Polyunsaturated Fatty Acids, Carnitine and Lactate as Biological Markers of Brain Energy in Autistic Children

Gehan A. Mostafa[1], Hanan A. El-Gamal[2], Amany S.E. El-Wakkad[3], Omar E. El-Shorbagy[2], Magda M. Hamza[2]

Department of Pediatrics[1], Faculty of Medicine, Ain Shams University; Department of Medical Studies, Institute of Postgraduate Childhood Studies[2], Ain Shams University; Department of Clinical Pathology[3], National Research Center

ABSTRACT

Background: *Aetiology of autism is speculative. Some cases has been associated with disturbance of brain energy. Carnitine is essential for transport of polyunsaturated fatty acids (PUFAs) across the inner mitochondrial membrane for β-oxidation and energy production. Thus, brain energy depends on adequate supply of PUFAs and carnitine in addition to normal mitochondrial function.* ***Objective:*** *This study was conducted to investigate brain energy metabolism in autistic children, through measuring plasma PUFAs, serum carnitine and plasma lactate, which is an index of mitochondrial function, in relation to autistic severity and clinical manifestations.* ***Methods:*** *Thirty autistic children were studied in comparison to 30 healthy children serving as controls. According to disease severity, assessed by Childhood Autism Rating Scale (CARS), autistic children were classified into patients with mild and moderate autism (n=18) and patients with severe disease (n=12). Besides full clinical and neuropsychiatric evaluation, measurements of plasma PUFAs (including linoleic, alphalinolenic, arachidonic "AA" and docosahexaenoic "DHA" acids), serum carnitine and plasma lactate were performed.* ***Results:*** *Autistic patients had significantly lower serum carnitine and plasma PUFAs (except linoleic acid) and significantly higher plasma lactate and ω6/ω3 ratio (AA/DHA) than controls (P<0.05). Low carnitine, DHA, AA, linolenic and linoleic acids were elicited in 90%, 76.6%, 53.3%, 52.2% and 33.3%, respectively of autistic children. On the other hand, 76.7% and 44.4% of autistic patients had elevated plasma lactate and ω6/ω3 ratio, respectively. Patients with severe autism had significantly lower serum carnitine and higher plasma lactate than patients with mild and moderate autism (P<0.05). Although severely autistic patients had lower values of plasma PUFAs and higher ω6/ω3 ratio than patients with mild and moderate autism, these differences were statistically insignificant. Patients with regressive autism (40%) had significantly higher ω6/ω3 ratio than those with non-regressive disease. On the other hand, hypotonic autistic children (20%) had significantly lower serum DHA than normotonic autistic ones. Although autistic children with gastrointestinal problems (50%) had higher ω6/ω3 ratio than patients without such problems, the difference did not reach statistical significance. There was a significant negative correlation between serum carnitine and plasma lactate levels.* ***Conclusion:*** *Brain energy metabolism of many autistic children may be suppressed due to summation of several factors including low serum carnitine, which may be attributed to disturbed mitochondrial function as evidenced by lactatemia, and/or low plasma PUFAs. Further studies are needed to prove these arguments and to assess the effect of new therapeutic modalities for autism (such as PUFAs, carnitine, and measures that augment the mitochondrial function) on amelioration of the autistic phenotype.* **(Int. J. Ch. Neuropsychiatry, 2005, 2(2): 179-188)**

INTRODUCTION

Autism is the most severe psychiatric disorder of childhood. Three main criteria are essential for its diagnosis which include; disturbed reciprocal social interaction, lack of communication and restriction of normal variation in behavior and interests[1]. Raising a mentally

deficient child is difficult, whereas raising an autistic child is heart breaking[2]. Some cases of autism has been associated with several different organic conditions including disturbance of brain energy metabolism which needs polyunsaturated fatty acids (PUFAs), carnitine and normal mitochondrial function[3].

