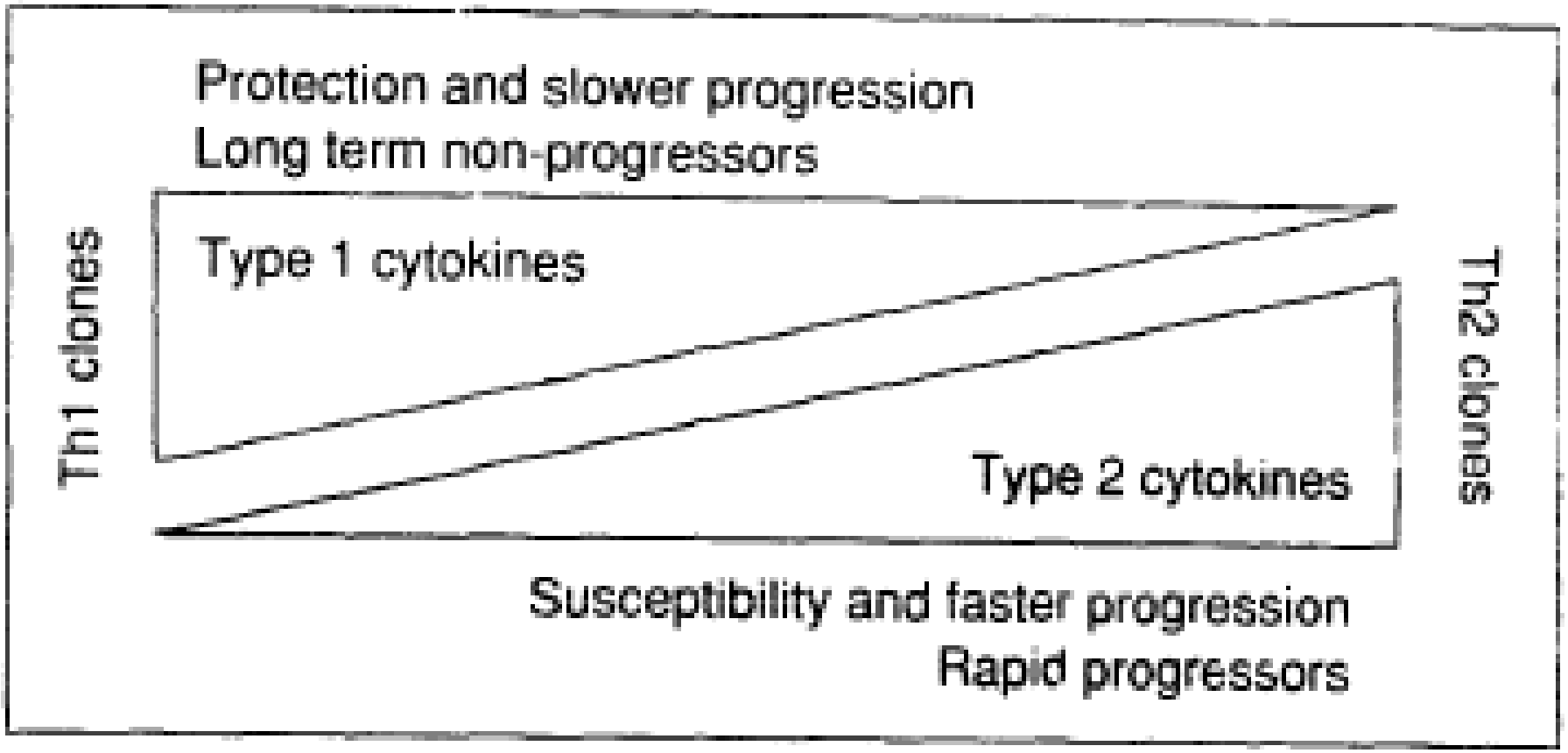
Abstract

Few immunological markers have been consistently reported in CFS. However, a shift to a T-helper 2 (Th2) type immune response has been hypothesized for individuals with CFS. The current study investigated whether individuals with CFS who exhibited a stronger shift towards a Th2 type of immune response would also exhibit more severe symptoms, poorer neurocognitive functioning, and poorer physical and psychosocial functioning. The current investigation measured the percentage of Th1-like and Th2-like memory cells using cell surface flow cytometry in 114 individuals with CFS. The associations between the ratio of Th1 and Th2 memory cells and various illness parameters measures were then examined, including symptom severity, psychiatric functioning, neurocognitive functioning, salivary cortisol levels, and chronic pain status. Results indicated that individuals who exhibited a more extreme shift towards a Th2 immune response also exhibited poorer sleep and high levels of basal salivary cortisol. The implications of these findings are discussed.

Keywords

chronic fatigue syndrome; t-helper 2 shift; immunology; salivary cortisol; cognitive functioning

TH1-TH2 Shift in HIV Determines Progression of Illness



97. Clerici M, Shearer, GM. The Th1-Th2 hypothesis of HIV infection: new insights. Immunology Today 1994;15(12):575-581

Component 4
(treat the immune dysfunction)

- Important to diagnose and treat the immune dysfunction when treating the infectious component
- You are probably using immune modulators in your practice
- But novel immune modulators are a major breakthrough in the treatment of CLD
- Biggest change in treatment strategy for CLD
- Immune dysfunction and inflammation are major underlying causes of a wide-range of illnesses, including CLD, CFS, FM, depression, anxiety, CV disease, neurodegenerative diseases, aging (use for preventive medicine), metabolic illness, pain syndromes, autoimmune diseases, fertility issues, autism, diabetes, obesity, mitochondria dysfunction, inflammation, toxic exposure and addictions (many more)

Chronic Lyme Disease

- After months or years, see significant immune breakdown of TH1 and overstimulation of TH2
- May never become symptomatic or continue to be misdiagnosed as other conditions, including CFS, FM, MS, autoimmune disease, migraines, depression, bipolar, pain syndromes, Parkinson's, ALS, Alzheimer's, CHF, obesity, OCD, ADHD, DM, lupus, Crohn's, PCOS, PMS, IBS, IC, prostatitis, sleep disorder, etc.
- The longer the duration, generally the worse the immune dysfunction, the worse the symptoms and the more treatment resistant.
- Coinfections make symptoms worse and harder to treat.

Immune Modulating Therapies

- Increase TH1 and decrease TH2
- Boosting NK cell and lowering inflammatory cytokines
  - Peptides (Thymosin alpha-1/Thymosin B4/AMP (LL-37)/BPC 157/Nootropics)
  - Stem Cells (Umbilical Cord)
  - LDN (addendum)
  - Heparin(addendum)
  - Transfer factors (addendum)
  - IVIG (low dose or high dose-give 10-20 iu pitocin IV with each treatment if possible)
  - Ozone (MAH/Direct/High-dose/10-pass)
  - UVBI
  - LDA/LDI
  - Allergy elimination (gluten)
  - Antivirals (The deterioration in HIV is in direct correlation to the TH 1/TH2 balance)
  - Antibiotics (do provocated WB, ECP and other immune markers whenever possible
  - Mushroom extracts
  - Isoprinosine
  - High dose B12
  - GcMAF/Leukine/Neupogen
  - Probiotics
  - Silver
  - Antioxidants/Glutathione (low glutathione decreases TH 1 and increases TH2)
  - Chelation (heavy metals stimulate TH2 and lower TH1)
  - Bee Venom

Peptides
(the master regulators)

A greater understanding of the underlying mechanisms of aging and chronic illness has shown that seemingly simple peptides are found to be involved with and regulate most every known process and system in the body in a tissue and cellular specific manner.

The body uses a vast array of highly specific signaling peptides to regulate different parts and functions of tissues throughout the body. Thus, peptide therapy offers the potential to achieve specific desired responses of select tissues rather than less precise and broad effects of hormone therapy or trying to achieve a desired effect by altering physiologic processes with synthetic medications.

Currently, peptides are available that are shown to safely and effectively improve and modulate specific parts of hormone production, immune function, the sleep cycle, the production of inflammatory mediators, DNA replication, cell division and renewal, cancer cell destruction and apoptosis, libido and sexual arousal, weight loss and other metabolic activities, tissue healing and specific biological functioning of the brain, skin, eyes and urinary and reproductive systems.

