My Experiences and Understanding of VLCAD Deficiency and its Treatment

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This disorder is characterized by the deficiency of the VLCAD enzyme that is required for converting long-chain fatty acids (*either endogenous or from dietary intake*) into energy. It is an autosomal recessive disorder that has only been recognized and characterized since 1994 when, for the first time, the VLCAD enzyme was discovered. Prior to that time, during the early 1980's, a disorder was recognized that was incorrectly called "Long-Chain Acyl-CoA Dehydrogenase" (LCAD) deficiency and subsequently found to be actually a deficiency of the newly discovered VLCAD enzyme. Prior to 1994 many so-called "LCAD" patients that were identified by skin biopsy enzyme assay died during infancy or early childhood with severe cardiac involvement. To my knowledge, there have not been any deaths due to metabolic decompensation and cardiomyopathy of VLCAD patients as adolescents or adults despite subsequent diagnosis of new patients in those age groups.

This difference between affected infants who often died with cardiomyopathy and the apparently less severe form of VLCAD deficiency in older surviving children, adolescents, and adults prompted investigation to explain why there were two very different clinical forms of the disorder. I will refer to these two phenotypes as "VLCAD-C" for the severe infantile form with **C**ardiomyopathy and "VLCAD-H" for the milder phenotype of older patients whose initial problems in infancy and childhood was **H**ypoglycemia (low blood sugar). The descriptions of the clinical courses of these two phenotypes is as follows:

"**VLCAD-C**": These patients characteristically have episodes of hypoglycemia in the neonatal period that is followed at 3-5 months of age by cardiac failure, and evolution of "hypertrophic or dilated cardiomyopathy" requiring Intensive Care often for 1 or more months. Without an immediate diagnosis and appropriate dietary management, they usually died on that admission. They would also be found to have very high levels of creatine phosphokinase (CPK) signaling involvement of skeletal muscle (*Rhabdomyolysis*). In summary, this phenotype involves metabolic compromise of heart, liver, and skeletal muscle.

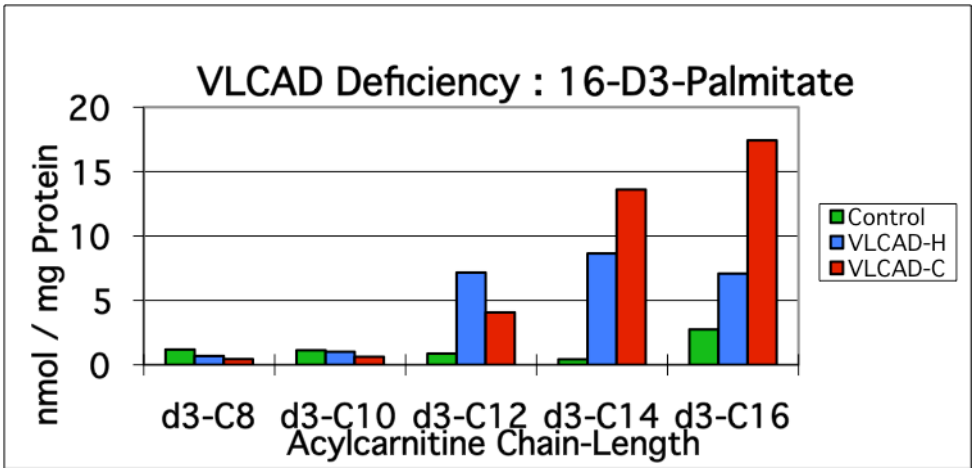
"**VLCAD-H**": These patients also have episodes of hypoglycemia in the neonatal period and subsequently during childhood and adolescence when they ***will have recurrent bouts of muscle pain that may require hospitalizations for a few days to control the rhabdomyolysis***. However, they do not have cardiac involvement and do not evolve hypertrophic cardiomyopathy as seen in the more severe VLCAD-C phenotype.

