The effect of several antiepileptic treatments on the fatty acid metabolism of Sprague-Dawley rats

Adrienne Ubbink

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Supervisor:	Dr. G. Terre'Blanche
Co-Supervisor	Prof. L.J. Mienie
Assistant Supervisor:	Prof. J.J. Bergh

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List of abbreviations

3-HB	3-Hydroxy butyrate
3-HB-CAR	3-Hydroxy butyrate carnitine
A	Adenosine
AA	Arachidonic acid
AC	Adenylate cyclase
ACA	Aminoacetic acid
ACS	Acyl-CoA synthetase
ADHD	Attention deficit hyperactivity disorder
ADP	Adenosine diphosphate
AKD	α-ketoglurate dehydrogenase
ALC	Acetyl-L-carnitine
AMDIS	Automated Mass Spectral Deconvolution and Identification System
AMPA	α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate
ATPase	Adenosine triphosphatase
ATP	Adenosine 5'-triphosphate
BDNF	Brain-derived neurotrophic factor
BHB	β-hydroxybutyric acid
BSA	Body surface area
BSTFA	*N,O*-Bis(trimethylsilyl)trifluoroacetamide
CACT	Carnitine acylcarnitine translocase
cAMP	Adenosine 3',5'-cyclic monophosphate
CAR	Carnosine
CBM	Carbamazepine
CoA	Coenzyme A
CPT	Carnitine pamtoyltransferase
CT	Carnitine/acylcarnitine translocase
CU	Carnitine uptake
DCFA	Dicarboxylic fatty acid
DCFAC	Dicarboxylic fatty acylcarnitine
DHA	Docosahexaenoic acid
DMSO	Dimethyl sulfoxide

EDTA	Ethylenediaminetetraacetic acid
EPA	Eicosapentaenoic acid
FFA	Free fatty acids
GABA	Gamma-aminobutyric acid
GABA-T	GABA transaminase
GAD	Glutamic acid decarboxylase
GAT-1	GABA-transport
GC/MS	Gas chromatography-mass spectrometry
GEPR	Genetically epilepsy prone rats
GHB	γ-hydroxybutyrate
GLUT	Glucose transporter
GOT	Glutamic-oxaloacetic transaminase
H	Histamine
HDC	Histidine decarboxylase
HDC-KO	HDC knockout
HED	Human equivalent dose
IS	Internal standard
K	Kainate
K_{ATP}	ATP-sensitive potassium
KD	Ketogenic diet
LACS	Long acyl-CoA synthetase
LCFA	Long chain fatty acids
LCFA-CoA	Long chain fatty acyl-CoA
LCFAC	Long chain fatty acylcarnitine
LC-MS/MS	Liquid Chromatography/Mass Spectrometry/Mass Spectrometry
MCFA	Medium chain fatty acids
MCFA-CoA	Medium chain fatty acyl-CoA
MCFAC	Medium chain fatty acylcarnitine

NMDA	N-methyl-D-aspartate
PCr:Cr	Phosphor-creatine:creatine
PPARα	Peroxisome proliferator-activated receptor-α
PTZ	Pentylenetetrazol
PUFA	Polyunsaturated fatty acid
ROS	Reactive oxygen species
SCS	Succinyl CoA synthetase
SRF	Reduce sustained repetitive firing
SSA	Succinate semi-aldehyde
SSA-DH	Succinate semi-aldehyde dehydrogenase
SSAR	Succinate semi-aldehyde reductase
TCA	Tricarboxylic acid
TMCS	Trimethylchlorosilane
UCP	Uncoupling proteins
VPA	Valproate

Uittreksel

Antiepileptiese behandeling sluit 'n wye reeks geneesmiddels en dieetaanvullings in. Vir die effektiewe behandeling van epilepsie is 'n begrip van die meganismes wat by die onderskeie behandelings betrokke is belangrik. Talle strukture en prosesse is betrokke by die ontstaan van 'n epileptiese aanval, naamlik neurone, ioonkanale, reseptore, glia as ook inhiberende en eksiterende sinapse. Antiepileptiese geneesmiddels is ontwikkel om hierdie funksies sodanig te wysig dat daar eerder remming (inhibisie) as eksitasie plaasvind sodat epileptiese aanvalle voorkom word. Valproaat en karbamasepien is twee algemene geneesmiddels wat gebruik word vir die behandeling van epilepsie, alhoewel hulle chemiese strukture van mekaar verskil. Asetiel-L-karnitien is 'n dieetaanvulling wat vetsuur transport na die mitochondria bemiddel. Karnosien is 'n potensiële antiepileptiese geneesmiddel wat epileptiese aanvalle kan onderdruk deur die histaminergise sisteem. Die ketogeniese dieet is 'n behandeling wat algemeen gebruik word in weerstandige epilepsie.