Thus, increasing numbers of peptides are becoming clinically available that can safely improve, optimize or normalize specific functions of the body.

Peptides

- Thymic peptides
  - Thymosin Alpha 1 (TA1)
  - Thymosin Beta 4 (Tβ4)
  - Thymulin
- Pineal proteins
  - Epithalon (addendum)
- Nootropics
  - Semax
  - Selank
  - Cerebrolysin
- Antimicrobial peptides
  - LL-37
- Others
  - Follistatin
  - BPC-157

Peptides for CFS/Lyme

- **Thymic peptides:** Thymosin alpha-1 (boost TH1), thymosin beta-4/thymulin (TH1-TH2 modulation)
- **BPC-157**
  - Reduces inflammation and increases healing in most every tissue, including gut (probably best treatment for leaky gut), brain, skin, muscle, degenerative joints, cardiac (prevents and treats arrhythmia, & heart failure in Lyme myocarditis), neuropathic pain and protective against neuro and endotoxins.
- **Noortopics-Cerebrolysin/Semax/Selank**
  - Improves brain function, memory, depression and anxiety
- **GHRP/GHRP**-increases growth hormone
- **GH Frag 174-191**-fragment of growth hormone
- **PT 141**-libido for men and women & erectile dysfunction
- **Epithalon** (see Addendum Slides)
  - Pineal peptide-increases longevity with significant reduction in CVD and cancer
  - Increases telomere length
  - Can reverse infertility
- **Delta Sleep Inducing Peptide (DSIP)-**induces deep sleep and reduces pain
- **Follistatin**-weight loss, muscle gain
- **Antimicrobial peptides (LL-37)**

Thymic/Pineal Peptides and Age

- According to the U.S. Center for Disease Control (CDC), approximately 80 % of aged individuals are afflicted with at least one chronic disease as a result of a declination of thymic-related immune function.[73]
- Obesity and calorie intake are strongly associated with thymic involution
- The majority of people have pineal gland calcification by age 30.

72. Consolini R, Legitimo A, Calleri A, et al. Distribution of age-related thymulin titres in normal subjects through the course of life. Clin Exp Immunol 2000; 121:444-447
73. Gui J, Mustachio LM, Su DM, et al. Thymus Size and Age-related Thymic Involution: Early Programming, Sexual Dimorphism, Progenitors and Stroma. Aging Dis 2012;3(3):280-90

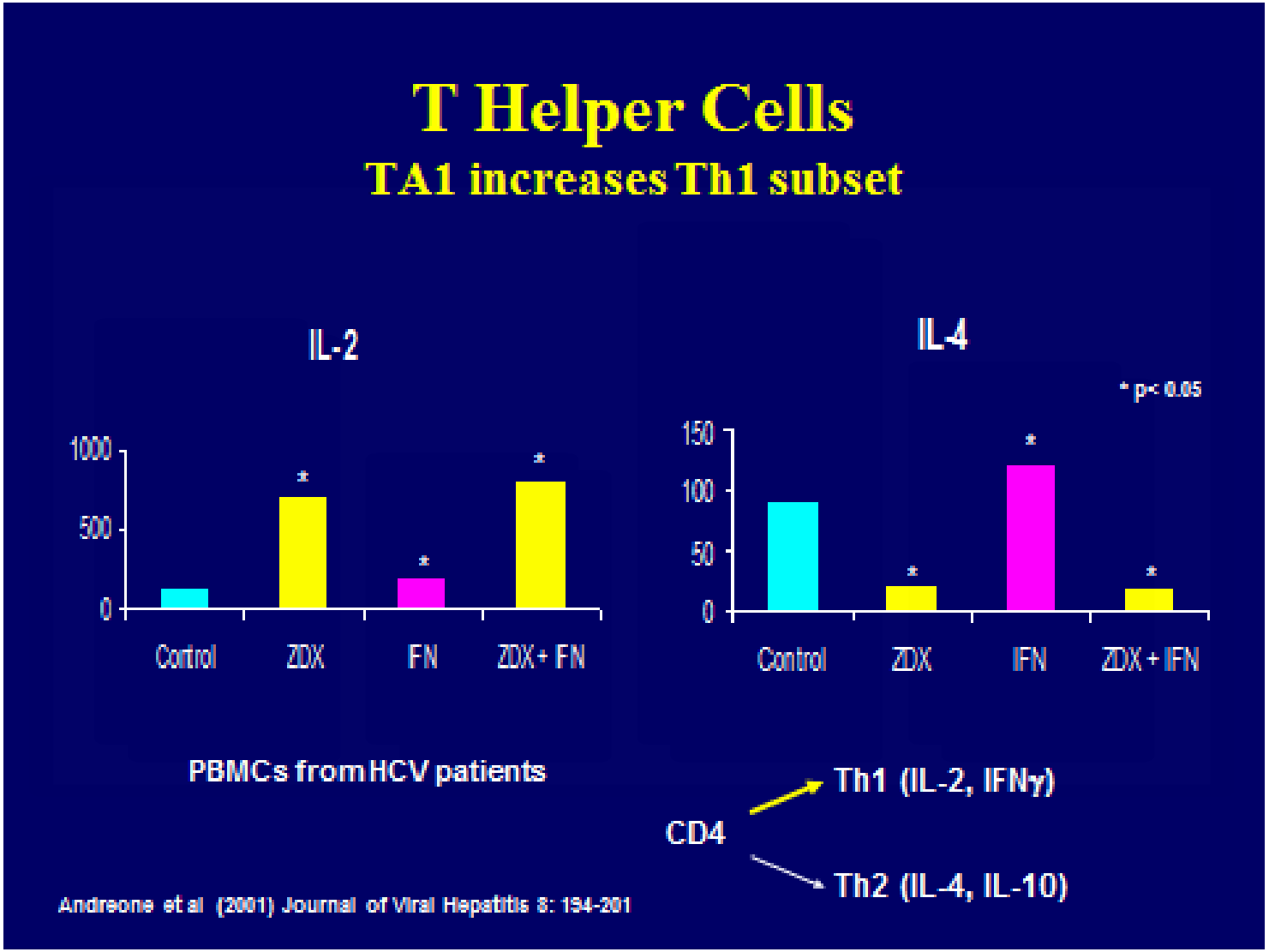
Thymic Peptides – Clinical Effects
(Thymosin alpha-1, thymosin beta-4, thymulin)

- Improved tissue repair and healing[35,36,37,41]
- Improved host defense to infection[26,27,28,29,30]
- Reverse immunosuppression of chronic infection (Lyme)[24,26,27,28,29,33,34,37,]
- Increases antioxidant and glutathione production[26,27,28,31,28,29,63,66]
- Boost NK function[26,35]
- TH2-TH1 immune modulation (infections, cancer, herxheimer, autoimmune)[26,27,28,30,31,33]
- Bind neuro/endotoxins[111]
- Cardiac regeneration and protection post-MI, CHF, etc.[39,65,98,99,100]
- Neurologic regeneration and protection post-stroke, TBI, Lyme, Alzheimer's, neuropathy, Parkinson's, etc.[63,67,42]
- Stimulate stem cell activity and proliferation[32,34,36,38,40,41,42]
- Increases longevity[89,90,91]
- Almost non-existent side effects at 100-fold dose+ excess[103,104]
- Excellent safety profile with large therapeutic window (over 1000 fold)[103,104]

