

Chapter 13

**Endosymbiotic Actinidic Archaea and
Viroidal Induced Warburg Phenotype Can
Be Reversed by a Modified Vegetarian High
Fibre, High Medium Chain Triglyceride
Ketogenic Diet**

Introduction

Actinidic archaea has been related to the pathogenesis of schizophrenia, malignancy, metabolic syndrome X, autoimmune disease and neuronal degeneration. An actinide dependent shadow biosphere of archaea and viroids in the above mentioned disease states is described. The actinidic archaeal and viroid induced Warburg phenotype contributes to the pathology of the disease states mentioned. The possibility of administration of high medium chain triglyceride, high fibre ketogenic diet on actinide based primitive organism like archaea with a mevalonate pathway and cholesterol catabolism was considered in these disease states.¹⁻¹⁰ The effect of a high medium chain triglyceride and a high fibre modified vegetarian ketogenic diet on the Warburg phenotype was also studied.

The ketogenic diet is a high-fat, adequate-protein, low-carbohydrate diet that in medicine is used primarily to treat difficult-to-control (refractory) epilepsy in children. The diet mimics aspects of starvation by forcing the body to burn fats rather than carbohydrates. However, if there is very little carbohydrate in the diet, the liver converts fat into fatty acids and ketone bodies. The ketone bodies pass into the brain and replace glucose as an energy source. An elevated level of ketone bodies in the blood, a state known as ketosis, leads to a reduction in the frequency of epileptic seizures. The ketogenic diet results in adaptive changes to brain energy metabolism that increases the energy reserves; ketone bodies are a more efficient fuel than glucose, and the number of mitochondria is increased. This may help the neurons to remain stable in the face of increased energy demand during a seizure, and may confer a neuroprotective effect.¹⁰⁻¹⁵

Dietary fibre and medium chain triglycerides have antiviral and antibacterial effects. A low carbohydrate diet generates lesser glucose for the body and inhibits glycolysis. Dietary fibre generates short chain fatty acids butyrate and propionate which are immunosuppressive. The decrease in cytokines has inhibitory effect on

the generation of the Warburg phenotype. The results of the study on the effect of a high fibre, high MCT vegetarian ketogenic diet on the actinidic archaea and viroid induced Warburg phenotype are presented in this paper.¹⁰⁻¹⁵

Materials and Methods

The following groups were included in the study: - multiple sclerosis, rheumatoid arthritis and SLE. There were 10 patients in each group and each patient had an age and sex matched healthy control selected randomly from the general population. The blood was drawn from the (1) in freshly diagnosed cases in the fasting state before treatment was initiated, and (2) after a 15-days modified high fibre, high MCT vegetarian ketogenic diet of medium chain triglycerides (150 g of coconut oil), fibre (45 g of banana stem fibre) and vegetable proteins (black gram protein 100 g/day) with 50 g of carbohydrate (black gram polysaccharide). The blood samples were drawn in the fasting state before treatment was initiated. Plasma from fasting heparinised blood was used and the experimental protocol was as follows (I) Plasma+phosphate buffered saline, (II) same as I+cholesterol substrate, (III) same as II+rutile 0.1 mg/ml, (IV) same as II+ciprofloxacin and doxycycline each in a concentration of 1 mg/ml. Cholesterol substrate was prepared as described by Richmond.¹⁶ Aliquots were withdrawn at zero time immediately after mixing and after incubation at 37 °C for 1 hour. The following estimations were carried out: - Cytochrome F420, free RNA, free DNA, hexokinase activity and archaeal cholesterol oxidase activity as measured by hydrogen peroxide liberation.¹⁷⁻¹⁹ Cytochrome F420 was estimated fluorimetrically (excitation wavelength 420 nm and emission wavelength 520 nm). Informed consent of the subjects and the approval of the ethics committee were obtained for the study. The statistical analysis was done by ANOVA.

