RELATIONSHIP BETWEEN ANTIEPILEPTIC DRUGS AND BIOLOGICAL MARKERS AFFECTING LONG-TERM CARDIOVASCULAR FUNCTION IN CHILDREN AND ADOLESCENTS

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ABSTRACT

Background
Epilepsy is a neurological disorder, relatively common in the paediatric population. These children are often treated with antiepileptic drugs (AEDs) for several years. The consequence of such long-term exposure may lead to variations in plasma homocysteine and serum lipoprotein concentrations.

Objective(s)
To review the cardiovascular effects of anticonvulsant therapy and their use in childhood epilepsy with special reference to homocysteine and lipoprotein.

Methods
A literature search was conducted on PubMed (1966-May 2009) and MEDLINE (1966-May 2009). Key terms included antiepileptic drugs, epilepsy, homocysteine, cardiovascular events, and children.

Results
Certain AEDs including carbamazepine, phenobarbital, phenytoin and valproic acid, as well as the presence of a homozygous 5-methylenetetrahydrofolate reductase polymorphism in the genotype, are potential causes of elevation in plasma homocysteine and serum lipoprotein concentrations.

Conclusions
Persistent elevation in these biochemical markers has shown to be associated with the development of long-term sequelae such as cardiovascular diseases, prompting concerns about the long-term implications of chronic AED use in children and cardiovascular risk. Further research is needed to assess the relationship between specific chronic AED use, homocysteine and lipoprotein concentrations, the influence of genotype, as well as the risk of long-term sequelae in the paediatric population.

Key Words: *Anticonvulsant/antiepileptic drugs; epilepsy; homocysteine; cardiovascular events; children*

Epilepsy is a common and chronic neurological disorder characterized by apparently unprovoked recurrent paroxysmal events or seizures that are associated with a sudden alteration in motor activity and behaviour with or without alteration in conscious awareness.[1] The alteration in state is the result of an abnormal and excessive hypersynchronous firing within a group of epileptic neurons in the brain.[1] According to Epilepsy Canada, epilepsy affects approximately 0.6% of the Canadian population. More specifically, epilepsy affects approximately 0.3% of children between the ages of 0-11, 0.6% of children between the ages of 12-14, and 0.6% of adolescents between the ages of 16-24.[2,3] The most common form of treatment for seizure

control involves the use of antiepileptic drugs (AEDs). These drugs are often used for variable time intervals ranging from a minimum of 1-2 years to lifelong therapy for certain epilepsies. While many of the dose-related effects and hypersensitivity reactions are well documented, the effects of long-term use of AEDs on metabolism and metabolic adaption remain poorly understood and are not frequently investigated. Some studies have found that AEDs are associated with an elevation in the plasma concentration of total homocysteine.[4-10] Elevation in serum concentration of lipoprotein has also been reported in patients receiving chronic AED therapy.[11-16] In addition, children affected may have variations in genes encoding the enzymes involved in homocysteine metabolism that may also cause an elevation in homocysteine concentration. Therefore, a combination of risk factors may further elevate the plasma concentration of homocysteine. These elevated concentrations can be toxic to the vascular structure and function by affecting both the clotting system and endothelium of blood vessels, leading to microvascular changes and a higher likelihood of long-term sequale such as cardiovascular and atherosclerotic disease developing later in life.[5-7,17]

A study done by McCully in 1969[18] was the first to suggest a link between the increase in plasma total homocysteine concentration and vascular disease. Following this, several studies have found that elevated plasma concentrations of total homocysteine, even if the concentrations are within reference range,[19] are an independent risk factor for cardiovascular and cerebrovascular disorders,[20] ischemic stroke, transient ischemic attack in chidren,[21,22] thrombosis, atherosclerosis, and stroke.[15,19,23,24] High concentrations of total homocysteine have also been related to potential teratogenic effects as there is a threefold increased risk for major congenital malformations including fetal cardiovascular incidents and neural tube defects in children whose mothers receive AEDs[22,25] during the first trimester.[26]

Similarly, elevated serum lipoprotein (a) concentration has been identified as a secondary risk factor for the development of atherosclerotic cardiovascular disease and is also an independent risk factor for vascular diseases including myocardial infarction and stroke.[11,12] Individuals with lipoprotein (a) concentrations over the threshold value of 30 mL/dL, have an increased risk for the development of early atherosclerotic vessel disease.[12,13]

Given that children diagnosed with epilepsy are often placed on treatment with AEDs for several years, possibly leading to vascular diseases as a consequence of elevated total homocysteine and lipoprotein (a) concentrations, it would be beneficial to examine the relationship between the use of such AEDs and these biological factors as well as possible effect(s) they may have on vasculature structure and function. Therefore, the objective of this paper is to review the literature on children and adolescents with epilepsy who are being treated with AEDs and their effects on homocysteine and lipoprotein concentrations. In addition, the influence of genotype on concentrations of these biochemical markers in epileptic children is reviewed. Possible interventions to reduce the risk of vascular disease will also be considered. Finally, limitations of prior studies and the value of carefully designed studies in the future will be examined.

METHODS

A literature search was conducted systematically using PubMed (1966-May 2009) and MEDLINE (1966-May 2009). Key terms included antiepileptic drugs, epilepsy, homocysteine, lipoprotein, cardiovascular events, and children. These studies examined the effects AEDs have on plasma homocysteine and serum lipoprotein concentrations, and thus in turn, on cardiovascular effects, as well as the influence of polymorphisms. Selection criteria included any original article reporting on a paediatric population ranging from the ages of 1 to 18 years and consisting of relatively equal proportions of males and females. These children also had a diagnosis of epilepsy of various types (idiopathic epilepsy, partial epilepsy, partial epilepsy with secondary generalized seizures and generalized epilepsy), were receiving AED monotherapy, and were otherwise healthy. In contrast, selection criteria excluded any article reporting on non-human patients and those not in English. Other exclusion criteria included children on other medications or supplements known to interfere with the

metabolism of homocysteine, children with nutritional problems, or abnormalities in the liver, kidney or heart. Additional references were identified through cross-references of selected articles and going back to the original article, if they had not been identified through the PubMed or MEDLINE search.

RESULTS

Homocysteine and Antiepileptic Drugs

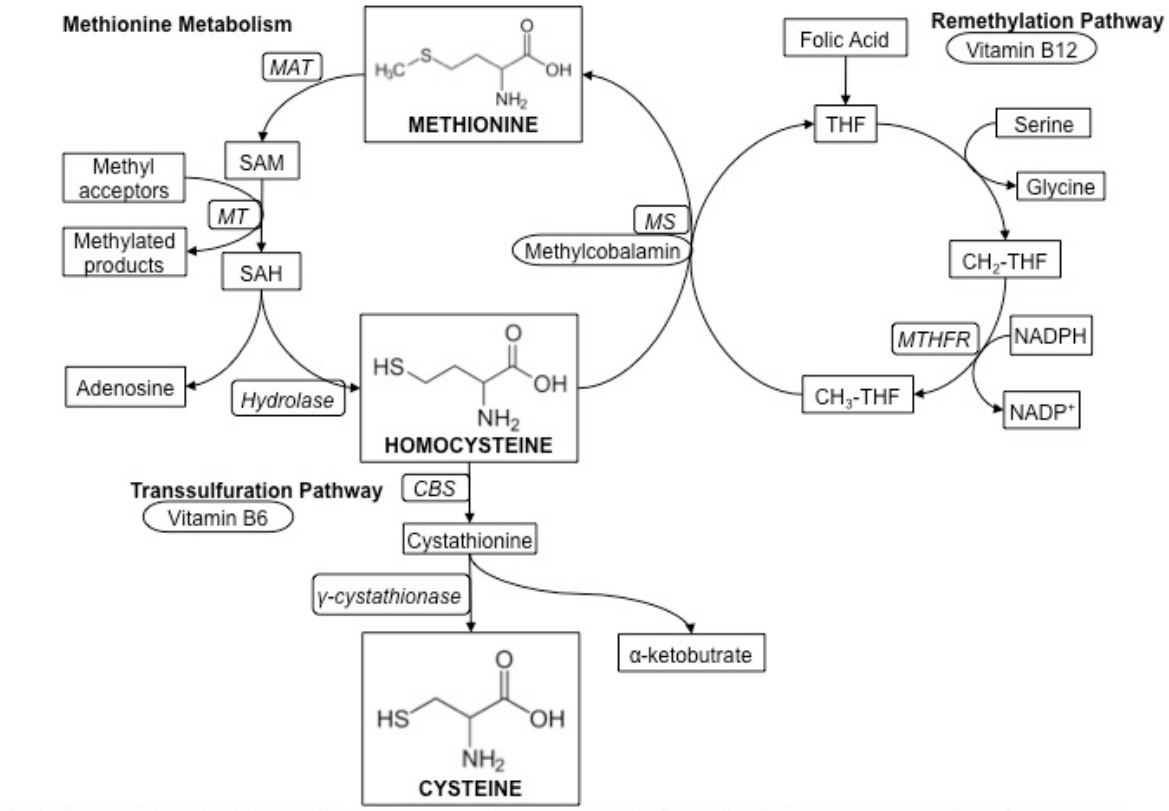
1.0 Homocysteine

Homocysteine is a thiol-containing amino acid formed as an intermediate product during the metabolism of methionine (Figure 1). Methionine is derived from the diet and is activated by methionine S-adenosyltransferase (MAT) to form S-adenosylmethionine (SAM).[27,28]

The significance of the formation of SAM is that it serves as the principal methyl donor in numerous reactions including methylation of DNA, RNA, hormones, lipids and neurotransmitters, as well as serving as a substrate for methyl transferase (MT).[27,29,30]

Once SAM is demethylated as a by-product of these methylation reactions, the S-adenosylhomocysteine (SAH) produced is subsequently hydrolyzed in a reversible reaction by SAH hydrolase to form homocysteine and adenosine.[27-31] Homocysteine can now participate in two metabolic pathways: the remethylation pathway and the transsulfuration pathway.[6,24,27,30,32-37] It is the intracellular concentration of SAM, and thus methionine, that act as variables to determine the pathway through which the metabolic fate of homocysteine is cycled.[34]

Figure 1. Metabolism of Homocysteine.



CBC = cystathionine β-synthase; CH_2-THF = 5,10-methylenetetrahydrofolate; CH_3-THF = 5-methyltetrahydrofolate; MAT = methionine S-adenosyltransferase; MS = methionine synthase; MT = methyl transferase; MTHFR = methylentetrahydrofolate reductase; $NADP^+$ = nicotinamide adenine dinucleotide phsphate; NADPH = nicotinamide adenine dinucleotide phosphate-oxidase; SAM = S-adenosylmethionine; SAH = S-adenosylhomocysteine; THF = tetrahydrofolate.

The remethylation pathway is favoured in situations where there is a relative deficiency in methionine and is the most common pathway which recycles homocysteine back to methionine.[23,30,34] This pathway requires vitamin B12 (cobalamin) and folic acid as cofactors and is catalyzed by methionine synthase (MS), which serves as a methyl transfer enzyme (Figure 1).[24,27,29,34,36,37] A methyl group comes from the donor 5-methyltetrahydrofolate (CH_3-THF), which is formed upon the reduction of 5,10-methylenetetrahydrofolate (CH_2-THF) catalyzed by methylentetrahydrofolate reductase (MTHFR) in the presence of NADPH.[7,29,34,36,37] This methyl group is then transferred to cobalamin forming methylcobalamin and tetrahydrofolate (THF), the biologically active forms of cobalamin and folic acid, respectively.[8,24,27,29,33] Methylcobalamin may now serve as a methyl carrier with the help of the MS by transferring its methyl group to homocysteine, thus resulting back to methionine.[33]

The transsulfuration pathway on the other hand, is favoured in situations where there is an excess of methionine and leads to the conversion of homocysteine to cysteine.[7,23,37] This pathway requires vitamin B6 (pyridoxal 5'-phosphate) as a cofactor. Homocysteine condenses with serine in an irreversible reaction catalyzed by cystathionine β-synthase (CBS) forming cystathionine.[24,31] Cystathionine is subsequently hydrolyzed by γ-cystathionase to form cysteine and α-ketobutyrate (Figure 1).[7,24,29,30,32,36,37] Cysteine can be incorporated into glutathione or further metabolized to sulfate and excreted into the urine.[24,28]

There are various forms of circulating homocysteine existing as both free and protein-bound. The sulfhydryl (reduced) form of homocysteine is oxidized in the plasma to the disulfide (oxidized) forms: homocystine and mixed disulfides. These mixed disulfides involve homocysteine and include homocysteine-cysteine and protein-bound homocysteine. The sum of all forms of homocysteine that exist in plasma or serum is collectively known as total homocysteine. The reduced form of homocysteine comprises about 1%, while the oxidized forms comprise about 98-99% of total homocysteine. More specifically, homocystine comprises about 5-10%, homocysteine-cysteine comprises about 5-10%, and protein-bound homocysteine comprises about 80-90% of total homocysteine.[29]

In physiological conditions, homeostasis is usually maintained between the formation and degradation of homocysteine; however, concentrations vary between individuals and several trends have been identified with respect to total homocysteine concentration. Total homocysteine increases significantly with age;[8,10,22,35,38,39] Ono *et al*.[8] reported that the plasma concentration of homocysteine in a group consisting of patients aged 15 years and older were significantly higher than those in the group aged 1 to 14 years. Total homocysteine also varies with respect to ethnic background[9] (a significant higher mean homocysteine concentration was found in black compared with white or Hispanic children),[10] is higher in adult men and menopausal women, and is lowest in children.[9,10] There is controversy as to whether or not the total homocysteine concentration is gender dependent as some studies[10,39] found that there was a significantly higher mean homocysteine concentration for boys than girls, while other studies[20,35,38,39] could not confirm these findings. Other interactions, including gene-environment, gene-gene and other variables, may play a role in the final determination of plasma homocysteine concentration.[27] These variables include: treatment with AEDs, a deficiency of the enzymes or cofactors necessary for the remethylation or transsulfuration pathway, genetic polymorphisms in genes coding for enzymes involved in the two pathways, dietary habits, personal lifestyle, and disease.[5-7,17,24,38,40-45]

When homeostasis is not maintained, elevated concentrations of homocysteine accumulate in the plasma and result in a condition known as hyperhomocysteinemia. Although severe hyperhomocysteinemia is rare, mild hyperhomocysteinemia occurs in approximately 5-7% of the general population.[19,24,36] Due to the recognition of age as an influence on normal plasma homocysteine concentrations within children, hyperhomocysteinemia in children is often defined as the total homocysteine values above the 95th percentile within a specific distribution of values for a paediatric population defined according to their gender and age.[10,38] Therefore, cut-offs for hyperhomocysteinemia in children usually range from 8.3-11.3 μmol/L,[10,38] compared to that of adult values of fasting plasma total homocysteine concentrations greater than 15 μmol/L.[46]

1.1 Antiepileptic Drugs Influencing Plasma Homocysteine Concentrations

AEDs are known to interfere with the metabolism of homocysteine causing a disturbance in the equilibrium among the various proteins, vitamins, and lipids. As the vitamins serve as necessary cofactors for homocysteine metabolism, it would be beneficial to examine their role to better understand the metabolism of homocysteine itself. Several first generation AEDs have been associated with folic acid deficiency. Numerous studies[6-10] have demonstrated that prolonged treatment with several first generation AEDs may potentially cause depletion in serum folic acid concentration. This will affect the downstream metabolic pathway, which includes the synthesis of 5-methyltetrahydrofolate necessary for the transmethylation of homocysteine back to methionine in the remethylation pathway. The result is an elevation in plasma homocysteine concentration, increasing by as much as 11.4-40%,[17,42,43] that in turn may lead to hyperhomocysteinemia. In addition, Huemer *et al.*[40] reported 15.4% of the children receiving AEDs were found to have significantly elevated homocysteine concentrations and thus hyperhomocysteinemia.