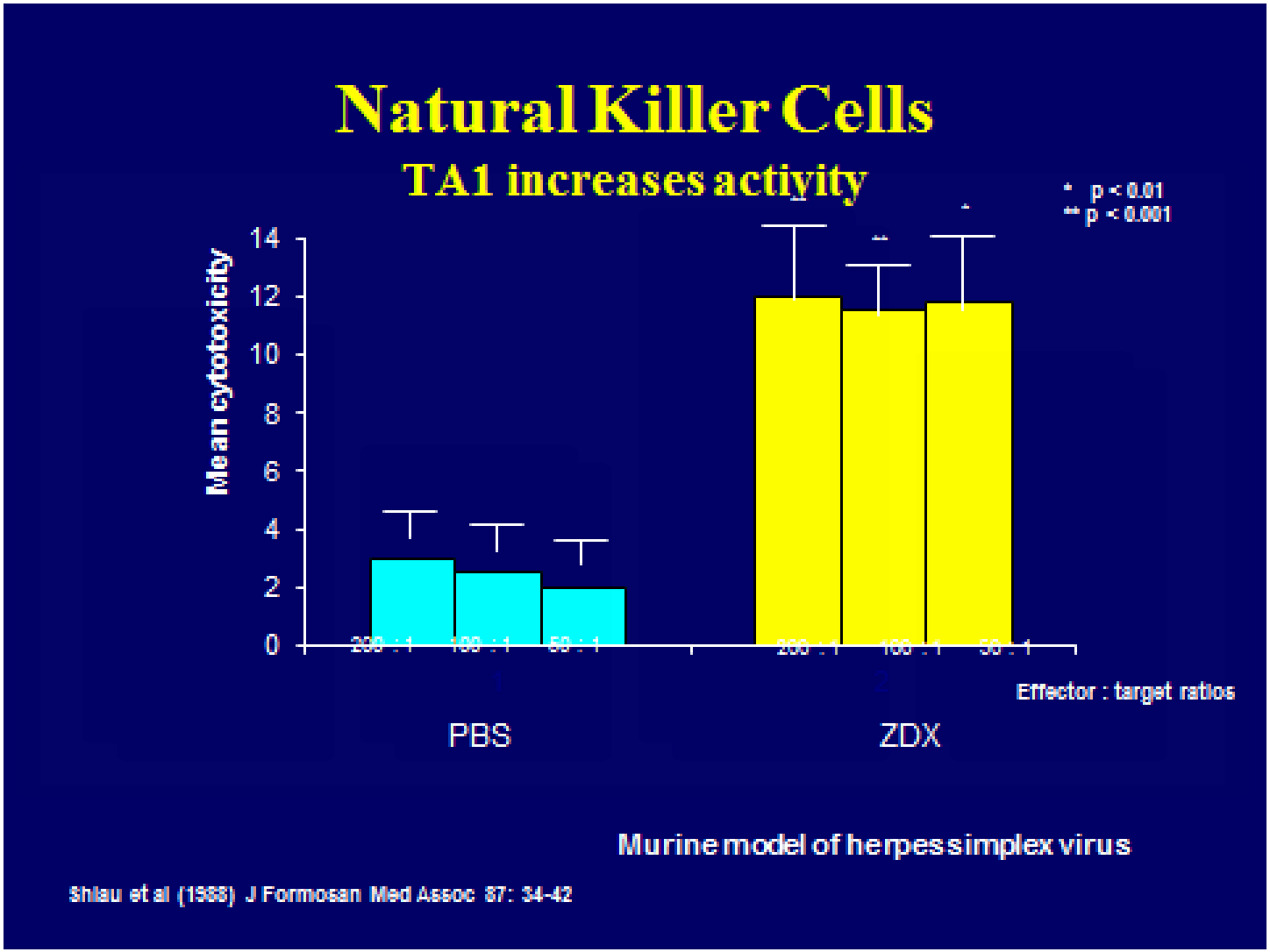
TA1 Applications

- Approved in over 30 countries
  - Cancer treatment/chemotherapy adjunct[29,30,31,33]
  - Treatment of Hepatitis B and C[28,30]
  - Treatment of AIDS[28]
  - Approved in USA as orphan drug
  - Vaccine adjunct[29]

TA1 Effects


T Helper Cells
TA1 increases Th1 subset
IL-2
1000
500
0
Control
ZDX
FN
ZDX + FN
IL-4
* p< 0.05
150
100
50
0
Control
ZDX
IFN
ZDX + IFN
PBMCs from HCV patients
CD4
Th1 (IL-2, IFNγ)
Th2 (IL-4, IL-10)
Andreone et al (2001) Journal of Viral Hepatitis 8: 194-201

TA1 Effects


Natural Killer Cells
TA1 increases activity
* p < 0.01
** p < 0.001
Mean cytotoxicity
14
12
10
8
6
4
2
0
200 : 1
100 : 1
50 : 1
200 : 1
100 : 1
50 : 1
1
2
Effector : target ratios
PBS
ZDX
Murine model of herpes simplex virus
Shiau et al (1988) J Formosan Med Assoc 87: 34-42

Tβ4 and Neurologic Regeneration

Editorial

EXPERT OPINION

Thymosin β4 as a restorative/ regenerative therapy for neurological injury and neurodegenerative diseases

Michael Chopp† & Zheng Gang Zhang
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Thymosin β4 (Tβ4) promotes CNS and peripheral nervous system (PNS) plasticity and neurovascular remodeling leading to neurological recovery in a range of neurological diseases. Treatment of neural injury and neurodegenerative disease 24 h or more post-injury and disease onset with Tβ4 enhances angiogenesis, neurogenesis, neurite and axonal outgrowth, and oligodendrogenesis, and thereby, significantly improves functional and behavioral outcomes. We propose that oligodendrogenesis is a common link by which Tβ4 promotes recovery after neural injury and neurodegenerative disease. The ability to target many diverse restorative processes via multiple molecular pathways that drive oligodendrogenesis and neurovascular remodeling may be mediated by the ability of Tβ4 to alter cellular expression of microRNAs (miRNAs). However, further investigations on the essential role of miRNAs in regulating protein expression and the remarkable exosomal intercellular communication network via exosomes will likely provide insight into mechanisms of action and means to amplify the therapeutic effects of Tβ4.

Keywords: microRNA, oligodendrocyte progenitor cells, thymosin beta 4, tissue plasminogen activator, traumatic brain injury

Expert Opin. Biol. Ther. (2015) 15(Suppl.1):S9-S12

BPC (Body Protection Compound) Overview

- Protects and heals inflamed intestinal epithelium
  - key treatment for leaky gut
- Significantly beneficial for inflammatory bowel disease, TBI, gastric ulcers, neurdegenerative diseases, pain syndromes as well as skin, muscle, tendon and ligament damage.
- Protects liver from toxic insults (alcohol, antibiotics, etc.) and promotes healing
- Protects against negative effects of acute and chronic stress, including increases cell survival under stress
- Prevents and reverses toxic damage from environmental, neuro and endotoxins (true of a number of peptides)
- Increases growth hormone receptors and effects of GH
- Prevents and inhibits arrhythmias

43. Sikiric P, Seiwerth S, Rucman R, et al. Stable gastric pentadecapeptide BPC 157: novel therapy in gastrointestinal tract. Curr Pharm Des 2011;17(16)1612-32
44. Boban-Blagaic A, Vladimir Blagaic, et al. The influence of gastric pentadecapeptide BPC 157 on acute and chronic ethanol

AMP Overview

- AMPs serve as endogenous antibiotics that are able to rapidly kill an unusually broad range of bacteria, fungi, parasites and viruses.[76,80]
- Direct effects involve selective disruption of prokaryotic cell membrane[106]
- In addition to their direct antimicrobial activity, also have multiple modes of action, including immune modulating effects[106]
- LL-37 boosts mesenchymal stem cell migratory behavior and immunomodulatory effects.[77]
- Significant anti-biofilm activity at low levels[78,105]
- The MIC of LL-37 is much lower in vivo than in-vitro due to its immune modulating effects.
- AMPs are synergistic with antibiotics and reduce the development of drug resistance.[79]
- Effective against free borrelia spirochetes and cystic forms[76]
- Even low doses block the bioactivity of endotoxins and neurotoxins[109,110]
- Well-tolerated, very safe with little side effects and a large therapeutic window
- Caution with autoimmune disease at high doses, as with any immune modulator

78. Fuente-Nunez C, Hancock. Using anti-biofilm peptides to treat antibiotic-resistant bacterial Infections. Postdoc J 2015;3(2):1-8

AMP Mechanism of Action

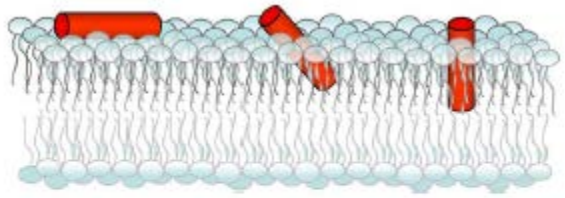

Fig. 2. Association of amphipathic α-helical peptides (cylinders) with a lipid bilayer can occur in three general orientations: parallel to the membrane surface, at an oblique angle, or perpendicular to the membrane surface (i.e., along the bil

