CHRONIC FATIGUE SYNDROME-RELATED CONDITIONS (CFSRCs) AND THE ROLE OF THE NEUROENDOCRINE, IMMUNE, METABOLIC AND METAGENOMIC SYSTEMS: *TOWARDS BETTER DIAGNOSIS AND TREATMENT*

Chris J. Newton Ph.D.

Director of Bio-Medical Research, CytoGenex Ltd and Centre for Immuno-Metabolism and Microbiome Studies; Senior Visiting Scientist Max Planck Institute of Psychiatry Munich

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Introduction

Chronic Fatigue Syndrome (CFS)-related conditions (CFSRCs), as defined on the next page of this article, represent a broad range of conditions of ill health for which an element of the symptom complex is fatigue resulting from altered/diminished physiological function. Quite apart from the human suffering that these conditions inflict, financially, CFS/ME has been estimated to cost the UK economy £100M each year[1]. Under the wider definition of CFSRCs as presented here, this is likely to be a large under estimate of the true cost.

It is probable that phenomena that are associated with the chronic fatigue process are also shared with other chronic illnesses, most particularly, those that involve infectious agents. In support of this, studies are now suggesting that the body's own microbiota or metagenome plays a fundamental role in CFSRCs, such as insulin resistance and obesity. These observations are likely to represent 'the tip of the iceberg' as there is a growing appreciation that a myriad of microorganisms resident in our bodies, play a major role in a range of situations of chronic ill health.

Some of the conditions that lie within the symptom complex of CFSRCs can also be classified under another symptom complex known as affective spectrum disorders (ASDs)[2]. A common element that is emerging for these is chronic inflammation, involving the neuro-endocrine, immune, and metabolic systems and also the microbiota/metagenome of the body[3].

This article develops the idea that a factor controlling or modulating the inflammatory condition is the body's stress sensing and management pathways. Stress as defined on the next page is any situation to which the body must respond in order to maintain a state of equilibrium. No distinction is made here as to mode of stress; therefore stress can be physical as well as emotional and therefore can be induced by infectious agents and other environmental factors, including diet. Given the epidemic proportion that obesity is assuming in Western Europe and the United States, this article also discusses the implications of current dietary practices in the light of a stress hypothesis and also the role of the metagenome. Finally, the article concludes with an outline of potential diagnostic and therapeutic interventions that might be made for CFSRCs.

Chronic Fatigue Syndrome-Related Conditions (CFSRC)

- Coeliac disease/other autoimmune conditions
- Fibromyalgia
- Gulf War Syndrome
- Insulin resistance/obesity
- Lyme disease/infection
- Multiple sclerosis (MS)
- Myalgic encephalomyelitis (ME)
- Physical trauma
- Post chemo/radiotherapy
- Post operative malaise
- Post viral infection

Symptoms of Fatigue-Related Conditions

- Lasting exhaustion after physical or mental exertion
- Poor and un-refreshing sleep
- Cognitive decline ('brain fog')
- Muscle weakness/cramps/pain /tingling in extremities
- Joint/lymph node pain/sore throat
- Abdominal pain/ nausea/bloating constipation/ diarrhea/
- Intolerance to light, noise, alcohol and certain foods
- Depression/ irritability/ panic attacks/poor temperature control/sweating/headaches/ dizziness/weight gain

The common physiological connection for CFSRC

To a certain extent, fatigue is a fact of life. For some, the degree of fatigue is such that normal work and socializing routines are disrupted. At the extreme, an individual might be so physically and mentally disabled that bed rest is required to allow some kind of recovery. Given the many conditions that have an element of CF, the challenge for the diagnostician/ biomedical scientist is to determine whether the symptoms shared by these conditions have a common physiological root. On the following pages of this journal article, common roots will be explored. As ideas are developed, research that should be undertaken in order to develop new diagnostic and treatment strategies, will be outlined

Physiological stress and CFSRCs

As a word commonly used to describe our emotional state, 'stress' has a far broader meaning when it comes to physiology and biochemistry. Stress might be defined as any physical, psychological/neurological stimulus that places a biochemical/bio-energetic demand on a cell/organ system. A stressful condition is only problematic when the body's organ and cell systems have insufficient resources to counter the stress; in other words, to bring the system back to a normal working equilibrium condition. At the level of the body-mind (brain) interaction, stressful events can be either physical or emotional. Physical events might be related to an enhanced physical activity, such as exercise, trauma (accident and surgery), to chemical stress (food substances/over-indulgence/ deficiency, noxious chemical agents, temperature, ionizing radiation) or biological agents (microbiological agents, prions, allergens). Whilst physical stressing agents might appear to have little association with emotional stress, it appears that the same area of the brain that processes emotional stress, also senses body systems that are running outside normal parameters. This area is the limbic system (amygdala, hippocampus, hypothalamus, thalamus and other brain areas) - pituitary (L-P axis). Indeed, this region of the brain is responsible for processing 'fight or flight responses', where alarm or fear leads to enhanced production of adrenal steroids (cortisol) and adrenalin. The L-P axis also integrates stress signals from immune cells. The hypothalamus and pituitary have receptors for molecules produced by immune cells[4]. Therefore cytokines such as interleukin-6 (IL-6), produced by activated immune cells, are sensed by the hypothalamus and pituitary and these systems signal the adrenal gland to produce cortisol (see Fig 1).

Acute and chronic stress

Whilst the acute stress response pathway serves an important role in maintaining homeostasis in response to acute physical and psychological stress, prolonged stress of contemporary living or long-term physical 'stimuli' appear to be damaging. Cortisol is produced in a biphasic manner by the adrenal gland in response to adrenocorticotrophic hormone (ACTH) secreted by the pituitary. Peak blood levels occur around 8:00 am and fall to a nadir around midnight to 2:00 am. Whilst cortisol increases blood glucose and enhances the metabolism of fats and proteins, other systems such as the immune system are both positively and negatively regulated. As stress levels increase, ACTH pulses from the pituitary increase in frequency and amplitude, raising the overall average daily production of cortisol[5]. Such an up-regulation of cortisol over a sustained period of time can result in increased activity of one part of the immune system and a decrease in the activity of another[6]. The effect of this could be the loss of normal homeostatic control within the body.