Most reviews of this deficiency describe the importance of the VLCAD enzyme in the "beta oxidation" spiral in **mitochondria** but do not address the degradation of fatty acids in other cell bodies such as the **peroxisome**. The peroxisome and mitochondrial pathways for fatty acid conversion to energy are linked together. The first thing to understand is the structure of fatty acids: dietary fats and stored fats, relevant to this discussion, contain 16-18 carbon atoms (C16 – C18) linked in a chain with the first carbon being the acid group. The oxidation cycle of fatty acids results in the reduction of carbon atoms, two at a time producing from C16 a C14 fat plus the two carbon unit known as "acetyl-CoA". This cycle is repeated from C14 to C4, each time releasing another acetyl-CoA. *When produced in the mitochondrion*, acetyl-CoA is utilized by the "Citric acid cycle" finally producing the high-energy compound called **ATP**. Clearly, when the VLCAD enzyme is deficient, it is not possible to go from C16 to C4 and acetyl-CoA is not available for adequate energy production. This is why a severe energy deficit occurs when a VLCAD patient is fasted or receiving a high fat diet. The peroxisome contains different enzymes for beta oxidation and represents ~ 30% of total cellular fat oxidation, is limited to going from C16 down to C8. The C8 fatty acid is converted into an acylcarnitine, exported from the peroxisome, taken up by the mitochondrion, reactivated and oxidized down to C4 and ketone bodies in the liver. Normal mitochondrial oxidation can go from C16 or C18 down to C4 with the latter producing "ketone bodies" in the liver that can be exported to other organs as a source of acetyl-CoA for energy production from their citric acid cycle. In VLCAD deficiency the ability to produce and export these "ketone bodies" is severely diminished thus causing energy deficits in other organs that depend on them for energy production. This is especially true for skeletal muscle and the heart and results in muscle cell breakdown (*rhabdomyolysis*) and cardiomyopathy with heart failure during illness in the severe form of VLCAD deficiency.

Can these two phenotypes be recognized biochemically ?
Why does the milder phenotype of VLCAD deficiency never have heart involvement but has intermittent muscle breakdown (*rhabdomyolysis*) with illness?

The **biochemical distinction** between these two phenotypes was demonstrated with special studies of cultured skin cells to which was added stable isotope (non-radioactive) labeled palmitic acid in three publications between 1998 and 2001. [References 1-3]. Palmitic acid is the preferred substrate for the VLCAD enzyme that was deficient in fibroblasts (cultured skin cells) from biopsies of patients representing both "VLCAD-C" and "VLCAD-H". When these cultured cells were analyzed following 72 hours of incubation with palmitic acid, the metabolites representing the attempted metabolism of this fatty acid could be measured. One could observe the metabolism of palmitic acid (having 16 carbon atoms) down to metabolites having

14, 12, 10, etc. down to 4 carbon atoms. The figure below illustrates the successful 100% recognition and differentiation of the metabolic profiles for "VLCAD-C" and "VLCAD-H". Skin cells from patients with "VLCAD-H" reveal a ratio of C16 : C12 of ~ 1.0 in contrast to the ratio of C16 : C12 of ~ 4.0 observed with patients with the severe "VLCAD-C" phenotype.
These results suggested that in the milder form of VLCAD, the peroxisome was contributing more to the oxidation C16 and C18 fatty acids than in the severe cardiac form.



Until recently, this method of identifying which form of VLCAD deficiency was present could be performed only at Duke University, Mayo Clinic, Baylor Medical Center in Dallas and and the Academic Medical Center in Amsterdam. Although some reviews have claimed that this test is "invasive" and takes too long, I have found it to be of enormous help in early management and of excellent prognostic value often providing great relief to parents when the milder form was identified. When a blood (*not plasma*) acylcarnitine profile detected an affected infant but could not identify the phenotype, the infant was treated with a high MCT containing formula such as Portagen and Carnitine supplementation while awaiting the skin fibroblast analysis to clarify the phenotype.
[*For more detailed clinical and therapeutic aspects of these two VLCAD phenotypes, see Reference 4*]