First generation AEDs used to treat epilepsy can be classified metabolically into three basic categories: (1) those that induce cytochrome P450 isozymes including carbamazepine (CBZ), phenytoin (PHT), phenobarbital (PB), and oxcarbazepine (OXCZ); and (2) those that inhibit cytochrome P450 isozymes including valproic acid (VPA). The remaining substances can be referred to as (3) other AEDs. Some of these AEDs are described and summarized in Table 1.[47-50] To examine the effect of AEDs on plasma homocysteine, serum folic acid, vitamin B12 and vitamin B6 concentrations, eight relatively recent studies were identified and summarized in Tables 2a and 2b.[5-7,12, 42,51-53]

Studies comparing the plasma homocysteine, serum folic acid, vitamin B12 and vitamin B6 concentrations in epileptic children with healthy controls prior to commencing AED monotherapy treatment with either CBZ or VPA, found no significant difference in these paramaters.[5,51] These baseline evaluations suggest that it is the AED rather than the convulsive disorder or other situations (genetic abnormalities or metabolism of homocysteine), that play a major role in the early development of hyperhomocysteinemia in epileptic patients.[5,51,54]

1.2 Drugs that Induce Cytochrome P450 Isozymes

CBZ, PHT, PB, and OXCZ, as mentioned above, are all drugs that induce specific isoforms of cytochrome P450. Table 2a shows seven studies conducted in children receiving CBZ, PB, or OXCZ for treatment of epilepsy. Five of these studies[5,12,42,51,52] demonstrated a significant elevation in plasma concentration of homocysteine when compared to baseline data and control values, while Kurul *et al.*[6] and Vurucu *et al.*[7] showed no significant difference. In contrast, five studies[5,7,42,51,52] demonstrated a significant reduction in serum folic acid concentration compared to baseline data and control values, while Kurul *et al.*[6] and Tumer *et al.*[12] showed no significant difference. As for serum vitamin B12 concentration, six studies[5-7,12, 51,52] demonstrated no significant difference, while Karabiber *et al.*[42] showed a significant reduction when compared to patients receiving VPA, baseline data and control values. Finally, of the three studies[5,51,52] that examined serum vitamin B6 concentration, all showed a significant reduction when compared to baseline data and control values.

The studies which showed no statistical significance could possibility be explained by the small patient population, as in the study conducted by Kurul *et al.*,[6] or the shorter duration of treatment, as in the studies conducted by Vurucu *et al.*[7] and Tumer *et al.*[12] This is demonstrated in the studies conducted by Kurul *et al.*[6] and Vurucu *et al.*,[7] which did observe a slight elevation in plasma homocysteine in patients receiving CBZ or OXCZ in comparison to baseline and control values, however, this was not enough to be considered statistically significant. Kurul *et al.*[6] reported that although the plasma homocysteine concentrations between the study and control groups were not significantly different, 27.2% demonstrated hyperhomocysteinemia. Similarly, Tumer *et al.*[12] showed a slight but insignificant reduction in serum folic acid, and observed that in patients receiving CBZ or PH, folic acid concentrations declined significantly when plasma homocysteine concentrations were high. Kurul *et*

al.[6] also noted that although serum folic acid concentrations were found to be normal, folic acid concentrations decreased among patients with hyperhomocysteinemia receiving CBZ or OXCZ.

CBZ and PB are known to be potent cytochrome P450 isozyme inducers. They can directly modulate the activity of different hepatic enzymes. Induction of these cytochrome P450 isozymes in the liver may cause a depletion of the cofactors necessary for the metabolism of homocysteine, such as folic acid, vitamin B12 and vitamin B6.[5] This in turn may lead to the alterations observed in homocysteine concentrations, and thus hyperhomocysteinemia. Therefore, hyperhomocysteinemia in these patients can be secondary to the reduced concentrations of cofactors.[51,54] Potential mechanisms through which this may occur include: the hepatic induction of these cytochrome P450 isozymes impairing intestinal absorption of folic acid through a competitive interaction between folic acid coenzymes and drugs, the accelerated degradation or depletion of folic acid, the increase or dysfunction of homocysteine metabolism in the liver, the acceleration of vitamin metabolism, and the interference in the metabolism of folic acid coenzymes.[8,34,42]

In summary, children taking AEDs that induce cytochrome P450 isozymes (CBZ, PB and OXCZ), are associated with a significant elevation in homocysteine concentration, as well as a significant reduction in folic acid and vitamin B6 concentrations in serum, while the effects on the serum concentration of vitamin B12 remain unclear. This also implies a negative correlation between plasma homocysteine and serum folic acid concentrations,[12,52,54] as well as vitamin B6 concentrations, but only later on in the treatment period.[52]

1.3 Drugs that Inhibit Cytochrome P450 Isozymes

VPA is a broad spectrum drug that inhibits specific cytochrome P450 isozymes and is the most commonly administered AED in children alongside CBZ.[13] Table 2b shows eight studies conducted in children receiving VPA for treatment of epilepsy. Six of these studies[5,7,12,42,51,52] demonstrated a significant elevation in total plasma homocysteine concentration when compared to baseline data and the control values, while Kurul *et al.*[6] and Unal *et al.*[53] showed no significant difference. The literature on serum folic acid concentration in patients taking VPA appear to be controversial as three studies[5,42,52] showed a significant reduction, one study[51] showed a significant elevation and three studies[6 7,12] showed no significant difference in folic acid concentration when compared to the baseline data and control values. As for serum vitamin B12 concentration, three studies[7,51,52] demonstrated a significant elevation while four studies[5,6,12,42] showed no significant difference following treatment with VPA. Finally, of the three studies[5,51,52] that examined serum vitamin B6 concentration, two studies[5,52] demonstrated a significant reduction when compared to baseline data and control values, while Attilakos *et al.*[51] showed no statistically significant difference after treatment with VPA. Furthermore, Verrotti *et al.*[5] found that the concentration of serum folic acid and vitamin B6 was slightly lower in patients treated with CBZ than those treated with VPA.

The studies which showed no statistical significance could possibly be explained by the small patient population, as in the studies conducted by Kurul *et al.*,[6] Unal *et al.*[53] and Attilakos *et al.*[51] The two studies conducted by Kurul *et al.*[6] and Unal *et al.*[53] did observe a slight elevation in homocysteine concentration in patients receiving VPA compared to baseline and control values, however, this was not enough to be considered statistically significant. Kurul *et al.*[6] reported that although the plasma homocysteine concentrations between the study and control group were not significantly different, 12.5% of the study group demonstrated hyperhomocysteinemia. Similarly, Attilakos *et al.*[51] observed a slight reduction in vitamin B6 concentration in patients after receiving VPA treatment, however this was not enough to be considered statistically significant.

As for the discrepancy in serum folic acid concentration, a possible reason for the significant reduction in folic acid concentration in the study conducted by Attilakos *et al.*,[51] might be because of the shorter, 20-week duration of treatment. Comparable studies included minimum treatment periods of six months or more. In addition, of the three studies showing no significant difference, Vurucu *et al.*[7] and Tumer *et al.*[12] showed a slight but insignificant reduction in serum folic acid,

while Tumer *et al*.[12] further indicated that in the patient group, folic acid concentrations were observed to decline significantly when plasma homocysteine concentrations were high. The third study conducted by Kurul *et al.*[6] also noted that although serum folic acid concentrations were found to be normal, of the patients with hyperhomocysteinemia, folic acid concentrations were lower.

Little is known about how VPA exerts its effects on the metabolism of homocysteine. The variability in vitamin cofactor concentrations could result from VPA having a lower degree of isozyme-inducing activity than CBZ and PB; therefore, the reduced degree of reductions on folic acid and vitamin B6 concentration may lead to only minor alterations observed in homocysteine concentrations.[5,7] In studies where the concentration of cofactors such as serum folic acid, vitamin B6 and vitamin B12 were not reduced, the findings could be indicative of another pathway taking precedence over the metabolism of homocysteine; a pathway that is independent of cofactors.[51]

Therefore, in summary, children taking AEDs that inhibit cytochrome P450 isozymes, such as VPA, experience a significant elevation in homocysteine concentration, and a significant reduction in vitamin B6 concentration while the effects on the concentrations of folic acid and vitamin B12 remain unclear.

In general, adolescents receiving AEDs, whether it be a cytochrome P450 isozyme inducer or inhibitor, seem to have an elevation in plasma homocysteine concentration and a reduction in vitamin concentrations, showing an inverse correlation;[8,40,43] homocysteine bearing a stronger relationship with folic acid than with vitamin B12 or vitamin B6.[5,8,12,17,24,25,40,42,43,51,52,55]

This may lead to the conclusion that the status of serum folic acid concentration predominantly influence total homocysteine concentration in patients with vascular disease, as well as healthy controls.[12,24,40] The fact that folic acid is the most important dietary determinant of total homocysteine concentration is fitting with its role in the metabolism of homocysteine. Since folic acid itself is required to initiate the methyl transfers leading to the conversion of homocysteine to methionine, it is used as a substrate. Conversely, vitamins B12 and B6 are not utilized themselves when homocysteine is metabolized; they only function as cofactors of enzymes involved in homocysteine metabolism. Possible mechanisms by which AEDs can cause hyperhomocysteinemia may be through the dysfunction of homocysteine metabolism, the acceleration of vitamin metabolism, and the interference in the metabolism of folic acid coenzymes.[5,8,24,34,40,42]

TABLE 1 Properties of Antiepileptic Drugs in Children and Adolescents

AED	Use of Drug	Major Mechanism of Action	Pharmacokinetics	Pharmacokinetic Interaction(s)	Adverse Effect(s)
Carbamazepine	Partial and generalized seizures (excluding absence and myoclonus) Childhood epilepsy syndromes	Blocks voltage-gated sodium channels	*Bioavailability (%):* 75-85 *Time to Peak Level (hours):* 4-8 *Volume of Distribution (L/kg):* 0.93-1.28 *Protein Binding (%):* 75 (CBZ) and 50 (CBXE) *Biotransformation:* Epoxidation, hydroxylation, conjugation, autoinduction *Hepatic Enzymes:* CYP3A4 (major), CYP1A2, CYP2C8, UDPGT family enzymes *Active Metabolite(s):* CBZE *Elimination Half-life (years):* 5-36[a]	Potent broad spectrum *inducer* of hepatic cytochrome P450 enzymes including CYP3A4, CYP2C9, CYP2C19, and CYP1A2	Drowsiness, somnolence, dizziness, sedation, diplopia, nausea, ataxia, blurred vision, weight gain, nystagmus, nystagmus, gastrointestinal symptoms, gait problems, change in mood, tremor, cognitive disturbances, rash
Lamotrigine	Partial and generalized epilepsy Lennox-Gastaut syndrome Other generalized epilepsy syndromes	Blocks voltage-gated sodium channels	*Bioavailability (%):* ~100 *Time to Peak Level (hours):* 2-4 *Volume of Distribution (L/kg):* 1.24-1.47 *Protein Binding (%):* 55 *Biotransformation:* Glucuronidation without phase 1 reaction *Hepatic Enzymes:* UDPGT family enzymes; UGT1A4 *Active Metabolite(s):* - *Elimination Half-life (hours):* 32[a]	Hepatic glucuronidation is not via the P450 enzymes, therefore, has little effect on the metabolism of other AEDs	Dizziness, vomiting, headache, asthenia, rash, nausea, dizziness, somnolence, insomnia, flu-like syndrome, rhinitis, vomiting
Levetiracetam	Partial seizures with or without secondarily generalized seizures	Binds selectively, and with high affinity, to a synaptic vesicle protein known as SV2A, which is involved in synaptic vesicle exocytosis and presynaptic	*Bioavailability (%):* >95 *Time to Peak Level (hours):* 1-2 *Volume of Distribution (L/kg):* 0.5-0.7 *Protein Binding (%):* <10 *Biotransformation:* Hydrolysis *Hepatic Enzymes:* Not metabolized by hepatic enzymes *Active Metabolite(s):* -	Has no inhibitory effect on hepatic cytochrome P450 enzymes	Headache, infection, anorexia, somnolence, aggression, emotional lability, oppositional behaviour, psychosis

		neurotransmitter release	*Elimination Half-life (hours):* 6[a]		
Oxcarbazepine[c]	Used in the treatment of epilepsy with partial onset seizures Partial and secondary generalized seizures	Acts through its 10-monohydroxy metabolite. Blocks voltage-gated sodium channels.	*Bioavailability (%):* ~100 *Time to Peak Level (hours):* 4-12 (OXCZ) and 7 (MHD) *Volume of Distribution (L/kg):* 0.3-0.8 *Protein Binding (%):* 60 (OXCZ) and 40 (MHD) *Biotransformation:* Reduction, conjugation *Hepatic Enzymes:* CYP450, UDPGT family enzymes *Active Metabolite(s):* MHD *Elimination Half-life (hours):* 1-2.5 (OXC) and 8-10 (MHD)	*Inducer* of hepatic cytochrome P450 enzymes including CYP2C19, CYP3A4, CYP3A5	Dizziness, headache, diplopia, ataxia, fatigue, somnolence, rash, exacerbate seizures, lower incidence of allergic reactions, causes symptomatic hyponatremia
Phenobarbital	Partial or generalized seizures (including absence and myoclonus) Status epilepticus Lennox-Gastaut syndrome Other childhood epilepsy syndromes Febrile convulsions Neonatal seizures	Enhances GABA-A receptor activity	*Bioavailability (%):* 85-100 *Time to Peak Level (hours):* 2-4 *Volume of Distribution (L/kg):* 0.45-0.7 *Protein Binding (%):* ~50 *Biotransformation:* Hydroxylation, *N*-glucosidation, conjugation *Hepatic Enzymes:* CYP2C9, CYP2C19, CYP2E1 *Active Metabolite(s):* none *Elimination Half-life (hours):* 53-140	Potent *inducer* of hepatic cytochrome P450 enzyme activity and increases the metabolism of other drugs	Sedation, disturbances of mood and behaviour and possibly cognition, exacerbation of seizures, serious allergic reactions