Results

Plasma of control subjects showed increased levels of the above mentioned parameters with after incubation for 1 hour and addition of cholesterol substrate resulted in still further significant increase in these parameters. The plasma of patients showed similar results but the extent of increase was more. The addition of antibiotics to the control plasma caused a decrease in all the parameters while addition of rutil increased their levels. The addition of antibiotics to the patient's plasma caused a decrease in all the parameters while addition of rutil increased their levels but the extent of change was more in patient's sera as compared to controls. The results are expressed in tables 1-5 as percentage change in the parameters after 1 hour incubation as compared to the values at zero time. The patients on modified ketogenic diet showed a decrease in all the parameters. Vegetarian ketogenic diets based on high fibre and high medium chain triglycerides has a inhibitory effect on the growth of archaea and viroids as well as archaeal cholesterol oxidase activity. The vegetarian ketogenic diet with its high fibre and high MCT content reversed the Warburg phenotype has indicated by a reduction in hexokinase activity.

Table 1. Effect of rutil, antibiotics and ketogenic diet on cytochrome F420.

| Group | CYT F420 % (Increase with Rutil) | | CYT F420 % (Decrease with Doxy+Cipro) | | CYT F420 % (Decrease with Ketogenic diet) | |
|---------|-------------------------------------|------|--|------|--|------|
| | Mean | ±SD | Mean | ±SD | Mean | ±SD |
| Normal | 4.48 | 0.15 | 18.24 | 0.66 | 18.25 | 0.72 |
| MS | 22.12 | 1.81 | 61.33 | 9.82 | 59.84 | 7.62 |
| SLE | 22.79 | 2.13 | 55.90 | 7.29 | 66.07 | 3.78 |
| RA | 22.59 | 1.86 | 57.05 | 8.45 | 65.77 | 5.27 |
| F value | 306.749 | | 130.054 | | 257.996 | |
| P value | < 0.001 | | < 0.001 | | < 0.001 | |

Table 2. Effect of rutile, antibiotics and ketogenic diet on free RNA.

| Group | RNA % change (Increase with Rutile) | | RNA % change (Decrease with Doxy+Cipro) | | RNA % change (Decrease with Ketogenic diet) | |
|---------|--|------|--|------|--|------|
| | Mean | ±SD | Mean | ±SD | Mean | ±SD |
| Normal | 4.37 | 0.13 | 18.38 | 0.48 | 18.15 | 0.58 |
| MS | 23.29 | 1.98 | 67.46 | 3.96 | 65.46 | 5.79 |
| SLE | 23.11 | 1.52 | 66.68 | 3.97 | 62.49 | 7.26 |
| RA | 22.29 | 2.05 | 67.03 | 5.97 | 58.70 | 7.34 |
| F value | 427.828 | | 654.453 | | 203.651 | |
| P value | < 0.001 | | < 0.001 | | < 0.001 | |

Table 3. Effect of rutile, antibiotics and ketogenic diet on DNA.

| Group | DNA % change (Increase with Rutile) | | DNA % change (Decrease with Doxy+Cipro) | | DNA % change (Decrease with Ketogenic diet) | |
|---------|--|------|--|------|--|------|
| | Mean | ±SD | Mean | ±SD | Mean | ±SD |
| Normal | 4.37 | 0.15 | 18.39 | 0.38 | 18.78 | 0.11 |
| MS | 22.62 | 1.38 | 63.82 | 5.53 | 67.05 | 3.00 |
| SLE | 23.30 | 1.42 | 65.07 | 4.95 | 66.67 | 4.21 |
| RA | 22.29 | 2.05 | 58.70 | 7.34 | 63.97 | 3.62 |
| F value | 337.577 | | 356.621 | | 673.081 | |
| P value | < 0.001 | | < 0.001 | | < 0.001 | |

Table 4. Effect of rutile, antibiotics and ketogenic diet on hexokinase activity.

