Adult polyglucosan body disease (APBD): Anaplerotic diet therapy (triheptanoin) and demonstration of defective methylation pathways

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ABSTRACT

APBD is a rare disorder most often affecting adults of Ashkenazi Jewish origin due to partial deficiency of the glycogen brancher enzyme (GBE). It is characterized by progressive involvement of both the central and peripheral nervous systems and deposition of amylopectin-like polyglucosan bodies. There have been no metabolic derangements that might suggest effective therapy nor have there been any clinical improvements for control of its relentless progression. The APBD patients, in this study, experienced stabilization of disease progression, and limited functional improvement in most patients with dietary triheptanoin. Due to a plateau in clinical improvement, the reduced plasma creatinine and methionine levels prompted evaluation of other plasma methylation intermediates in this complex integrated pathway system: decreased S-adenosylmethionine (SAM) ($p < 0.002$), increased S-adenosylhomocysteine ($p < 0.001$), elevated creatine ($p = 0.001$) and increased free choline ($p < 0.001$). Plasma levels of homocysteine and guanidinoacetate were normal. Impaired metabolism of choline and creatine may relate to the progressive dysmyelination and progressive muscle weakness associated with APBD. The partial deficiency of GBE appears to produce a secondary energy deficit possibly related to inadequate reserves of normal glycogen for efficient degradation to free glucose. Dysfunctional regulation of glycogen synthase (GS) may result in continued synthesis and deposition of polyglucosan bodies. This investigation has demonstrated, for the first time, arrest of clinical deterioration with limited functional recovery with triheptanoin diet therapy and the existence of significant derangement of methylation pathways that, when corrected, may lead to even greater therapeutic benefits.

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Introduction

Adult polyglucosan body disease (APBD) is a late-onset autosomal-recessive metabolic disorder due to deficiency of the glycogen brancher enzyme (GBE). It occurs most frequently in patients of Ashkenazi Jewish origin and, most commonly, is due to a TYR329-SER mutation in the GBE gene [1]. The disorder is characterized by a gradual progression of involvement of both the central and peripheral nervous systems with a variable phenotype that often includes pyramidal tetraparesis, sensory neuropathy in the lower extremities, neurogenic bladder, and, occasionally, cognitive impairment. The disease progresses, relentlessly, from difficulty walking, impaired balance, progressive weakness, eventually involving the upper body, and can lead to early death [1–3]. Polyglucosan bodies (*intracellular inclusions of amylopectin-like polysaccharide that has fewer branched points*) are greatly increased in neurons and glia [3–5]. As with other glycogenoses, the prevailing rationale for the mechanism of the disease is "cellular mechanical disruption" caused by the progressive increase and deposition of this abnormal polysaccharide.

Currently, there is no effective therapy for recovery of function or relieving the progression of this disease. Similarly, there is very little information relative to primary or secondary abnormalities in intermediary metabolism. The purpose of this report is to examine the possibility that there is a significant secondary impairment of energy metabolism and disruption of methylation in these patients. Encouraging clinical results with anaplerotic diet therapy with the odd-carbon triglyceride (triheptanoin – C7TG) appears to interrupt the progression of symptoms and provides some functional recovery.

Methods

All of the analytical methods for blood levels were performed on plasma from fasting blood samples.

Quantitative blood acylcarnitine, urinary organic acid, and plasma amino acid analyses have been described previously [6–8].

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Quantitative analysis of amino acids in plasma was determined by ion-exchange HPLC with post-column derivatization using ninhydrin. The amino acids were detected by UV–vis at 570 nm and data integration performed with PeakNet software version 6.30 (Dionex, Sunnyvale, CA) [8].

Blood levels of glucose, BUN, creatinine, sodium, potassium, chloride, CO_2, calcium, total protein, albumin, bilirubin, alkaline phosphatase, SGOT (AST), SGPT (ALT), Creatine Kinase (CPK), Lipid panels (triglycerides, cholesterol, HDL, and LDL) and complete blood count and platelets were obtained on each visit to detect metabolic abnormalities as well as for safety monitoring during the dietary protocol.

Total plasma homocysteine (tHcy) was determined using a high performance liquid chromatography (HPLC) method with fluorescence detection [9]. Plasma levels of s-adenosyl-methionine (SAM), and s-adenosyl-homocystine (SAH) were measured by a modification of the stable-isotope dilution liquid chromatography–electrospray injection tandem mass spectrometry (LC–ESI–MS/MS) previously described [10]. Plasma levels of guanidinoacetic acid (GAA), and creatine and free and bound levels of plasma choline were measured in the Clinical Chemistry Department of the Free University of Amsterdam by previously described methods [11,12].

All of the diagnostic assays for levels of GBE from fibroblasts or sural nerve biopsies and targeted DNA analysis for the Tyr329Ser mutation in the GBE gene were provided by the referring physicians prior to entry into this protocol (See patient descriptions for results).