Phenytoin	Partial and primary and secondarily generalized seizures (excluding myoclonus and absence) Status epilepticus Childhood epilepsy syndromes	Blocks voltage-gated sodium channels	*Bioavailability (%):* 95. Zero-order kinetics can affect the apparent extent of absorption. *Time to Peak Level (hours):* 3-10 *Volume of Distribution (L/kg):* 0.7-0.9 *Protein Binding (%):* 90 *Biotransformation:* Metabolize to arene oxides via zero-order kinetics, *N*-glucosidation, hydroxylation *Hepatic Enzymes:* CYP2C9, CYP2C19, UDPGT family enzymes *Active Metabolite(s):* none *Elimination Half-life (hours):* 7-42[a,b]	Potent *inducer* of many hepatic cytochrome P450 enzymes; therefore, increasing clearance and decreasing concentrations of most other AEDs that are eliminated by hepatic metabolism	Ataxia, dysarthria, motor slowing, lethargy, sedative mental changes, rash, fever, anaemia
Valproic Acid	Primary and secondarily generalized seizures (including myoclonus and absence) and partial seizures Lennon-Gastaut syndrome Idiopathic generalized epilepsy Childhood epilepsy syndromes Febrile convulsions	Unclear. Acts at the GABA-A receptor.	*Bioavailability (%):* 80-100 *Time to Peak Level (hours):* 0.5-8 *Volume of Distribution (L/kg):* 0.20-0.30 *Protein Binding (%):* 70-93 *Biotransformation:* glucuronidation, beta-oxidation, hydroxylation, ketone formation, and desaturation *Hepatic Enzymes:* CYP2C9, CYP2A6, CYP2B9, UDPGT family enzymes *Active Metabolite(s):* none *Elimination Half-life (hours):* 11.6 and 1.0 receiving monotherapy and polytherapy, respectively	*Inhibitor* of some hepatic cytochrome P450 enzymes. Metabolism of VPA is sensitive to enzymatic induction. VPA has a high affinity for serum proteins; therefore, can displace other drugs.	Tremor, drowsiness, lethargy, confusion, reversible dementia, brain atrophy, encephalopathy, nausea, vomiting, anorexia, gastrointestinal distress, hepatic failure, pancreatitis, thrombocytopenia, decreased platelet aggregation, fibrinogen depletion,hyperammonemia , hypocarnitinemia, hyperinsulinism, menstrual irregularities, polycystic ovaries, major malformations including neural tube defect and possible developmental delay in offspring, hair loss, edema, nocturnal enuresis, decreased bone mineral density

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AED = antiepileptic drug; CBZ = carbamazepine; CBXE = carbamzepine-10-11-epoxide; CYP = cytochrome P450; GABA-A = Gamma-Aminobutyric Acid; MHD = 10-monohydroxy derivative; OXCZ = oxcarbazepine; PB = phenobarbital; PHT = phenytoin; SV2A = Synaptic vesicle glycoprotein 2A; UDPGT = Uridine Diphosphate Glucuronosyltransferase; VPA = valproic acid
*There are many drug- and patient-related factors which can affect the pharmacologic properties of these drugs including age-related differences between newborns, infants, children, adolescents, and adults.
[a]varies with age and comedication
[b]has nonlinear elimination kinetics
[c]10-keto analogue of carbamazepine

TABLE 2A
Results of Studies Evaluating the Relationship between Cytochrome P450 Isozyme Inducers and Homocysteine and Vitamin Concentrations in Children

Study	Methodology	Study Population	Key Findings (data presented as mean ± S.D.)
Results of Studies Showing no Change in Hcy Concentration			
Kurul (2007)[6]	*Method:* Fasting venous blood samples obtained *Study Design:* Cross-sectional *Assay Used:* NR (plasma Hcy concentration, serum folic acid and vitamin B12 concentration)	*Total Number of Patients:* 25 (13 Female, 12 Male) *Mean Age (range) years:* 11.44 ± 3.33 (6-18) *Location of Study:* İzmir (Turkey) *Type of Epilepsy Diagnosed:* Idiopathic epilepsy *Drug Studied (dosage):* CBZ (NR), OXCZ (NR) *Mean Duration of Treatment (range) years:* 4.78 ± 2.07 (1.5-11)	→ Hcy between patient and control group (p=0.522) → Folic acid between patient and control group (p=0.855)[a] → Vitamin B12 between patient and control group (p=0.798) Hyperhomocysteinemia (Hcy concentrations > normal cut-off) found in 27.2% of on CBZ
Vurucu (2008)[7]	*Method:* Blood samples collected after overnight fasting, stored at -80°C until Hcy levels were measured *Study Design:* Cross-sectional *Assay Used:* HPLC (plasma Hcy concentration), autoanalyzer by immune-assay (serum folic acid and vitamin B12 concentrations)	*Total Number of Patients:* 93 (34 Female, 59 Male) *Mean Age (range) years:* 9.33 ± 4.95 (3-15) *Location of Study:* Ankara (Turkey) *Type of Epilepsy Diagnosed:* Idiopathic epilepsy *Drug Studied (dosage):* CBZ (NR) *Mean Duration of Treatment (range) years:* 2.68 ± 2.82 (≥0.5)	→ Hcy concentration between patient and control group (p>0.05) ↓ Folic acid by 2.04 ± 3.97 ng/mL in patient compared to control group (p=0.001)[a] → Vitamin B12 between patient and control group (p>0.05)

Results of Studies Showing Significant Changes in Hcy Concentration			
Verrotti (2000)[5]	*Method:* Venous blood sample obtained after overnight fasting, plasma and serum aliquots were quickly separated and frozen at -80°C for batched analysis. Done before onset of therapy and after one year of treatment. *Study Design:* Prospective *Assay Used:* HPLC and fluorescence detection (plasma Hcy concentrations), commercial kits (serum folic acid and vitamin B12 concentrations)	*Total Number of Patients:* 60 (29 Female, 31 Male) *Mean Age (range) years:* 16.2 ± 2.7 (14.2-17.9) *Location of Study:* Chieti (Italy) *Type of Epilepsy Diagnosed:* Various types of epilepsy *Drug Studied (dosage):* CBZ (16.8 ± 7.2 mg/kg/day) *Mean Duration of Treatment (range) years:* NR	→ Hcy, folic acid and vitamin B12 between patient and control group at beginning of the study ↑ Hcy by 6.0 ± 9.0 μmol/L and 6.2 ± 9.3 μmol/L in patient compared to baseline and control group, respectively, after one year of treatment ($p<0.01$) ↓ Folic acid by 4.7 ± 4.2 nmol/L and 3.5 ± 5.0 nmol/L in patient compared to baseline and control group, respectively, after one year of treatment ($p<0.01$), slightly more decrease than VPA treated patients → Vitamin B12 between patient and baseline and control group ↓ Vitamin B6 by 12.0 ± 7.3 nmol/L and 12.0 ± 8.2 nmol/L in patient compared to baseline and control group, respectively, after one year of treatment ($p<0.001$), slightly more decrease than VPA treated patients
Vilaseca (2000)[52] **numbers shown as medians not means*	*Method:* Fasting blood was obtained during the course of AED monitoring *Study Design:* Cross-sectional *Assay Used:* HPLC and fluorescence detection (plasma tHcy, vitamin B6 concentrations), competitive protein binding chemiluminescence assay (serum total folic acid and vitamin B12 concentrations)	*Total Number of Patients:* 136 *Mean Age (range) years:* NR (1-18) *Location of Study:* Barcelona (Spain) *Type of Epilepsy Diagnosed:* Partial epilepsy, partial epilepsy with secondary generalized seizures and generalized epilepsy *Drug Studied (dosage):* CBZ (20-30mg/kg/day) *Mean Duration of Treatment (range) years:* NR (≤3 and >3)	↑ tHcy by 1.9, 1.3, and 1.3 in patient compared to control group aged 1-10, 11-15, 16-18 years, respectively ($p<0.05$) ↓ Folic acid by 8.1 nmol/L in patient compared to control group ($p<0.00001$) → Vitamin B12 between patient and control group ↓ Vitamin B6 by 19 nmol/L in patient compared to control group ($p<0.0001$) Hyperhomocysteinemia (Hcy >95th percentile) found in 41.9% of patients taking CBZ
Tumer (2002)[12]	*Method:* Blood samples were obtained after 12h fasting *Study Design:* Cross-sectional *Assay Used:* HPLC and fluorescence detection (plasma Hcy concentrations), competitive protein binding chemiluminescence assay (folic	*Total Number of Patients:* 111 (49 Female, 62 Male) *Mean Age (range) years:* 10.28 ± 4.51 (NR) *Location of Study:* Ankara (Turkey) *Type of Epilepsy Diagnosed:* Epilepsy *Drug Studied (dosage):* CBZ (15-20 mg/kg), PB (5-10 mg/kg) *Mean Duration of Treatment (range) years:*	↑ Hcy by 1.38 ± 3.21 μmol/L in patient compared to control group ($p<0.05$) → Folic acid between patient and control group ($p>0.05$)[a] → Vitamin B12 between patient and control group ($p>0.05$) ↓ Folic acid when Hcy concentrations high ($r=-0.23$, $p<0.05$)

	acid and vitamin B12 concentrations)	3.08 ± 1.04 (NR)	
Karabiber (2003)[42]	*Method:* Venous blood samples collected after overnight fasting, immediately centrifuged for separation of serum and stored at -20°C until analyses *Study Design:* Cross-sectional *Assay Used:* micro ELISA (serum Hcy concentrations), autoanalyzer by immune-assay (serum folic acid and vitamin B12 concentrations)	*Total Number of Patients:* 66 (34 Female, 32 Male) *Mean Age (range) years:* NR (2-16) *Location of Study:* Ankara (Turkey) *Type of Epilepsy Diagnosed:* Epilepsy *Drug Studied (dosage):* CBZ (NR) *Mean Duration of Treatment (range) years:* NR (≥1)	↑ Hcy by 6.8 ± 13.4 µmol/L in patient compared to control group (p<0.05) ↓ Folic acid by 1.5 ± 3.9 ng/mL in patient compared to control group (p<0.05) ↓ Vitamin B12 by 58 ± 226 ρg/mL in patient compared to control group (p<0.05) Hyperhomocysteinemia (Hcy >15µmol/L) found in 23.3% of patients taking CBZ
Attilakos (2006)[51]	*Method:* Blood samples were obtained after 10-12h fasting, plasma and serum aliquots were quickly separated and frozen to -80°C until analysis. Done before and after a 20-week period of therapy. *Study Design:* Prospective *Assay Used:* HPLC (plasma tHcy and vitamin B6 concentrations), radioassay kit (serum folic acid and vitamin B12)	*Total Number of Patients:* 52 (34 Female, 32 Male) *Mean Age (range) years:* 9.2 ± 4.9 (4.5-14) *Location of Study:* Athens (Greece) *Type of Epilepsy Diagnosed:* Various types of epilepsy *Drug Studied (dosage):* CBZ (NR) *Mean Duration of Treatment (range) years:* NR	→ Hcy, folic acid, vitamin B12 and vitamin B6 between the two groups of patients and between the patient and control group at beginning of study ↑ Hcy by 0.7 ± 2.3 µmol/L during 20-week period of treatment in patient compared to pretreatment group (p<0.01) ↓ Folic acid by 1.7 ± 3.7 nmol/L during 20-week period of treatment in patient compared to pretreatment group (p<0.01) → Vitamin B12 between patient and control group ↓ Vitamin B6 by 6.3 ± 14.3 nmol/L during 20-week period of treatment in patient compared to pretreatment group (p<0.001) Hyperhomocysteinemia (Hcy >95th age percentile) found in 15.0% of patients taking CBZ after 20-week period of treatment
Tan (2009)[56]	*Method:* Blood samples collected after overnight fasting, centrifuged and analyzed immediately *Study Design:* Cross-sectional *Assay Used:* FPIA (plasma Hcy concentration), radioassay kit (serum folic acid)	*Total Number of Patients:* 195 (94 Female, 101 Male) *Mean Age (range) years:* 36.0 ± 11.3 (18-65) *Location of Study:* Kaohsiung (Taiwan) *Type of Epilepsy Diagnosed:* Idiopathic, cryptogenic, generalize, partial epilepsy *Drug Studied (dosage):* CBZ (400-1,800 mg/kg/day), PHT (200-400 mg/kg/day), PB (90-240 mg/kg/day)	↑ Hcy by 11.99 ± 7.81 µmol/L between patient and control group (p<0.001) ↓ Folic acid by 10.32 ± 15.06 nmol/L between patient and control group (p<0.001)

		Mean Duration of Treatment (range) years: 18.1 ± 10.1 (2-39)	

↑ = increase; ↓ = decrease; → = no difference; AED = antiepileptic drug; CBZ = carbamazepine; ELISA = enzyme-linked immunosorbent assay; FPIA = fluorescence polarization immunoassay; Hcy = homocysteine; HPLC = high-performance liquid chromatography; NR = none reported; OXCZ = oxcarbazepine; PB = phenobarbital; PHT = phenytoin; tHcy = total homocysteine; VPA = valproic acid
P values < 0.05 considered statistically significant
[a]Folic acid concentrations decreased when plasma Hcy concentrations high
*Values are of total homocysteine median (range) in µmol/L

TABLE 2B
Results of Studies Evaluating the Relationship between Cytochrome P450 Isozyme Inhibitors and Homocysteine and Vitamin Concentrations in Children

Study	Methodology	Study Population	Key Findings (data presented as mean ± S.D.)
Results of Studies Showing no Change in Hcy Concentration			
Kurul (2007)[6]	*Method:* Fasting venous blood samples obtained *Study Design:* Cross-sectional *Assay Used:* NR (plasma Hcy concentration, serum folic acid and vitamin B12 concentration)	*Total Number of Patients:* 25 (13 Female, 12 Male) *Mean Age (range) years:* 11.44 ± 3.33 (6-18) *Location of Study:* İzmir (Turkey) *Type of Epilepsy Diagnosed:* Idiopathic epilepsy *Drug Studied (dosage):* VPA (NR) *Mean Duration of Treatment (range) years:* 4.78 ± 2.07 (1.5-11)	→ Hcy between patient and control group (p=0.522) → Folic acid between patient and control group (p=0.855) → Vitamin B12 between patient and control group (p=0.798) Hyperhomocysteinemia (Hcy concentrations > normal cut-off) found in 12.5% of patients on VPA
Unal (2009)[53]	*Method:* Fasting blood samples obtained and analyzed before onset of therapy and re-evaluated after 9 months or 1 year of therapy *Study Design:* Prospective *Assay Used:* Nephelometric assay (mean Hcy concentration)	*Total Number of Patients:* 21 (9 Female, 12 Male) *Mean Age (range) years:* 7.75 ± 2.21 (1-13) *Location of Study:* Ankara (Turkey) *Type of Epilepsy Diagnosed:* Epilepsy *Drug Studied (dosage):* VPA (NR) *Mean Duration of Treatment (range) years:* 0	→ Hcy after treatment for 9 months to 1 year (p=0.09)
Results of Studies Showing Significant Changes in Hcy Concentration			
Verrotti (2000)[5]	*Method:* Venous blood sample obtained after overnight fasting, plasma and serum aliquots were quickly separated and frozen at -80°C for batched analysis. Done	*Number of Patients:* 60 (29 Female, 31 Male) *Mean Age (range) years:* 16.2 ± 2.7 (14.2-17.9) *Location of Study:* Chieti (Italy)	→ Hcy, folic acid and vitamin B12 between patient and control group at beginning of the study ↑ Hcy by 5.1 ± 8.4 µmol/L and 4.8 ± 8.4 µmol/L in patient compared to baseline and control