Polyunsaturated fatty acids (PUFAs) include linoleic, alpha linolenic, arachidonic (AA) and docosahexaenoic acids (DHA). Human brain is 60% fat of which 25% is DHA. PUFAs modulate membrane fluidity and hence function of the neuronal cell[4]. They are needed for normal and healthy brain development and function especially during early infancy, and they influence the function of powerful neurotransmitters[5]. PUFAs supplementation constitutes an environmental factor able to alter the brain function[6].

Carnitine, a non-essential nutrient synthesized in the liver and kidney, is essential for transport of long chain fatty acids across inner mitochondrial membrane for β- oxidation and energy production[7]. Synaptic transmission of multiple neurotransmitters needs the neurobiological effect of acetyl carnitine[8]. Mitochondrial defect, which could be assessed by measuring plasma lactate, may be the origin of carnitine deficiency in some autistic children[9]. So, strategies to augment the mitochondrial function by stimulating mitochondrial enzymes activity or decreasing the production of endogenous toxic metabolites may be beneficial in treatment of autism[10].

This study was conducted to investigate brain energy metabolism in autistic children, through measuring plasma polyunsaturated fatty acids, serum carnitine and plasma lactate which is an index of mitochondrial function, in relation to autistic severity and clinical manifestations. This may add new biological indices and therapeutic implications for autism.

METHODS

Study population

This case-control study was conducted on 30 autistic children recruited from Outpatients Pediatric Clinic, Faculty of Medicine, Ain Shams University and the outpatient special needs clinic in the Institute of Postgraduate Childhood Studies, Ain Shams University over a period of 15 months. Patients were fulfilling the criteria for the diagnosis of autism according to the DSM IV diagnostic criteria for research[11]. They were 23 males and 7 females. Their ages ranged from 4 to 12 years (mean±SD: 8±2.13 years). Patients were classified into two groups according to disease severity assessed by Childhood Autism Rating Scale (CARS)[12]; group I included 18 patients with mild and moderate autism and group II included 12 patients with severe autism. Patients were excluded from the study if they have associated neurological diseases as cerebral palsy or significant medical diseases as hepatic and renal involvement.

Patients were subjected to clinical evaluation with special emphasis on history of sleep disorders, convulsions, developmental milestones and symptoms suggesting gastrointestinal (GI) disorders (such as epigastric pain, constipation, diarrhea and bloating) and full neurological examination.

Autistic children were studied in comparison to 30 age- and sex- matched healthy children, who had no history suggesting medical, neurological or psychiatric disorders, serving as controls. They were 23 males and 7 females. Their ages ranged from 4 to 12 years (mean±SD = 8.17±2.90 years). An informed consent was obtained from the parents or caregivers of each child before enrollment.

Study measurements

* *Sample collection*

Five millimeters of venous blood were collected and equally subdivided into two clean tubes, the first tube was dry and the blood was left to clot, then the serum was separated and stored at -20°C until assay of carnitine. The second tube was containing fluoride EDTA as an anticoagulant, then centrifugation of the samples was done immediately and plasma was separated and stored in sterile aliquoutes at -20°C until assay of lactate and PUFAs.

* *Assessment of serum carnitine and plasma lactate*
  Serum carnitine was measured by using enzymatic ultraviolet test from Roche Diagnostics Gmbh, Cat. No. 1' 242008, Germany [12]. Plasma lactate was measured by using enzymatic determination from BioMerieux REF 61 192, France. according to manfucturer instructions[13].
* *Determination of plasma polyunsaturated fatty acids (Linoleic, Linolenic, Arachidonic and Docosahexaeinoic acids)*
  The principle was the injection of separated fatty acids from the plasma into the gas chromatography instrument. Separation of fatty acids from plasma was done by addition of 3 ml of 3 g/L solution of nonanoic acid in 100 µL of acetyl chloride slowly with magnetic stiring for 45 min at room temperature. Three ml of 6% potassium carbonate solution was added in water with stiring while cooling the mixture in an ice bath. After that, 300 µL of hexan and vortex were added and the sample was cooled at 4ºc for 30 minutes. Then, centrifugation was done for 10 minutes. Finally, 100 µ of the upper layer was removed and 1.1 µL was subjected to Gas Chromatography H5890 (Hewlett-Pakard, Palo Alto, CA)[14].
* *Interpretation of serum carnitine, plasma PUFAs and lactate results*
  As data distribution was non-parametric, patients were considered to have decreased serum carnitine, plasma DHA, linolenic, arachidonic and linoleic acids if their levels were below the 5th percentile of the control values (5.56 mg/ml, 1.30 µg/ml, 1.44 µg/ml, 2.34 µg/ml, and 1.14 µg/ml respectively). On the other hand, patients were considered to have elevated plasma lactate and ω6/ω3 ratio if their levels were above the 95th percentile of the control values (12.04 mg/dl and 3.55 respectively).