| Group | Hexokinase % change (Increase with Rutile) | | Hexokinase % change (Decrease with Doxy+Cipro) | | Hexokinase % change (Decrease with Ketogenic diet) | |
|---------|---|------|---|------|---|------|
| | Mean | ±SD | Mean | ±SD | Mean | ±SD |
| Normal | 4.21 | 0.16 | 18.56 | 0.76 | 18.43 | 0.82 |
| MS | 22.81 | 1.91 | 63.47 | 5.81 | 60.28 | 9.22 |
| SLE | 22.47 | 2.17 | 65.97 | 4.62 | 63.90 | 7.13 |
| RA | 21.66 | 1.94 | 67.03 | 5.97 | 62.37 | 5.05 |
| F value | 292.065 | | 317.966 | | 115.242 | |
| P value | < 0.001 | | < 0.001 | | < 0.001 | |

Table 5. Effect of rutile, antibiotics and ketogenic diet on cholesterol oxidase activity.

| Group | Cholesterol oxidase activity % (Increase with Rutile) | | Cholesterol oxidase activity % (Decrease with Doxy+Cipro) | | Cholesterol oxidase activity % (Decrease with Ketogenic diet) | |
|---------|---|------|---|------|---|------|
| | Mean | ±SD | Mean | ±SD | Mean | ±SD |
| Normal | 4.43 | 0.19 | 18.13 | 0.63 | 18.48 | 0.39 |
| MS | 21.14 | 1.20 | 60.53 | 4.70 | 67.10 | 3.82 |
| SLE | 22.86 | 1.91 | 63.66 | 6.88 | 68.53 | 2.65 |
| RA | 23.29 | 1.67 | 60.52 | 5.38 | 61.91 | 7.56 |
| F value | 380.721 | | 171.228 | | 556.411 | |
| P value | < 0.001 | | < 0.001 | | < 0.001 | |

Discussion

There was increase in cytochrome F420 indicating archaeal growth. The archaea can synthesize and use cholesterol as a carbon and energy source as indicated by cholesterol oxidase activity.²⁰⁻²² The archaeal origin of the enzyme activities was indicated by antibiotic induced suppression. The study indicates the presence of actinide based archaea with an alternate actinide based enzymes or metalloenzymes in the system as indicated by rutile induced increase in enzyme activities.²⁰⁻²² The archaeal cholesterol oxidase activity was increased resulting in generation of hydrogen peroxide.²⁰⁻²² The archaeal glycolytic hexokinase activity were increased. The archaea can undergo magnetite and calcium carbonate mineralization and can exist as calcified nanoforms.¹⁷ There was an increase in free RNA indicating self replicating RNA viroids and free DNA indicating generation of viroid complementary DNA strands by archaeal reverse transcriptase activity. The high fibre and high MCT modified vegetarian ketogenic diet can block archaeal and viroidal multiplication. Fibre and MCT have a antiarchaeal and antiviroidal effect.¹¹⁻¹⁵

Archaea can induce the host AKT PI3K, AMPK, HIF alpha and NFkB producing the Warburg metabolic phenotype.¹⁰ The increased glycolytic hexokinase activity indicates the generation of the Warburg phenotype. A high

fibre and high MCT modified vegetarian ketogenic diet can inhibit hexokinase activity and glycolysis and reverse the Warburg phenotype. The generation of the Warburg phenotype is due to activation of HIF alpha. This stimulates anaerobic glycolysis, inhibits pyruvate dehydrogenase, inhibits mitochondrial oxidative phosphorylation, stimulates heme oxygenase, stimulates VEGF and activates nitric oxide synthase. The low carbohydrate diet generates less of glucose and inhibits the glycolytic pathway. This reverses the Warburg phenotype. The high fibre intake generates short chain fatty acids butyrate and propionate. Short chain fatty acids bind to lymphocyte GPCR receptors and are immunosuppressive. The reduction in cytokine generation inhibits the Warburg phenotype. The antiarchaeal and antiviral action of MCT and dietary fibre also inhibits the generation of the Warburg phenotype.¹¹⁻¹⁵