	before onset of therapy and after one year of treatment. *Study Design:* Prospective *Assay Used:* HPLC and fluorescence detection (plasma Hcy concentrations), commercial kits (serum folic acid and vitamin B12 concentrations)	*Type of Epilepsy Diagnosed:* Various types of epilepsy *Drug Studied (dosage):* VPA (21.7 ± 6.8 mg/kg/day) *Mean Duration of Treatment (range) years:* NR	group, respectively, after one year of treatment ($p<0.01$) ↓ Folic acid by 3.0 ± 5.2 nmol/L and 3.1 ± 5.7 nmol/L in patient compared to baseline and control group, respectively, after one year of treatment ($p<0.01$) → Vitamin B12 between patient and baseline and control group ↓ Vitamin B6 by 10.6 ± 6.7 nmol/L and 10.0 ± 7.8 nmol/L in patient compared to baseline and control group, respectively, after one year of treatment ($p<0.001$)
Vilaseca (2000)[52] **numbers shown as medians not means*	*Method:* Fasting blood was obtained during the course of AED monitoring *Study Design:* Cross-sectional *Assay Used:* HPLC and fluorescence detection (plasma tHcy, vitamin B6 concentrations), competitive protein binding chemiluminiscence assay (serum total folic acid and vitamin B12 concentrations)	*Total Number of Patients:* 136 *Mean Age (range) years:* NR (1-18) *Location of Study:* Barcelona (Spain) *Type of Epilepsy Diagnosed:* Partial epilepsy, partial epilepsy with secondary generalized seizures and generalized epilepsy *Drug Studied (dosage):* VPA (20-40 mg/kg/day) *Mean Duration of Treatment (range) years:* NR (≤3 and >3)	↑ tHcy by 1.6, 2.7, and 1.8 in patient compared to control group aged 1-10, 11-15, 16-18 years, respectively ($p<0.05$) ↓ Folic acid by 3.3 nmol/L in patient compared to control group ($p<0.05$) ↑ Vitamin B12 by 382 ρmol/L in patient compared to control group ($p<0.0001$) ↓ Vitamin B6 by 18.3 nmol/L in patient compared to control group ($p<0.0001$) Hyperhomocysteinemia (Hcy >95th percentile) found 39.2% of patients taking VPZ
Tumer (2002)[12]	*Method:* Blood samples were obtained after 12h fasting *Study Design:* Cross-sectional *Assay Used:* HPLC and fluorescence detection (plasma Hcy concentrations), competitive protein binding chemiluminescence assay (folic acid and vitamin B12 concentrations)	*Total Number of Patients:* 111 (49 Female, 62 Male) *Mean Age (range) years:* 10.28 ± 4.51 (NR) *Location of Study:* Ankara (Turkey) *Type of Epilepsy Diagnosed:* Epilepsy *Drug Studied (dosage):* VPA (15-30 mg/kg) *Mean Duration of Treatment (range) years:* 3.08 ± 1.04 (NR)	↑ Hcy by 1.38 ± 3.21 μmol/L in patient compared to control group ($p<0.05$) → Folic acid between patient and compared to control group ($p>0.05$) → Vitamin B12 between patient and compared to control group ($p>0.05$) ↓ Folic acid when Hcy concentrations high ($r=-0.23$, $p<0.05$)

Karabiber (2003)[42]	*Method:* Venous blood samples collected after overnight fasting, immediately centrifuged for separation of serum and stored at -20°C until analyses *Study Design:* Cross-sectional *Assay Used:* micro ELISA (serum Hcy concentrations), autoanalyzer by immune-assay (serum folic acid and vitamin B12 concentrations)	*Total Number of Patients:* 66 (34 Female, 32 Male) *Mean Age (range) years:* NR (2-16) *Location of Study:* Ankara (Turkey) *Type of Epilepsy Diagnosed:* Epilepsy *Drug Studied (dosage):* VPA (NR) *Mean Duration of Treatment (range) years:* NR (≥1)	↑ Hcy by 4.8 ± 7.3 µmol/L in patient compared to control group (p<0.01) ↓ Folic acid by 1.7 ± 3.5 ng/mL in patient compared to control group (p<0.05) → Vitamin B12 between patient and control group (p>0.05) Hyperhomocysteinemia (Hcy above 15µmol/L) found in 30.5% of patients taking VPA
Attilakos (2006)[51]	*Method:* Blood samples were obtained after 10-12h fasting, plasma and serum aliquots were quickly separated and frozen to -80°C until analysis. Done before and after a 20-week period of therapy. *Study Design:* Prospective *Assay Used:* HPLC (plasma tHcy and vitamin B6 concentrations), radioassay kit (serum folic acid and vitamin B12)	*Total Number of Patients:* 52 (34 Female, 32 Male) *Mean Age (range) years:* 9.2 ± 4.9 (4.5-14) *Location of Study:* Athens (Greece) *Type of Epilepsy Diagnosed:* Various types of epilepsy *Drug Studied (dosage):* VPA (NR) *Mean Duration of Treatment (range) years:* NR	→ Hcy, folic acid, vitamin B12 and vitamin B6 between the two groups of patients and between the patient and control group at beginning of study ↑ Hcy by 0.9 ± 2.7 µmol/L during 20-week period of treatment in patient compared to pretreatment group (p<0.001) ↑ Folic acid by 2.0 ± 6.2 nmol/L during 20-week period of treatment in patient compared to pretreatment group (p<0.01) ↑ Vitamin B12 by 375.1 ± 610.8 pmol/mL during 20-week period of treatment in patient compared to pretreatment group (p<0.01) → Vitamin B6 between patient and control group Hyperhomocysteinemia (Hcy >95th age percentile) found in 25.0% of patients taking VPA after 20-week period of treatment
Vurucu (2008)[7]	*Method:* Blood samples collected after overnight fasting, stored at -80°C until Hcy levels were measured *Study Design:* Cross-sectional *Assay Used:* HPLC (plasma Hcy concentration), autoanalyzer by immune-assay (serum folic acid and vitamin B12 concentrations)	*Total Number of Patients:* 93 (34 Female, 59 Male) *Mean Age (range) years:* 9.33 ± 4.95 (3-15) *Location of Study:* Ankara (Turkey) *Type of Epilepsy Diagnosed:* Idiopathic epilepsy *Drug Studied (dosage):* VPA (NR) *Mean Duration of Treatment (range) years:* 2.68 ± 2.82 (≥0.5)	↑ Hcy by 1.36 ± 3.38 µmol/L in patient compared to control group (p=0.003) → Folic acid between patient and control group (p=0.105) ↑ Vitamin B12 by 281 ± 391.21 pg/mL in patient compared to control group (p=0.001)

Can J Clin Pharmacol Vol 17 (1) Winter 2010:e5-e46; January 4, 2010

Tan (2009)[56]	*Method:* Blood samples collected after overnight fasting, centrifuged and analyzed immediately *Study Design:* Cross-sectional *Assay Used:* FPIA (plasma Hcy concentration), radioassay kit (serum folic acid)	*Total Number of Patients:* 195 (94 Female, 101 Male) *Mean Age (range) years:* 36.0 ± 11.3 (18-65) *Location of Study:* Kaohsiung (Taiwan) *Type of Epilepsy Diagnosed:* Idiopathic, cryptogenic, generalize, partial epilepsy *Drug Studied (dosage):* VPA (500-2,000 mg/kg/day) *Mean Duration of Treatment (range) years:* 18.1 ± 10.1 (2-39)	↑ Hcy by 11.99 ± 7.81 μmol/L between patient and control group (p<0.001) ↓ Folic acid by 10.32 ± 15.06 nmol/L between patient and control group (p<0.001)

↑ = increase; ↓ = decrease; → = no difference; AED = antiepileptic drug; CBZ = carbamazepine; ELISA = enzyme-linked immunosorbent assay; FPIA = fluorescence polarization immunoassay; Hcy = homocysteine; HPLC = high-performance liquid chromatography; NR = none reported; OXCZ = oxcarbazepine; PB = phenobarbital; tHcy = total homocysteine; VPA = valproic acid
*Values are of total homocysteine median (range) in μmol/L
P values < 0.05 considered statistically significant

1.4 Duration of Treatment

As previously mentioned, AEDs are often used for variable time intervals that can range from a minimum of 1-2 years to lifelong therapy for certain epilepsies. Prolonged treatment is often associated with a wide range of adverse effects including vascular endothelial dysfunction, thus leading to an increased risk of vascular disease because of elevated plasma homocysteine concentration[4-10] and elevated serum lipoprotein concentration.[11-16]

Three studies[8,40,52] have demonstrated that the duration of AED treatment positively correlates with total homocysteine concentration, especially in children being treated with CBZ.[52] Vilaseca *et al.*[52] found significant differences in total homocysteine concentrations between three different age ranges (1-10, 11-15 and 16-18 years), with the greatest difference seen among the oldest children. This positive correlation could be explained by the physiological trends of increasing homocysteine concentrations in children as they age.

Similarly, Ono *et al.*[8] found that low folic acid concentrations appear to be associated with duration of AED therapy. The reduction in folic acid concentration was observed to increase with age of child. This effect was more pronounced in the older group of children (15-35 years of age) compared to the younger group of children (1-14 years of age). It is likely that the older group of children started AED therapy during early childhood when their first seizure occurred, and therefore would have a longer duration of treatment. This raises the possibility that long-term AED treatment might lead to a progressive increase in total homocysteine concentration while folic acid concentration remain low,[8, 52] putting older children at a greater risk for elevated homocysteine concentrations.

More recently, Tan *et al.*[56] conducted a study examining the effect of long-term AED exposure. This study was based on 195 patients diagnosed with epilepsy between three months to 55 years of age and had received prolonged cytochrome P450 isozyme-inducing and/or isozyme inhibiting AED mono- or poly-therapy for 2-39 years. Consistent with previous studies, these authors also found a significant increase in plasma total homocysteine and a significant decrease in serum folic acid concentrations in the epileptic patients compared to the control group. It seems therefore, that the duration of treatment may add to the risks of hyperhomocysteinemia during chronic AED treatment.

Conflicting results in the study conducted by Attilakos *et al.*[51] revealed that children receiving CBZ or VPA for a short-term treatment, that is, a 20-week period, already show significant increases in plasma total homocysteine concentrations. Therefore, elevated concentrations of homocysteine can be seen in paediatric patients undergoing anticonvulsant therapy for a short- or longer-term. This leads to the need for additional better designed prospective studies to address this important question.

1.5 Monotherapy vs. Polytherapy

Several monotherapeutic treatments with either cytochrome P450 inducers or inhibitors interfere with the metabolism of homocysteine in various ways creating a change in the concentrations of proteins and vitamins involved. Studies have shown that there is no significant difference between the different monotherapies with respect to total homocysteine concentration, but these did carry a significant impact on folic acid, vitamin B12 and vitamin B6 concentrations.[40,52] Not surprisingly, when two or more drugs are given simultaneously during multidrug treatments, a synergistic effect may occur.[40] Children who are on multidrug therapy for epilepsy show a significantly positive correlation with total homocysteine and a negative correlation with folic acid concentrations in comparison with the baseline and control groups.[40]

1.6 Effect of AED Therapy on Vascular Diseases

Elevated concentrations of plasma total homocysteine could lead to long-term changes in vascular structure and function, potentially adding to the risk of further progression of vascular disease.[5-7,17,19] Homocysteine is thought to cause endothelial dysfunction by damaging endothelial cells through the increased generation of endothelium-derived vasoconstrictors and the depleted production of endothelial-derived vasodilators.[57] As previously mentioned, homocysteine is rapidly oxidized in the plasma forming homocystine and its mixed disulfides. During this oxidation, several events occur which

further promote the progression of vascular disease including: the production of potent reactive oxygen species (hydrogen peroxide and superoxide anion; both of which are capable of causing endothelial toxicity), oxidation of low-density lipoproteins, initiation of lipid peroxidation and impairment of cellular oxidative defence mechanisms.[23] Furthermore, the elevated concentrations of total plasma homocysteine enhances the release of proinflammatory mediators that could further amplify the risks of vascular diseases.[58]

Several studies have shown that the relationship between total homocysteine concentration and risk of vascular disease is continuous and graded.[59] Increments of homocysteine by 5 μmol/L was associated with a 60-80% higher risk of coronary artery disease, a 50% higher risk of cerebrovascular disease, and a sixfold higher risk of peripheral vascular disease.[59] It was also found that total homocysteine concentrations exceeding the 95th percentile are related to a fourfold increased risk for ischemic cerebrovascular disease in childhood.[33,60] This is comparable to the relative risk in adults with moderate hyperhomocysteinemia for ischemic vascular disease.[33] Reports indicate a higher prevalence of ischemic vascular disease in epileptic patients on AEDs than in the general population.[61]

Lipoprotein and Antiepileptic Drugs

2.0 Lipoproteins

As the name suggests, lipoproteins are composed of both lipids and proteins whose function is to transport dietary fats, lipids and cholesterol via the bloodstream. There are four main classes of lipoproteins, each with its own specific role in transporting within the circulation: chylomicrons, very low-density lipoproteins, low-density lipoprotein (LDL), and high-density lipoprotein (HDL). LDLs carry cholesterol from the liver to the cells within the body, where it undergoes desertification and is utilized in a number of biochemical pathways. The liver is where some cholesterol, fats and lipids are synthesized and stored. HDLs on the other hand, collect excess or unused cholesterol from the tissues within the body and return it to the liver, where it is broken down to bile acids and then excreted.[47,62] Each class of lipoprotein contains distinctive apolipoproteins (A, B, C, D, E and/or H) which help organize the structure of a lipoprotein particle and determine its interactions with enzymes, extracellular lipid transfer proteins, and cell-surface receptors. In addition, several copies of different apolipoproteins are found in each of the other lipoprotein classes.[62]

Lipoprotein (a) is a species of LDL and is considered a risk factor in the development of atherosclerosis because of its similar structure to plasminogen.[47,63] Plasminogen is produced in the liver and serves as the normal substrate for plasminogen activator which is secreted by and bound to endothelial cells. Once plasminogen binds to plasminogen activator, it becomes active and produces the enzyme plasmin. Plasmin is found in blood and degrades the protein fibrin, in the process called fibrinolysis. With lipoprotein (a) having a similar structure to plasminogen, it can compete and therefore inhibit the binding of plasminogen to its receptors on the endothelial cells. When this occurs, less plasmin is produced, which leads to the inhibition of fibrinolysis and eventually an increased risk for cardiovascular events such as thrombosis.[47]

The concentration of serum lipoprotein (a) is relatively low at birth, but gradually increases to adult concentration and remains stable over the first few months of life.[63,64] The concentration of lipoprotein (a) compared to other lipoproteins are much less affected by age, gender, weight, and diet.[63] However, similar to plasma concentration of homocysteine, there are factors that may affect the serum concentration of lipoproteins, including drugs, physical exercise, disease, genetic defects, and hormones.