Statistical analysis

The data were analyzed by commercially available software package (Stat View, Abacus Concepts, Inc., Berkley, CA, USA). The data were presented as mean and standard deviation, in addition to median and interquartile range (IOR). Mann Whitney test was used for comparison between two groups as data distribution was non parametric. Spearman's correlation coefficient "r" was used to determine the relationship between different variables. Chi-Square test was used for comparison between qualitative variables of the studied groups. For all tests, a probability (p) of less than 0.05 was considered significant.

RESULTS

Serum carnitine, plasma lactate and PUFAs in autistic children

Autistic patients had significantly lower serum carnitine and plasma PUFAs (except linoleic acid) and higher plasma lactate and ω6/ω3 ratio (AA/DHA) than controls (table 1). Frequency of autistic patients with low serum carnitine and plasma PUFAs and high plasma lactate and ω6/ω3 (AA/DHA) ratio is shown in table (2).

Relationship between autistic severity and the studied laboratory markers of brain energy metabolism

Patients with severe autism had significantly lower serum carnitine and higher plasma lactate than patients with mild and moderate autism. Although severely autistic patients had lower values of plasma PUFAs and higher ω6/ω3 ratio than patients with mild and moderate autism, these differences were statistically insignificant (table 3).

Relationship between the important clinical findings elicited in some autistic children and laboratory parameters of brain energy metabolism:

Twelve out of the studied 30 autistic children (40%) had autistic regression i.e. they underwent regression in language and developmental milestones after a period of normal development. Patients with autistic regression had significantly higher ω6/ω3 ratio [median (IQR): 8.98 (14.05)] than patients without autistic regression. [(median

(IQR): 1.89 (2.16), z = 2.6, P<0.01]. In contrast, they had comparable results regarding serum carnitine, plasma lactate and PUFAs (P>0.05).

Mild hypotonia of the extremities (more distal than proximal) was elicited in 6 out of the studied 30 autistic children (20%). Autistic patients with hypotonia had significantly lower plasma DHA than normotonic autistic patients (median (IQR): 1.25 (1.28) versus 3.5 (0.69) µg/ml, z = 2.7, P<0.01). Hypotonic and normotonic autistic patients had comparable results of serum carnitine, plasma lactate and other PUFAs (P>0.05).

Twenty percent of autistic children (n= 6) had convulsions (generalized tonic clonic in 2 patients and focal in the other 4 patients). Sleep disorders were found in 8 autistic children (26.7%). Autistic children with and without convulsions and those with and without sleep disorders had comparable results of serum carnitine, plasma PUFAs and lactate (P>0.05).

Gastrointestinal (GI) problems (diarrhea, constipation or bloating) were found in 15 autistic children (50%) in comparison to 2 only of the healthy controls (6.7%). The difference between both groups was statistically significant (X2 = 13.9, P<0.05). Although autistic patients with GI problems had lower serum carnitine and plasma PUFAs and higher plasma lactate and ω6/ω3 ratio than patients without such problems, these differences did not reach statistical significance (P>0.05).

Correlation between PUFAs, carnitine and lactate

There was a significant negative correlation between serum carnitine and plasma lactate levels of autistic patients (r = -0.47, P<0.05) as shown in (Fig. 1). On the other hand, PUFAs levels of autistic patients did not significantly correlate with their plasma lactate and serum carnitine levels (P>0.05).