Dietary protocol

All patients signed informed consent under Baylor University Medical Center's Institutional Review Board protocol (#099-135) registered under the US Food and Drug Administration Investigational New Drug IND 59,303. Initially, patients were evaluated for 5 days when baseline clinical and laboratory data were obtained. The triheptanoin diet was started by the second day with dose adjustments as required and daily educational sessions regarding management of the disorder and rationale for diet therapy as well as current understanding of the disorder, the biochemical fate of C7TG in prior clinical studies, and the hypothesis of "energy deficiency" in APBD. Patients were re-evaluated with clinical and laboratory evaluations at approximately 2, 6, and 12 months in an open-label clinical trial due to the relentless progressive nature of APBD. Nutritional needs were based on recommended daily allowance guidelines. The average daily percent compositions of the diet during the protocol were as follows: protein 13.1%, carbohydrate 37.2%, and fat 20%. C7TG (8.3 kcal/g) represented 30–35% of daily caloric intake (equivalent to 1.0–1.5 g/kg/day). The patients consumed the daily amount of oil in four equal doses (three main meals and at bedtime) consumed over a 20- to 30-min, mixed in preferred foods, such as yogurt, pudding, or beverages, etc. Simple sugar intake was reduced to optimize oxidation of triheptanoin and to prevent undue weight gain.

Patient descriptions

APBD-1

This 66 year old Ashkenazi Jewish female's symptoms began over the past 20 years with increasingly poor balance with progressive difficulty walking that progressed from need for a cane, to a walker and, most recently, a wheelchair. At age 50 she was presumed to have "multiple sclerosis" and was treated accordingly without impact on the progression of the disease. Her symptoms include a long history of neurogenic bladder with urgency, incon-

tinence and poor emptying, burning pain in her legs, and ptosis of the right eye. Brain MRI documented diffuse non-confluent nodular leukoencephalopathy. Similar lesions were observed in the spinal cord. Diagnosis was confirmed at age 64 by sural nerve biopsy (revealing polyglucosan bodies), and glycogen brancher enzyme (GBE) deficiency in fibroblasts (GBE = 262 nmol/min/mg protein-normal: 1300 ± 390). Mutation analysis indicated that she was homozygous for the Tyr329Ser mutation in the GBE gene.

APBD-2

This 58 year old female is also of Ashkenazi Jewish origin. Her initial symptoms involved tingling in hands and feet that progressed. She developed a neurogenic bladder along with weakness in her legs with falling. She also had reduced dorsiflexion of her feet with exercise and hammer toes that also affected her gait and balance. Initially, she was also thought to have multiple sclerosis. She had also developed ptosis of the right eye. In summary, she had features of peripheral neuropathy with prominent myelopathy and neurogenic bladder at the time of entering the dietary protocol. Sural nerve biopsy revealed excess polyglucosan bodies and absence of glycogen brancher enzyme by histochemistry.

APBD-3

This 51 year old Ashkenazi Jewish male's symptoms began with increasing urinary urgency and poor bladder control that has required self-catheterization for relief. Initially, he was thought to have prostatic hypertrophy with dilated ureters due to urinary retention and plasma elevations of BUN and creatinine. Simultaneously, he had increasing balance problems and is ambulatory without a strict requirement for assistive devices. The diagnosis was established by fibroblast enzyme assay for the GBE (125 nmol/min/mg protein - controls: 1300 ± 390). Sural nerve biopsy revealed multiple polyglucosan bodies.

APBD-4

He was a 61 year old Ashkenazi male whose symptoms began 8 years earlier with bladder urgency and poor bladder control that required self-catheterization. This was followed by development of numbness in his toes that progressed to weakness and foot drop and a progressive gait disorder. Over the next 5 years this progressed to requiring a cane, then a walker, and two years ago he required and continues to use a wheelchair and is no longer ambulatory. A sural nerve biopsy and mutation analysis 7 years previously documented GBE deficiency and homozygosity for the Tyr329Ser mutation in the GBE gene.

APBD-5

He is a 59 year old male of Ashkenazi Jewish origin and the younger sibling of patient APBD-4. His symptoms began ~8 years ago with progressive bladder urgency and impaired bladder control with subsequent difficulties walking but remained ambulatory. He has evidence of both peripheral neuropathy and myelopathy. Blood DNA mutation analysis revealed that he was also homozygous for the Tyr329Ser mutation in the GBE gene.

Results

At baseline and on each visit, while on the diet, there were no persistent or consistent abnormalities in any blood studies except for variations in serum levels of CPK, creatinine, and lipid profiles. Isolated elevated fasting CPK levels, including pre-diet samples,

were observed, consistently in all samples from patients APBD-3–5, and ranged from 143–328 IU/L (normal: 30–135 IU/L). There were no associated increases in AST or ALT with these CPK levels.

Blood creatinine levels were consistently below or at the lower normal range (e.g. 0.6, Normal: 0.7–1.2), with normal BUN levels, in all patient samples except for APBD-3 who had mild chronic renal disease with elevated BUN in all of his samples.