2.1 Antiepileptic Drugs Influencing Serum Lipoprotein Concentrations

As mentioned above, an increase of lipoprotein (a) concentration has been identified as an important independent risk factor for cardiovascular diseases and atherosclerosis,[10-13,41] resulting possibly from the use of AEDs, or genetic defects. Some AEDs, particularly the ones which are hepatic enzyme inducers, are also known to effect serum lipid and apolipoprotein concentrations.[12,15,65] Serum lipoprotein (a) concentrations over 30 mg/dL carry an increased risk for the development of early atherosclerotic vessel disease.[12,13] In 2002, Tumer *et al.*,[12] showed that 28.8% of epileptic children taking CBZ, PB, VPA or a combination

of these, have serum lipoprotein (a) concentrations above the threshold, in comparison to age matched controls. No significant correlations were found between serum lipid values and apolipoprotein concentrations with age, gender, duration of therapy, or serum drug concentrations in children with epilepsy.[14,16] The concentration of plasma lipoprotein (a) in children treated with AEDs, however, were found to be significantly higher than those of the children without epilepsy.[12] Individuals with high serum high-density lipoprotein cholesterol (HDL-C), HDL_2-C or apolipoprotein A1 (apo A1) concentrations have a low risk of coronary heart disease, whereas those with high serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) or apolipoprotein B (apo B) concentrations have an increased risk.[14] To examine the effect of AEDs on serum lipoprotein concentrations, nine studies were identified and summarized in Tables 3a and 3b.[12-16,53,63,65,66]

Studies that compared the serum concentration of lipoproteins at the baseline of the study, before the initiation of AED therapy, with either CBZ, PB or VPA, excluded the possibility that these changes could be the result of genetics or of the convulsive disorder itself. Instead, the data suggests that the changes are a consequence of the AED administration.[13, 63,65, 66]

2.2 Drugs that Induce Cytochrome P450 Isozymes

These antiepileptic drugs (CBZ and PB) are metabolized in the liver and induce—thereby increasing—cytochrome P450 isozyme activity, which can lead to alterations in the metabolism of lipids, bile acids, and other endogenous molecules.[7,12,14,65,67] Table 3a shows eight and six studies conducted in children receiving CBZ and PB, respectively, for treatment of epilepsy. Three[12,13,65] of the eight studies have demonstrated a significant elevation in lipoprotein (a) concentration in patients receiving CBZ monotherapy when compared to pretreatment and control concentrations, while Aynaci *et al.*[63] and Verrotti *et al.*[66] showed no significant difference. Three other studies[12,63,65] also showed a significant elevation in lipoprotein (a) concentration in patients receiving PB monotherapy when compared to pretreatment and control concentrations, while Verrotti *et al.*[66] showed no significant difference.

Apo A1 and apo B concentrations were also looked at in patients receiving CBZ or PB, however, the literature on these apolipoprotein concentrations appears controversial. Serum apo A1 and apo B concentrations were found significantly reduced in one study,[14] significantly elevated in another study,[13] while the remaining four studies[15,63,65,66] found no significant difference in children receiving CBZ monotherapy in comparison to those receiving VPA, the pretreatment values and control group. Similarly, serum apo A1 concentrations were found significantly reduced in one study,[14] significantly elevated in another study,[65] while the remaining two studies[63,66] showed no significant difference in children receiving PB monotherapy when compared to the pretreatment and control group. Serum apo B concentrations were found to have no statistically significant difference in children receiving PB monotherapy when compared with the pretreatment and control groups.[14,63,65,66]

When serum TC concentrations were examined in patients receiving CBZ monotherapy, five studies[13-16,66] were found to be significantly elevated, while Aynaci *et al.*[63] and Sonmez *et al.*[65] showed no significant difference compared to those receiving VPA as well as the pretreatment values and control group. Similarly in patients receiving PB monotherapy, serum TC concentrations were found to be significantly elevated in four studies,[14,16,65,66] while Aynaci *et al.*[63] showed no significant difference compared to the pretreatment and control group.

Finally, serum HDL-C and LDL-C concentrations were examined in children receiving CBZ and PB. Of the seven studies that looked at children receiving CBZ monotherapy, four studies[13,14,16,66] were found to have significantly higher HDL-C concentrations, while the remaining three studies[15,63,65] showed no significant difference compared to those receiving VPA, the pretreatment and control groups. Similarly, of the five studies which examined children receiving PB monotherapy, three studies[16,63,65] were found to have significantly higher HDL-C concentrations, while the remaining two studies[14,66] showed no significant difference compared to the pretreatment and

control groups. Serum LDL-C concentrations were found to be significantly elevated in five studies,[13-16,66] while Aynaci *et al.*[63] and Sonmez *et al.*[65] showed no significant difference in patients receiving CBZ monotherapy compared to those receiving VPA, the pretreatment and control groups. As for children receiving PB monotherapy, serum LDL-C concentrations were found to be significantly elevated in three studies,[14,65,66] while Aynaci *et al.*[63] and Eiris *et al.*,[16] showed no significant difference compared to the pretreatment and control groups. In addition, a significant decrease was found in the HDL_2-C/HDL_3-C ratio in patients receiving either CBZ or PB monotherapy.[14]

Of the studies conducted above that showed no significant difference, it was noted that a slight elevation in serum lipoprotein (a), TC, HDL-C, and LDL-C concentrations were observed in patients receiving CBZ or PB compared to pretreatment values and control values, however, this was not enough to be considered statistically significant. These insignificant values could possibility be explained by the small patient population or the short-term duration of monotherapy treatment in the prospective study conducted by Aynaci *et al.*[63] As for the discrepancy in serum apo A1 and apo B concentrations, the study conducted by Eiris *et al.*[14] showing a significant decrease in apo A1 and apo B concentrations in patients treated with CBZ or PB and CBZ respectively, may be due to a longer mean duration of treatment. These patients endured 4.7 ± 5.1 years of treatment, while in other studies the mean duration of treatment was less than four years. This could imply that only after long-term periods of treatment with CBZ or PB will result in decreased apo A1 and apo B concentrations.

The mechanistic underpinnings of the relationship between microsomal enzyme function and its relationship to increased lipid concentrations in serum as well as the precise isoenzymes of cytochrome P450 that are involved in increasing serum lipid concentration remain unclear. Few mechanisms have been proposed however, as to the cause of increase in lipoprotein (a) concentration in children on CBZ therapy. These include the isozyme-inducing properties of CBZ, the AED-induced changes in kidney function, and the changes in the rates of production of lipoprotein (a).[11,65] In addition, the increase in TC concentration has been thought to be due to the effect of CBZ competing with cholesterol in the utilization of cytochrome P450 isozymes which can lead to a reduction in the transformation of cholesterol in biliary acids.[65,67] Despite the uncertainty in mechanism, it appears that children taking AEDs that induce cytochrome P450 isozymes, that is CBZ and PB, experience a significant increase in lipoprotein (a), TC, HDL-C and LDL-C concentrations, while the concentrations of apo A1 and apo B remain controversial.

TABLE 3A
Results of Studies Evaluating the Relationship between Cytochrome P450 Isozyme Inducers and Lipoprotein Concentration in Children

Study	Methodology	Study Population	Key Findings (data presented as mean ± S.D.)
Results of Studies Showing no Change in Lipoprotein Concentration			
Aynaci (2001)[63]	*Method:* Venous blood samples were obtained after overnight fasting, serum samples stored at -70°C until the time of analyses up to 6 months. Blood samples taken before and at 3, 6, and 12 months of therapy. *Study Design:* Prospective *Assay Used:* Cholesterol oxidase enzymatic method (serum cholesterol concentrations), cholesterol oxidase enzymatic method (HDL-C), Friedewald formula (LDL-C), immunonephelometric methods [plasma apo A1, apo B, Lp (a)]	*Total Number of Patients:* 22 *Mean Age (range) years:* 6.8 ± 0.8 (1-13) *Location of Study:* Trabzon (Turkey) *Type of Epilepsy Diagnosed:* Recently diagnosed epilepsy (atonic, simple partial, and generalized tonic-clonic epileptics) *Drug Studied (dosage):* CBZ (20 mg/kg/day, twice daily) *Mean Duration of Treatment (range) years:* NR	→ Lp (a) between patients at pretreatment, 3 and 6 months → Apo A1 between patients at pretreatment, 3 and 6 months → Apo B between patients at pretreatment, 3 and 6 months → TC between patients at pretreatment, 3 and 6 months → HDL-C between patients at pretreatment, 3 and 6 months → LDL-C between patients at pretreatment, 3 and 6 months
Results of Studies Showing Significant Changes in Lipoprotein Concentration			
Eiris (1995)[16]	*Method:* Blood sample taken after overnight fasting, stored at -40°C until analysis *Study Design:* Cross-sectional *Assay Used:* Colorimetry with automated analyzer (serum TC concentrations), electrophoretic separation and subsequent enzymatic quantification of cholesterol and cholesterol esters associated with each lipoprotein fraction (HDL-C, LDL-C)	*Total Number of Patients:* 119 (49 Female, 70 Male) *Mean Age (range) years:* 10.1 ± 6.8 (NR) *Location of Study:* Galicia (Northwest Spain) *Type of Epilepsy Diagnosed:* Epilepsy *Drug Studied (dosage):* CBZ (NR), PB (NR) *Mean Duration of Treatment (range) years:* 4.3 ± 5.5 (NR)	↑ TC by 21.36 ± 40.3 mg/dL in patient on CBZ compared to control group ($p<0.001$) ↑ TC by 18.3 ± 23.8 mg/dL in patients on PB compared to the control group ($p<0.05$) ↑ HDL-C by 8.65 ± 22.5 mg/dL in patient on CBZ compared to control group ($p<0.001$) ↑ HDL-C by 7.85 ± 24.6 mg/dL in patients on PB compared to the control group ($p<0.05$) ↑ LDL-C by 10.6 ± 37.7 mg/dL in patient on CBZ compared to control group ($p<0.05$) → LDL-C between patients on PB and control group

Sozuer (1997)[15]	*Method:* Venous blood samples were obtained after 12h fasting *Study Design:* Cross-sectional *Assay Used:* Enzyme calorimetric (end point) assay (serum TC and HDL-C concentration), Friedewald formula (LDL-C concentration), radial immunodiffusion (apo A1 and apo B concentration)	*Total Number of Patients:* 39 (22 Female, 17 Male) *Mean Age (range) years:* 11.05 (2-17) *Location of Study:* Istanbul (Turkey) *Type of Epilepsy Diagnosed:* NR *Drug Studied (dosage):* CBZ (25.5 mg/kg/day) *Mean Duration of Treatment (range) years:* 1.46 (0.25-5)	→ Apo A1 between patients receiving CBZ, VPA and control group → Apo B between patients receiving CBZ, VPA and control group ↑ TC in patients receiving CBZ compared to those receiving VPA and control group ($p<0.01$) → HDL-C between patients receiving CBZ, VPA and control group ↑ LDL-C in patients receiving CBZ compared to those receiving VPA and control group ($p<0.005$)
Verrotti (1998)[66]	*Method:* Blood samples were obtained after fasting and before first drug dose of day, evaluated before beginning of AED therapy, after at least 3.5 years and again at 1-1.67 years after end of therapy *Study Design:* Prospective *Assay Used:* Enzymatic methods with analyzer (serum lipid and cholesterol concentrations), two-site radioimmunometric assay [serum Lp (a)], Friedewald formula (LDL-C concentration)	*Total Number of Patients:* 54 (27 Female, 27 Male) *Mean Age (range) years:* 15.17 ± 6.6 1(NR) *Location of Study:* Siena (Italy) *Type of Epilepsy Diagnosed:* Epilepsy *Drug Studied (dosage):* CBZ (16.7 ± 8.2 mg/kg), PB (3.2 ± 0.5 mg/kg) *Mean Duration of Treatment (range) years:* NR (≥3.5)	→ between control and epileptic children before start of therapy → Lp (a) between patients on CBZ or PB and control group → Apo A1 between patients on CBZ or PB and control group → Apo B between patients on CBZ or PB and control group ↑ TC by 1.18 ± 0.78 mmol/L in patient on CBZ compared to control group ($p<0.05$) ↑ TC by 1.11 ± 1.46 mmol/L in patients on PB compared to control group ($p<0.01$) ↑ HDL-C by 0.82 ± 0.64 mmol/L in patients on CBZ compared to control group ($p<0.01$) → HDL-C between patients on PB and control group ↑ LDL-C by 0.31 ± 0.86 mmol/L in patients on CBZ compared to control group ($p<0.01$) ↑ LDL-C by 0.28 ± 1.88 mmol/L in patients on PB compared to control group ($p<0.01$) After end of therapy, serum levels of lipids and lipoproteins had completely returned to normal
Eiris (2000)[14]	*Method:* Blood samples were obtained after overnight fasting and stored at -40°C until analysis *Study Design:* Cross-sectional	*Total Number of Patients:* 320 *Mean Age (range) years:* 9.5 ± 5.6 (NR) *Location of Study:* Galicia (Northwest Spain) *Type of Epilepsy Diagnosed:* Epilepsy *Drug Studied (dosage):* CBZ (NR), PB (NR) *Mean Duration of Treatment (range) years:* 4.7	↓ Apo A1 by 7.6 ± 26.9 mg/dL in patients on CBZ compared to control group ($p<0.01$) ↓ Apo A1 by 13.2 ± 28.3 mg/dL in patients on PB compared to control group ($p<0.05$) ↓ Apo B by 9.9 ± 16.1 mg/dL in patients on CBZ compared to control group ($p<0.005$)

	Assay Used: Colorimetry with automated analyzer (serum TC concentrations), electrophoretic separation and subsequent enzymatic quantification of cholesterol and cholesterol esters associated with each lipoprotein fraction (HDL and LDL concentrations), immunologic-turbidimetric measure with automated analyzer (apolipoprotein concentrations)	± 5.1 (NR)	→ Apo B between patients on PB and control group ↑ TC by 17.5 ± 38.7 mg/dL in patients on CBZ compared to control group (p<0.005) ↑ TC by 17.4 ± 33.2 mg/dL in patients on PB compared to control group (p<0.005) ↑ HDL-C by 7.4 ± 21.8 mg/dL in patients on CBZ compared to control group (p<0.005) → HDL-C between patients on PB and control group ↑ LDL-C by 10.1 ± 36.4 mg/dL in patients on CBZ compared to control group (p<0.005) ↑ LDL-C by 15.1 ± 38.2 mg/dL in patient compared to control group (p<0.005)
Aynaci (2001)[63]	*Method:* Venous blood samples were obtained after overnight fasting, serum samples stored at -70°C until the time of analyses up to 6 months. Blood samples taken before and at 3, 6, and 12 months of therapy. *Study Design:* Prospective *Assay Used:* Cholesterol oxidase enzymatic method (serum cholesterol concentrations), cholesterol oxidase enzymatic method (HDL-C), Friedewald formula (LDL-C), immunonephelometric methods [plasma apo A1, apo B, Lp (a)]	*Total Number of Patients:* 22 *Mean Age (range) years:* 6.8 ± 0.8 (1-13) *Location of Study:* Trabzon (Turkey) *Type of Epilepsy Diagnosed:* Recently diagnosed epilepsy (atonic, simple partial, and generalized tonic-clonic epileptics) *Drug Studied (dosage):* PB (5 mg/kg/day, twice daily) *Mean Duration of Treatment (range) years:* NR	↑ Lp (a) in patients at 3 and 6 months compared to pretreatment (p<0.025) → Apo A1 between patients at pretreatment, 3 and 6 months → Apo B between patients at pretreatment, 3 and 6 months → TC between patients at pretreatment, 3 and 6 months ↑ HDL-C in patients at 6 months compared to pretreatment (p<0.025) → LDL-C between patients at pretreatment, 3 and 6 months
Tumer (2002)[12]	*Method:* Blood samples were obtained after 12h fasting *Study Design:* Cross-sectional *Assay Used:* HPLC and fluorescence detection (plasma Hcy concentrations),	*Total Number of Patients:* 111 (49 Female, 62 Male) *Mean Age (range) years:* 10.28 ± 4.51 (NR) *Location of Study:* Ankara (Turkey) *Type of Epilepsy Diagnosed:* Epilepsy *Drug Studied (dosage):* CBZ (15-20 mg/kg), PB (5-10 mg/kg)	↑ Lp (a) by 1.38 ± 3.21 mg/dL in patient compared to control group (p<0.05)