There was a significant negative association between carnitine and lactate among autistic children as 23 out of the 27 patients with low serum carnitine levels (85.2%) had elevated plasma lactate as well. In addition, all the 3 patients with normal serum carnitine had also normal plasma lactate levels (Fig. 2).

Table 1. Comparison between autistic patients and controls regarding the studied laboratory markers.

	Autistic patients (n=30)		**Controls (n=30)**		**Z**	**P**
	Mean ± SD	**Median (IQR)**	**Mean± SD**	**Median (IQR)**		
Carnitine (mg/ml)	3.38 ±1.31	3.500 (2.08)	6.40± 0.61	6.45 (1.12)	6.30	**< 0.0001****
Lactate (mg/dl)	18.87± 8.33	19.16 (12.83)	8.05± 2.18	7.86 (3.84)	5.43	**< 0.001****
DHA (ω3) (µg/ml)	0.59± 0.76	0.17 (1.06)	2.37± 0.55	2.26 (0.91)	6.25	**< 0.001****
Linolenic (µg/ml)	1.68±0.95	1.44 (1.09)	2.92± 1.79	1.92 (3.20)	2.57	**<0.01****
Arachidonic (ω6)(µg/ml)	2.67± 2.17	2.33 (3.59)	4.30± 2.0	3.44 (3.77)	3.02	**<0.01****
Linoleic (µg/ml)	2.24± 1.78	2.21 (2.78)	2.37± 1.17	2.01 (1.09)	0.64	>0.05
ω6/ω3	42.02±154.23	2.89 (9.06)	1.88± 0.86	1.44 (1.46)	2.11	**<0.05***

P >0.05 = Non significant, P < 0.05* = Significant, P < 0.01, 0.001, 0.0001** = highly significant
DHA = Docosahexaenoic acid

Table 2. Frequency of autistic patients with low serum carnitine, plasma PUFAs and high plasma lactate and ω6/ω3 (AA/DHA) ratio.

Laboratory markers	All autistic patients	Patients with mild and moderate autism	Patients with severe autism
%Low carnitine	90	83.3	100
%High lactate	76.7	66.7	91.7
%Low DHA	76.6	72.2	83.3
%Low linolenic	52.2	40.0	75.0
%Low AA	53.3	50.0	58.3
%Low linoleic	33.3	41.7	22.2
%High ω6/ω3 (AA/DHA)	44.4	36.4	57.1

DHA=Docosahexaenoic acid; AA=Arachidonic acid

Table (3): Comparison between autistic patients with mild and moderate autism and those with severe autism regarding the studied laboratory markers.

	Patients with mild and moderate autism (n=18)		Patients with severe autism (n=12)		Z	P
	Mean±SD	Median (IQR)	Mean ± SD	Median (IQR)		
Carnitine (mg/ml)	3.83±1.26	3.88 (1.25)	2.69±1.09	2.53 (2.02)	2.18	**<0.05***
Lactate (mg/dl)	16.15±7.43	15.80 (10.95)	22.95±8.20	23.80 (16.19)	2.16	**<0.05***
DHA (ω3) (µg/ml)	0.67±0.81	0.17 (1.69)	0.48±0.69	0.10 (0.74)	0.46	>0.05
Linolenic (µg/ml)	1.81±0.91	1.78 (0.79)	1.43±1.04	1.12 (1.48)	1.36	>0.05
Arachidonic (ω6)(µg/ml)	2.97±2.37	2.32 (3.57)	2.21±1.83	2.33 (3.53)	0.47	>0.05
Linoleic (µg/ml)	1.97±1.88	1.15 (2.69)	2.64±11.65	2.33 (3.52)	1.04	>0.05
ω6/ω3	5.94±7.42	2.75 (7.29)	98.7±247.3	3.95 (16.89)	0.41	>0.05