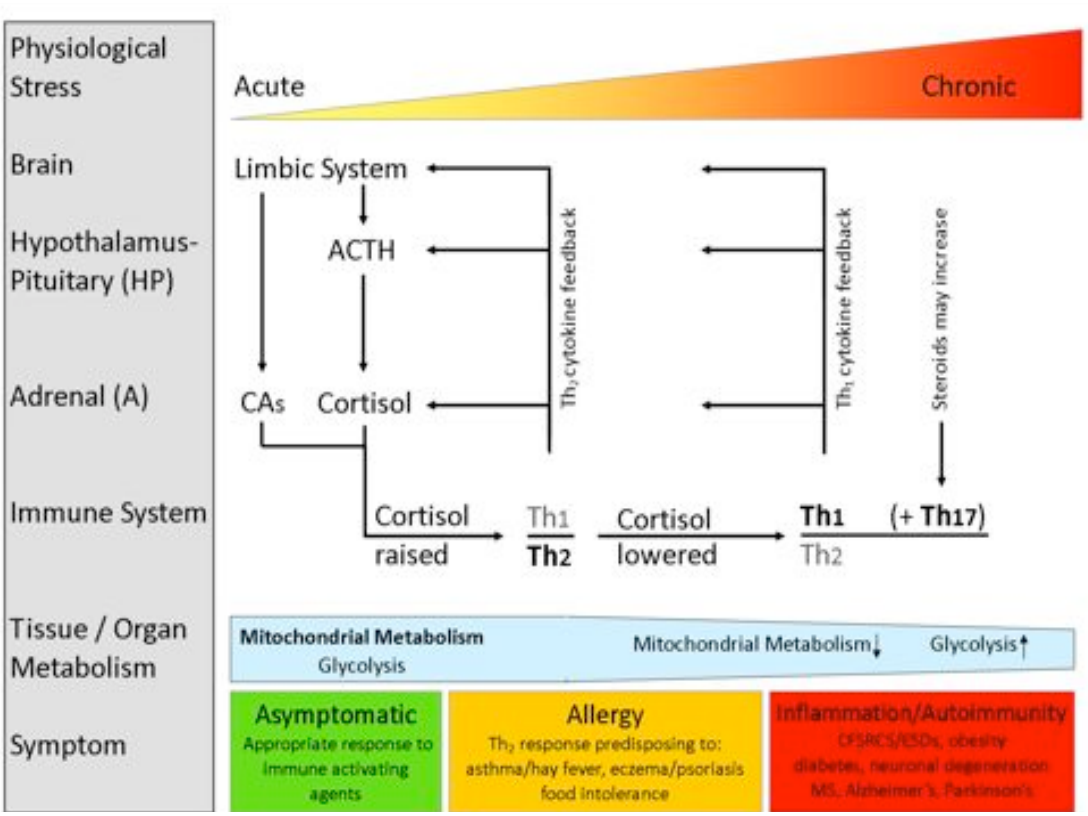

Figure 1 The role of physiological stress in altering the balance of the immune system. This model predicts that sustained stress increases blood levels of CAs and cortisol. This favours an antibody-mediated Th2 response, classically associated with allergy. As the stressful situation continues, adrenal function may diminish, lowing cortisol levels. The balance now swings towards Th1 reactivity, increasing susceptibility to inflammatory activators such as microorganisms. Although feedback systems exist to dampen the over activation of the HPA, these will fail if the adrenal is 'exhausted'. Synthetic steroids (cortisol mimics) are often given to lower inflammation. In some conditions, theses can exacerbate problems, especially if the response is TH17 driven as in the case of infection with Borrelia *burgdorferi*. Unlike Th1 responses, recent observations suggest that glucocorticoids can increase signaling pathways for TH17-driven autoimmunity (see Figure 2 and Prado C et al. 2011 Rhumatology 50:194-1801

Neuroendocrine-Immune-Metabolic Interaction in CFS

Heightened production of cortisol from the adrenal gland due to stress can lead to changes in the production of cytokines critical to mounting appropriate responses to invading pathogens[7]. Therefore stressing situations that raise adrenal cortisol and also the catecoleamines (CAs) adrenaline and noradrenaline, predispose the immune system to a more allergen prone reactivity. Indeed allergic conditions such as asthma, allergic rhinitis (hay fever), eczema and food allergies appear to be characterized by dominant Th2 responses[8]. In contrast to isolated episodes of acute stress, when stressful situations become the norm, there is evidence that adaptation can occur, where the stress response is blunted[9]. This may serve the physiological role of protection against acute stressing events when the stress is often repeated (for example performance stress). Despite the potential to adapt to stressing events, in states of negative emotional stress, such as during long-term unemployment, cortisol levels are significantly higher[10]. In the condition of extreme anxiety, there is evidence for decreased cortisol output by the adrenal gland[11]. Similarly, there also appears to be a deficit in the hypothalamic-pituitary-adrenal axis (HPA) in CFSRCs[12,13]. To what extent these deficits can be linked with prior hyper-stimulation of the adrenal with subsequent 'adrenal fatigue' is difficult to ascertain. However, from non-PubMed web-based literature sources, there is agreement that 'adrenal burnout' contributes to many

Intervention 1

Measurement of the diurnal range of cortisol in samples of saliva throughout the day

CFSRCs. From an immunological perspective, diminished cortisol output from the adrenal would appear to alter the balance of the immune system towards Th1, a swing that is associated with an inflammatory response[14]. The significance of this with regard to CFSRCs is that evidence has been accumulating to suggest that these conditions all involve chronic inflammation[2] (see Figure 1). In parallel with this alteration, there is a change in metabolism of immune cells. This metabolic switch is facilitated by the up-regulation of a fundamental control protein termed Hypoxia-Inducible Factor-1 (HIF-1)[15]. This protein becomes active when tissue concentrations of oxygen decrease below a critical threshold and it is also responsive to pro- inflammatory mediators such as interferon gamma (IFN-γ), interleukin-6 (IL-6), IL-1β and TNFα[16]. Most recently, a new arm of the immune system has been characterized that is also thought to play a fundamental role in the inflammatory response and in particular, in the phenomenon of autoimmunity. This new component involves the Th17 cell[17]; a particular T-lymphocyte helper cell that secretes interleukin-17 (IL-17)[17,18,19]. Recent studies show that HIF-1 also plays a fundamental role in the way inflammatory initiators direct the fate of T cells towards Th17 activity. In two parallel papers, Shi et al. (2011)[20] and Dang et al. (2011)[21]

(Neuroendocrine.....continued)

show that HIF-1 controls the balance between the production of Th17 and anti-inflammatory regulatory T cells (Treg)[22]. Of great significance is the further observation made by Shi et al., that pharmacologically altering the metabolic consequences of HIF-1 activation, reduces the production of Th17 pro-inflammatory cells in favour of anti-inflammatory Treg cells[23]. As an ascorbate (Vit C)-requiring prolyl-hydroxylase targets HIF-1 for destruction[24] it might be predicted that high dose Vit C would reduce inflammation and autoimmunity.