The Carnitine Controversy:
Despite several reviews in the literature that suggest carnitine supplementation may be dangerous possibly producing heart rhythm problems from long chain acylcarnitines. This concern originated from a single LCHAD patient in Canada who had a rhythm disturbance while receiving carnitine. To my knowledge, there have been no other examples of this in any publications up to the present. In unpublished studies between myself and North Shore hospital, several VLCAD patients in the ICU in crisis were given intravenous carnitine in very high doses while being monitored for heart rhythm ***without any evidence of rhythm disturbance***. In my 30 year experience with carnitine supplementation in all forms of fat oxidation and organic acidurias, Carnitine's only side effects have included only a bad fishy smell or diarrhea but ***never*** any other dangerous effects. The fishy smell is due to bacterial breakdown in the intestine producing a fragment that is normally destroyed by the liver but if that action is reduced, the fragment (trimethylamine) circulates in the blood and may be found in breath, sweat, etc. giving the fishy smell. Both side effects which only occur rarely or due to very high doses can be eliminated by stopping or reducing the dose.

So, what is my rationale for using carnitine supplementation?
Most physicians consider it important to provide carnitine only when the blood levels indicate a significant deficiency and do not explain why it might have other important benefits to the patient's metabolism. **My rationale is *very* different and based on compromised metabolism inside the mitochondrion during illness:** In 1981, we documented that carnitine could act as a "scavenger" by combining with short chain fatty acids as "acylcarnitines" and eliminating them in the urine. This was felt to be a good way to remove potentially toxic short chain acids such as propionic acid found in the organic acidurias: propionic acidemia and methylmalonic aciduria. Although this scavenging role occurs with SCAD and MCAD deficiency, it could not eliminate fatty acid metabolites containing more than 12 carbons such as in LCHAD, Trifunctional Protein, VLCAD, Translocase or CPT II deficiencies.

However, carnitine has another important metabolic effect inside the mitochondrion. During illness in VLCAD and other long chain deficiencies, toxic long chain acyl CoA compounds accumulate. An acyl-CoA compound like C16-CoA consumes and sequesters Co-enzyme A (CoA) and compromises oxidation of amino acids etc., thus interfering with energy (ATP) production. CoA cannot pass through the mitochondrial membrane so that when it is trapped inside as acyl-CoA compounds from defects

such as VLCAD, metabolism and energy generation are severely compromised. When carnitine is supplemented during this crisis, the acyl-CoA compounds are converted to non-toxic acylcarnitines and CoA is made available again for mitochondrial metabolism and energy generation. This is the most important contribution of carnitine supplementation for long chain fat defects.

Treatment Considerations: Fat mobilization during illness and why it occurs …
When a patient with VLCAD and other long-chain deficiencies becomes ill and is unable to eat, there is an energy deficiency that can only be approached by ***mobilizing body stores*** of fat, amino acids and carbohydrate in an attempt to correct the energy deficiency. However, this is ineffective because due to VLCAD deficiency. Long chain fats cannot be oxidized to provide needed acetyl-CoA for ATP and ketone generation. All organs are therefore affected.

Since I have always been fascinated by basic science publications that might provide a better understanding of what we observe clinically, I discovered that Dr. Grahame Hardie had characterized a mechanism that regulates metabolism either in the direction of "catabolism" (mobilization of body stores for energy production) versus "anabolism" (reversing catabolism and favoring "synthesis"). This is like a "switch" existing in each cell. This switch can be activated by reduced cell levels of cellular ATP and is an enzyme called AMP-mediated protein kinase (**AMPK**) resulting in enhanced catabolism in an attempt to relieve the energy deficit. ***Activated*** AMPK enhances catabolism of precursors for the Citric Acid Cycle and ATP synthesis while inhibiting the mammalian target of rapamycin (mTOR) that is responsible for activation of synthetic pathways such as protein synthesis and cellular proliferation (reference 6). AMPK activation is known to enhance β-oxidation (for energy) while impairing fatty acid synthetic pathways. AMPK can only be ***inactivated*** by treatments designed to increase ATP concentration in the cell. In summary, decreased energy production with activation of AMPK is responsible for mobilization of stored fat, glycogen, and amino acids from body protein creating severe illness in long-chain fat disorders including VLCAD.