P >0.05 = Non significant, P < 0.05* = Significant; DHA = Docosahexaenoic acid

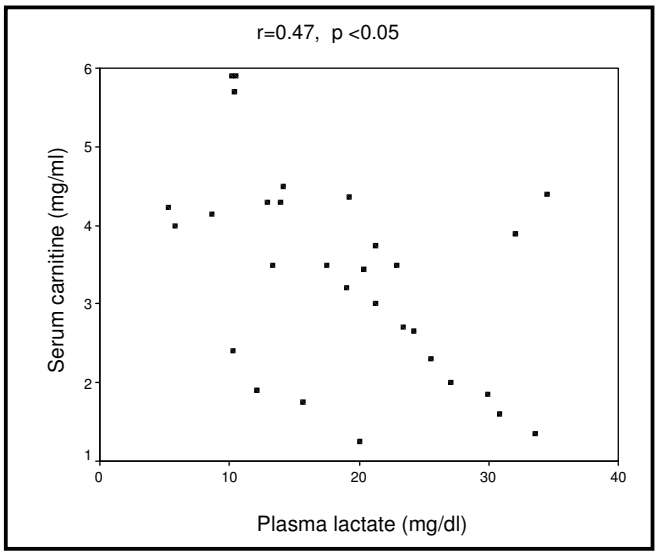

Fig. (1) Correlation between plasma lactate and serum carnitine levels of autistic patients.

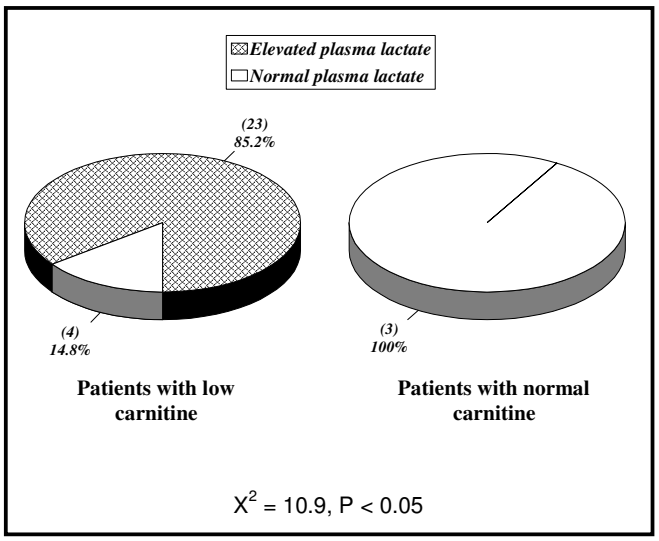

Fig (2) Association between serum carnitine and plasma lactate levels of autistic children.

DISCUSSION

Etiology of autism is speculative. A variety of biochemical, anatomical and neuroradiological studies imply a disturbance of brain energy metabolism in some autistic patients. A likely etiological possibilities may involve mitochondrial dysfunction[9] and reduced PUFAs in some autistic children[4]. In the present study, autistic patients had significantly lower serum carnitine than healthy controls. These results were in accordance with a previous recent study[9] conducted on autistic children. Acetyl carnitine is needed for mitochondrial transfere of PUFAs and synaptic transmission of multiple neurotransmitters. It affects attention, antagonizes deterioration of ability to learn and improves long term memory[8].

Certain mitochondrial point mutatioins could be the basis for autism in some individuals[16]. In a recent study, 4 out of the studied 5 autistic patients had A3243G mutation in the mtDNA tRNA Leu(UUR) gene[17]. Low serum carnitine in autism may be secondary to mitochondrial dysfunction resulting in a defect in β-oxidation of PUFAs causing accumulation of carnitine esters that are excreted in the urine draining the body carnitine[9]. This argument was supported by our findings of significantly higher plasma lactate levels of autistic patients than healthy children in addition to a significant negative correlation between carnitine and lactate levels. This indicated that the more was the severity of the mitochondrial dysfunction, measured by plasma lactate levels, the more was the degree of reduction of serum carnitine levels. Similarly, Filipeck and associates[9] have reported more elevation of serum lactate levels in autistic patients than healthy children. Elevated plasma lactate levels were elicited in 76.6% of our autistic children. In contrast, Oliveria and associates[3] reported that 14 only out of their studied 69 autistic children (14.6%) had elevated plasma lactate levels. This discrepancy may be explained by the differences in the chosen cut-off value above which the patient was considered to have lactatemia, number and age of the studied autistic patients.