The inflammatory response, autoimmunity ASDs and CFS

Inflammation and autoimmunity are now thought to be at the root of a whole range of conditions that lie within what is being termed, 'affective spectrum disorders'[2] These disorders range from neurodegeneration to CFS. Together these observations suggest that an environment is established within the body that allows for the over activation of the inflammatory arms (Th1 and Th17) of the immune system. As indicated above, this change could be caused by stress-induced deregulation of the adrenal gland. Alternatively, inflammation could be a consequence of acute or chronic infection with a pathogenic agent or environmental toxins like mercury. The bacterium causing Lyme disease, Borrelia *burgdorferi* produces biotoxins and these have been shown to induce both Th1 and Th17 activation[25,26]. Some methods for the detection of Borrelia infection measure the production of Th1 cytokines such as IFN-γ. However, improvements in the measurement systems for infective agents may come from the recognition that Borrelia and other pathogenic agents (Babesia, Bartonella, Ehrlichia and Mycoplasma) might induce a Th17 response in addition to the recognized Th1 response. As data exist to suggest that steroids (glucocorticoids) increase the Th17 arm of the immune system, this should be taken into consideration before steroids are prescribed for infective agents like Borrelia (see Fig 1).

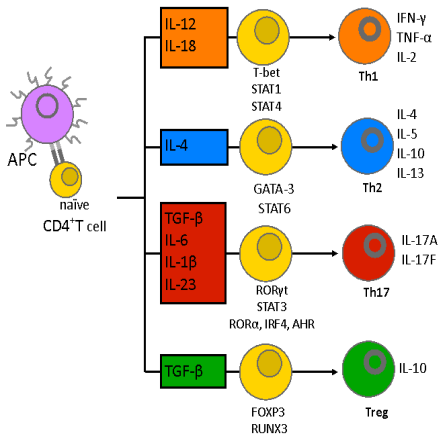

***Figure 2** The activation of the Th cell system.* **Antigen presenting cells (APC) such as macrophages and dendritic cells interact with naïve CD4+ T cells to direct their transformation to a Th cell sub-type. Antigens presented to Th cells, together with the activation of APC receptors for bio-molecules (pathogen-associated molecular patterns , PAMPs), alter the expression of cytokines. These in turn guide the transformation of naïve Th cells by changing intracellular signaling pathways (eg STAT3/4/6).**

Intervention 2
The use of better methods for the detection of pathogenic agents that are associated with or cause CFSRC

Inflammation and cell stress

Inflammation is associated with an increase in cytokines such as IL-1β, IL-6, IL-12, INF-γ and TGFβ. In particular, IL-6 is integral to the inflammatory phenotype (Th1/Th17)[27]. Blood levels of IL-6 are raised in illness and this is thought to increase intracellular oxidative stress through the activation of the NADPH oxidase enzyme pathway[28] (see Fig 3). This results in the depletion one of the most essential antioxidants, glutathione (GSH). The loss of major antioxidant capacity results in further functional damage to cells and in particular, to energy transfers systems. The effect of the latter is widespread. A reduced ability to generate the energy transfer compound, ATP, leads to an array of cellular defects, not least of which are impaired membrane transport systems and synthetic capacity.

Studies have shown that the thyroid hormone system is particularly affected. The transport of thyroxine (T_4) and tri-iodothyrone (T_3) into the cell is an energy dependent process[29] and most recently, studies have shown that in states of increased proinflammatory cytokine production, T_4 is preferentially converted to reverseT_3 (rT_3) at the expense of T_3[28]. It should also be noted that the enzyme responsible for rT_3 is a target for HIF-1 activity[30]. As rT_3 is an inactive hormone, it blocks the actions of T_3 rendering tissues hypothyroid despite apparently normal blood levels of T_4 and T_3 (see Fig 3). The question that must be addressed is whether this is a common scenario in the inflammatory condition. Given that infective agents like Borrelia *burgdorferi* induce a Th17 response, particular attention should be paid to signs of autoimmunity. There is evidence that thyroid function can be altered in Lyme disease in a potential Hashimoto's-like autoimmune attack[31,32]. For this reason, careful attention should be paid to blood levels of thyroid stimulating hormone (TSH), as an increases outside the normal range might be one of the first signs of auto-immune damage to the thyroid gland.

Immune-metabolic interactions: energy transfer systems

As outlined above, inflammation involves a change in the metabolic pathways within cells. Immune cells that are part of the Th1/Th17 inflammatory mechanism generate more of the 'high energy' compound

Intervention 3
T_3: rT_3 ratio should be measured for subjects with CFSRCs and ASDs