Lipid profiles

Triglyceride and HDL levels were normal in all patient samples. Increased cholesterol and LDL levels were not consistently altered during diet therapy in APBD-1–3. Isolated LDL elevations without increased cholesterol were observed in APBD-4 and 5 (siblings). The diet did not alter their abnormalities. Hematologic parameters were normal and unaffected by the diet. (See Table E-1 for more details).

Metabolic parameters

Free carnitine levels were essentially normal in all patients. Quantitative acylcarnitine levels indicated that propionylcarnitine (C3) was below average (<1.4 μM) at baseline for all patients. The levels of long-chain acylcarnitines (C16, C18:1, C18:2, and C18:3) were also decreased in samples from four of the patients: APBD-2–5 (e.g. palmitoylcarnitine: 0.38–0.87 μM (average normal's: 1.20–1.97 μM). All other acylcarnitine levels were unremarkable either prior to or with the diet.

Quantitative urinary organic acid analysis failed to reveal any abnormalities that could be considered "markers" for this disease or that were perturbed by the diet. However, when patients were ketotic (APBD-1 and 3–5), the ratio of 3-OH-butyrate: acetoacetate (normally ~3:1) was inverted (<1.0) suggesting an intra-mitochondrial redox alteration of the NAD:NADH ratio that could also indicate reduced citric acid flux and decreased energy production. The cytosolic redox (*lactate:pyruvate ratio*) was normal in all patients. There were no significant abnormalities of other analytes. Serial plasma amino acids were unrevealing except for methionine levels in the low normal range in four of these patients (APBD-1–4).

Since creatinine and methionine were decreased, fasting plasma levels of other methylation components were measured at baseline and after receiving triheptanoin supplementation (Table 1). tHcy and guanidinoacetate (GAA) were normal at baseline and on the diet in all patient samples. At baseline, SAM was extremely low ($p \leqslant 0.002$), SAH was markedly elevated ($p \leqslant 0.001$) and the SAM:-SAH ratio was decreased in all patients ($p \leqslant 0.001$). Both creatine and free choline were elevated ($p = 0.001$) and bound choline was reduced but not significant ($p = 0.07$) (Fig. 1). Exclusion of APBD #3 with kidney disease increased the significance of bound choline to $p \leqslant 0.01$.

After 6–8 months on the diet, plasma SAM levels increased to normal levels in three of the five patients and SAH levels decreased significantly in four of the patients resulting in significant increases in the SAM:SAH ratio ($p = 0.03$). Plasma creatine levels decreased in three patients while it increased in the other two patients. Plasma free and bound choline did not show any consistent changes although bound choline appeared to increase in three of the five patients. Methionine levels were unaffected while receiving the diet (Fig. 2).

Subsequent clinical course with the triheptanoin diet

(*APBD-1*) (*C7TG Diet for 16 mo*): After 96 h of diet therapy, ptosis of the right eye disappeared, stuttering, a life long problem, was improved, bladder function improved, the burning leg pain was eliminated, and she was able to extend her quadriceps against gravity. After 6 months, she was able to cross her legs and put on shoes as well as less difficulty in standing from the sitting position. At this time, she was able to walk on the treadmill for 5 min with her legs supporting her weight without any restraining straps. At 12 months of diet therapy, walking on the treadmill had increased to 30 min. Prior to the diet treatment, she could not walk any significant distance. On three occasions (*before, at 7 months, and at 14 months on the diet*) her 6 min walk test increased from 0 to 167 to 192 feet, respectively. The triheptanoin dose schedule was interrupted for 10 days due to antibiotic therapy for an infection that in combination with the oil resulted in chronic diarrhea. Weakness ensued within 3–5 days but resolved with completion of the antibiotic therapy and resumption of dietary oil. Although, currently, she still has difficulty with bladder control, she is now able to go to the bathroom at night using a rolling walker rather than her wheelchair. She does physical therapy for 3 h twice a week, exercises at home with a stationary bike three times per week, and is starting to lose weight from the increased physical activity. Despite these functional improvements, she was concerned that the rate of improvement had "leveled off".

(*APBD-2*) (*C7TG Diet for 7.5 months*): Ptosis of the right eye disappeared after 96 h of diet therapy. By 7 months, she perceived improvement in muscle strength and endurance as well as improved balance. She now walks independently but will use a cane with prolonged walking distances. Her urinary frequency and incontinence have also decreased. Her 6 min walk test increased from 1468 feet before the diet, to 1680 feet (gain of 212 feet) after 7 months of diet therapy.

(*APBD-3*) (*C7TG Diet for 10 months*): He has experienced increased muscle strength and endurance with mildly improved balance. He feels that his disorder, currently, is no longer progressing and that he has experienced some functional recovery. His 6 min walk test increased from 1338 feet before the diet, to 1594 feet (gain of 256 feet) after 7 months of diet therapy. His BUN and creatinine levels remain mildly increased.