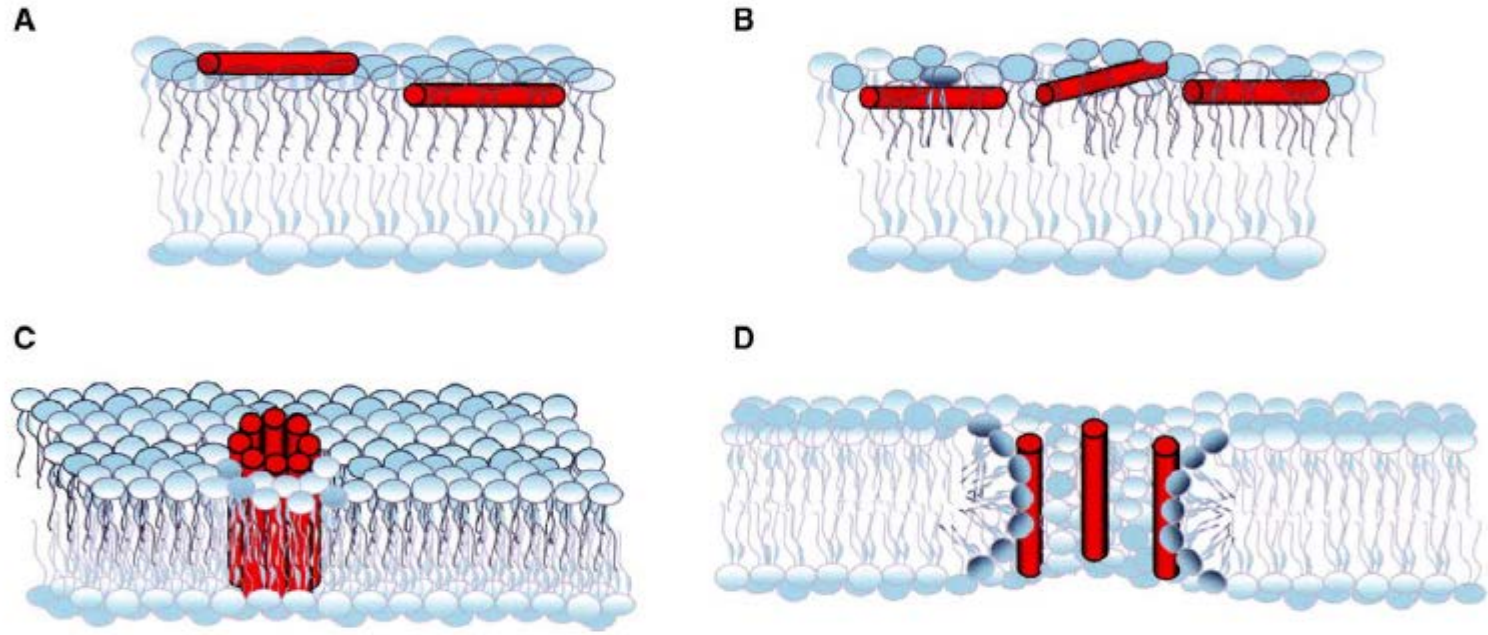

Fig. 8. Models of transmembrane channel formation. (A) Peptide α-helices (cylinders) initially associate parallel to the membrane surface, either superficially (left) or embedded just below the aqueous interface. (B) Peptides continue to accumulate at or near the bilayer surface, disrupting lipid packing and causing membrane thinning. This step may or may not involve peptide–peptide aggregation. Once a critical peptide/lipid ratio is reached, peptides either (C) insert into the membrane as a barrel-stave type pore, or (D) induce the localized formation of toroidal pores.

106. Sato H, Feix JB. Peptide–membrane interactions and mechanisms of membrane destruction by amphipathic α-helical antimicrobial peptides. Biochim Biophys Acta 2006;1758(9):1245-56.

AMP-Rapid Onset of Effect (1 minute)

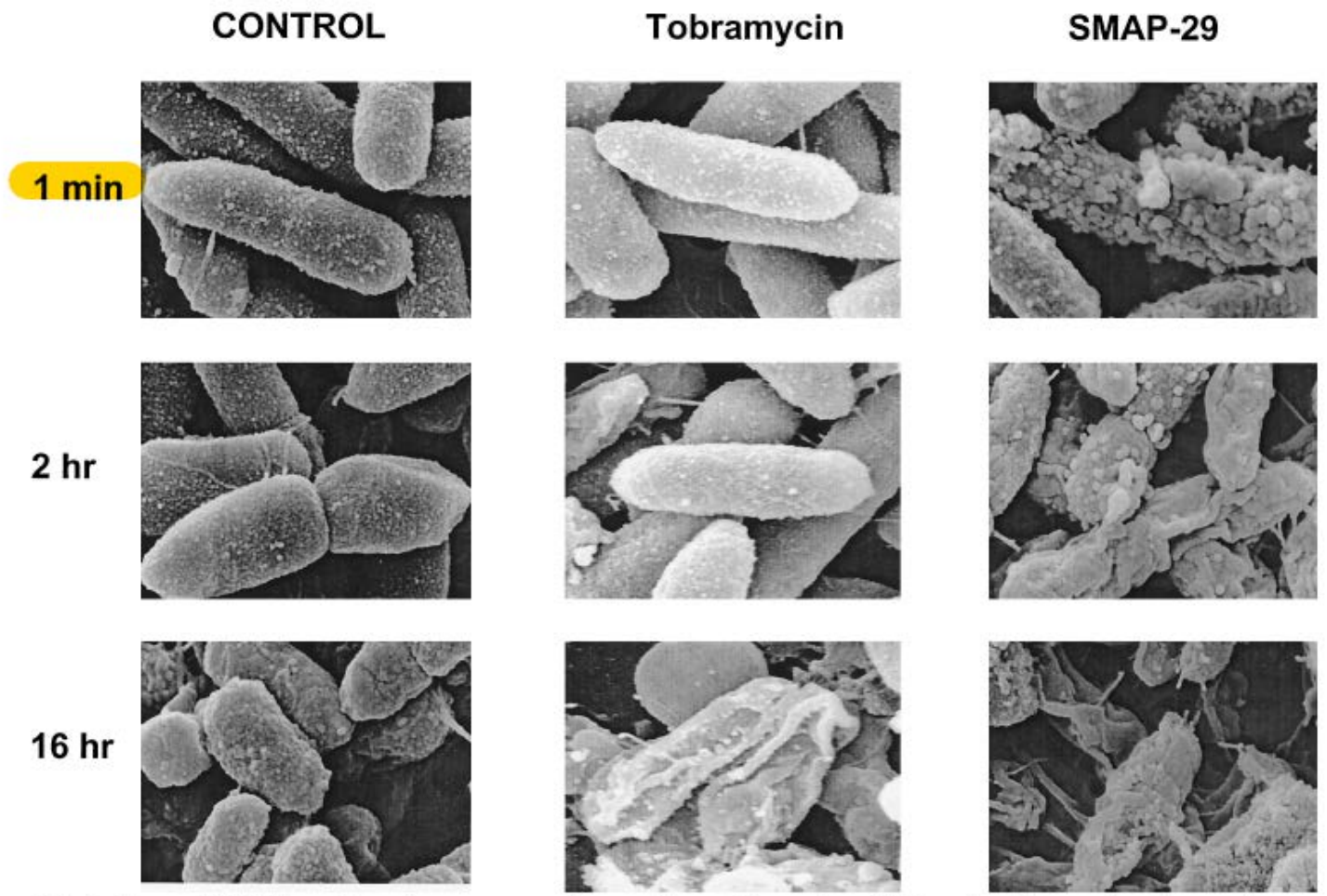

FIG. 3. Effects of SMAP29 or tobramycin treatment on the morphology of *P. aeruginosa* PAO1 evaluated by scanning electron microcopy. PAO1 was treated with media alone, tobramycin (5 μg/ml), or SMAP29 (0.5 μg/ml). At 1 min, 2 h, and 16 h the bacteria were processed for scanning electron microscopy. Within one minute, treatment with SMAP29 resulted in blebbing or blistering of the outer cell wall. The effects of tobramycin were much slower in their onset.