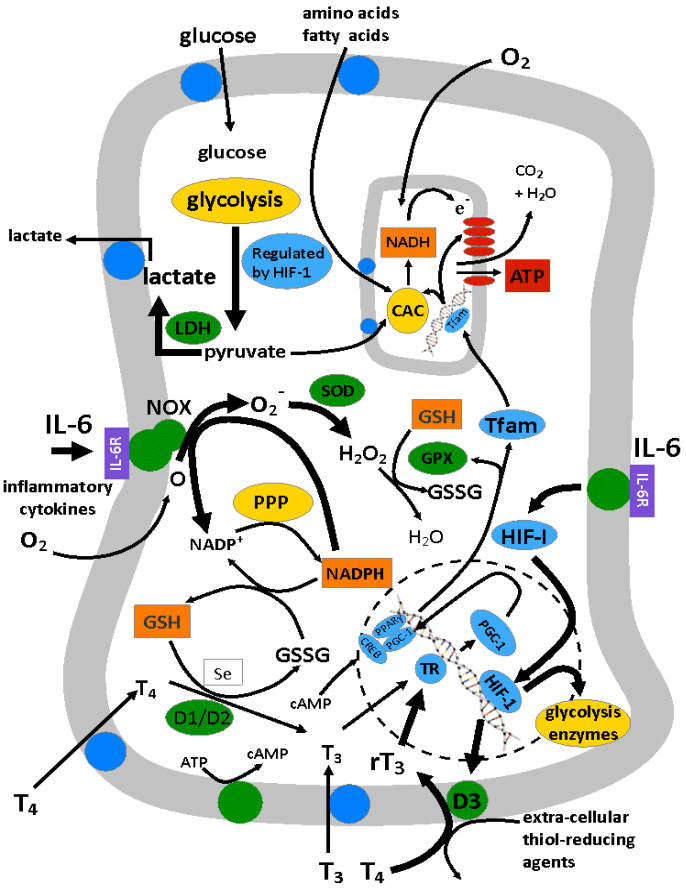

Figure 3 Response of the cell to inflammatory cytokines. IL-6 has been shown to increase the activity of hypoxia-inducible factor 1 (HIF-1) and to increase oxidative stress via activation of NADPH oxidase (NOX). These events lead to the up-regulation of glycolysis and the depletion of intracellular thiol antioxidant glutathione (GSH) respectively. As the intracellular pool of reducing agents (GSH and NADPH) becomes depleted, T_3 production from T_4 is reduced. As the D3 de-iodinase has a membrane location it has access to extracellular reducing agents and is able to convert T_4 to rT_3. This blocks the function of thyroid hormone receptor (TR) and diminishes the expression of the transcription factor, PGC-1; co-activators of which are the peroxisome proliferator receptor γ (PPARγ) and CREB. By an increase in HIF-1, mitochondrial metabolism is reduced. The overall effect is that flux through glycolysis is increased and lactic acid accumulates in the cell. For some cells, this may lead to death and the release of lactate dehydrogenase (LDH) into the blood. (CAC, citric acid cycle; SOD, superoxide dismutase; GPX, glutathione peroxidase; PPP, pentose phosphate pathway; CREB, cyclic AMP responsive element, Se, selenium; D1/D2/D3 T_4 de-iodinases).

(Immune-metabolic .....continued)

ATP, by glycolysis. This process is inefficient and it leads to the generation of large amounts of lactic acid within the tissues (see Fig 3). Lactic acid is toxic and unless it is transported out of cells by the monocarboxylic acid transporters [33, 34] it can build up and damage cells. This phenomenon is responsible for muscle stiffness and cramp after strenuous exercise has been undertaken without sufficient training. When cells die, they release the enzyme lactate dehydrogenase (LDH) and this can be detected in blood samples. The enhanced release of LDH into blood has been noted after exercise training and endurance events[35]. It is also apparent to a similar extent in individuals with a poorly functioning thyroid gland[36].

Given that thyroid hormones play a major role in regulating mitochondria energy transfer systems[37], these observations suggest that LDH is released from cells as a result of the accumulation of lactic acid due to higher glycolytic activity. Since higher glycolytic metabolism is apparent in immune cells that have the Th1/Th17 inflammatory[20,21] phenotype, it is possible that LDH activity is raised in CFSRCs (see Fig 3). In a preliminary study, LDH concentrations in blood have been observed to be raised at least 2 fold in a group of chronically fatigued individuals in comparison to non- fatigued individuals[38]. These observations suggest that the alteration in energy transfer systems in subjects with CFSRCs extends beyond cells of the immune system. It is likely that LDH is released from cells of major tissues structures such as muscle; this idea is entirely consistent with the symptoms of muscle pain, fatigue and exercise intolerance in chronically fatigued individuals.

Mitochondrial defects in CFSRCs

Recent observations on immune cells suggest that inhibitors of glycolysis are able to block the development of inflammatory Th17 cells[20]. In view of these findings, it is likely that reduced mitochondrial efficiency is associated with a swing to the Th1/Th17 inflammatory phenotype. Although this idea has yet to be tested experimentally, studies have demonstrated that there are defects in mitochondrial function in individuals with chronic fatigue/ME[39]. Whether the defects in mitochondrial function are primary events in chronic fatigue or whether they are secondary to those that activate an inflammatory response has yet to be determined. Despite this, mitochondrial activity is a further aspect of cell function that might be explored as a diagnostic and therapeutic target for CFSRCs.

Intervention 4 and 5

Blood CO_2, LDH and lactate could be measured in combination with mitochondrial function in macrophages and neutrophils, under various conditions and in particular, after therapeutic supplementation

Immuno-metabolism in obesity

Recent studies suggest that obesity is as much a function of immune modulation as it is a dietary condition. Whilst nutritional excess combined with a lack of physical activity are fundamental initiating factors, progression to a state of insulin resistance and morbid obesity involves adipocyte (fat cell) - immune cell interactions. In the lean state, macrophages make up 4-10% (on a cell number basis) of the bulk of adipose tissue[40,41]. With increasing fat synthesis (lipogenesis) from high carbohydrate uptake and excessive dietary fats, storage mechanisms within adipocytes become overwhelmed.

(*Immuno-metabolism..... continued)*

This can lead to adipocyte cell death and the release of free fatty acids into extracellular spaces and into the circulation[41]. This process is pro-inflammatory and it leads to a large increase in the number of macrophages and other leukocytes. Macrophages can increase to a cellularity of 20- 40% and the macrophage-adipocyte interaction substantially influences insulin sensitivity of the tissues as a whole[41]. Indeed, due to the pro-inflammatory environment, macrophages are of the M1 phenotype, paralleling the Th1 phenotype of lymphocytes. Saturated fatty acids released from adipocytes and lipopolysaccharde (LPS) from intestinal microflora can bind Toll-4 and induce HIF-1. This in turn up-regulates components of the glycolytic pathway and decreases the activity of mitochondrial energy transfer systems. These metabolically skewed, M1 macrophages, secrete cytokines such as such as TNFα and IL-1β and the macrophage attracting chemokine, CCL2[41]. The outcome of this process is that inflammation spreads throughout the expanding adipose tissue mass and insulin sensitivity is decreased further. There is evidence that inflammation does not remain restricted to adipose tissues; the process becomes systemic, affecting tissues throughout the body[42]. It has been suggested that, 'adipose tissue of obesity resembles an organ chronically infected by an intracellular organism, despite the presence of pathogen'[41] (an idea that will be returned to later). In support of this, serious infection is associated with insulin resistance. The common link is the production of pro-inflammatory factors such as TNF-α, IL-1β and IL-6. A further mechanism by which the, pro- inflammatory state, could contribute to metabolic change is via the thyroid hormone system. As mentioned earlier, cytokines such as TNFα and IL-6 induce oxidative stress and deplete intracellular glutathione. This diminishes the capacity of cells of peripheral tissues to convert the thyroid hormone T_4, to T_3. Instead, T_4 is preferentially converted to rT_3. More directly, HIF-1 has been shown to induce the activity of the enzyme that converts T_4 to rT_3[30]. As rT_3 is unable to activate the thyroid hormone receptor, it blocks T_3-induced transcription (RNA synthesis) and translation (protein synthesis) of a mitochondrial control protein, PGC-1[43]. This protein is also induced by the Th2 cytokine IL-4 [44] and in a complex with the (PPARγ)/retinoid X receptor, PGC-1 activates the transcription of genes involved in oxidative metabolism within mitochondria[43].