Table 1
Plasma methylation indices in adult polyglucosan body disease patients.

| Patient	Normal range	Methionine 21–35 μM	SAM 64–125 nmol/L	SAH 5–20 nmol/L	SAM/SAH 2.1–5.6	Creatine 6–50 μM	Creatinine 0.6–1.2 mg/dl	Choline free 6.2–8.8 μM	Choline bound 1826–2462 μM
APBD-1	BASELINE	22.0	22.7	68.5	0.33	75	0.5	13.4	1453
	C7 Diet: 6–8 months	22.0	59.3	45.5	1.30	58	0.6	23.3	1950
APBD-2	BASELINE	21.0	44.7	72.4	0.62	62	0.5	17.6	1946
	C7 Diet: 6–8 months	25	44.0	52.1	2.09	81	0.5	12.8	2150
APBD-3	BASELINE	16.0	36.9	59.5	0.90	45	1.1	22.8	2520
	C7 Diet: 6–8 months	14.0	77.9	37.3	0.49	41	1.1	9.2	2520
APBD-4	BASELINE	27.0	43.4	48.1	0.90	78	0.6	17.7	2500
	C7 Diet: 6–8 months	19.0	30.0	60.8	0.49	94	0.7	18.1	1565
APBD-5	BASELINE	27	45.9	78.4	0.59	65	0.6	16.2	1601
	C7 Diet: 6–8 months	20	65.4	48.4	1.35	56	0.6	15.1	2080
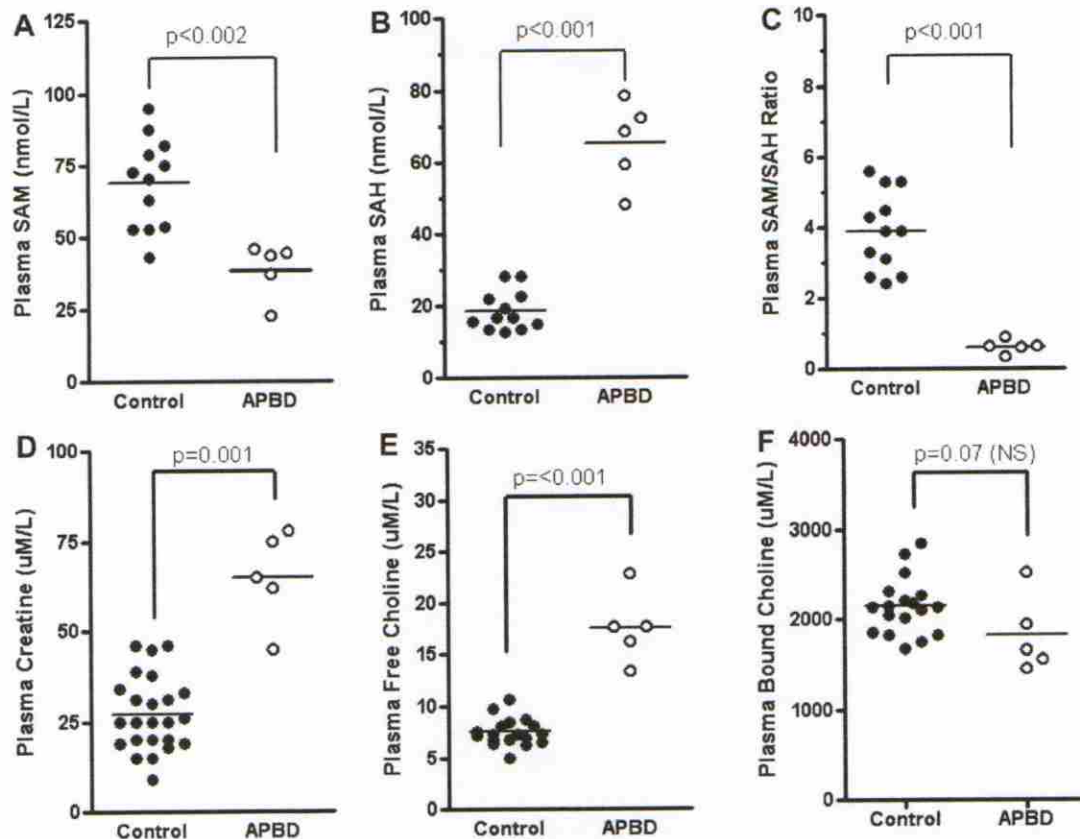
Fig. 1. Plasma methylation metabolites observed in five patients with APBD deficiency compared to normal controls: (A) S-adenosylmethionine (SAM), (B) S-adenosylhomocysteine (SAH), (C) ratio of SAM:SAH, (D) creatine, (E) free choline, and (F) bound choline. Except for the latter, all other metabolites support the existence of defective methylation in the APBD patients.

(*APBD-4*) (*Diet for 7 mo*): He is the most severely affected of these patients and has not improved significantly on the diet therapy. However, after 7 months of the diet, he feels he has gained upper body strength and is no longer deteriorating but feels that the improvements have "plateaued". His "6 min walk" tests (performed while in his wheelchair for 12 min) have increased from 1888 feet before the diet, to 1988 feet at 7 months of diet therapy (gain of 100 feet).