When the role of activated AMPK due to energy deficiency is considered, it focuses our attention on the fundamental goal of effectively treating VLCAD and other serious long chain fat disorders. The diet is usually low in long chain fat with enhanced carbohydrate and supplements of essential fatty acids such as linoleic and linolenic acids. We have shown that these essential fats are mainly involved in synthesis of important compounds and are poorly involved in beta oxidation where they might contribute to toxic compounds due to a defect such as VLCAD. In contrast, saturated fats (such as palmitate) and oleate are effectively oxidized by beta oxidation and produce the toxic metabolites that accumulate in VLCAD deficiency and are converted to the acylcarnitines that are diagnostic for this deficiency (C16 and C14:1 respectively) from blood analysis and expanded newborn screening. (Reference 7).

MCT oil is of definite value in the therapy because its medium chain fats do not require VLCAD or other long chain enzymes for beta oxidation. They slip in under the block providing needed energy and ketone bodies for export from the liver to other organs including muscle and heart ! However, they only provide acetyl-CoA for the citric acid cycle and ATP generation. For the citric acid cycle to function *optimally*, it requires a source of oxaloacetate in addition to acetyl-CoA for maximum ATP production. With VLCAD patients referred to me, I often found that they were receiving MCT supplement as little as a tablespoon (15 grams) ! For effective inactivation of the "switch" 35% of total caloric intake is more reasonable (for a 20 Kg [44 lbs] child this would equal ~ 65-80 grams of MCT per day).

During crisis, 10% intravenous glucose as a source of acetyl-CoA is always started. However, sometimes the glucose is not effectively entering the cells and additional insulin is required to "force" the glucose into the cell while also inhibiting fat mobilization. As described above, carnitine supplementation also has a role for normalizing mitochondrial metabolism.

My Experience with VLCAD Patients:
From January 2000 until May 2009, 23 VLCAD patients were managed and investigated under an FDA IND treatment protocol at Baylor University Medical Center. As of August 2010, the age of these patients ranged from 7 to 52 years.

Twelve patients had the VLCAD-C phenotype and 11 had the VLCAD-H phenotype. All 23 patients were diagnosed by direct enzyme assay in fibroblasts, DNA mutation analysis, and identification of the phenotype in cultured fibroblasts as described above (*See figure* above). Results of the enzyme assay as performed by Dr. Vianey-Saban suggested that VLCAD-C patients had little if any residual activity while those with VLCAD-H had more residual activity. These findings did not allow for a clear distinction of the phenotypes but clearly documented the deficiency. There was no reliable correlation between DNA mutation analyses by Dr. Andersen in Denmark and the 2 phenotypes as was reliably identified by analysis of palmitate oxidation in cultured fibroblasts. Although blood acylcarnitine analysis confirmed VLCAD deficiency, one could not differentiate the phenotypes from those profiles. ***When I refer to a "milder phenotype", I am referring to VLCAD-H in which there is no heart involvement but recurrent "rhabdomyolysis" requiring multiple hospitalizations will occur***. Parents ***must*** pay attention to onset of episodes of skeletal muscle pain and seek help evewn before any darkening of urine (myoglobinuria). Blood levels of

CPK will be markedly elevated with onset of muscle pain and if not treated will evolve into frank myoglobinuria. (*See use of fructose for early treatment of muscle pain-below*).
Since 2000, there were only two deaths. One (*VLCAD-C*) was due to non-compliance with therapy. The other (*VLCAD-H*) was due to a torn superior vena cava during a routine mediport replacement. All of these patients received a diet containing triheptanoin – an odd carbon number MCT (7 carbon atoms instead of 8 & 10 carbons in normal MCT oil). The metabolism of this compound provides both compounds required by the citric acid cycle (acetyl-CoA, oxaloacetate and produces ketone bodies for other organs that can provide both acetyl-CoA & oxaloacetate to these organs. There were remarkable improvements in these patients and the mortality rate as describe above was only 2 of the 23 patients (7%) compared to 75% of VLCAD patients studied in France receiving conventional MCT oil diets. (See References 8 & 9 for more information.) Now that I am retired, I have successfully encouraged Dr. Vockley in Pittsburgh to continue evaluating the utility of triheptanoin.