Our finding of a significant decrease of serum carnitine and a significant increase of plasma lactate levels in patients with severe autism than those with mild and moderate disease is another important clue for the pathogenic role of mitochondrial dysfunction in autism. This means that the extent of the reduction of serum carnitine and elevation of plasma lactate were closely linked to the autistic severity assessed by CARS. Thus, the relationship of mitochondrial dysfunction, measured by high plasma lactate and low serum carnitine, to the autistic severity is possibly a causal one in which mitochondrial dysfunction not only plays a role in the pathogenesis of autism but also determines its clinical severity.

The benefits of studying the mitochondrial function in autism is the identification of the subgroup of autistic patients with an identified metabolic derangement. Palliative therapeutic interventions aiming at minimizing the consequences of mitochondrial dysfunction in these patients should be subsequently done. These interventions include decreasing lactate levels pharmacologically, supplementing components of the respiratory chain and cofactors of respiratory chain enzymes, and administering oxygen radicals scavengers[10].

In the present study, autistic children had significantly lower plasma PUFAS (DHA, linolenic and arachidonic acids) and significantly higher omega6/omega3 ratio than healthy children. In addition, reduced plasma levels of DHA, linolenic, arachidonic and linoleic acids were found in 76.6%, 52.2%, 53.3% and 33.3%, respectively of autistic children and elevated omega6/omega3 ratio was elicited in 44.4% of autistic children. Researches on PUFAs in autism are very limited. One preliminary study found that average total levels of omega-3 (n-3 PUFAs) in the autistic children were about 20% lower than mentally retarded children used as controls. Levels of DHA were 23% lower. These deficiencies, resulted in a significantly higher ratio of n-6 to n-3 in 25% of autistic children[4].

Regarding the relation between the autistic severity and PUFAs, patients with severe autism had lower plasma values of PUFAs and higher ω6/ω3 ratio than patients with mild and moderate autism, but these differences were insignificant. No data regarding the relation between the autistic severity and PUFAs are available. Thus, further studies are warranted regarding this issue. Increasing evidence suggests that essential fatty acids, which are critical nutrients for the brain, may be especially important for children suffering from developmental disorders like autism. In fact, some researchers theorize that psychiatric diseases with defect in communication as attention deficit hyperactivity disorder, dyslexia, dyspraxia, schizophrenia and autism can be characterized as "phospholipid spectrum of disorders. This could explain why these conditions often overlap, why they tend to cluster in families, and why they often share similar clinical features[18]. PUFAs imbalances in autism could arise from many possible causes, which include dietary imbalances (the modern diet is often n-3 deficient), enzyme dysfunction, genetic mutations and/or a reduced ability of PUFAs to incorporate themselves or remain intact inside the cell membrane[4].

The optimal range of the ratio of omega6/omega3 varies from 1/1 to 4/1. High ω6/ω3 ratio promotes the pathogenesis of many diseases. Whereas low ω6/ω3 ratio exerts a suppressive effect. A ratio of ω6/ω3 of 4/1 is the optimal ratio of brain mediated function[19]. An important clue for the possible etiopathogenic role of PUFAs deficiency in the pathogenesis of some cases of autism is our finding of the significant increase of ω6/ω3 ratio in patients with regressive autism than those with non-regressive disease. This may suggest that in a normally developed infant with an adequate supply of PUFAs in the breast milk, a weaning on omega-3 PUFAs poor diet may result in expression of the autistic phenotype in presence of genetic susceptibility.