80. Sato H, Feix JB. Peptide–membrane interactions and mechanisms of membrane destruction by amphipathic α-helical antimicrobial peptides. Biochim Biophys Acta 2006;1758(9):1245-56.

Nootropics

- Neurotrophic action similar to that of nerve growth factors [72-74]
- Peripheral and central nervous system stimulation
- Neuroprotective effects
- Shields neurons from neurotoxins, inflammation and injury
- Protects from stress and depression
- Improves memory (even in healthy adults)
- Neurological regeneration
  - TBI[69]
  - Alzheimer's[15,69-72]
  - Parkinson's'
  - Stroke[69,73]
  - Toxin induced
- No significant side effects reported [15,69,70,72,73]

Stem Cell Therapy for CFS/Lyme Disease

- Mesenchymal stem cell (MSC) treatment effects include:
  - Direct replacement of the injured tissue with growth of the MSCs
  - Paracrine action on injured cells and supporting tissue
  - Activation of stem cells in and around the injured tissue
  - Indirect effects on tissue support
  - Stimulation of revascularization
  - Protection of tissue from stress-induced apoptosis
  - Protection of tissue and cells from neurotoxins
  - Anti-inflammatory effects
  - Immune modulatory effects
  - Secretion of antimicrobial peptides
- MSC paracrine effects are bidirectional , resulting in reprogramming of their exosomes and secretome to respond to specific need of the tissue.
- MSCs will secrete different products in presence of infection (maybe not if stem cells have been chronically exposed to insult or invader)

93. Kalaszczynska I, Ferdyn K. Wharton's Jelly Derived Mesenchymal Stem Cells: Future of Regenerative Medicine? Recent Findings and Clinical Significance. BioMed Res Int 2015;1-11

UCTMSC vs. Autologous MSCs

- Autologous stem cells derived from one's bone marrow or fat tissue have served as the mainstay of rejuvenate stem cell therapy for the last decade.
- The aging of MSCs, as well as their microenvironment, dramatically affect the function, viability and rejuvenating potential of MSCs, especially over many years.
- The use of autologous MSCs of CFS/Lyme patients often have limited regenerative capacity due to age, chronic illness, inflammation, infections and cumulative negative effects of environmental toxins and endotoxins, unhealthy lifestyle and medications.
- Studies show that chronic use of seemingly safe, commonly used medications, such as NSAIDS, will dramatically reduce the body's MSC function.

93. Kalaszcynska I, Ferdyn K. Wharton's Jelly Derived Mesenchymal Stem Cells: Future of Regenerative Medicine? Recent Findings and Clinical Significance. BioMed Res Int 2015;1-11
94. Hsieh J-Y, Wang H-W, Chang S-J, Liao K-H, Lee I-H, et al. (2013) Mesenchymal Stem Cells from Human Umbilical Cord Express Preferentially Secreted Factors Related to Neuroprotection, Neurogenesis, and Angiogenesis. PLoS One 2013;8(8):e72604.

UCTMSC vs. Autologous MSCs

- Aged autologous MSCs from chronically ill patients, such as Lyme, often suffer from:
  - Loss of ability to secrete numerous cytokine, peptide regulators, growth factors, and antimicrobial peptides
  - Reduced anti-inflammatory effects
  - Reduced immune modulatory capacity
  - Reduced self-renewal and differentiation capacity
  - Lower telomerase levels and shorter telomere length
  - A reduction of chemokine receptors, limiting their effectiveness to home-in on the damaged target tissue.
  - Overall loss of viability and increased apoptosis with stress (don't survive the transplant)
  - Reduced paracrine effects
- Umbilical cord tissue MSCs do not suffer from the above limitations.

76. Alcayaga-Miranda F, Cuenca J, Khoury M. Antimicrobial Activity of Mesenchymal Stem Cells: Current Status and New Perspectives of Antimicrobial Peptide-Based Therapies. Front Immuno 2017;8(339):1-15

77. Oliveria-Bravo M, Sanglorgi BB, Schavinato S, et al. LL-37 boosts immunosuppressive function of placenta-derived mesenchymal stromal cells. Stem Cell Resch & Ther 2016;7-189

Mesenchymal Stem Cells

Systematic Review

Umbilical Cord Tissue Offers the Greatest Number of Harvestable Mesenchymal Stem Cells for Research and Clinical Application: A Literature Review of Different Harvest Sites

C. Thomas Vangsness Jr., M.D., Hal Sternberg, M.D., and Liam Harris, B.S.

Purpose: Recent years have seen dramatic increases in the techniques used to harvest and isolate human mesenchymal stem cells. As the potential therapeutic aspects of these cells further develop, informative data on the differences in yields between tissue harvest sites and methods will become increasingly valuable. We collected and compared data on cell yields from multiple tissue harvest sites to provide insight into the varying levels of mesenchymal stem cells by tissue and offer primary and alternative tissue types for harvest and clinical application. **Methods:** The PubMed and Medline databases were searched for articles relating to the harvest, isolation, and quantification of human mesenchymal stem cells. Selected articles were analyzed for relevant data, which were categorized according to tissue site and, if possible, standardized to facilitate comparison between sites. **Results:** Human mesenchymal stem cell levels in tissue varied widely according to tissue site and harvest method. Yields for adipose tissue ranged from 4,737 cells/mL of tissue to 1,550,000 cells/mL of tissue. Yields for bone marrow ranged from 1 to 30 cells/mL to 317,400 cells/mL. Yields for umbilical cord tissue ranged from 10,000 cells/mL to 4,700,000 cells/cm of umbilical cord. Secondary tissue harvest sites such as placental tissue and synovium yielded results ranging from 1,000 cells/mL to 30,000 cells/mL. **Conclusions:** Variations in allogeneic mesenchymal stem cell harvest levels from human tissues reflect the evolving nature of the field, patient demographic characteristics, and differences in harvest and isolation techniques. At present, Wharton's jelly tissue yields the highest concentration of allogeneic mesenchymal stem cells whereas adipose tissue yields the highest levels of autologous mesenchymal stem cells per milliliter of tissue. **Clinical Relevance:** This comparison of stem cell levels from the literature offers a primer and guide for harvesting mesenchymal stem cells. Larger mesenchymal stem cell yields are more desirable for research and clinical application.

TISSUE ENGINEERING: Part B
Volume 00, Number 00, 2014
© Mary Ann Liebert, Inc.
DOI: 10.1089/ten.teb.2013.0664

Umbilical Cord Mesenchymal Stem Cells: The New Gold Standard for Mesenchymal Stem Cell-Based Therapies?

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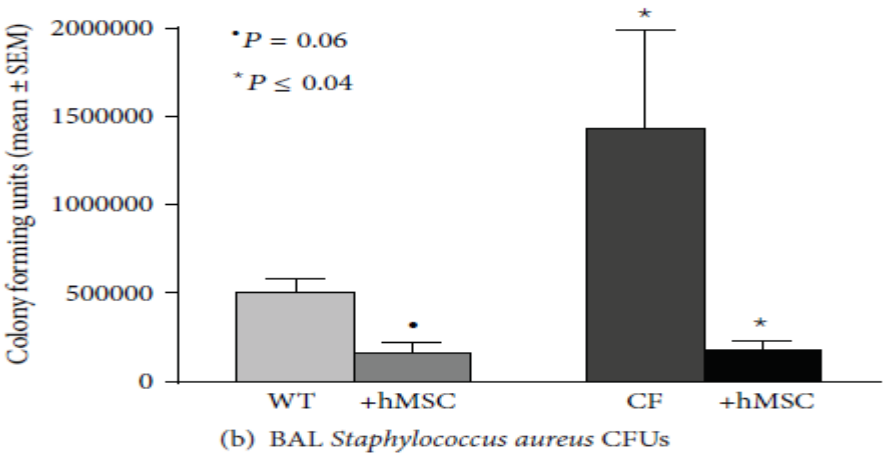
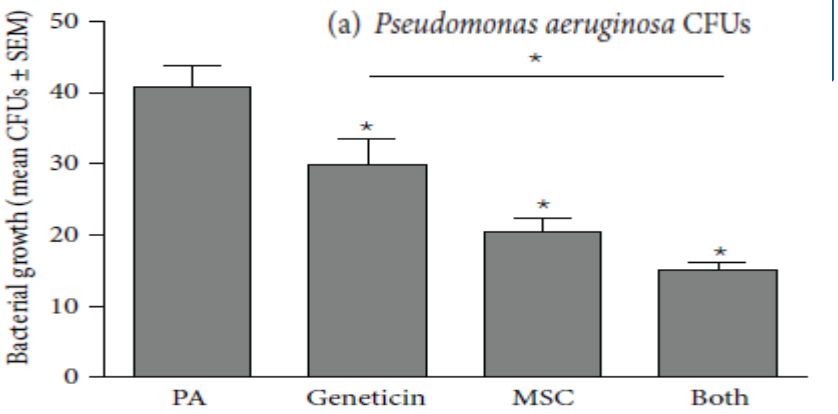
Due to their self-renewal capacity, multilineage differentiation potential, paracrine effects, and immunosuppressive properties, mesenchymal stromal cells (MSCs) are an attractive and promising tool for regenerative medicine. MSCs can be isolated from various tissues but despite their common immunophenotypic characteristics and functional properties, source-dependent differences in MSCs properties have recently emerged and lead to different clinical applications. Considered for a long time as a medical waste, umbilical cord appears these days as a promising source of MSCs. Several reports have shown that umbilical cord-derived MSCs are more primitive, proliferative, and immunosuppressive than their adult counterparts. In this review, we aim at synthesizing the differences between umbilical cord MSCs and MSCs from other sources (bone marrow, adipose tissue, periodontal ligament, dental pulp,...) with regard to their proliferation capacity, proteic and transcriptomic profiles, and their secretome involved in their regenerative, homing, and immunomodulatory capacities. Although umbilical cord MSCs are until now not particularly used as an MSC source in clinical practice, accumulating evidence shows that they may have a therapeutic advantage to treat several diseases, especially autoimmune and neurodegenerative diseases.