Krüger (2006), het aangetoon dat daar 'n toename in plasma omega-3 vetsuurvlakke is in epileptiese patiënte wat behandel word met karbamasepien of valproaat. Die hipotese het ontstaan dat die reedsgenoemde geneesmiddels die effek van die ketogeniese dieet op vetsuurmetabolisme kan naboots.

Die studie wat in hierdie verhandeling gerapporteer word, is ontwerp om die effek van verskillende antiepileptiese behandelings op vetsuurmetabolisme te meet, soos bepaal deur urienuitskeinding van asielkarnitiene, glisienkonjugate en glisien. Ses groepe manlike Sprague Dawley rotte (n = 10, vir elke groep) is onderskeidelik met valproaat, karbamasepien, karnosien, asetiel-L-karnitien en 'n ketogeniese dieet behandel. Die sesde groep het as kontrole gedien. Die rotte is vir 28 dae behandel waarna hulle onthoof is. Bloed- en urinemonsters is versamel. Die glisienkonjugate en glisien is met behulp van gaschromatografie-massa-spektrometrie (GC/MS) bepaal en die asielkarnitiene deur middel van isotoopverdunnings-tandem-massa-spektrometrie (LC-MS/MS).

Die ketogeniese dieet het 'n stadiger toename in liggamsmassa getoon, asook betekenisvolle toenames in die urienuitskeidings van langkettingasielkarnitien ($p < 0.001$), dikarboksielasielkarnitine ($p < 0.001$), asetielkarnitien ($p < 0.001$), vrye karnitien ($p < 0.001$) en 3-hidroksie-butiraat-karnitien ($p < 0.001$).

Valproaat-, karbamasepien- en karnosienbehandeling het geen statistiese betekenisvolle effekte op die parameters van vetsuurmetabolisme, getoon nie. Die data toon aan dat die genoemde antiepileptiese middels se antikonvulsiewe effekte nie plaasvind as gevolg van 'n meganisme soortgelyk aan dié van die ketogeniese dieet nie.

Rotte wat met met asetiel-L-karnitien behandel, is het 'n toename getoon in die urienuitskeiding van asielkarnitien ($p < 0.001$), karnitien ($p < 0.001$) en 3-hidroksie-butiraat-karnitien ($p < 0.001$), wat daarop dui dat astiel-L-karnitine kan moontlik 'n effektiewe detoksiefikasie middel kan wees vir pasiënte met mediumketting asiel-KoA dehidrogenase ontoereikendheid.

Abstract

Treatments for epilepsy encompass a wide range of drugs and dietary supplements and understanding their mechanisms of action is important for effective clinical use. Many structures and processes are involved in the development of a seizure, including neurons, ion channels, receptors, glia, as well as inhibitory and excitatory synapses. Antiepileptic drugs are designed to modify these processes to favour inhibition over excitation in order to stop or prevent seizure. Valproate and carbamazepine are two widely used antiepileptic drugs although the chemical structures differ from each other. Acetyl-L-carnitine is a dietary supplement, which assists in fatty acid transportation into the mitochondria. Carnosine is a potential antiepileptic drug that could inhibit seizures through the histaminergic system. The ketogenic diet is a treatment generally used to treat refractory epilepsy.

Based on a study by Krüger (2006), who reported increased plasma omega-3-fatty acid levels in epileptic patients treated with carbamazepine or valproate, it was hypothesized that aforementioned drugs could mimic the effects of the ketogenic diet on fatty acid metabolism.

The study reported in this dissertation was designed to assess the effect of several anti-epileptic treatments on fatty acid metabolism as monitored by urinary excretion of acylcarnitines, glycine conjugates and glycine. Six groups of male Sprague Dawley rats ($n = 10$, for each group) were respectively treated with valproate, carbamazepine, carnosine, acetyl-L-carnitine, and a ketogenic diet. The sixth group served as a control. Rats were treated for 28 days, decapitated, and blood and urine samples were collected. For the determination of glycine conjugates and glycine, a standardised method, employing gas chromatography-mass spectrometry (GC/MS), was used. Acylcarnitines was deterrnined using isotope-dilution tandem mass spectrometry (LC-MS/MS).

The ketogenic diet resulted in slower body mass gain, as well as increased urinary long chain fatty acylcarnitines ($p < 0.001$), dicarboxylic fatty acylcarnitines ($p < 0.001$), acetylcarnitine ($p < 0.001$), free carnitine ($p < 0.001$) and 3-hydroxybutyrate-carnitine ($p < 0.001$) excretion.