(*APBD-5*) (*Diet for 7 mo*): Since starting the diet, he has noted improvement in generalized strength, he has less weakness and fatigue, and feels his gait has improved. However at 7 months, he also feels he has "plateaued" but is definitely not progressing. The results of his 6 min walk test were 1371 feet before the diet and 1385 feet after 7 months (insignificant gain).

Discussion

APBD is a rare disorder most often affecting adults of Ashkenazi Jewish origin due to a deficiency of the glycogen brancher enzyme (GBE) to a residual level of ~10% of normal. Symptoms of peripheral neuropathy, myelopathy, neurogenic bladder, and occasional cognitive impairment usually are manifest, initially, in the third to fifth decade. The disorder is characterized by progressive involvement of both the central and peripheral nervous systems and is associated with deposition of amylopectin-like polyglucosan bodies [1–5]. Since normal glycogen cannot be synthesized, adequately, the availability of glucose for energy is compromised for both the central and peripheral nervous systems. Although the mechanism is not well understood, glycogen synthase may be chronically activated due to an associated energy deficit limiting ATP availability required for inactivation, by phosphorylation, of serine/threonine residues on the enzyme [13,14]. If glycogen synthesis is unregulated, polyglucosan bodies would accumulate. Neural tissue is highly dependent on import of glucose from gluconeogenesis and glycogenolysis and ketone bodies from β-oxidation of fatty acids by the liver. Current thinking has favored "mechanical disruption" of cells by accumulating polyglucosan bodies as the main mechanism for the progressive symptomatology. However, it may be more appropriate to consider that a cellular energy deficit due to inadequate normal glycogen reserves and unregulated glycogen synthase (GS) may be also relevant.

There have been no previous reports describing metabolic derangements that might suggest effective therapy nor have there been any improvements in function or control of the progression of this disease with any therapy. As with most glycogenoses, the assumption has been that restriction of carbohydrate with increased protein intake would be the logical dietary recommendation. The probability that lack of glucose from normal glycogen stores might produce an energy deficit prompted the current investigation. The inability to maintain normal glycogen reserves, as a source of glucose has a significant metabolic consequence: Glucose is metabolized to pyruvate and is, therefore, an intra-mitochondrial source of not only acetyl-CoA but also oxaloacetate. These compounds are required for citrate synthesis, flux through the CAC, and ATP production via the respiratory chain. For these reasons, the anaplerotic compound, triheptanoin, was chosen as a dietary supplement to increase fueling of the CAC and enhanced
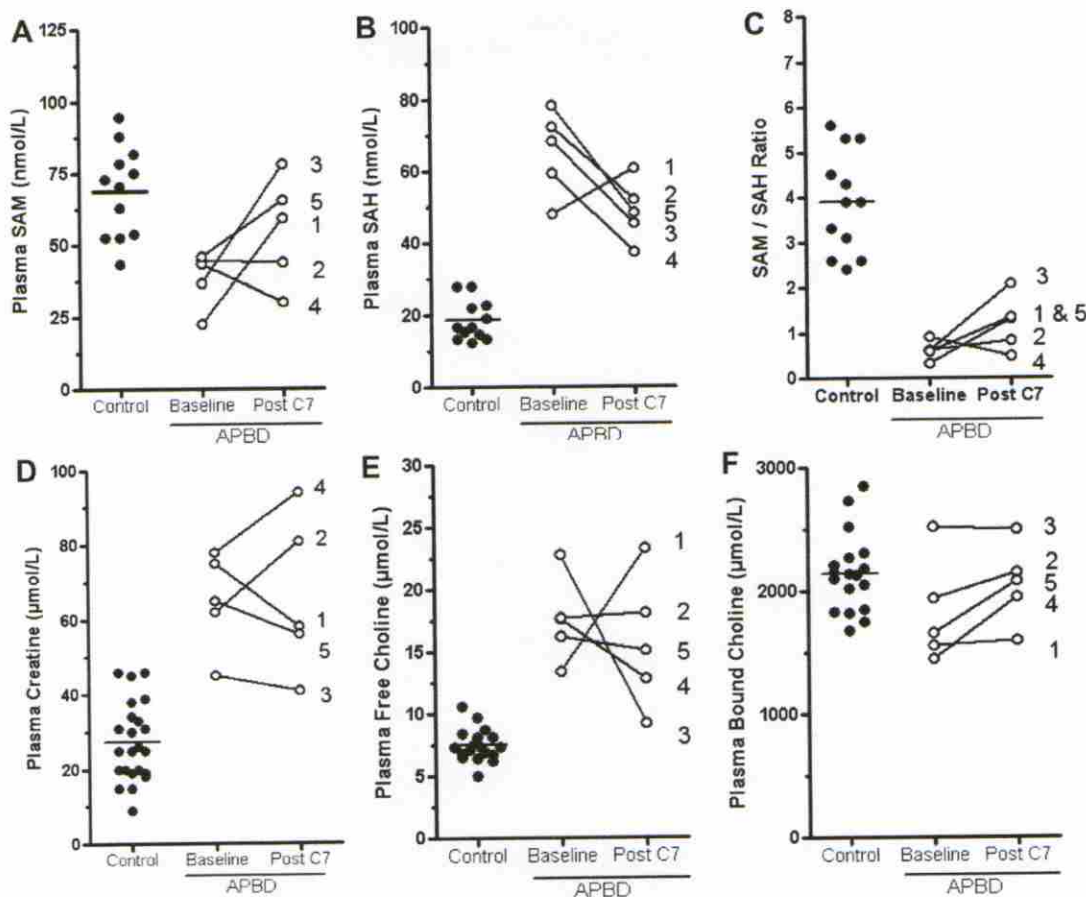
Fig. 2. Effects of triheptanoin diet therapy on the plasma levels of methylation metabolites compared to the pre-treatment baselines for APBD patients #1–5. Panels A–F are the same as in Fig. 1.