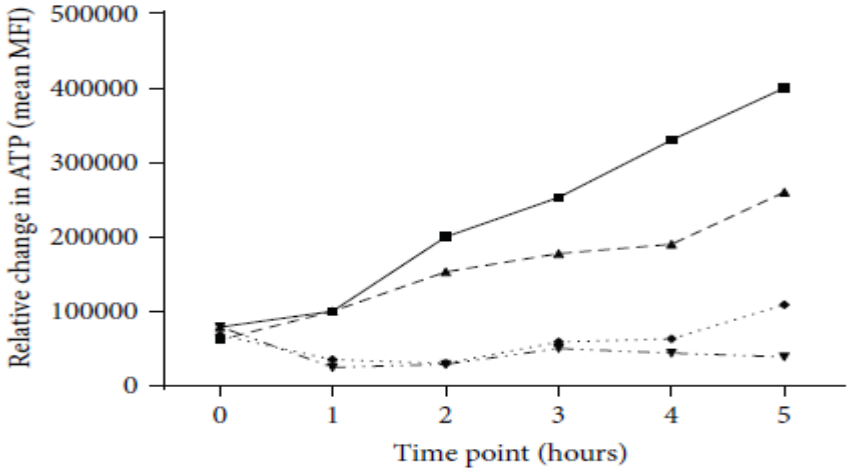
Antimicrobial Effects of MSCs



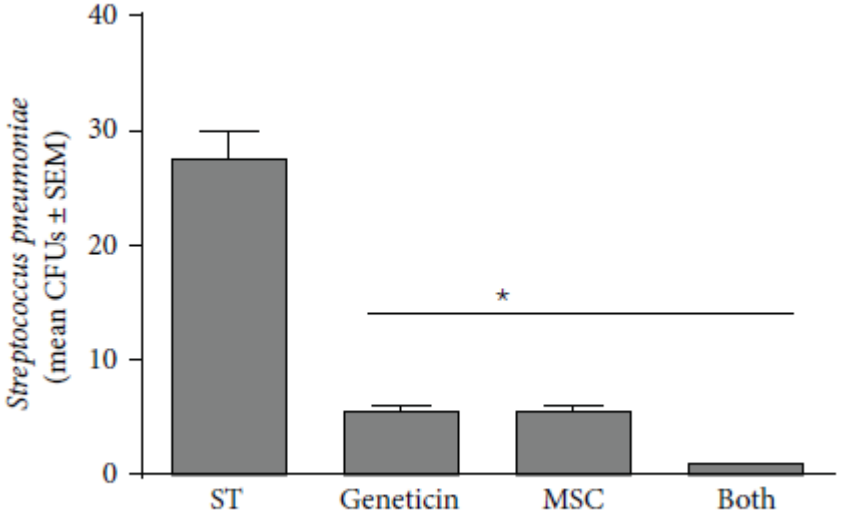
(b) BAL *Staphylococcus aureus* CFUs



(a) *Pseudomonas aeruginosa* CFUs



(b) *Pseudomonas aeruginosa* growth rate



Sutton MT, Fletcher D, Ghosh SK, et al. Antimicrobial Properties of Mesenchymal Stem Cells: Therapeutic Potential for Cystic Fibrosis Infection, and Treatment. Stem Cells Int 2016; 2016:5303048:1-10.

Stem Cell and Peptide Synergy

- Mesenchymal stem cells (MSCs) secrete LL-37[76,77]
- Younger MSCs secrete significantly more antimicrobial and immune modulating peptides as well as growth factors, cytokines and signaling molecules.[93,94,99,108]
- MSCs shown to enhance bacterial clearance and increase survival in bacterial or LPS induced sepsis and cystic fibrosis, which is shown to be mediated by the antimicrobial and endotoxin binding of LL-37[76,109,110]
- Thymic peptides and LL-37 are shown to enhance the proliferation, differentiation and migration of MSCs into target tissues as well as increase their immune modulating effects.[38,40,77,99]
- Thus, in addition to being used as stand-alone therapies, the peptides can multiply the already profound regenerative and healing effects of UCTMSCs

73. Alcayaga-Miranda F, Cuenca J, Khoury M. Antimicrobial Activity of Mesenchymal Stem Cells: Current Status and New Perspectives of Antimicrobial Peptide-Based Therapies. Front Immuno 2017;8(339):1-15

74. Oliveria-Bravo M, Sanglorgi BB, Schavinato S, et al. LL-37 boosts immunosuppressive function of placenta-derived mesenchymal stromal cells. Stem Cell Resch & Ther 2016;7-189

Tβ4 and MSC Synergy

Cell Transplantation and Tissue Regeneration

Thymosin β4 Increases the Potency of Transplanted Mesenchymal Stem Cells for Myocardial Repair

Lei Ye, MD, PhD; Pengyuan Zhang, MD, PhD; Sue Duval, PhD; Liping Su, BSc; Qiang Xiong, PhD; Jianyi Zhang, MD, PhD

Background—Thymosin β4 (Tβ4) has been shown to enhance the survival of cultured cardiomyocytes. Here, we investigated whether the cytoprotective effects of Tβ4 can increase the effectiveness of transplanted swine mesenchymal stem cells (sMSCs) for cardiac repair in a rat model of myocardial infarction (MI).

Methods and Results—Under hypoxic conditions, cellular damage (lactate dehydrogenase leakage), apoptosis (terminal deoxynucleotidyl transferase dUTP nick end labelingc cells), and caspase-8 activity were significantly lower, whereas B-cell lymphoma-extra large protein expression was significantly higher, in sMSCs cultured with Tβ4 (1 μg/mL) than in sMSCs cultured without Tβ4, and Tβ4 also increased sMSC proliferation. For in vivo experiments, animals were treated with basal medium (MI: n=6), a fibrin patch (Patch: n=6), a patch containing sMSCs (sMSC: n=9), or a patch containing sMSCs and Tβ4 (sMSC/Tβ4: n=11); Tβ4 was encapsulated in gelatin microspheres to extend Tβ4 delivery. Four weeks after treatment, echocardiographic assessments of left-ventricular ejection fraction and fractional shortening were significantly better ($P<0.05$) in sMSC/Tβ4 animals (left-ventricular ejection fraction=51.7±1.1%; fractional shortening=26.7±0.7%) than in animals from MI (39±3%; 19.5±1.7%) and Patch (43±1.4%; 21.6±0.9%) groups. Histological assessment of infarct wall thickness was significantly higher ($P<0.05$) in sMSC/Tβ4 animals (50%, [45%, 80%]) than in animals from MI (25%, [20%, 25%]) group. Measurements in sMSC (left-ventricular ejection fraction=45±2.6%; fractional shortening=22.9±1.6%; TH=43% [25%, 45%]), Patch, and MI animals were similar. Tβ4 administration also significantly increased vascular growth, the retention/survival of the transplanted sMSCs, and the recruitment of endogenous c-Kit$^+$ progenitor cells to the infarcted region.