Valproate, carbamazepine and carnosine treatments did not cause in any statistically significant effects on the parameters of fatty acid metabolism as mentioned above. The data indicate that aforementioned anticonvulsant drugs do not exert their anticonvulsant effects by mechanisms similar to that of the ketogenic diet.

Rats treated with acetyl-L-carnitine demonstrated increased urinary acylcarnitine ($p < 0.001$), carnitine ($p < 0.001$) and 3-hydroxybutyrate-carnitine ($p < 0.001$) excretion, suggesting that

acylcarnitine may be an effective detoxifying agent for patients suffering from medium-chain acyl-CoA dehydrogenase deficiency (MCADD).

Chapter 1

1 Introduction

Approximately one-third of epilepsy patients do not achieve seizure control with available drugs, and many patients experience adverse drug effects. Antiepileptic drugs are imperfect and their effects are not always communicated to the patient. Often, no single drug is adequate to provide control and one has to add to the cocktail of antiepileptic drugs to control seizures. Then the patient is more likely to suffer from side effects. In the last decade, new anticonvulsants have been introduced, but so far, they are not completely effective, because altogether they result in a seizure-free status in no more than 15-20% of drug-resistant epilepsy patients (Perucca, 2000; Bialer and Yagen, 2007). Valproic acid is the least potent of the established antiepileptic drugs, but has potentially life-threatening side effects.

Dietary treatments comprise an intriguing and novel approach to epilepsy treatment. The ketogenic diet is certainly the best-known dietary treatment for refractory epilepsy. The ketogenic diet, a high-fat, low-protein, and low-carbohydrate diet with a ratio of 4:1 of fat : carbohydrate and protein, has been modified to include medium-chain triglycerides and the Atkins diet protocol (Freeman *et al.,* 2007; Hartman and Vining, 2007). The ketogenic diet is also used as a treatment option for various seizures, including infantile spasms, myoclonic seizures, and tonic-clonic seizures. The uses of the diet have been expanded to treat disorders of energy metabolism, such as glucose transporter one (GLUT-I) deficiency and pyruvate-dehydrogenase-complex deficiency, by enabling the body to use an alternative energy source.

Few topics in nutrition have caused as much controversy as fats (Taubes, 2001). Omega-3 polyunsaturated fatty acids (PUFAs) are essential for normal brain development and function (Yuen and Sander, 2004). A lack of dietary omega-3 essential fatty acids, especially docosahexaenoic acid (DHA), has been implicated in several neurological disorders such as ADHD (attention deficit/hyperactivity disorder), peroxisomal disorders (X-linked adrenoleukodystrophy, adrenomyeloneuropathy, neonatal adrenoleukodystrophy, and Refsumis disease), schizophrenia, depression, Parkinson's disease, stroke, and Alzheimer disease (Horrocks and Farooqui, 2004). Several studies in animal models have shown that omega-3 PUFAs can raise the threshold of epileptic seizures (Voskuyl *et al.,* 1998; Yehuda *et al.,* 1994) and prevent status epilepticus-associated neuropathological changes in the hippocampal formation of epileptic rats (Ferrari *et al.,* 2008).

The β-oxidation of long-chain fatty acids (saturated and unsaturated) in the mitochondria is important for the provision of energy and is of particular importance for cardiac and skeletal

muscle. The toxicity of valproate has long been considered to be due to its interference with mitochondrial β-oxidation.

Antiepileptic drugs interfere in fatty acid metabolism, which may (at least partially) explain drug action. A study done by Krüger in 2006 indicated an increase in several of the omega-3 fatty acids, especially DHA, also an increase in several of the acylcarnitines, especially the long-chain acylcarnitines (adipyl, suberyl and octanoylcarnitine) in both the drug-responsive and the refractory groups. In aforementioned study, no statistical differences in the PUFA or long chain acylcarnitine levels of the valproate and carbamazepine treated groups, were found. It was therefore thought that the elevated DHA-levels were due to the epileptogenic status of the patients, and not the result of anticonvulsant therapy.

The aim of the study presented here, was to determine the fatty acylcarnitine profiles in rats subjected to several anticonvulsant treatments, which included valproate, carbamazepine, acetyl-L-carnitine, carnosine, and the ketogenic diet, in order to establish the effect of these interventions on the fatty acid metabolism.

In chapter 2, epilepsy treatments for this disease and possible mechanisms by which these treatments control epilepsy, as well as the effects of said treatments on fatty acid metabolism, are discussed. Chapter 3 describes all the experimental procedures employed to test the effect of the treatments on the fatty acid metabolism. Chapter 4 contains the results obtained together with short discussions on the results. A conclusion is drawn in chapter.5.