energy production for the APBD patients as described in Fig. 3. Nearly all of the heptanoate (C7) is taken up by the liver from the portal vein and is converted to the 5-carbon intermediate β-ketopentanoyl-CoA (BKP-CoA). BKP-CoA can be utilized within the liver as a source of both acetyl-CoA and oxaloacetate thus fuel-
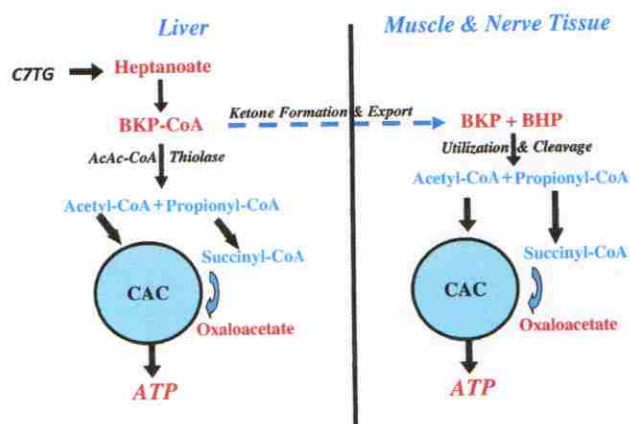

Fig. 3. Current understanding of the fate of enteral triheptanoin (C7TG) and heptanoate metabolism in liver with export of 5-carbon ketone bodies for providing substrate to the citric acid cycle (anaplerosis) in muscle and nerve tissue. BKP, beta-ketopentanoate; BHP, beta-hydroxypentanoate; CAC, citric acid cycle.

ing the hepatic CAC. Additionally, BKP-CoA can proceed through the HMG-pathway producing the 5-carbon ketone bodies: BKP and β-hydroxypentanoate (BHP) for export, uptake by other organ systems (*including the nervous system*) as a source of acetyl-CoA and oxaloacetate for the CAC in those organs [15–18]. The final metabolic products of heptanoate are CO_2, HOH, and ATP.

The APBD patients all perceived stabilization of the disease progression, increased strength, and in most patients, decreased urinary frequency, elimination of ptosis and burning leg pain within 96 h on the diet along with improvement in the 6 min walk during the protocol. There was no history, prior to the diet protocol, for increasing physical performance in physical therapy programs in which four of the five patients attempting. Despite functional improvement, all patients sensed a plateau in the rate of improvement, without loss, after 6–8 months of the diet. Although the dose of triheptanoin at 30–35% of total calories per day was effective for these patients and compliance was excellent, Patient 1 experienced return of weakness in days while unable to take triheptanoin during a 10 day course of antibiotic therapy. This may be a warning to not interrupt the diet therapy during clinical investigation.

Evaluation of chemical and metabolic testing at baseline and during therapy revealed the following: First, routine blood chemistries, blood acylcarnitines, and urine organic acids failed to show any disease-specific markers of APBD deficiency either before or following treatment with dietary triheptanoin. All patients had abnormal lipid profiles at baseline (increased LDL) that were not influenced by the diet therapy. Acylcarnitine analysis revealed

decreased levels of propionylcarnitine (C3AC) and long-chain species (*palmitoyl- to linolenoyl-carnitine*). Reduced levels of (C3AC) are often observed in patients with inherited defects reflecting overconsumption of propionyl-CoA to enhance succinyl-CoA in the CAC [16]. There was no other evidence for disturbed propionate metabolism or β-oxidation either before or while on the triheptanoin diet based on normal excretion of 3-OH-propionate, methylcitrate, methylmalonate, and malonate in all five patients.