In the insulin resistant state and in tissues chronically infected by pathogens, it might be expected that metabolism is further skewed towards glycolysis by inflammatory cytokine- induced damage to the thyroid hormone system.

Intervention 6
In the insulin resistant state, thyroid function could be checked by measuring the T3:rT3 ratio. In CFSRCs, glucose tolerance and insulin sensitivity should be monitored

Fatty acids and inflammation

The commonality for a wide range of fatiguing conditions is that physiological stress leads to an inflammatory response. Principal mediators in this response are the inflammatory cytokines and transcription factors like HIF-1. Inflammatory activators lead to a heightened state of oxidative stress and the depletion of critical antioxidants such as glutathione (see Fig 3). A strategy that may prove beneficial under such conditions is to replenish these systems by way of nutritional supplementation.

Diet should also be considered for its content of inflammatory substances. Most Western diets have a high content of carbohydrates and fat. Carbohydrates when supplied as simple sugars or complex carbohydrates, lead to a rise in blood glucose and the release of insulin. This process is stressing for the body and our tolerance to glucose or our insulin sensitivity, can vary. In experimental systems, saturated fatty acids (SFAs) such as lauric, myristic and palmitic acid have been shown to bind to the pattern recognition receptors, Toll-2 and Toll-4[41]. Activation of the Toll-4 receptor complex induces inflammatory cytokines such as TNFα and these cytokines have been shown to reduce the sensitivity of the insulin signaling process within target cells[41]. The role that Toll-4 plays in activating inflammatory pathways[45], combined with raised concentrations of SFAs in obesity[46], has been taken as evidence for their deleterious effects when consumed in the diet. Also, the link between SFAs and our intake of cholesterol from animal fats, together with the potential pro-inflammatory effect of SFAs has provided the rational for the current dietary advice that we should replace most SFAs in the diet with polyunsaturated fatty acids. There are indeed certain polyunsaturated fatty acids (PUFAs), like the omega 3 and omega 6 fatty acids that the body cannot synthesize (unlike SFAs) and these so-called essential fatty acids (EFAs) are required from our diets. Evidence is also accumulating that the omega 3s are anti-inflammatory by way of binding to the peroxisome proliferator receptor gamma (PPARγ) and by blocking the binding of SFAs to Toll-4[45]. Further to this, there are suggestions that we should maintain a ratio of omega 6 to omega 3 of around 4:1 or lower[47]. If this is indeed correct, a potential problem with our diets is that one of the widely used vegetable oils, sunflower oil, has virtually no

Fatty acids and inflammation continued)

omega 3 PUFA. A further potential problem in the current dietary scenario is that PUFAs like omega 6, are highly prone to oxidation during the cooking process[48]. In this respect, many pre-cooked food items are now prepared using PUFAs. Recently published work shows that administration of moderately oxidized PUFAs (ox-PUFAs) depletes pig livers of vitamin E reserves and increases the expression of genes within the liver, that are known to be sensitive to oxidative stress[49]. As porcine physiology is similar to that of the human, these observations suggest that human exposure to ox-PUFAs will results in uptake by the liver. The question remains as to whether ox-PUFAs will then be incorporated into lipid (fat)-protein transport vesicles, VLDL and then into LDL, HDL and Lipoprotein(a) Lp(a).

Intervention 7
Given the chemical instability of PUFAs and the pro-inflammatory properties of oxidized PUFAs, the practice of cooking with mainly omega 6 sunfower oils requires investigation

When PUFAs and cholesterol within LDL become oxidized (ox-LDL), this leads to the oxidative modification of a protein component of LDL, apoB. In this form, the ox-LDL cannot be taken up by muscle and fat cells. Instead, it is taken up by macrophages[50]. This process is inflammatory and ox-LDL and ox-Lp(a) are thought to have a major role in both insulin resistance and the atherosclerosis[50,51]. Indeed, the oxidative modification of PUFAs results in the production of reactive carbonyl compounds (RCCc). These can enhance inflammation due to their ability to modify and inactivate peptides/proteins[52].

Oxidation of lipids in CFSRCs

The evidence presented above suggests that CFSRCs and obesity involve the inflammatory, Th1/Th17 arms of the immune system. In the obese condition, there is evidence for the direct activation of inflammatory pathways by fatty acids and also of enhanced oxidation of LDL to yield the inflammatory activator, ox-LDL. Given that an oxidative environment is associated with inflammation, it is perhaps not surprising there are reports of the enhanced oxidation of LDL and the production of RCCs in chronic fatigue states in the absence of obesity[53]. To what extent dietary factors exacerbate this situation is uncertain. However, as suggested above, the influence of the type dietary fatty acid, together with the adequacy of the supply of dietary antioxidant compounds, should be further investigated.

Immune-microbiota interaction

Reference was made above to the idea that, 'adipose tissue in obesity resembles a tissue chronically infected with an intracellular organism'[41]. Most recently, evidence has been presented that this may literally be the case. Compelling work using the mouse model shows that after only one week of a high fat diet, live intestinal bacteria are present in large numbers, in adipose tissue and in blood[54]. This translocation into adipose tissue is prevented in mice lacking the pattern recognition receptors Nod1 or CD14 (Nod1 is an intracellular bacterial pattern recognition receptor and CD14 is a co-receptor protein for Toll-4). This startling study further demonstrated that the 'metabolic bacteremia' was associated with an influx of dendritic cells within adipose tissue and also that the probiotic, *Bifidobacterium animalis could* reverse the high fat diet-induced translocation of bacteria. This work is now supported by a study showing that the presence of 16S rDNA (a gene marker for bacteria) was higher in samples of blood from people who later went on to develop diabetes[55]. These findings are of great significance as they support a role for bacteria in a far wider range of human illnesses than previously thought. One might speculate that in addition to a spirochete-type invasion of tissues (as in Lyme disease), various transport mechanisms could deliver micro-organisms (or components of) to a wide range of tissues. It is highly likely that this phenomenon contributes to chronic inflammation observed in CFRCs and ASDs and it may even have a role in cancer.