	competitive protein binding chemiluminescence assay (folic acid and vitamin B12 concentrations)	*Mean Duration of Treatment (range) years:* 3.08 ± 1.04 (NR)	
Sonmez (2006)[65]	*Method:* Venous blood samples were obtained after overnight fasting, serum samples stored at -70°C until the time of analyses up to 12 months. Blood samples taken before and at 3, 6, and 12 months of therapy. *Study Design:* Prospective *Assay Used:* Cholesterol oxidase enzymatic method (serum cholesterol levels), cholesterol oxidase enzymatic method (HDL-C), Friedewald formula (LDL-C), immunonephelometric methods (apo A1, apo B), tint Elize Lipoprotein (a) kit by antigen-antibody reaction [serum Lp (a)]	*Total Number of Patients:* 72 *Mean Age (range) years:* 6.9 ± 4.8 (1-13) *Location of Study:* Trabzon (Turkey) *Type of Epilepsy Diagnosed:* History of new-onset seizure and history of epilepsy. Generalized tonic-clonic, myoclonic, atonic, and simple and complex partial seizures. *Drug Studied (dosage):* CBZ (20 mg/kg/day, twice daily), PB (5 mg/kg/day, twice daily) *Mean Duration of Treatment (range) years:* NR	↑ Lp (a) by 8.1 ± 27.5 mg/dL, 13.7 ± 29.9 mg/dL, and 25.8 ± 35.3 mg/dL in patients on CBZ at 3, 6 and 12 months, respectively, compared to pretreatment (p<0.05) ↑ Lp (a) by 4.1 ± 17.0 mg/dL, 12.3 ± 21.1 mg/dL, and 19.4 ± 22.7 mg/dL in patients on PB at 3, 6 and 12 months, respectively, compared to pretreatment (p<0.05) → Apo A between patients on CBZ at pretreatment, 3, 6 and 12 months ↑ Apo A by 33.1 ± 28.9 mg/dL, 28.9 ± 32.6 mg/dL, and 35.0 ± 31.5 mg/dL in patients on PB at 3, 6 and 12 months, respectively, compared to pretreatment (p<0.05) → Apo B between patients on CBZ or PB at pretreatment, 3, 6 and 12 months → TC between patients on CBZ at pretreatment, 3, 6 and 12 months ↑ TC by 24.5 ± 54.6 mg/dL, 21.0 ± 46.3 mg/dL, and 20.6 ± 44.0 mg/dL in patients on PB at 3, 6 and 12 months, respectively, compared to pretreatment (p<0.05) → HDL-C between patients on CBZ at pretreatment, 3, 6 and 12 months ↑ HDL-C by 8.1 ± 15.4 mg/dL, 11.7 ± 14.7 mg/dL, and 9.1 ± 18.0 mg/dL in patients on PB at 3, 6 and 12 months, respectively, compared to pretreatment (p<0.05) → LDL-C between patients on CBZ at pretreatment, 3, 6 and 12 months ↑ LDL-C by 17.9 ± 47.9 mg/dL, 14.3 ± 39.3 mg/dL, and 14.9 ± 38.9 mg/dL in patients on PB at 3, 6 and 12 months, respectively, compared to pretreatment (p<0.05)

Voudris (2006)[13]	*Method:* Blood samples were obtained after 12h fasting. Done before onset of monotherapy, after 6, 12 and 24 months of treatment. *Study Design:* Prospective *Assay Used:* Immunoephelometric method [serum Lp (a), apo A1, apo B concentrations], enzymatic colorimetric methods (serum TC and HDL-C concentrations), Friedewald formula (LDL-C concentration)	*Total Number of Patients:* 48 (22 Female, 26 Male) *Mean Age (range) years:* 8.95 ± 5.01 (2-15) *Location of Study:* Athens (Greece) *Type of Epilepsy Diagnosed:* Epilepsy *Drug Studied (dosage):* CBZ (NR) *Mean Duration of Treatment (range) years:* NR (≤2 years)	↑ Lp (a) by 5.43 ± 11.74 mg/dL, 6.30 ± 12.44 mg/dL, and 5.1 ± 12.06 mg/dL in patients at 6, 12, and 24 months, respectively compared to pretreatment (p=0.002, 0.002, 0.014) ↑ Apo A1 by 17.89 ± 34.82 mg/dL, and 16.83 ± 37.00 mg/dL in patients at 12, and 24 months, respectively compared to pretreatment (p=0.01, 0.017) ↑ Apo B by 14.5 ± 34.85 mg/dL, 11.50 ± 33.09 mg/dL, and 6.78 ± 29.38 mg/dL in patients at 6, 12, and 24 months, respectively compared to pretreatment (p=0.004, 0.004, 0.022) ↑ TC by 24.33 ± 60.83 mg/dL, 29.05 ± 59.39 mg/dL, and 16.94 ± 50.35 mg/dL in patients at 6, 12, and 24 months, respectively compared to pretreatment (p=0.012, 0.001, 0.014) ↑ HDL-C by 8.50 ± 25.99 mg/dL, and 2.72 ± 24.46 mg/dL in patients at 12, and 24 months, respectively compared to pretreatment (p=0.002, 0.047) ↑ LDL-C by 21.72 ± 52.44 mg/dL, and 21.05 ± 51.63 mg/dL in patients at 6, and 12 months, respectively compared to pretreatment (p=0.004, 0.004)
Tan (2009)[56]	*Method:* Blood samples collected after overnight fasting, centrifuged and analyzed immediately *Study Design:* Cross-sectional *Assay Used:* FPIA (plasma Hcy concentration), radioassay kit (serum folic acid)	*Total Number of Patients:* 195 (94 Female, 101 Male) *Mean Age (range) years:* 36.0 ± 11.3 (18-65) *Location of Study:* Kaohsiung (Taiwan) *Type of Epilepsy Diagnosed:* Idiopathic, cryptogenic, generalize, partial epilepsy *Drug Studied (dosage):* CBZ (400-1,800 mg/kg/day), PHT (200-400 mg/kg/day), PB (90-240 mg/kg/day) *Mean Duration of Treatment (range) years:* 18.1 ± 10.1 (2-39)	↑ TC by 0.35 ± 1.38 mmol/L in patients compared to control group (p=0.001) → HDL-C between patients and control group (p=0.123) → LDL-C between patients and control group (p=0.241)

↑ = increase; ↓ = decrease; → = no difference; AED = antiepileptic drug; Apo A1 = apolipoprotein A1; Apo B = apolipoprotein B; CBZ = carbamazepine; HDL-C = high-density lipoprotein cholesterol; HPLC = high-performance liquid chromatography; LDL-C = low-density lipoprotein cholesterol; Lp (a) = lipoprotein (a); NR = not reported; PB = phenobarbital; PHT = phenytoin TC = total cholesterol; VPA = valproic acid
P values < 0.05 considered statistically significant

TABLE 3B
Results of Studies Evaluating the Relationship between Cytochrome P450 Isozyme Inhibitors and Lipoprotein Concentration in Children

Study	Methodology	Study Population	Key Findings (data presented as mean ± S.D.)
Results of Studies Showing no Change in Lipoprotein Concentration			
Sozuer (1997)[15]	*Method:* Venous blood samples were obtained after 12h fasting *Study Design:* Cross-sectional *Assay Used:* Enzyme calorimetric (end point) assay (serum TC and HDL-C concentration), Friedewald formula (LDL-C concentration), radial immunodiffusion (apo A1 and apo B concentration)	*Total Number of Patients:* 39 (22 Female, 17 Male) *Mean Age (range) years:* 10.05 (2-17) *Location of Study:* Istanbul (Turkey) *Type of Epilepsy Diagnosed:* NR *Drug Studied (dosage):* VPA (22 mg/kg/day) *Mean Duration of Treatment (range) years:* 1.46 (0.25-5)	→ Apo A1 between patient and control group → Apo B between patient and control group → TC between patient and control group → HDL-C between patient and control group → LDL-C between patient and control group
Aynaci (2001)[63]	*Method:* Venous blood samples were obtained after overnight fasting, serum samples stored at -70°C until the time of analyses up to 6 months. Blood samples taken before and at 3, 6, and 12 months of therapy. *Study Design:* Prospective *Assay Used:* Cholesterol oxidase enzymatic method (serum cholesterol concentrations), cholesterol oxidase enzymatic method (HDL-C), Friedewald formula (LDL-C), immunonephelometric methods [plasma apo A1, apo B, Lp (a)]	*Total Number of Patients:* 22 *Mean Age (range) years:* 6.8 ± 0.8 (1-13) *Location of Study:* Trabzon (Turkey) *Type of Epilepsy Diagnosed:* Recently diagnosed epilepsy (atonic, simple partial, and generalized tonic-clonic epileptics) *Drug Studied (dosage):* VPA (20 mg/kg/day, twice daily) *Mean Duration of Treatment (range) years:* NR	→ Lp (a) between patients at pretreatment, 3 and 6 months → Apo A1 between patients at pretreatment, 3 and 6 months → Apo B between patients at pretreatment, 3 and 6 months → TC between patients at pretreatment, 3 and 6 months → HDL-C between patients at pretreatment, 3 and 6 months → LDL-C between patients at pretreatment, 3 and 6 months

Results of Studies Showing Significant Changes in Lipoprotein Concentration			
Eiris (1995)[16]	*Method:* Blood sample taken after overnight fasting, stored at -40°C until analysis *Study Design:* Cross-sectional *Assay Used:* Colorimetry with automated analyzer (serum TC concentrations), electrophoretic separation and subsequent enzymatic quantification of cholesterol and cholesterol esters associated with each lipoprotein fraction (HDL-C, LDL-C)	*Total Number of Patients:* 119 (49 Female, 70 Male) *Mean Age (range) years:* 10.1 ± 6.8 (NR) *Location of Study:* Galicia (Northwest Spain) *Type of Epilepsy Diagnosed:* Epilepsy *Drug Studied (dosage):* VPA (NR) *Mean Duration of Treatment (range) years:* 4.3 ± 5.5 (NR)	↓ TC by 18.38 ± 32.6 mg/dL in patient compared to control group (p<0.001) → HDL-C between patient and control group ↓ LDL-C by 18.60 ± 28.1 mg/dL in patient compared to control group (p<0.001)
Verrotti (1998)[66]	*Method:* Blood samples were obtained after fasting and before first drug dose of day, evaluated before beginning of AED therapy, after at least 3.5 years and again at 1-1.67 years after end of therapy *Study Design:* Prospective *Assay Used:* Enzymatic methods with analyzer (serum lipid and cholesterol concentrations), two-site radioimmunometric assay [serum Lp (a)], Friedewald formula (LDL-C concentration)	*Total Number of Patients:* 54 (27 Female, 27 Male) *Mean Age (range) years:* 15.17 ± 6.61 (NR) *Location of Study:* Siena (Italy) *Type of Epilepsy Diagnosed:* Epilepsy *Drug Studied (dosage):* VPA (52.7 ± 12.9 mg/kg) *Mean Duration of Treatment (range) years:* NR (≥3.5)	→ between control and epileptic children before start of therapy → Lp (a) in patient compared to control group → Apo A1 in patient compared to control group → Apo B in patient compared to control group → TC in patient compared to control group ↑ HDL-C by 0.62 ± 0.90 mmol/L in patient compared to control group (p<0.05) ↓ LDL-C by 0.28 ± 1.23 mmol/L in patient compared to control group (p<0.05) After end of therapy, serum levels of lipids and lipoproteins had completely returned to normal
Eiris (2000)[14]	*Method:* Blood samples were obtained after overnight fasting and stored at -40°C until analysis *Study Design:* Cross-sectional *Assay Used:* Colorimetry with automated analyzer (serum TC concentrations), electrophoretic separation and subsequent enzymatic quantification of	*Total Number of Patients:* 320 *Mean Age (range) years:* 9.5 ± 5.6 (NR) *Location of Study:* Galicia (Northwest Spain) *Type of Epilepsy Diagnosed:* Epilepsy *Drug Studied (dosage):* VPA (NR) *Mean Duration of Treatment (range) years:* 4.7 ± 5.1 (NR)	↓ Apo A1 by 21 ± 25.0 mg/dL in patient compared to control group (p<0.005) ↓ Apo B by 13.1 ± 17.1 mg/dL in patient compared to control group (p<0.005) ↓ TC by 12.7 ± 36.6 mg/dL in patient compared to control group (p<0.005) → HDL-C between patient and control group ↓ LDL-C by 10.5 ± 33.1 mg/dL in patient compared to control group (p<0.005)

	cholesterol and cholesterol esters associated with each lipoprotein fraction (HDL and LDL concentrations), immunologic-turbidimetric measure with automated analyzer (apolipoprotein concentrations)		
Tumer (2002)[12]	*Method:* Blood samples were obtained after 12h fasting *Study Design:* Cross-sectional *Assay Used:* HPLC and fluorescence detection (plasma Hcy concentrations), competitive protein binding chemiluminescence assay (folic acid and vitamin B12 concentrations)	*Total Number of Patients:* 111 (49 Female, 62 Male) *Mean Age (range) years:* 10.28 ± 4.51 (NR) *Location of Study:* Ankara (Turkey) *Type of Epilepsy Diagnosed:* Epilepsy *Drug Studied (dosage):* VPA (15-30 mg/kg) *Mean Duration of Treatment (range) years:* 3.08 ± 1.04 (NR)	↑ Lp (a) by 1.38 ± 3.21 mg/dL in patient compared to control group ($p<0.05$)
Sonmez (2006)[65]	*Method:* Venous blood samples were obtained after overnight fasting, serum samples stored at -70°C until the time of analyses up to 12 months. Blood samples taken before and at 3, 6, and 12 months of therapy. *Study Design:* Prospective *Assay Used:* Cholesterol oxidase enzymatic method (serum cholesterol levels), cholesterol oxidase enzymatic method (HDL-C), Friedewald formula (LDL-C), immunonephelometric methods (apo A1, apo B), tint Elize Lipoprotein (a) kit by antigen-antibody reaction [serum Lp (a)]	*Total Number of Patients:* 72 *Mean Age (range) years:* 6.9 ± 4.8 (1-13) *Location of Study:* Trabzon (Turkey) *Type of Epilepsy Diagnosed:* History of new-onset seizure and history of epilepsy. Generalized tonic-clonic, myoclonic, atonic, and simple and complex partial seizures. *Drug Studied (dosage):* VPA (20 mg/kg/day, twice daily) *Mean Duration of Treatment (range) years:* NR	↑ Lp (a) by 4.7 ± 15.4 mg/dL, 10.7 ± 17.5 mg/dL, and 17.7 ± 22.4 mg/dL in patients at 3, 6 and 12 months, respectively, compared to pretreatment ($p<0.05$) → Apo A between patients at pretreatment, 3, 6 and 12 months → Apo B between patients at pretreatment, 3, 6 and 12 months → TC between patients at pretreatment, 3, 6 and 12 months → HDL-C between patients at pretreatment, 3, 6 and 12 months → LDL-C between patients at pretreatment, 3, 6 and 12 months