On occasion, VLCAD patients in our studies who experienced muscle pain before a real crisis, were relieved by taking a dose of Fructose every 4 hours. [***unpublished data***] This supplement was ~3-4 tablespoons of Fructose powder in ~ 4 ounces of water. Relief was usually noted within one hour of that dose and aborted the attack thus avoiding hospitalization. Fructose is another form of anaplerotic therapy like triheptanoin. (*fructose is in most grocery stores where powdered sugar is located.*)

Other Observations:
To my knowledge, no deaths due to metabolic deterioration in patients with VLCAD-H have been experienced in my clinical experience or reported by other physician investigators before or after 1994. The prognosis for this phenotype is very good with, currently, no basis for concerns regarding lifespan. Now that physicians dealing with adult patients with recurrent rhabdomyolysis are aware of this disorder as a part of that differential diagnosis, more adult patients are being identified with this phenotype. As described, these patients do not have any cardiac involvement and are successfully managed with dietary therapy. [See Reference 5]

Two of my patients with VLCAD-H are good examples of the clinical course and tolerance for surgical procedures:

A 43 year old woman (DOB 12-29-1966) experienced recurrent rhabdomyolysis since adolescence. She has had two successful pregnancies (cesarean) since 2001 without complications. Since 2003, she has experienced only two episodes of rhabdomyolysis but only one required hospitalization for intravenous therapy. During that episode, she had cardiac evaluation with EKG, Echocardiogram, and 2 weeks on a Holter monitor that excluded any cardiac abnormalities. She continues to be healthy and active as of the date of this report.

A 41 year old woman (DOB: 8/24/1964) experienced recurrent hypoglycemia in childhood and subsequently recurrent rhabdomyolysis without cardiac involvement. She was diagnosed with VLCAD-H at the age of 18 years. She successfully completed three pregnancies (cesarean deliveries), underwent gall bladder surgery for stones, and had had 3 mediport placements prior to her death at age 41 years due to a tear in the superior vena cava during that mediport replacement procedure. Her pregnancies were complicated by repeated bouts of rhabdomyolysis. She was on no diet therapy during her pregnancies.

A 52 year old male: (DOB 9-25-58. 52 years old in 2010). He also experienced recurrent hypoglycemia in childhood and subsequently recurrent rhabdomyolysis without cardiac involvement. He has had only three episodes of rhabdomyolysis since 2002 all with good recovery with intravenous fluid therapy. He is a successful salesman. His surgical history was not available.

All other VLCAD-H patients in my practice have undergone general surgical procedures with anesthesia without incident (appendectomy, hernia repair, cholecystectomy, etc.). Many of the younger patients had tonsillectomy, adenoidectomy, wisdom teeth removed, and immunizations without consequences. All of my remaining VLCAD-H patients are well.

My experience with the more severe VLCAD-C patients has been very good. Of note is that following successful treatment of a cardiomyopathy between 2-5 months of life, only one of my 12 patients had a recurrence of cardiomyopathy in later life. That occurred in a remote location where the treatment protocol was not followed. These patients are plagued by recurrent rhabdomyolysis in later life and are often confused with adult CPT II patients. All in all, they have had a good result and were able to participate in sports especially when taking a dose of fructose prior to exercise.

I apologize for, perhaps, excessive information but from following the literature and the e-mails to the FOD support group, I have been impressed (or depressed) by the confusion regarding VLCAD deficiency and its current management. I have always felt that the more understanding of the disease and reasons for its management are very important for families as well as their physicians. I remain a firm believer that "we are all in this together" !!

My best wishes to all of you. Sincerely, Charles R. Roe, MD

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