In the context of the delivery of microorganisms to 'extra-digestive system' sites, one must consider the barrier function of the intestinal epithelium. Due to inflammation in the lining of the small intestine in Coeliac disease (and gluten intolerance) and other food allergies, it is highly likely that bacteria (or their components) are able to cross the gut and find their way into the systemic circulation. This situation most probably amplifies, and may even be an initiating event for CFSRCs and ASDs.

Non-biological inflammatory drivers

Our exposure to industrial pollutants has diminished over the past 25 years mainly due to more stringent regulation. Despite this, we are now exposed to a large number of chemical agents and electromagnetic radiation by stealth. Various literature sources suggest that exposure to these agents can induce low-grade inflammation. Some of these chemical agents act by de-regulating the endocrine system and some act by modifying the structure of body bio-molecules, food components or gut microorganisms to produce antigens capable of activating T-lymphocytes. Amongst the many substances shown to interact with the endocrine system Bisphenol A (BPA),

Environmental inflammatory drivers continued)

and phthalates, have been shown to have oestrogen-like activity[56] and from structural analysis, BPA is also likely to bind to the glucocorticoid receptor (GR)[57]. As oestrogens, these compounds could increase the concentration of cortisol binding globulin (CBG) and decrease the concentration of free cortisol[58]. This could deregulate the immune system as discussed above. As a compound that binds to the GR, BPA could act as an agonist like cortisol to increase Th2 immune responses or it could block the action of cortisol to increase the Th1 inflammatory responses. In addition to endocrine disruptors, compound like polychlorinated biphenyls (PCBs), dioxins and organochlorine pesticides are detected in blood samples. Concentrations of these substances have been shown to correlate with body mass index and studies reveal that adipose tissue is a target for these substances[59]. By a mechanism not yet known, they can increase the concentration inflammatory cytokines such as IL-1 and IL-8[59].

Organic molecules are not the only substances that can initiate an inflammatory response or indeed bind to receptor proteins. Mercury, nickel and cadmium are inorganic molecules that seem to do both. Mercury and cadmium have been shown to activate oestrogen responses[60,61] and nickel can bind to Toll-4[62] and also stabilize HIF-1[24]. By acting as haptens to change protein structures, by Toll-binding, or via HIF-1 stabilization, these and other heavy metals share an ability to activate a Th1/Th2/Th17 responses. In this way, heavy metals such as mercury might be involved in autoimmunity[63]. Indeed a large amount of research has accumulated to suggest that metal exposure from amalgam dental work and from food and other environmental sources could contribute to CFSRCs [63, 64]. Due to reactivity with thiol groups within proteins (sulphur groups), mercury can inactivate antioxidant pathways such as glutathione/glutathione peroxidase[65] This will increase oxidative stress and amongst other deleterious effects, it could block peripheral T_3 synthesis as discussed previously (see Fig 3).

This article began by discussing 'stress' under the most catholic definition of the term. In this way, stress should be considered as an imposed change to body systems. This might be caused by a psychological event such as chronic mental or physical exertion or rather more subtly, by exposure to environmental agents as described in the last few paragraphs. Returning to the theme that was introduced in the opening paragraphs of this article, we live in a symbiotic relationship with the micro-organisms that constitute our microbiome. This relationship is probably maintained by the ability of immune system to regard these organisms as part of the body (self tolerant). However, de-regulation of our immune system brought about by a set of hormonal changes may de-stabilize our microbiome leading to inflammatory symptoms. Also, acquisition of new environmental agents such in the case of the Lyme disease (Borrelia, Mycoplasma, Babesia, Bartonella etc.) and exposure to chemical and biological agents as exemplified in the case of Gulf War illness [66] may be sufficient to upset the delicate balance and induce inflammatory symptoms.

Summary

Although CFRCs have been reported for over 200 years, debilitating fatigue came to prominence in the late 1970s and early 1980s. Since then, the prevalence of CFSRCs has apparently increased, perhaps as a result of the acceptance that these illnesses are 'real medical conditions'. CFSRCs may also be increasing due to current societal practices. As a global society we expose ourselves to a large number of stressing agents, both inorganic and organic and also electromagnetic. The former category includes polluting agents, such heavy metals and latter includes physical and psychological stress, infectious agents, diet, fat-soluble compounds and endocrine disruptors. In addition to these, we are now bathed in a sea of electromagnetic radiation. The outcome for an individual where the body's defense systems have become overwhelmed may be the appearance of the numerous symptoms of CFSRCs. Our challenge now is to correctly identify the condition and to provide the appropriate advice to restore health.

Intervention 8 Inflammatory cytokines such as IL-6 and TNFα, should be monitored together with stimulus-specific Th cells and serum glutathione

This document now concludes with a summary of some of the research that might be undertaken to develop more appropriate diagnostic systems and therapeutic regimens.

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Interventions and research questions

Intervention 1 (Endocrine function):

Basal endocrine investigations should be made on individuals where indices of HPA function are determined. Of particular importance is the determination of salivary cortisol throughout the day. This contrasts with the currently accepted synacthen test (synthetic ACTH) that looks for adrenal inadequacy that is indicative of Addison's disease. In CFSRCs, cortisol concentrations may not be grossly disturbed, rather, they may not be optimal and show inadequate HPA function at certain times of day. Cortisol binding globulin (CBG) is influenced by circulating oestogen concentrations and CBG concentrations may prove informative in the obese state and after exposure to potential oestrogenic endocrine disruptors[56].

Intervention 2 (Infectious agents):

The current detection methods for infectious agents such as Babesia, Bartonella, Ehrlichia and Micoplasma (and other bacteria, fungi and viruses) are poor to say the least. Most systems rely on serology techniques and some of the best systems only detect the infective agent in 50-60% of clinically diagnosed cases. As an alternative, the response of Th1/Th2/Th17 cells to an antigen can be informative. These responses are measured by systems termed lymphocyte transformation tests (LTT), the archetype examples of which are the Elispot® and the MELISA®. In a cohort of individuals with suspected infections, a comparison of the best available serology techniques will be made with the LTT tests.

Treatments for most bacterial pathogens currently involve the use of antibiotics. Often treatment effects diminish with time due to metabolic adaption of the organism or an inability of the subject to tolerate the antibiotic. A new treatment paradigm is needed that avoids the use of antibiotics. To this end, investigations will be conducted on a novel non-invasive pulsed electromagnetic therapy.