Voudris (2006)[13]	*Method:* Blood samples were obtained after 12h fasting. Done before onset of monotherapy, after 6, 12 and 24 months of treatment. *Study Design:* Prospective *Assay Used:* Immunoephelometric method [serum Lp (a), apo A1, apo B concentrations], enzymatic colorimetric methods (serum TC and HDL-C concentrations), Friedewald formula (LDL-C concentration)	*Total Number of Patients:* 48 (22 Female, 26 Male) *Mean Age (range) years:* 8.95 ± 5.01 (2-15) *Location of Study:* Athens (Greece) *Type of Epilepsy Diagnosed:* Epilepsy *Drug Studied (dosage):* VPA (NR) *Mean Duration of Treatment (range) years:* NR (≤2)	↑ Lp (a) by 2.09 ± 16.61 mg/dL, 2.45 ± 17.12 mg/dL, and 2.65 ± 17.42 mg/dL in patients at 6, 12, and 24 months, respectively compared to pretreatment (p=0.005, 0.040, 0.002) → Apo A1 between patients pretreatment, 6, 12 and 24 months → Apo B between patients pretreatment, 6, 12 and 24 months ↓ TC by 8.23 ± 53.19 mg/dL in patients at 24 months compared to pretreatment (p=0.036) → HDL-C between patients pretreatment, 6, 12 and 24 months → LDL-C between patients pretreatment, 6, 12 and 24 months
Unal (2009)[53]	*Method:* Fasting blood samples obtained and analyzed before onset of therapy and re-evaluated after 9 months or 1 year of therapy *Study Design:* Prospective *Assay Used:* Nephelometric assay (mean Hcy concentration)	*Total Number of Patients:* 21 (9 Female, 12 Male) *Mean Age (range) years:* 7.75 ± 2.21 (1-13) *Location of Study:* Ankara (Turkey) *Type of Epilepsy Diagnosed:* Epilepsy *Drug Studied (dosage):* VPA (NR) *Mean Duration of Treatment (range) years:* 0	↑ Lp (a) by 4.89 ± 44.07 mg/dL after treatment for 9 months to 1 year (p=0.002)
Tan (2009)[56]	*Method:* Blood samples collected after overnight fasting, centrifuged and analyzed immediately *Study Design:* Cross-sectional *Assay Used:* FPIA (plasma Hcy concentration), radioassay kit (serum folic acid)	*Total Number of Patients:* 195 (94 Female, 101 Male) *Mean Age (range) years:* 36.0 ± 11.3 (18-65) *Location of Study:* Kaohsiung (Taiwan) *Type of Epilepsy Diagnosed:* Idiopathic, cryptogenic, generalize, partial epilepsy *Drug Studied (dosage):* VPA (500-2,000 mg/kg/day) *Mean Duration of Treatment (range) years*: 18.1 ± 10.1 (2-39)	↑ TC by 0.35 ± 1.38 mmol/L in patients compared to control group (p=0.001) → HDL-C between patients and control group (p=0.123) → LDL-C between patients and control group (p=0.241)

↑ = increase; ↓ = decrease; → = no difference; AED = antiepileptic drug; Apo A1 = apolipoprotein A1; Apo B = apolipoprotein B; CBZ = carbamazepine; HDL-C = high-density lipoprotein cholesterol; HPLC = high-performance liquid chromatography; LDL-C = low-density lipoprotein cholesterol Lp (a) = lipoprotein (a); NR = not reported; PB = phenobarbital; TC = total cholesterol; VPA = valproic acid
P values < 0.05 considered statistically significant

2.3 Drugs that Inhibit Cytochrome P450 Isozymes

Although VPA has less isozyme-inducing activity than CBZ, it can still impair the intestinal absorption of dietary folic acid[12] and modify lipid parameters.[14] Table 3b shows nine studies conducted in children receiving VPA for treatment of epilepsy. Four of these studies[12,13,53,65] show a significant elevation in lipoprotein (a) concentration in patients receiving VPA when compared to pretreatment and control values, while Aynaci *et al.*[63] and Verrotti *et al.*[66] show no significant difference. Serum apo A1 and apo B concentrations were found to be significantly lower in one study conducted by Eiris *et al.*,[14] while five studies[13,15,63,65,66] showed no significant differences when compared to the pretreatment and control group. When serum TC concentrations were examined in patients receiving VPA monotherapy, three studies[13,15,16] were found to be significantly lower, while four studies[15,63,65,66] showed no significant differences compared to the pretreatment and control group. Finally, serum HDL-C concentrations were found to be significantly higher in one study conducted by Verrotti *et al.*,[66] while the remaining six studies[13-16,63,65] showed no significant differences compared to the pretreatment and control group. Serum LDL-C concentrations were found to be significantly lower in the three studies[14,16,66] while in the remaining four studies,[13,15,63,65] no significant differences emerged in patients receiving VPA monotherapy compared to the pretreatment and control group. In addition, a significant reduction was seen in HDL_2-C/HDL_3-C ratio.[14]

Of the studies conducted above that show no significant difference, it was noted that a slight increase in lipoprotein (a) concentration was observed in patients receiving VPA compared pretreatment and control values, however this was not enough to be considered statistically significant. These insignificant values could possibly be explained by the small patient population in the study conducted by Aynaci *et al.*,[63] or the differences in methodology of assays used to measure lipoprotein (a) concentrations. As for the serum apo A1 and apo B concentrations, the difference between these two findings may be because of the much larger sample size in the study conducted by Eiris *et al.*,[14] causing the statistical analysis to have very different values.

The literature on serum lipoprotein and apolipoprotein concentrations has been conflicting in children receiving AED monotherapy. The general consensus however, is that the isozyme-inducing AEDs (CBZ and PB) often increase the concentrations of serum lipids, whereas isozyme-inhibiting drugs (VPA) decrease or do not appear to significantly alter the concentrations of serum lipids.[13,15] These findings may be indicative of serum lipid status being modified by chronic isozyme-inducing AEDs but not as much by isozyme-inhibiting AED therapy. In addition, when comparing the concentrations of homocysteine, lipoprotein (a), folic acid and vitamin B12 between monotherapy and combined drug treatment groups, no significant differences were found.[12]

2.4 Duration of Treatment

When considering the duration of therapy as an effect on vascular function, the study conducted by Tan *et al.*[56] also examined serum concentrations of TC, HDL-C and LDL-C. Again, these authors reported finding an increase in TC, HDL-C and LDL-C concentrations in the epileptic patients compared to the control group. Furthermore, atherosclerosis was assessed by looking at each patient's common carotid artery intima media thickness (CCA IMT) and was found that the mean CCA IMT was significantly increased in patients with epilepsy. The mean CCA IMT also appeared to be significantly correlated with duration of AED therapy, age, body mass index as well as other vascular risk factors: blood levels of high sensitivity C-reactive protein, and thiobarbituric acid reactive substances or thiols. Therefore, Tan *et al.* concluded that the duration of AED therapy was significantly associated with the progression of vascular diseases, particularly atherosclerosis, in patients with epilepsy.

2.5 Effect of AED Therapy on Vascular Diseases

According to the definition above regarding risk of coronary heart disease, children receiving CBZ or PB seem to be at a higher atherogenic risk than healthy children serving as controls, in contrast to children receiving VPA which do not exhibit an

increased risk.[14,15] In addition, increased concentrations of HDL_2-C/HDL_3-C ratio have been shown to be a powerful protective factor against coronary heart disease, however, children treated with CBZ, PB, or VPA, all showed decreased ratios.[14] The American Academy of Pediatrics[68] has stated that serum LDL-C may also be a good predictor of coronary vascular disease when serum TC concentrations are high. Concentrations of LDL-C within the range of 110-129 mg/dL are defined as "borderline", while concentrations in excess of 130 mg/dL are defined as "high".[68] About 30% of children receiving CBZ or PB were reported to have "high" LDL-C concentrations,[14,15] compared to the 10% of children receiving VPA[14,15] and 10% of controls.[14-16] Therefore, the reduction of LDL-C concentration in childhood may decrease the risk of coronary heart disease.[15]

AED monotherapy in children seem to produce a significant increase in serum lipoprotein (a) concentration[63,65] occurring early in the course of treatment and persisting thereafter.[13,65] It may therefore be useful to measure serum lipoprotein (a) concentrations routinely in children taking these AEDs, as atherosclerosis starts in childhood and progresses with age.[13] Verrotti *et al.*[66] demonstrated that upon completion of AED monotherapy, serum lipid and lipoprotein concentrations had completely returned to normal. This implies that the changes seen in paediatric patients during AED monotherapy are reversible and no permanent modification of lipid metabolism occurs. The cause of the elevation in lipoprotein (a) concentration in children with epilepsy treated with CBZ or VPA monotherapy is unclear, however, possible mechanisms have been proposed including a biochemical effect of AEDs on the synthesis of lipoprotein (a), as well as differences in the lifestyle of epileptic compared to non-epileptic individuals.[11] It is also possible that multiple factors including diet, exercise and genotype to name a few, are involved. Although no significant relationship was found between homocysteine and lipoprotein (a) concentrations, high concentrations of both these risk factors in children taking AEDs suggest that the risk of atherosclerotic cardiovascular disease is higher in these patients.[12]

Genetic Polymorphisms

3.0 Genotype, AED and Relationship to Epilepsy and Birth Defects

Individuals born with epilepsy are likely to have a phenotype that is influenced by the interaction of several genes, as well as non-inherited environmental factors. A few studies have implicated the MTHFR gene as a susceptibility factor for epilepsy.[43,69,70] These authors found that the frequencies of the genotypes in the common C677T mutation in the MTHFR gene were significantly different from a group of healthy controls.[69] More specifically, individuals with epilepsy had increased frequencies of the MTHFR 677TT genotype.[43,69,70]

Epileptic women of childbearing age carrying a polymorphism in the C677T MTHFR gene have the potential to expose their fetus to AEDs during pregnancy, which may lead to malformations.[71] These malformations occur in about 6-14% of children exposed to AEDs, compared to about 3% of children who were not exposed to AEDs, but still had mothers who were epileptic.

Children exposed to AEDs prenatally have an increased risk of suffering from fetal anticonvulsant syndromes (FACS)[45] compared to their siblings who serve as their controls.[72] This association was more prevalent in mothers with the MTHFR 677TT genotype who were more likely to give birth to children with FACS.[45] FACS are specific combinations of major and minor malformations including patterns of congenital malformation, neurodevelopmental impairment, neonatal withdrawal symptoms, and craniofacial dysmorphisms.[45,72,73]

Major congenital malformations occurred in a significantly higher frequency in fetuses exposed to multidrug therapy while minor congenital malformations had significantly higher frequencies in children exposed to CBZ, PB, VPA, PHT or multidrug therapy compared to children of mothers who did not receive AED treatment.[72] The most frequent major congenital malformation was inguinal hernia, most commonly seen after exposure to CBZ.[72] Mothers with the MTHFR 677TT genotype were more likely to give birth to children with major congenital malformations compared to mothers with the MTHFR 677CC genotype.[45] The risk of such congenital malformation affects 5-14% of

children exposed to PB, PHT, CBZ and VPA, but may be higher in those exposed to multiple therapies.[74] Neurodevelopmental impairment was significantly more frequent in children exposed to CBZ, VPA, PHT, or multidrug therapy compared to children of mothers who did not receive AED treatment.[72] These impairments include speech delay, learning, as well as behavioural difficulties.[72,73] The most frequent neurodevelopmental delay was speech delay; most commonly seen after exposure to VPA, CBZ or multidrug therapy.[72] The genotype of the mother, however, does not appear to be a risk factor in giving birth to children with neurodevelopmental delay who have been exposed to AEDs.[45] Neonatal withdrawal symptoms were significantly more frequent in children exposed to VPA, PHT or multidrug therapy compared to children of mothers who did not receive AED treatment.[72] These symptoms included jitteriness and seizures.[72,73] Finally, craniofacial dysmorphisms were significantly more frequent in children exposed to CBZ, VPA or multidrug therapy.[72]

The malformations associated with FACS have been shown to be associated not only with a polymorphism in the C677T MTHFR gene, but also with lower folic acid concentrations in the mother.[71] As mentioned above, MTHFR is a key enzyme in the production of 5-methyltetrahydrofolate, which is required by MS in the remethylation of homocysteine to methionine. The MTHFR 677TT gene interacts with dietary folic acid to cause hypomethylation of DNA in leukocyte in healthy adults.[75-77] Therefore, polymorphisms in the C677T MTHFR gene could inadvertently affect fetal development through hypomethylation of DNA or protein in mother folic acid deficiency states due to AED therapy or dietary reasons.[45]

Although the focus in this section is on the first generation AEDs, it is important to keep in mind that there are other second generation AEDs that may also be associated with birth defects. Topiramate for example, can be used for the treatment of generalized tonic clonic seizures or partial seizures with or without secondary generalization. A recent study found that although women exposing their fetus to topiramate, either as monotherapy or polytherapy, resulted in a live birth, 17.4% of these pregnancies had an abnormality of some sort including major and minor congenital malformations.[78] While there is limited research on topiramate, results from this study are relevant in women with epilepsy of childbearing years. Hence, further investigation is required to determine the mechanism and safety of this drug.

3.1 Genetic Susceptibility Factors Influencing Homocysteine and Folic Acid Concentrations

Variations in genes encoding the enzymes—MTHFR, CBS, and MS—involved in homocysteine metabolism could alter homocysteine concentrations. Kang *et al.*[79] found a thermolabile variant in the MTHFR gene which has been associated with a 50% reduction in the activity of the reductase enzyme. It is the most commonly inherited cause of mild hyperhomocysteinaemia, occurring in 5-10% of the white population[37] and was found in 17% of patients in North America with coronary artery disease in contrast to the 5% found in normal controls.[80] This thermolabile variant is caused by a missense mutation of the basepair 677 where cytosine (C) is transitioned to thymine (T) leading to an amino acid substitution of alanine to valine.[37,81] The three genotypes associated with the thermolabile MTHFR allele are 677TT homozygosity, which is associated with decreased enzyme activity and mild elevations of plasma homocysteine concentrations, 677CT heterozygosity, and 677CC negative homozygosity, which is associated with the wild-type allele.[22]

A few studies have shown that there is no significant difference in the prevalence of each genotype according to age or gender of children.[7,51,82] A study done by Alessio *et al.*[82] revealed that in a population of Brazilian children, 9.5% had the MTHFR 677TT, 44% had the MTHFR 677CT, and 46.5% had the MTHFR 677CC genotype. Furthermore, the frequency for the MTHFR 677TT polymorphism is higher in epileptic patients compared to the controls who did not have epilepsy.[43] More recently however, studies have shown that the prevalence of each genotype is not significantly different when comparing epileptic children receiving AED treatment to a healthy control group.[52,83]

Since 5-methyltetrahydrofolate is a major form of circulating folic acid, the decrease in production is associated with elevated total homocysteine concentration.[43] Sure enough, there is a highly significant association of plasma total

homocysteine concentration related to C677T MTHFR polymorphisms that demonstrate a progressive increase from the MTHFR 677CC, to the MTHFR 677CT to MTHFR 677TT genotype.[22,43] The mean total homocysteine concentration in the MTHFR 677TT genotype is significantly higher in epileptic patients compared to healthy controls[22] and is usually associated with a twofold increase in hyperhomocysteinaemia.[22,42,43] No significant difference was observed in plasma total homocysteine concentration between the MTHFR 677CT and MTHFR 677CC genotype, and the reference group clearly had lower concentrations.[22,82]

In contrast, Alessio *et al.*[82] analyzed the C677T MTHFR polymorphism in isolation and found that the concentrations of homocysteine and folic acid was in the normal range in all infants between the ages of 1-8 years with the MTHFR 677TT genotype and that there was no correlation with homocysteine concentration, even though this particular genotype exhibited slightly lower folic acid concentrations when compared to other groups. From this, they concluded that the C677T MTHFR polymorphism does not seem to be a risk factor for increased plasma homocysteine concentrations during childhood.