Conclusions—Extended-release Tβ4 administration improves the retention, survival, and regenerative potency of transplanted sMSCs after myocardial injury. (*Circulation*. 2013;128[suppl 1]:S32-S41.)

Key Words: angiogenesis ■ microsphere ■ myocardial infarction ■ stem cell ■ tissue engineering

Using Immune Dysfunction to Diagnose Lyme

- When to expect Lyme
  - Meets criteria for CFS/FM/ME or MSIDS
  - More severe forms of CFS/FM
  - More neurologic/autonomic symptoms and brain fog
  - The more "strange" symptoms, the more likely Lyme disease
  - The more immune dysfunction, the more likely LD

Identification of Immune Dysfunction

- Low NK cell function < 30 LU
- Low immune cell function (ATP production) (Quest)
- Low CD 57 (Labcorp)
- Elevated C4a (Quest-Nat. Jewish on dry ice)
- VEGF (increase with bartonella, suppressed by molds)
- Eosinophil cationic protein (Babesia—may only increase after treatment)
- ACE above 30
- Immune activation of coagulation (D-dimer, soluble fibrin monomer, prothrombin fragment 1+2, thrombin antithrombin complex, PAI-1)(Lyme/babesia)
- Low Igg subclasses (Secondary to TH1-TH2 shift (low IFgamma))
- Leptin above 12
- Elevated human transforming growth factor beta (HTGFB)
- Most any band on Quest WB, (even a 41 kd—selection bias on my part)
  - Provocate with antibiotics before doing indirect testing and/or treat the immune system before testing
  - Will know with a high probability who will or should test positive on further Lyme testing

Review of Key Labs:

(red: key tests, blue important, green useful)

- Low or low normal NK cell function <30 (Quest)
- Elevated C4a (Quest)
- Elevated HTGF-beta
- ECP-Eosinophil cationic protein (high sensitivity for Babesia with provocated test)
- Immune activation of coagulation (D-dimer, soluble fibrin monomer, prothrombin fragment 1+2, thrombin antithrombin complex, PAI-1(often with Babesia)
- Low immune cell function (ATP production)(Quest)
- Low CD 57 (Labcorp)
- Elevated VEGF with bartonella (if zero, not a valid test)
- Low Igg subclasses
- ACE above 30

- Importance is based on combination of sensitivity, specificity and logistics (ability to get useful results due to difficult processing, complex processing needed or send out required and based on over 10 years of clinical practice (mostly with Quest for the standard tests).
- Do not include specialty tests or specialty laboratories, which can be extremely useful

Peptide Usage Overview

Holtorf, Kent. National Academy of Hypothyroidism and Integrated Sciences (NAHIS)

	Class	Pain	Immunity	Inflammation	Libido	Anti-aging	Weight Loss	Cognitive	Antioxidant	Sleep	Dose (1)	Conditions
GH FRAG 176-191	Truncated HGH		+	+		+	+	+		+	150-500 mcg qd (pulse)	Healing, body fat,
Semax	Nootropic	+	+	++	+	+++		+++	++	+	300-1000 mcg/day	Cognitive dysfun memory, stroke, d depression, TBI, A
Cerebrolysin	Nootropic		+	++		++		+++	++	+	200-1000 mg qAM	Cognitive dysfun memory, stroke, d depression, TBI, A
Selank	Nootropic		+			+		++		+	250-1500 mcg/day	Cognitive dysfun memory, stroke, d depression, TBI, A ety, depression
CJC 1295 + Ipamorelin	GHRH/GHRP		+			+	+	+		+	200/200-500/500 qd (pulse)(1b)	Growth hormone
Follastatin	Myostatin Blocker		++	++			+++				100-200mcg 1-2 x/ week	Weight loss, musc
Melanotan II	MSH Analog				++		++				200 mcg q 1-2 weeks	Tanning, weight lo
PT141/Bremelanotide	MSH Analog				+++		+				1-2 mg q 1-3 days prn	Libido, weight los
Thymosin Alpha 1	TH1 Stimulation		+++	+		+		+	+		300-1000 mcg qd	Immune boosting infection, cancer
Thymulin	TH1-TH2 Balance	++	+++	+++		+++		++	++	+	500-1000 mcg/day	Immune modulati rejuvination, neuro tion, muscle pain
Thymosin Beta 4	TH1-TH2 Balance	++	+++	+++		++		++	++	+	300-1000 mcg qd	Immune modulati rejuvination, neuro tion, muscle pain
BCP 157	Reduce TH2 (Inflamation)		++	+++		++		+	+		500 mcg qd to q week oral; 200-1000 mcg qd - q week	Systemic or GI inf helaing, rejuvinaiti
DISP	Sleep Peptide	++				+			++	+++	300-1000 mcg/day (may increase to 3000 mcg/day)	Sleep
Epithalon	Pineal Gland Peptide		+			+++		++	++	++	300-1000 mcg/day	Immunity, cancer, ti-aging, telomere ing, DNA repair
LL-37	Anti-Microbial Peptide		+	+					+		200-1000 cmg/day	Anti-biofilm, chro tions, Lyme, anti-b anti-viral, anti-par

Thank You

Questions?

(can email kholt@holtorfmed.com or office phone # 310-375-2705)

Conclusions

Summary:

- Lyme disease is a multisystem disease with a key component being immune dysfunction.
- The immune dysfunction (TH1-TH2 shift) is a key component for understanding the ability for effective diagnosis and treatment of LD.
- Failure to address these immune abnormalities are a common cause of treatment failure.
- Treatments that address these abnormalities, including peptide therapies and umbilical cord stem cells can be very effective and may be replacing the standard of long-term antibiotics.

Markers of CFS

- Low NK cell function < 30 LU (low TH1)
- low CD 57 (Labcorp)(low TH1)
- Elevated C4a (high TH2)
- Elevated HTGFB (High TH2)
- ECP (provocated)
- Immune activation of coagulation (D-dimer, soluble fibrin monomer, prothrombin fragment 1+2, thrombin antithrombin complex, PAI-1)(high TH2)

Peptide Therapy

- Short chain of amino acids
- Generally < 70 AA (> 70 becomes a protein)
- Natural, bioidentical or altered (synthetic)
- Seemingly simple peptides are found to be regulate most every known process and system in the body in a tissue specific manner
- While hormone therapy and optimization was a mainstay of antiaging medicine, it is being understood that regulatory peptides are the master controls of many functions of the body, including hormone production

Immune Modulating Therapies

- Increase TH1 and decrease TH2
- Boosting NK cell and lowering inflammatory cytokines
  - **Peptides (Thymosin alpha-1/BPC 157/Epithalon)**
  - LDN
  - IVIG (low dose or high dose-give 10-20 iu pitocin IV with each treatment if possible)
  - Ozone (MAH/Direct/High-dose/10-pass)
  - UVBI
  - LDA/LDI
  - Allergy elimination (gluten)
  - Antivirals (The deterioration in HIV is in direct correlation to the TH1/TH2 balance)
  - Antibiotics (do provoked WB, ECP and other immune markers whenever possible
  - Transfer factors
  - Mushroom extracts
  - Isoprinosine
  - High dose B12
  - GcMAF/Leukine/Neupogen
  - Probiotics
  - Silver
  - Heparin
  - Antioxidants/Glutathione (low glutathione decreases TH1 and increases TH2)
  - Chelation (heavy metals stimulate TH2 and lower TH1)
  - Bee venom
  - Acupuncture

Thymosin alpha 1 (TA1)

- Immune Effects[1,10,12,13,14,66]
  - Stimulates T cell production
  - Assists in the development of B cells to plasma cells
  - Increased mitogen response by lymphocytes
  - Decreased production of proinflamatory cytokines
  - Increased chemotactic response and phagocytosis by neutrophils
  - Normalizes immune balance and response
  - Normalizes immune dysfunction
  - Promotes TH2 to TH1 shift