The reduced levels of the longer-chain species may represent increased β-oxidation of fatty acids to enhance availability of ketone bodies as a source of acetyl-CoA for other organ systems when glycogen degradation is limited. When ketotic, a mitochondrial redox abnormality was revealed by urine organic acid analysis (*ratio of 3-OH-butyrate to acetoacetate, normally ~3:1, was reversed indicating inadequate NADH for reduction of acetoacetate to 3-OH-butyrate*). These abnormalities are consistent with a chronic catabolic state and abnormal fueling of the CAC.

However, the reduced plasma methionine and creatinine levels that did not respond to triheptanoin therapy (Table 1) prompted examination of the integrity of methylation in these patients. Fig. 4 summarizes the observed methylation abnormalities as they relate to the integrated pathways of methionine, choline, and creatinine. The significant reduction of SAM, increased SAH, and abnormal SAM:SAH ratio at baseline ($p < 0.002$) improved after 6–8 months of diet therapy (Figs. 1 and 2). Presumably, increased availability of ATP from heptanoate oxidation would also increase synthesis of SAM. The baseline plasma levels for SAM, SAH, and the SAM:SAH ratio observed with the APBD patients may be unique to their "systemic" energy deficiency. These methylation parameters were no different from normal controls over the same age ranges for patients with multiple sclerosis, stroke, and peripheral neuropathy. Plasma creatinine levels for these patients were seen to gradually increase with age, as expected, but, as with SAM levels, no reduced levels of creatinine were observed [19].

Plasma creatine and free choline were significantly elevated ($p = 0.001$) at baseline but did not consistently improve with triheptanoin supplementation. The low levels of methionine and elevated free choline may indicate inadequate mitochondrial oxidation of choline to betaine that would lead to the observed reduction of both methionine and SAM at baseline. Guanidinoacetate and bound choline (*mainly phosphatidylcholine [lecithin]*) were normal at baseline and, also, were not consistently altered by the diet. The increased creatine levels ($p = 0.001$) may reflect impaired conversion of creatine + ATP to creatine-PO$_4$ by CPK resulting in low plasma creatinine levels. Creatine-PO$_4$ is the primary source of reserved high-energy PO$_4$ for both muscle and nerve tissue and, if limited, may be another contributor to the progressive muscle weakness characteristic of APBD patients.

The increased plasma free choline, ($p \leqslant 0.001$) may also relate to the dysmyelination associated with APBD. Choline is a major source of methyl groups and is essential for the integrity and function of cellular membranes (phosphatidylcholine [PC]), cholinergic transmission, and normal muscle function [20]. Fig. 5 briefly summarizes the direct and phosphoethanolamine methyltransferase (PEMT) pathways of choline metabolism. In addition to the important functions of providing acetylcholine and betaine, these pathways produce the phosphatidylcholine necessary for sphingomyelin synthesis. Turnover and degradation of sphingomyelin recovers phosphatidylcholine that restores choline reserves for renewed synthesis of acetylcholine and betaine. As described, a specific transport carrier exists for free choline transport across the blood–brain barrier. Phosphatidylcholine [PC] is carried into neurons as an ApoE lipoprotein [20]. For these reasons, although the mechanism is unclear, we hypothesize that the elevated plasma choline ($p = 0.001$) may relate to the dysmyelination observed in APBD patients.

The partial deficiency of GBE in APBD deficiency appears to produce a secondary energy deficiency due to inadequate reserves of normal glycogen that cannot be efficiently degraded to free glucose. There also appears to be a dysfunctional regulation of glycogen synthase (GS) resulting in continued synthesis of abnormal poorly branched glycogen (polyglucosan bodies). Inactivation of this cytosolic enzyme involves protein kinases, requiring ATP, for phosphorylation of serine/threonine residues. A chronic catabolic state appears to exist in this disorder (*and possibly other defects*) that may reflect chronic activation of GS, decreased propionylcarnitine (*over utilization- an indicator of endogenous amino acid turnover*), ongoing and, perhaps, enhanced β-oxidation with diminished long-chain fatty acids (*over utilization*) as plasma acylcarnitines. The need for substrate for the CAC is indicated by the
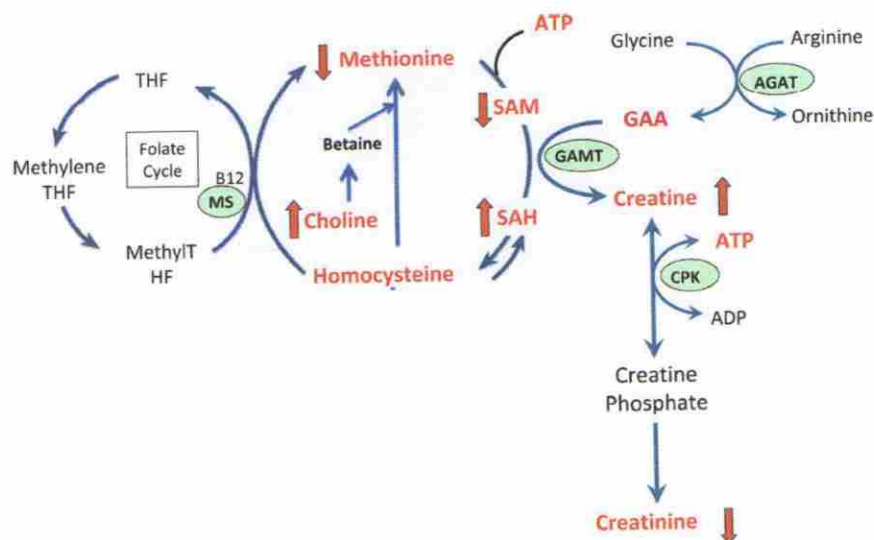