In epileptic patients receiving AED treatment, hyperhomocysteinemia was more frequent in the MTHFR 677TT genotype versus MTHFR 677CT or MTHFR 677CC genotypes.[43,52] Ono *et al.*[83] showed that in patients receiving multidrug therapy with the MHTFR 677TT genotype, concentration of homocysteine was significantly higher than in patients receiving monotherapy with the MTHFR 677CT or MTHFR 677CC genotype. Patients receiving PHT[43] or CBZ with the MTHFR 677TT genotype, showed higher concentrations of homocysteine compared to the control group[43,52] and patients receiving VPA.[43] Patients receiving VPA on the other hand again had controversial findings. A couple of studies[42,52] reported hyperhomocysteinemia to be most frequent in individuals with the MTHFR 677TT genotype, while others[7,43] reported no significant differences among the three genotypes with reference to plasma homocysteine concentrations, and hence, had no significant contribution to hyperhomocysteinemia. If both CBZ and VPA treated patients have hyperhomocysteinaemia more frequently in the MTHFR 677TT genotype, then it would seem that either drug would have an effect on the prevalence of hyperhomocysteinaemia related to that genotype.[52]

Folic acid concentrations on the other hand, show a progressive increase from the MTHFR 677TT, to MTHFR 677CT to MTHFR 677CC genotype.[7,9,52,82] However, Vurucu *et al.*[7] found there were no significant differences among the three genotypes regarding serum folic acid and vitamin B12 concentrations. Ono *et al.*[83] showed that in patients receiving multidrug therapy with the MTHFR 677TT genotype, occurrence of folic acid deficiency was significantly higher than patients receiving monotherapy with the MTHFR 677CT or MTHFR 677CC genotype. Patients with the MTHFR 677TT genotype receiving PHT[43] or CBZ showed lower concentrations of folic acid compared to the control group and patients receiving VPA.[43] Therefore, both AEDs and the MTHFR 677TT genotype appear to contribute to hyperhomocysteinemia and folic acid deficiency.[42,43,52]

The prevalence of cardiovascular disease between patients with the various genotypes for the C677T MTHFR polymorphism has been reported by Prengler *et al.*[22] to have no significant difference even though the thermolabile form of the MTHFR was more common among cardiovascular disease patients than among control subjects. The MTHFR 677TT genotype may be a genetic risk factor for cerebrovascular diseases, transient ischemic attack (TIA), and stroke in children[22] due to the significantly higher concentrations of plasma total homocysteine.[81] In the study conducted by Prangler *et al.*[22] of a paediatric population with stroke or TIA and cerebrovascular disease, 14% had the MTHFR 677TT genotype and in a paediatric population with ischaemic stroke or TIA, 19% had the MTHFR 677TT genotype compared with 12% in the reference population.[22] In the same sample, elevated total plasma homocysteine concentrations were observed compared to those who had the MTHFR 677CT or MTHFR 677CC genotype and those in the control group.[22] It is possible that the homozygous genotype for the C677T MTHFR polymorphism is associated with a tendency to increase the concentrations of homocysteine under certain circumstances, that is, AEDs, resulting in damage to the endothelium and leading to cerebrovascular diseases and focal ischaemia.[22]

Interventions to Reduce the Risk of Vascular Disease

4.0 Intervention with Folic Acid

Many studies, as indicated above, have demonstrated that patients taking AEDs show a significant inverse correlation between plasma homocysteine and folic acid concentrations.[5,17,32,42,43,51,52] This relationship lead to the idea that perhaps intervention with folic acid may counter the effects of AEDs on the concentration of homocysteine in patients. This was indeed confirmed by studies showing that intervention with folic acid resulted in significantly higher folic acid concentrations (much higher than the normal range), and lower total homocysteine concentrations (concentrations the same as, or less than those of the control subjects) in children being treated with AEDs[8,29,40,43] at weeks six and 12 compared with patients receiving a placebo.[40]

Folic acid, folic acid combined with vitamin B12, B6, and vitamin B12 with vitamin B6 have all been shown to reduce homocysteine concentrations back to normal within four to six weeks after the initiation of therapy, but may occur in as little as two weeks.[36] Huemer *et al.*[40] found that children with hyperhomocysteinemia treated with AED therapy had their plasma homocysteine concentrations return to normal after a three month oral supplementation of folic acid.

Other studies have shown that vitamin B12 and folic acid supplementation reduced the risk of cardiovascular disease and prevention of stroke by decreasing homocysteine concentrations,[17,19] especially for children with extremely high concentrations of homocysteine. It was also reported, however, that treatment with vitamin supplementation was associated with improvement, but not normalization of endothelial function, suggesting that hyperhomocysteinemia induced endothelial dysfunction might not be entirely reversible.[84] A study done by Jacques *et al.*[85] found that homocysteine concentration was lowered in 7% of the general population and almost 50% of patients with hyperhomocysteinemia who took folic acid through foodstuff such as leafy vegetables, certain cereals and fruits. This is a relatively inexpensive, natural, and reliable method of increasing folic acid intake and reducing homocysteine concentrations that physicians can take into consideration when prescribing AEDs to patients to prevent cardiovascular diseases.

In 1988, the US Food and Drug Administration decided to enrich grain products including cereal, flour, rice, pasta, etc., with folic acid and recommended an increase in dietary folic acid intake in adults by approximately 100 µg/day as a preventative measure for neural tube defect as well as preventing hyperhomocysteinemia in the general population.[26,44] As mentioned above, the malformations associated with FACS have also been linked to lower concentrations of folic acid in mothers, therefore, the American Academy of Neurology recommended that all women of childbearing potential who are taking AEDs consume at least 0.4 mg/day of folic acid.[26] Studies have shown, however, that a low dose supplementation of folic acid did not prevent malformations associated with AED therapy,[86,87] even when taken during the first trimester.[88,89]

Administration of folic acid is also recommended in epileptic patients with hyperhomocysteinemia due to folic acid deficiency to prevent vascular disease.[8] The recommended dosage of folic acid supplementation in children nonetheless has yet to be defined,[33] but should be given regularly for the duration of the AED therapy.[40]

4.1 Switching AEDs from Inducers to Non-inducers of Cytochrome P450 Isozymes

As mentioned above, both CBZ and PHT are potent inducers of cytochrome P450 isozymes. The inducing properties of both CBZ and PHT on the cytochrome P450 enzyme system are likely the means of toxicity toward the progression of vascular disease. These isozyme inducers are known to affect several important aspects of the metabolism of drugs, steroids, vitamin D, and cholesterol.[90] CBZ has been shown to significantly increase the concentration of various risk factors for vascular disease including homocysteine,[5,12,42,51,52] lipoprotein (a),[12,13,65] TC,[13-15,66] HDL-C,[13,14,66] and LDL-C.[13-15,66]

Interestingly, a recent study conducted by Mintzer *et al.*[91] suggested that the effects of CBZ or PHT, could possibly be reversed by switching to one of the second generation AEDs: lamotrigine (LTG) or levtiracetam (LEV), which are non-inducers of the cytochrome P450 isozymes. They found that both the healthy

control group and the patients who switched from CBZ to either LTG or LEV showed no change in homocysteine concentration, however, patients who switched from PHT to either LTG or LEV showed a mean model-predicted decline in homocysteine concentration. In contrast, patients who switched from CBZ to either LTG or LEV showed a mean decline of about one third in lipoprotein (a) concentration, however, both the healthy control group and patients who switched from PHT to either LTG or LEV showed no change in lipoprotein (a) concentration. As for the remaining lipids, upon switching from either CBZ or PHT to LTG or LEV resulted in a decrease in TC, HDL-C and LDL-C concentrations. Because this study was conducted within six weeks time and consisted of a small sample size, only TC and HDL-C showed a significant decline when switched from CBZ or PHT to LTG or LEV and CBZ to LTG or LEV, respectively.

Additional studies that are better designed with larger sample sizes are needed to assess the long-term effects of switching AEDs. The implications of this study suggest the potential benefits of the second generation AEDs which are without the isozyme-inducing property.

Limitations and Future Outlook

The limitations of the studies described above may contribute to the sometimes confusing results. Some of these limitations might be related to the patients and methods used.

A sample size calculation should always be done to determine the appropriate number of patients needed in any given study. None of the studies above included such a calculation, which raises issues with respect to validity and reliability of the results; especially in the case of small sample sizes. These sample sizes should carry enough statistical power to avoid statistically erroneous conclusions. Two studies examined above, one conducted by Kurul *et al.*[6] and the other by Aynaci *et al.*,[63] had relatively small patient sample sizes of 25 and 22, respectively. The results from these studies did not show any statistical significance, but these results could be the underlying pilot study used to determine the numbers needed for a more definitive study. In addition, studies should consist of a sample size that is large enough to be representative of the majority of children who have epilepsy within a particular population. Therefore, it is important that both inclusion and exclusion criteria be defined prior to the start of the study. Samples of smaller sizes are often homogenous and consequently, having a small sample would skew the results of what is observed in the end.

The participants selected vary according to their geographical area and ethnic background. These factors may affect the lifestyles of patients including their dietary habits, which simultaneously determine concentrations of homocysteine and vitamins, and physical exercise, which may lead to an increased or decreased risk of developing cardiovascular diseases, and thus altering the final results observed in a study. An important variable that should be considered in the selection of participants for the above studies is the duration of anticonvulsant therapy treatment prior to the actual study, as well as dosage of drug. Patients who were exposed to treatment for a longer period of time or receiving larger dosages may show exaggerated effects on homocysteine concentrations compared to patients who were exposed to treatment for a shorter period of time.

Study methodology including which design, assay and statistical analysis used in studies may significantly influence end results. The majority of the studies looked at above were cross-sectional or prospective. A major disadvantage of the cross-sectional study design is the difficulties in distinguishing between cause and effect versus association. The benefit of longitudinal, prospective studies looking at patients before the start of AED therapy suggest that it is the AED they are receiving rather than the epilepsy itself or other situations (genetic abnormalities or metabolism of homocysteine) which cause changes in homocysteine[5,51,54] and lipoprotein concentrations.[13,63,65,66] The studies above examining homocysteine and lipoprotein concentrations used relatively similar and comparable assays. The predominate assay used to measure plasma total homocysteine concentration was the use of high-performance liquid chromatography (HPLC) and fluorescence detection,[5,7,12,51,52] although automated fluorescence polarization immunoassay (FPIA)[40] and enzyme-linked immunosorbent assay (ELISA)[42] were also used. A study done by Zighetti *et al.*[92] in 2002, compared the HPLC and FPIA methods in measuring plasma total homocysteine concentrations and found that the novel FPIA

method compares well with the established HPLC method. In addition, there is a good correlation between total homocysteine measurement obtained with both FPIA and HPLC (R^2 = 0.969, p=0.001).

As noted above, little research has been conducted in children with epileptic seizures; therefore, further studies involving more patients in the paediatric age group and of different ethnic groups are needed to confirm the above results. Although it is known that children receiving AEDs based on a short-term period, that is, a 20-week period, have demonstrated significant increases in plasma total homocysteine,[51] clarification is needed to determine exactly how early this increase will occur. Further studies are also required to determine the precise nature of anticonvulsant therapy (monotherapy, drug type and versus polytherapy) that leads to an increase in plasma total homocysteine and lipoprotein concentrations. Lastly, additional research is necessary to evaluate the long-term effects of folic acid and multivitamin supplementation in patients taking anticonvulsant drugs with regards to cardiovascular disease.

CONCLUSIONS

Numerous studies have demonstrated that children who have epileptic seizures and are undergoing anticonvulsant therapy seem to have altered concentrations of homocysteine, vitamins, and lipoprotein (Table 4). This is likely a result of AEDs interfering with the metabolism of homocysteine, vitamins, folic acid, lipids, bile acids, and other endogenous molecules. In particular, isozyme-inducing AEDs such as CBZ, PB and PHT, have been shown to increase plasma homocysteine and serum lipid concentrations while decreasing serum folic acid concentrations. Similarly, isozyme-inhibiting AEDs such as VPA, have also been shown to increase plasma homocysteine, but either decrease or not alter serum lipid concentrations.

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TABLE 4 Summary of the Effects of AEDs on Possible Biomarkers for Vascular Diseases in Children

Biomarkers	Antiepileptic Drug			
	Carbamazepine	Oxcarbazepine	Phenobarbital	Valproic acid
Homocysteine	↑: 12, 42, 51, 52 →: 5, 6, 7		↑: 12	↑: 7, 12, 42, 51, 52 →: 6, 53
Folic Acid	↓: 5, 7, 42, 51, 52 →: 6, 12	→: 6	→: 12	↑: 51 ↓: 5, 12, 42, 52 →: 6, 7, 12
Vitamin B12	↓: 42 →: 5, 6, 7, 12, 51, 52	→: 6	→: 12	↑: 7, 51, 52 →: 5, 6, 12 42 ,
Vitamin B6	↓: 5, 51, 52			↓: 5, 52 →: 51
Lipoprotein (a)	↑: 12, 13, 65 →: 63, 66		↑: 12, 63, 65 →: 66	↑: 12, 13, 65 →: 63, 66
Apolipoprotein A1	↑: 13 ↓: 14 →: 15, 63, 65, 66		↑: 65 ↓: 14 →: 63, 66	↓: 14 →: 13, 15, 63, 65, 66
Apolipoprotein B	↑: 13 ↓: 14 →: 15, 63, 65, 66		→: 14, 63, 65, 66	↓: 14 →: 13, 15, 63, 65, 66
Total Cholesterol	↑: 13, 14, 15, 16, 66 →: 63, 65		↑: 14, 16, 65, 66 →: 63	↓: 13, 14, 16 →: 15, 63, 65, 66
High-Density Lipoprotein Cholesterol	↑: 13, 14, 16, 66 →: 15, 63, 65		↑: 16, 63, 65 →: 14, 66	↑: 66 →: 13, 14, 15, 16, 63, 65
Low-Density Lipoprotein Cholesterol	↑: 13, 14, 15, 16, 66 →: 63, 65		↑: 14, 65, 66 →: 16, 63	↓: 14, 66 →: 13, 14, 15, 16, 63, 65

↑ = significant increase; ↓ = significant decrease; → = no difference

In addition to epileptic children receiving AED therapy, individuals who have the MTHFR 677TT genotype polymorphism also appear to have increased concentrations of homocysteine and decreased concentrations of folic acid. The elevated concentration of homocysteine may make this MTHFR 677TT genotype a genetic risk factor for cerebrovascular diseases, TIA, and stroke in children.

These elevated concentrations of both homocysteine and lipoprotein, whether they are caused by the chronic use of AEDs or polymorphisms, have been associated with increased risk of developing cardiovascular diseases, via endothelial dysfunction. The biological significance of homocysteine and folic acid deficiency can be profound in terms of altering methylation patterns. Methylation controls gene transcription and long-term epigenetic effects are likely given the chronic use of AEDs; research into these areas need to occur at the bedside and the bench.

Several studies investigated various interventions to reduce the risk of vascular disease. Supplementation with folic acid and multivitamins has shown to increase folic acid concentrations and decrease homocysteine concentrations, thereby preventing cardiovascular diseases. Switching AEDs from a cytochrome P450 isozyme inducer to a non-inducer has also shown to reverse the negative effects associated with the isozyme inducing AEDs. It is therefore important to measure and monitor both plasma homocysteine and serum lipoprotein concentrations as these are risk factors that can potentially be modifiable. As well, they can potentially serve as useful biochemical markers in identifying and perhaps preventing cardiovascular diseases from developing later in life.

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