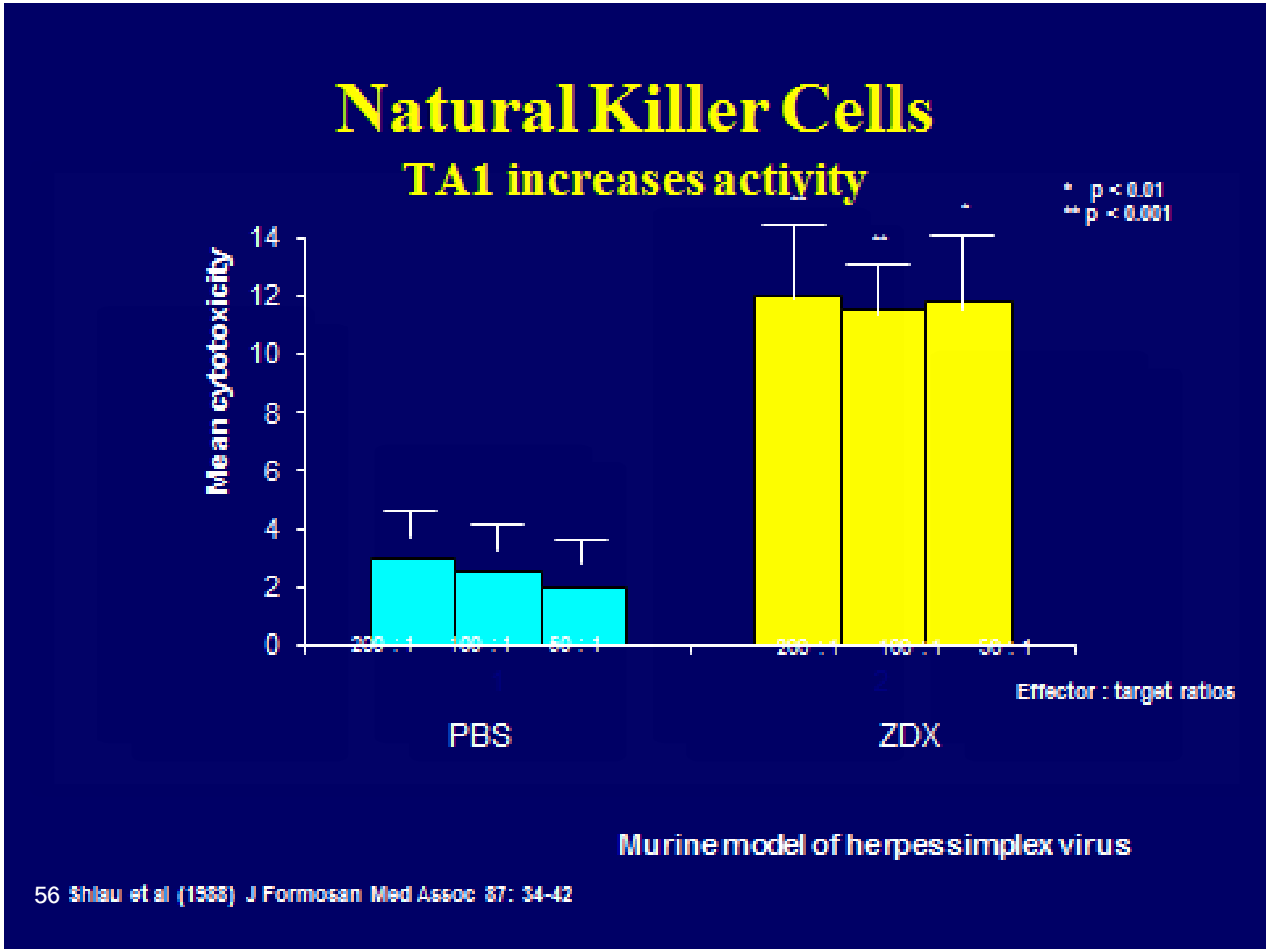
1. Lynch, H.E., et al., Thymic involution and immune reconstitution. Trends in Immunology, 2009. 30(7): p. 366-373.

10. Morozov, V.G. and V.K. Khavinson, *Natural and synthetic thymic peptides as therapeutics for immune dysfunction.* International. Journal of Immunopharmacology, 1997. **19**(9-10): p. 501-505.

Thymosin alpha 1 (TA1)

- Clinical Effects[1,19,12,14,66]
  - Improved tissue repair and healing
  - Improved host defense to infection
  - Improved microcirculation
  - Improves stress tolerance
  - Inhibits viral replication or growth of cancer
  - Improves cancer defense
  - Increases antioxidant and glutathione production
  - Reverse immunosuppression of CFS/FM/Lyme
  - Reduces inflammation
  - Anti-tumor effects

TA1 Effects


Natural Killer Cells
TA1 increases activity
* p < 0.01
** p < 0.001
Mean cytotoxicity
14
12
10
8
6
4
2
0
PBS
ZDX
Effector : target ratios
Murine model of herpes simplex virus
56 Shiau et al (1988) J Formosan Med Assoc 87: 34-42

Identification of immune dysfunction

- Elevated CRP, ESR
- Low testosterone/estrogen dominance
- Adrenal dysfunction (cortisol/DHEA)
- Low NK cell function < 30 LU
- Low immune cell function (ATP production) (Quest)
- low CD 57 (Labcorp)
- Elevated C4a (Quest-Nat. Jewish)
- Low Igg subclasses (Secondary to TH1-TH2 shift (low IFN-gamma))
- Leptin above 12
- Elevated human transforming growth factor-B (TGF-B)
- Any condition on slide 9

Initial Labs CFS/FM

- **TSH**
- **T4, Free**
- **T3, Free**
- **Reverse T3**
- **SHBG**
- **Cortisol**
- **ACTH**
- **DHEA-S**
- **Pregnenolone**
- **Vit D (25-OH)**
- **CBC**
- **Testosterone F&T**
- **IGF-1**
- **IGFBP-3**
- **Leptin**
- Thyroid Peroxidase AB
- Anti-thyroglobulin AB
- Comp Metabolic Panel
- CRP
- Lipid Panel
- Homocysteine
- Hemoglobin A1c
- Insulin
- **C4(a)** (National Jewish Quest)
- **NK cell activity** (Quest)
- **Thrombotic Marker panel**
  - **D-dimer**
  - **Prothrombin fragment 1+2**
  - **Thrombin-antithrombin complex**
  - **SFM**
- **Natural killer cell # (CD57)** (Labcorp)
- **ACE**
- **HTFG Beta -1**
- **Eosinophilic cationic protein (ECP)**
- **VEGF** (prone to error)
- **Lymphocyte subpanel #1)**
- **Igg subclasses**

Consider:

- RBC Folate
- B12
- Heavy Metals Panel
- RBC Magnesium
- Protein C/S Activity
- Estradiol
- IGG mold panel
- IGG foods
- FSH
- LH
- Progesterone
- CTX/NTX
- DHT
- Chlamydia Pneum
- Mycoplasma
- Candida
- Ehrlichia
- Babesia
- WAI-1 Babesia
- Fibrinogen

Initial Labs CFS/FM

(Sicker the patient)

- **TSH** (low)
- **T4, Free** (high)
- **T3, Free** (low)
- **Reverse T3** (high)
- **SHBG** (low)
- **Cortisol** (low)
- **ACTH** (low)
- **DHEA-S** (low)
- **Pregnenolone** (low)
- **Vit D (25-OH)** (low)
- **CBC** (low wbc low or high mcv)
- **Testosterone F&T** (low)
- **IGF-1** (low)
- **IGFBP-3** (low)
- **Leptin** (high)
- **C4(a)** (National Jewish-Quest) (high)
- **NK cell activity** (Quest) (low)
- **Immune cell ATP** (Quest) (low)
- **Thrombotic Marker panel**
  - **D-dimer** (high)
  - **Prothrombin fragment 1+2** (high)
  - **Thrombin-antithrombin** (high)
  - **SFM** (high)(+)
- **Natural killer cell # (CD57)** (Low) (Labcorp)
- **ACE** (> 35)
- **ECP** (> 8) stimulate with anti-malarial/anti-parasitics
- **VEGF** (high) stimulate with antibartonella meds/herbs

Thank You

Questions?