Neuropsychological and neurodegenerative diseases have been associated to impaired status of blood DHA and/or AA which might lead to decreased contents in neuronal membranes. The mechanisms by which DHA can impact on neuronal functions involve the modulation of membrane biophysical properties, the regulation of neurotransmitter release (such as dopamine and serotonin), the synthesis of oxygenated biologically active derivatives, and the nuclear receptor mediated transcription of lipid responsive genes[20]. Some, but not all studies of human infants suggest that dietary DHA may play a role in cognitive development and in some neurodevelopmental disorders like autism[21]. Our finding of significantly lower DHA in hypotonic autistic children than normotonic autistic patients is an important clue for the importance of DHA for neurological function. Also, autistic children with sleep problems had lower levels of DHA and higher levels of ω6/ωw3 ratio than autistic children without such problems, but these differences were insignificant. Recent studies reported that normal omega6/omega3 ratio is important for normal sleep with a maximum ratio of 4:1 [19].

In the present study, 50% of autistic children had GI problems. Autistic children with GI problems had higher ω6/ω3 ratio than those without such problems, but these differences were insignificant. In a previous study, over 90% of autistic children with GI symptoms had enterocolitis, such as lymphoid nodular hyperplasia (autistic enterocolitis) diagnosed by colonoscopy leading researchers to suspect a gut-brain connection in autism as GI problems may exacerbate some behavioral and sleep problems in autism. Also, fish oil may be useful in reducing the gastrointestinal problems commonly observed in children with autism[22].

PUFAs intake constitutes an environmental factor able to alter brain function[6] as evidenced by the decrease in the antisocial behaviour, including violence, with increase of PUFAs intake[23]. The only trial of PUFAs supplementation in autism is a 3-month open label ongowing study on 18 autistic children, ranging from 3 to 10 years of age. The preliminary results revealed that all autistic children displayed significant increases in their language and learning skills after supplementation

with complete omega (omega 3 and 6 for three months)[24]. The impressive results of this study supports the importance for design and implementation of future studies regarding the effect of PUFAs supplementation using larger sample size of autistic patients and placebo controlled formats. If this treatment is found to be effective, it would be possible to implement widely due to its low cost and safety.

In conclusion, brain energy metabolism of many autistic children may be suppressed due to summation of several factors including low serum carnitine, which may be attributed to disturbed mitochondrial function as evidenced by high plasma lactate levels, and/or low plasma PUFAs. The degree of suppression of brain energy was correlated positively with autistic severity. Further studies are needed to prove these arguments and to assess the effect of new therapeutic modalities for autism (such as PUFAs, carnitine and measures that augment the mitochondrial function) on amelioration of the autistic phenotype.