Fig. 4. Integrated methylation pathways emphasizing the relationships between choline, S-adenosylmethionine (SAM) and creatine synthesis. Observed plasma methylation metabolites and their levels at baseline are represented in red with block arrows (increased or decreased) compared to normal controls. ATP is indicated where required for these reactions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)
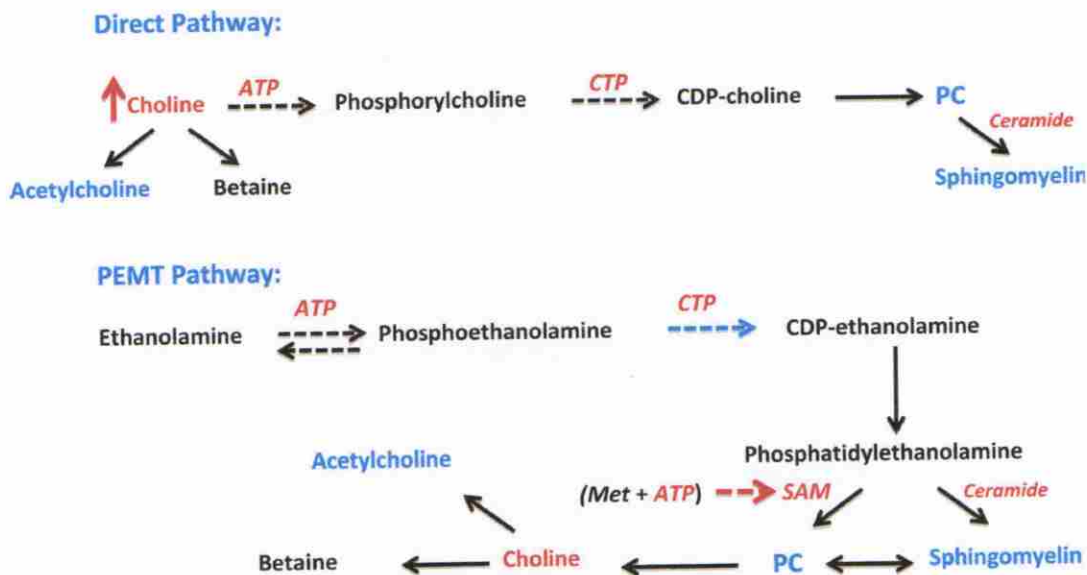
Direct Pathway:

PEMT Pathway:

Fig. 5. Choline metabolism by both the direct and phosphoethanolamine methyltransferase (PEMT) pathways. When compromised , this may underlie the defective myelination as observed in APBD patients. PC, phosphatidylcholine; Met, methionine; SAM, S-adenosylmethionine; CTP, cytidinetriphosphate.

increased NAD:NADH ratio that has caused a mitochondrial redox alteration as evidenced by the reversed ratio of 3-OH-butyrate:acetoacetate observed during ketosis. These findings suggest that the catabolic state may be due to an increased ratio of AMP:ATP that activates AMP-mediated protein kinase (AMPK) resulting in enhanced catabolism in an attempt to relieve an energy deficit. Activated AMPK enhances catabolism of precursors for CAC intermediates while inhibiting the mammalian target of apamycin (mTOR). The latter is responsible for activation of synthetic pathways such as protein synthesis and cellular proliferation [21]. AMPK activation is known to enhance β-oxidation (for energy) while impairing fatty acid synthetic pathways, possibly including synthesis of phosphatidylcholine and myelin synthesis.

In conclusion, this investigation has demonstrated, for the first time, the existence of an energy deficit in this disorder that is partially responsive to triheptanoin diet therapy with definite, but limited, clinical benefit arresting progression and providing functional recovery. In addition, the existence of secondary involvement of methylation pathways has also been clearly demonstrated, for the first time, in this glycogen disorder and is currently under investigation to determine if recovery can be accelerated. Without indications from decreased methylation products (plasma methionine and creatinine) this important secondary compromise would not have been demonstrated. It is likely that in other inherited disorders with significant energy insufficiency, that abnormal methylation during crisis, or chronically, may also be detected. As with APBD patients, the benefits of anaplerotic therapy may be enhanced by additional therapies designed to correct associated methylation abnormalities. The authors recognize the speculative aspects of this presentation but they hypothesize that correction of demonstrated methylation abnormalities in inherited defects is worthy of continued investigation for additional beneficial therapeutic management.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ymgme.2009.09.007.

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