The Warburg phenotype generates malignant, autoimmune, neurodegenerative, metabolic syndrome X and schizophrenic pathologies. The Warburg phenotype can lead to increased cell proliferation and malignant transformation. The mitochondrial PT pore hexokinase is increased leading onto cell proliferation. There is induction of glycolysis, inhibition of PDH activity and mitochondrial dysfunction resulting in inefficient energetics and metabolic syndrome. The archaea and viroid generated cytokines can lead to TNF alpha induced insulin resistance and metabolic syndrome X. The increase in glycolysis can activate glyceraldehyde 3-phosphate dehydrogenase which gets translocated to the nucleus after polyadenylation. The PARP enzyme is activated by glycolysis mediated redox stress. This can produce nuclear cell death and neuronal degeneration. The increase in the glycolytic enzyme fructose 1,6-diphosphatase increases the pentose phosphate pathway. This generates NADPH which activates NOX. NOX activation is related to NMDA activation and glutamate excitotoxicity. This leads onto neuronal degeneration.¹⁰

The increase in glycolysis activates the enzyme fructose 1,6-diphosphatase which activates the pentose phosphate pathway liberating NADPH. This increases NOX activity generating free radical stress and H₂O₂. Free radical stress is related to insulin resistance and metabolic syndrome X. Free radicals can activate NFκB producing immune activation and autoimmune disease. Free radicals can open the mitochondrial PT pore, produce release of cyto C and activate the caspase cascade. This produces cell death and neuronal degeneration. The free radicals can activate NMDA receptor and induce the enzyme GAD generating GABA. This activates the NMDA/GABA thalamo-cortico-thalamic pathway mediating conscious perception. Increased free radical generation can also initiate schizophrenia. Free radicals can also produce oncogene activation and malignant transformation. Free radicals can produce HDAC inhibition and HERV generation. The encapsulation of HERV particles in phospholipids vesicles can mediate the generation of the acquired immunodeficiency syndrome. Free radicals can also promote atherogenesis.¹⁰

The lymphocytes depend on glycolysis for its energy needs. The increase in glycolysis owing to the induction of Warburg phenotype can lead to immune activation. Immune activation can lead to autoimmune disease. TNF alpha can activate the NMDA receptor leading to glutamate excitotoxicity and neuronal degeneration. TNF alpha activating NMDA receptor can contribute to schizophrenia. TNF alpha can induce expression of HERV particles contributing to generation of acquired immunodeficiency syndrome. Immune activation has also been related to malignant transformation mediated by NFκB. TNF alpha can also act upon the insulin receptor producing insulin resistance. NOX activation consequent to the generation of the Warburg phenotype also activates the insulin receptor. Thus there is a hyperinsulinemic state leading on to metabolic syndrome X.¹⁰

Thus the induction of the Warburg phenotype can lead to malignancy, autoimmune disease, metabolic syndrome X, neuropsychiatric disease and neuronal degeneration. The Warburg phenotype leads to inhibition of pyruvate dehydrogenase and accumulation of pyruvate. The accumulated pyruvate enters the GABA shunt pathway and is converted to citrate which is acted upon by citrate lyase and converted to acetyl CoA, used for cholesterol synthesis. The pyruvate can be converted to glutamate and ammonia which is oxidised by archaea for energy needs. The increased cholesterol substrate leads to increased archaeal growth and further induction of the Warburg phenotype.¹⁰

A ketogenic diet is normal diet of the primitive hunter-gatherer humans. It is based upon a low carbohydrate, high saturated fat and high protein diet. In this study, a modified ketogenic diet was used. It included high medium chain triglycerides from coconut oil, high fibre from banana stem, high black gram protein and low black gram polysaccharide as source of carbohydrate. It was a modified vegetarian ketogenic diet high in MCT and fibre. This diet has got an antiviral and antiarchaeal activity and can reverse the Warburg phenotype, the basis of diverse malignant, autoimmune, neurodegenerative, metabolic syndrome X and schizophrenic pathologies.¹¹⁻¹⁵

References

- [1] Hanold D., Randies, J. W. (1991). Coconut cadang-cadang disease and its viroid agent, *Plant Disease*, 75, 330-335.
- [2] Valiathan M. S., Somers, K., Kartha, C. C. (1993). *Endomyocardial Fibrosis*. Delhi: Oxford University Press.
- [3] Edwin B. T., Mohankumaran, C. (2007). Kerala wilt disease phytoplasma: Phylogenetic analysis and identification of a vector, *Proutista moesta*, *Physiological and Molecular Plant Pathology*, 71(1-3), 41-47.
- [4] Kurup R., Kurup, P. A. (2009). *Hypothalamic Digoxin, Cerebral Dominance and Brain Function in Health and Diseases*. New York: Nova Science Publishers.