REFERENCES

1. Wolanczyk T, Ostrowska-Galemba K, Mikulska J, Komender J, Jagielska G, Tomaszewicz-Libudzic C. Features of autism, autistic traits, autism: retrospective analysis of clinical symptoms in children treated in the Pediatric Clinic. Psychiatria Polska 2001; 35(1) : 59-69.
2. Rapin I. Diagnosis and management of autism. Recent Advances in Pediatrics 2000; 8 :121-33.
3. Oliveira G, Diogo L, Grazina M, Garcia P, Ataide A, Marques C et al. Mitochondrial dysfunction in autism spectrum disorders : a population-based study. Dev Med Child Neurol 2005; 47(3) : 148.
4. Vancassel S, Durand G, Barthelemy C, Lejeune B, Marineau J, Guilloteau D, et al. Plasma fatty acids levels in autistic children. Prostaglandins Leukot Essent Fatty Acids 2001; 65:1-7.
5. Innis SM. The role of dietary n-6 and n-3 fatty acids in the developing brain. Dev Neurosci 2000; 22(5-6): 474-80.
6. Chalon S, Vancassel S, Zimmer L, Guilloteau D, Durand G. PUFA and cerebral function: focus on monoaminergic neurotransmitters. Lipids 2001; 36 : 937-44.
7. Virmani A, Gaetani F, Imam S, Binienda Z, Ali S. The protective role of L-carnitine against neurotoxicity evoked by drug of abuse, methamphetamine, could be related to mitochondrial dysfunction. Annals of the New York Academy of Sciences 2002; 965: 225-32.
8. Traina G, Valleggi S, Bernardi R, Rizzo M, Calvani M, Nicolai R et al. Identification of differentially expressed genes induced in the rat brain by acetyl-L-carnitine as evidenced by suppression subtractive hybridisation. Brain Res Mol Brain Res 2004; 132(1):57-63.
9. Filipek PA, Juranek J, Nguyen MT, Cummings C, Gargus JJ. Relative carnitine deficiency in autism. J Autism Dev Disord 2004 34(6) : 615-23.
10. Lombard J. Autism : a mitochondrial disorder ?. Medical Hypotheses 1998; 50 (6) : 497-500.
11. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 4th ed. Washington, DC: American Psychiatric Association; 1994.
12. Schopler E, Reichler RJ, Renner BR. *The Childhood Autism Rating Scale (CARS)*, for diagnostic screening and classification in autism. New York: Irvington; 1986.
13. Wieland OH. Determination of plasma carnitine by enzymatic ultraviolet test. In: Bergermeyer H.U, editor, Methods of Enzymatic Analysis. VCH Verlagsgesellschaft, weinheim, Deerfield Beach (Florida) 1965; p. 481-8.
14. Sacks DB. (editor) Carbohydrate In: Tietz Textbook of clinical chemistry Philadelphia: WB saunders company; 1999. p. 787-90.
15. Willem O, Vicki V, Peter F H , Van DP, Maria PM, Van DH, et al. Identification and quantification of intermediates of unsaturated fatty acids metabolism in plasma of patients with fatty acids oxidation disorder. Clin Chem 1995; 41(10).
16. Graf WD, Marin-Garcia J, Gao HG, Pizzo S, Naviaux RK, Markusic D, et al. Autism associated with the mitochondrial DNA G8363A transfer RNA (Lys) mutation. J Child Neurol 2000; 15: 357-61.
17. Pons R, Andreu AL, Checcarelli N , Vila MR, Engelstad K, Sue CM, et al. Mitochondrial DNA abnormalities and autistic spectrum disorders. J Pediatr 2004; 144 : 81-5.
18. Richardson AJ, Ross MA. Fatty acid metabolism in neurodevelopmental disorders: a new perspective on associations between attention-deficit hyperactivity disorders, dyslexia, dyspraxia and the autistic spectrum.

Prostaglandins Leukot Essent Fatty Acids 2000;63 (1/2): 1-9.
19. Simopoulos AP. Importance of the ratio of omega-6/ omega-3 essential fatty acids . Biomed Pharmacother 2002; 56: 365-79.
20. Alessandri JM, Guesnet P, Vancassel V, Astorg P, Denis I, Langelier B, et al. Polyunsaturated fatty acids in the nervous system: evolution of concepts and nutritional implications throughout life. Reprod Nutr Dev 2004; 44(6): 509-38.
21. Wainwright PE. Dietary essential fatty acids and brain function: a developmental perspective on mechanisms. Proceedings of the Nutrition Society 2002; 61 (1): 61-9.
22. Wakefield AJ, Anthony A, Murch SH, Thomson M, Montgomery SM, Davies S et al. Enterocolitis in children with developmental disorders. Am J Gastroenterol 2000; 95(9): 2285 - 95.
23. Gesch CB, Hammond SM, Hampson SE, Eves A, Crowder MJ. Influence of supplementary vitamins, minerals and essential fatty acids on the antisocial behaviour of young prisoners. Randomized, placebo-cotrolled trial. British Journal of Psychiatry 2002; 181 : 22-8.
24. Patrick L, Salik R. the effect of essential fatty acids supplementation on language development and learning skills in autism and Asperger's syndrome. Cited on: http: //www. joyfulgenuis.com., on Aug. 24th, 2005.