Intervention 3/6 (Tissue hypothyroidism):

Most cellular systems in the body respond to thyroid hormones. The binding of the active hormone T_3 to the thyroid hormone receptor (TR) induces the transcription of numerous genes, predominant amongst which are those that control metabolism. Although at the moment information is scarce from on-line literature sources such as PubMed, from other web-based sources there is a growing consensus of opinion that T_3, in addition to cortisol, is required for maintaining homeostasis in the immune system. Indeed, studies in animals strongly suggest that insufficient cortisol and T_3 production can leads to autoimmunity[58]. There is evidence (albeit somewhat anecdotal at present) that infectious agents, like Borrelia *burgdorferi* (Lyme disease spirochete), can induce an autoimmune attack of the thyroid gland. Further to this, there is new information to suggest that during inflammation, tissue concentrations of glutathione are depleted due to cytokine-induced oxidative stress. Since glutathione is required for the conversion of the pro-hormone T_4, to T_3, tissues may be depleted of T_3 due to the conversion of T_4 to an inactive hormone, rT_3. So despite apparently normal concentrations of T_4 and T_3 within the blood (within the normal range), tissues can be starved of the active hormone, T_3. Current routine blood analysis is only established to measure TSH, T_4 and T_3 (even the latter is not always measured) and therefore inflammation-induced tissue hypothyroidism is not identified. It is paramount therefore, that for individuals suffering from CFSRCs and other conditions where inflammation is a factor (diabetes and affective spectrum disorders, ASDs), that in addition to TSH andT_4, free T_3 and rT_3 are measured. With reference to current literature values for the ratio of T_3 to rT_3, the ratio for 'in-house' testing methodology should be established for non-fatigued individuals (non-diabetic). This will allow comparisons to be made with CFSRCs and ASDs such as depression. Given the observations made on some individuals with infectious diseases such as Lyme, it is most important that individuals are monitored at regular intervals (3-6 months) for the level of TSH. In this respect, it has been noted that TSH values can swing between

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Interventions and research question continued.....

normal and high after infection. This strongly suggests that an autoimmune attack on the thyroid can be established following bacterial (and most probably viral and fungal) infection. As mentioned above, although the evidence is not strong as yet from a human perspective, information from veterinary science suggests that low thyroid function deregulates the immune system, predisposing it towards autoimmunity[58].

Intervention 4 (Metabolic alteration hypothesis I):

Work conducted on subclinical hypothyroid and hypothyroid subjects, clearly demonstrates that the cytosolic enzyme, LDH, is raised two fold in comparison to euthyroid individuals. In our preliminary studies we have noted that fatigued individuals also demonstrate a raised LDH within the blood. This situation often goes undetected as the values still fall within the normal range of most testing establishments (in the UK at least). Not only does this highlight the problem of comparing values with normal ranges (ideally one needs serial measurements from late adolescence onwards with which values obtained during illness can be compared), it principally suggests that the metabolic state of major tissue is altered in CSFRCs; this could be due to tissue hypothyroidism and/or high HIF-1 activity. When tissue oxygen levels are sufficient, the final product from the cytosolic metabolism of glucose is pyruvate and this is transported into the mitochondria to be further metabolized. If tissues lack sufficient oxygen or have a defect in their mitochondria (as explained on the preceding pages), pyruvate is converted to lactic acid in the cytosol and it must be transported out of cells before it accumulates to cause damage. When cell damage does occur (as in the case of cardiac infarct, for which LDH has been used as an indicator) then blood levels of LDH can rise. Our studies suggest that a degree of tissue damage is occurring in the fatigued state and this may parallel the degree of inflammation within tissues. Based on these finding, it is important that for all fatigued individuals, LDH activity is measured in blood samples.

Intervention 5 (Metabolic hypothesis II): As alluded to above and on page 4, cellular metabolism under the influence of inflammatory factors swings towards glycolysis. This may be due to the influence that pro-inflammatory factors have on the transcription factor HIF-1. This factor is responsible for the up-regulation of enzymes involved in glycolysis and the down regulation of energetic processes in mitochondria. From a diagnostic perspective, it may be informative to test mitochondrial function. Methodology is currently being established to make these measurements on immune cells obtained from whole blood samples.

Intervention 6 (Glucose tolerance and insulin sensitivity)

The inflammatory condition is associated with a decrease in cell sensitivity to insulin. The outcome of this situation can be type II diabetes (progressing to Type I, insulin dependent diabetes, if corrective measures are not taken). A diminished sensitivity to insulin results in a decrease in the uptake of glucose into cells after a meal. A standard test for this is the glucose tolerance test (GTT) where after the measurement of basal blood glucose, a bolus dose of glucose is given; after 2 hours, the blood glucose is measured again. If the concentration is between 8 and 11mM, then the person is on their way to develop type II diabetes. As one of the diagnostic measurement for CFSRCs, a GTT test will therefore be performed and the results will be compared with non-fatigued individuals. As for many other measurements in the CF state, it is expected that the glucose tolerance (insulin sensitivity) will be in the normal range, but it is anticipated that tolerance to glucose will be lower than non-fatigued individuals.

Intervention 7 (Dietary fatty acid hypothesis):

Although much work has been undertaken on the association between saturated fatty acids (SFAs) and vascular/coronary health, the exact reason why saturated fat is deleterious to the body in the non-obese state is not entirely clear (certainly to the author of this document). The questions that have not been adequately addressed are 1), whether SFAs are damaging per se, 2), whether it is the quantity of SFAs consumed

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Interventions and research questions continued.....