- [5] Eckburg P. B., Lepp, P. W., Relman, D. A. (2003). Archaea and their potential role in human disease, *Infect Immun*, 71, 591-596.
- [6] Smit A., Mushegian, A. (2000). Biosynthesis of isoprenoids via mevalonate in Archaea: the lost pathway, *Genome Res*, 10(10), 1468-84.
- [7] Adam Z. (2007). Actinides and Life's Origins, *Astrobiology*, 7, 6-10.
- [8] Schoner W. (2002). Endogenous cardiac glycosides, a new class of steroid hormones, *Eur J Biochem*, 269, 2440-2448.
- [9] Davies P. C. W., Benner, S. A., Cleland, C. E., Lineweaver, C. H., McKay, C. P., Wolfe-Simon, F. (2009). Signatures of a Shadow Biosphere, *Astrobiology*, 10, 241-249.
- [10] Wallace D. C. (2005). Mitochondria and Cancer: Warburg Addressed, *Cold Spring Harbor Symposia on Quantitative Biology*, 70, 363-374.
- [11] Liu, Y. M. (2008). Medium-chain triglyceride (MCT) ketogenic therapy. *Epilepsia*,; 49S(8), 33-6.
- [12] Gasior, M., Rogawski, M. A., Hartman, A. L. (2006). Neuroprotective and disease-modifying effects of the ketogenic diet. *Behav Pharmacol*, 17(5-6), 431-9.
- [13] Maalouf, M., Rho, J. M., Mattson, M. P. (2009). The neuroprotective properties of calorie restriction, the ketogenic diet, and ketone bodies. *Brain Res Rev*, 59(2), 293-315.
- [14] Barbary, O. M., El-Sohaimy, S. A., El-Saadani, M. A., Zeitoun, A. M. A. (2010). Antioxidant, antimicrobial and anti-HCV activities of lignan extracted from flaxseed. *Research Journal of Agriculture and Biological Sciences*, 6(3), 247-256.
- [15] Lieberman, S., Enig, M. G., Preuss, H. G. (2006). A review of monolaurin and lauric acid. Natural virucidal and Bactericidal agents. *Alternative and Complementary Therapies*. 1, 310-314
- [16] Richmond W. (1973). Preparation and properties of a cholesterol oxidase from nocardia species and its application to the enzymatic assay of total cholesterol in serum, *Clin Chem*, 19, 1350-1356.
- [17] Snell E. D., Snell, C. T. (1961). *Colorimetric Methods of Analysis*. Vol 3A. New York: Van Nostrand.

- [18] Glick D. (1971). *Methods of Biochemical Analysis*. Vol 5. New York: Interscience Publishers.
- [19] Colowick, Kaplan, N. O. (1955). *Methods in Enzymology*. Vol 2. New York: Academic Press.
- [20] Van der Geize R., Yam, K., Heuser, T., Wilbrink, M. H., Hara, H., Anderton, M. C. (2007). A gene cluster encoding cholesterol catabolism in a soil actinomycete provides insight into *Mycobacterium tuberculosis* survival in macrophages, *Proc Natl Acad Sci USA*, 104(6), 1947-52.
- [21] Francis A. J. (1998). Biotransformation of uranium and other actinides in radioactive wastes, *Journal of Alloys and Compounds*, 271(273), 78-84.
- [22] Probian C., Wülfing, A., Harder, J. (2003). Anaerobic mineralization of quaternary carbon atoms: Isolation of denitrifying bacteria on pivalic acid (2,2-Dimethylpropionic acid), *Applied and Environmental Microbiology*, 69(3), 1866-1870.