in the diet that is the problem or 3), whether they are problematic due to their association with cholesterol and 4), under what physiological/biochemical/physical conditions do SFAs become a problem. Most of the original work on the link between cholesterol and atherosclerosis came from animal studies, often using guinea pigs and rabbits. In these studies, diet and living conditions could be controlled. With the exception of communities such as the Amish, where lifestyle is less variable, this sort of study is usually not possible and so experimental paradigms that suggest a particular outcome are most often flawed due to their inability to control all dietary and lifestyle variables. With regard to fatiguing conditions such as obesity, dietary SFAs are clearly not required, as they are synthesized from glucose (as they are in the non-obese state). In obesity however, excess dietary SFAs results in the activation of inflammatory pattern recognition receptors such as Toll-4 on macrophages, endothelial cells and adipocytes and this has been shown to exacerbate the insulin resistant state. What is not clear, however, is why saturated fatty acids are risk factors in the non-obese lean condition and why we should replace most saturated fatty acids with polyunsatuared (PUFAs). Whilst it is certain that we require PUFA such as the omega 3 and omega 6, what we don't know is exactly how much of these we need. Also, PUFA are highly susceptible to oxidative damage and reactive carbonyl compounds (RCCs) can form in the body such as 4-HNE and MDA. These and other RCCs can modify proteins in lipoproteins such as LDL and Lp(a) and inactivate glutathione decreasing the antioxidant capacity of cells and tissues. A further compounding factor is that in addition to modification within the body, PUFAs are oxidatively modified during the cooking process. The current dietary advice of using high omega 6 content vegetable oils in the cooking processes might be expected to increase the production of RCCs. Since there is evidence that in chronic fatigue states, oxidation of LDL is increased, one has to ask whether chronic low-grade inflammation and oxidative stress in CFSRCs are factors that increase the concentration of RCCs or, whether dietary and cooking practices increase RCCs and the production of advanced lipid peroxidation end products (ALEs). Assay systems exist that can measure the oxidation of phospholipids and apoproteins in both LDL and Lp(a). Using these systems, it is proposed to test the following: 1), the effect of ingesting oxidatively modified PUFAs (by thermal oxidation) on the oxidation of phospholipids and apoproteins in LDL and Lp(a) in non-fatigued individuals and in individuals with CFSRCs and 2), the effect on blood concentration of ox-phospholipids and ox-apoproteins in LDL and Lp(a) of a high PUFA diet (omega 6 in particular) in comparison to a diet where omega 6 PUFAs is restricted. In addition and dependent on the outcome of these studies, the effect of dietary supplementation with thiol antioxidants such as N-acetylcysteine, and MSM in combination with ascorbate and CoQ10 on the oxidation state of phospholipids in lipoproteins will be tested in fatigued and non fatigued individuals.

Intervention 8 (Blood inflammatory mediators, activated T cells and autoantibodies):

During inflammation, the cytokines TNFα, IFNγ and IL-1-β, IL-2, IL-6, IL-8,IL-12 and IL-17 are secreted by immune cells and other cell types that are part of the inflammatory environment (muscle, adipose tissue etc). Along with more 'traditional markers of inflammation' such as c-reactive protein, it is proposed that these cytokines are measured together with ox-LDL. The concentration of these inflammatory cytokines can also be monitored in blood samples in response to antibiotic therapy and in particular, in response to pulsed magnetic therapy. During the destruction of microorganisms, a large amount immune-activating bio-toxins are released from bacteria. Through pattern recognition receptors on immune cells and other cell types (adipocytes, endothelial, muscle etc) this can increase serum concentration of inflammatory cytokines such as TNFα, IFNγ, and IL-6 and also the matrix metalloproteinase MMP9, a tissue-remodeling enzyme. This phenomenon termed a

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Interventions and research questions continued.....

Jarisch-Herxheimer (Herx) reaction often manifests as flu-like symptoms and it has been reported to occur after pulsed magnetic therapy. In addition, the LTT methodology developed for the identification of bacterial infections, heavy metals and other antigens, will be used to monitor the response to antibiotics and the pulsed magnetic therapy. Autoimmune changes within the body are also often manifest by the appearance of antibodies to specific organs such as the thyroid or nerve tissues. In particular antibodies to thyroid peroxidase and thyroid binding globulin are raised in Hashimoto's thyroiditis and antinuclear antibodies (ANAs) are raised in multiple sclerosis (MS). Indeed, the latter is not specific to MS and the measurement of ANAs may prove useful as a general marker for autoimmune reactions within the body.

Diagnostic screening and treatment outcomes

Based on the discussion from previous pages and on the studies outlined above, the following diagnostic analytical methods will be developed:

- *Salivary cortisol, DHEA and CBG measurements*
- *Free T_3/rT_3 determinations (with TSH and thyroid antibodies)*
- *Glucose tolerance and insulin sensitivity*
- *Lactate, CO_2, LDH activity (and CK activity) and mitochondrial function*
- *Inflammatory markers (IFNγ, IL-1β, IL-2, IL-6, IL-8, IL-12, IL-17, Leptin, MMP9, TGFβ, TNFα, and αMSH)*
- *Auto-antibodies (thyroid peroxidase Ab, thyroid binding globulin Ab, myelin basic protein Ab (MBPAbs), gliadin Abs, cardiolipin Abs and ANAs)*
- *ox-LDL/ox-Lp(a)*
- *LTT testing for Borrelia, Babesia, Bartonella, Ehrlichia and Mycoplasma*
- *LTT testing for heavy metals, gliadin, other proteins*

New treatments that will be derived from the work outlined above are:

- *New anti-microbial therapy (monitored by measurement of inflammatory cytokines) and detection systems (Biochemical/Biophysical)*
- *Endocrine therapy - thyroid hormone (T_3/ mixed T_3/T_4) and low dose hydrocortisone replacement with the monitoring of autoimmune status*
- *Dietary monitoring and supplementation with antioxidants, B vitamins, transdermal Mg (and other minerals) and mitochondrial support factors (phospholipids, MAO inhibitors and PGC-1/PPAR/Tfam inducers)*

Note added after completion of main text

The physiological systems involved in CFSRCs and ASDs are many and various and it is not possible to adequately cover all here. However, one in particular has been omitted form the discussion and it is now thought that this system is too important not to be mentioned. This system is the vitamin D/ Vitamin D receptor (VDR) transcription complex as outlined in the various articles of the group of Trevor Marshall (Autoimmunity Research Foundation, Thousand Oaks , CA, USA). Many individuals with inflammation-related conditions have low plasma concentration of 25 (OH) vitamin D. This is not the active vitamin D metabolite. 1,25 (dihydroxy) vitamin D is the form that binds to the VDR. The work of Marshall et al. suggests that the VDR is down-regulated in CFSRCs that are related to chronic infection. Data exists for infections with the Lyme agent Borrelia, that the VDR is inactivated. Given that the VDR is extremely important in innate immunity (production of antimicrobial peptides) this is most probably one of the mechanisms by which Borrelia is able to evade the immune system. Also, it would appear that due to lack of feedback control, 1,25D levels paradoxically rise (given that 25D is diminished). Due to the high affinity of 1,25D for the thyroid receptor α and β, this may provide an addition mechanism by which the thyroid hormone system is de-regulated.