Chapter 2

2 Literature overview

2.1 Epilepsy

2.1.1 Definition

Epilepsy describes a group of neurological disorders, characterised by regular episodes of convulsive seizures or sensory disturbance, abnormal behaviour, loss of consciousness or all of the above. Generally, all types of epilepsy are caused by an uncontrolled, electrical discharge from the nerve cells of the cerebral cortex. Although most epilepsy is of unknown cause, it is sometimes associated with cerebral trauma or intracranial infection, brain tumours, vascular disturbances, intoxication or chemical imbalance. Epilepsy is mainly classified as either general or partial seizures (Anderson, 2002).

2.1.2 Epidemiology

New measured cases of epilepsy are between 40-70 per 100,000 in developed countries and 100-190 in developing countries. Ten percent of epileptic patients' deaths were directly related to seizures and status epilepticum (Guberman and Bruni, 1999).

Currently there are more than 40 types of epilepsy, making it an extremely variable disease and also complicating the effective treatment of this disorder. Despite progress in understanding the pathogenesis of seizures and epilepsy, the cellular basis of human epilepsy remains a mystery. Epilepsy treatments encompass a wide range of drugs and diet supplements and understanding their mechanism of action is important for effective clinical use.

2.1.3 Treatment

The goal of anticonvulsant treatment is to prevent epileptic seizures. Anticonvulsants act by various mechanisms. Voltage-activated sodium- and calcium-channels, glutamate receptors, and $GABA_A$ receptors represent the major targets for epilepsy treatment.

Conventional anticonvulsants generally inhibit sodium currents in the neuronal membrane (carbamazepine, phenobarbital, phenytoin, and valproate); while other drugs block the calcium-channels (valproate, ethosuximide). Some drugs inhibit the release of the excitatory neurotransmitter glutamate (lamotrogine, phenobarbitone, gabapentine, and topiramate). The most popular anticonvulsant drugs are those that potentiate the effects of GABA (benzodiazepines, phenobarbital, valproate, viabatrin, and tiagabine (Trevor *et al.*, 2002).

Approximately one-third of epilepsy patients do not achieve seizure control with available treatment for epilepsy (Kwan and Brodie, 2000) and therefore, alternative treatments are

considered. Treatments that received more attention for seizure control, especially in refractory epilepsy are the ketogenic diet or treatment with essential fatty acids.

The ketogenic diet is a high fat, low protein and low carbohydrate diet and is used as an anticonvulsant in a wide range of epileptic conditions, especially refractory epilepsy. In patients treated with the ketogenic diet, an increase in serum free fatty acids, including polyunsaturated fatty acids, can be expected (Fraser *et al.,* 2003). Neal *et al.* (2008) determined that the ketogenic diet is effective and should be included in the management of drug-resistant epilepsy in children.

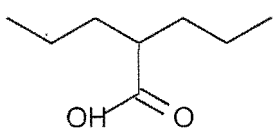
The findings of Bough *et al.* (2006) supported an energy preservation hypothesis for the anticonvulsant effect of the ketogenic diet, which might be particularly important for more metabolically active GABAergic interneurons. Schwartzkroin (1999) assumed that more information about the energy pathway and the mitochondrial function of the diet was required. The metabolic changes were still a key factor for the investigation for the mechanism of the ketogenic diet especially with respect to the energy substrates.

A study done by Ferrari *et al.* (2008) showed that omega-3 PUFAs had a neuroprotective effect in animals with epilepsy. Omega 3-PUFAs could improve symptoms of epilepsy in humans (Schlanger *et al.,* 2002) and animals (Yehuda, 1994; Voskuyl, 1998; Xiao and Li, 1999). However, other studies did not find convincing evidence for the antiepileptic effects of the PUFAs (Yuen *et al.,* 2005; Bromfield *et al.,* 2008).

The following antiepileptic treatments were studied in this investigation:

2.2 Valproate

Valproate, (2-n-propylpentanoic acid), is an uncomplicated, eight carbon, branched-chain, carboxylic acid, which is widely used to treat many types of epilepsy or seizures.



Valproate, a medium chain fatty acid, presumably enters the mitochondria by diffusion, as do other medium chain fatty acids. In the mitochondria, valproate forms valproyl-CoA. Valproyl-CoA may enter β-oxidation to form a conjugate with carnitine or glycine (Silva *et al.,* 2008). Valproate undergoes extensive biotransformation: mitochondrial β-oxidation, microsomal ω- and ω-1-hydroxylation, glucuronidation and conjugation reactions. These metabolites are mainly excreted in the urine (Silva *et al.,* 2008).

Aires *et al.* (2002) suggested that valproate enters the mitochondria, not only by passive diffusion, but also by carnitine transport. This insight on valproate biotransformation might have important implications for endogenous fatty acid oxidation of valproate.

Valproate may effectively be used to treat epilepsy, however, its exact mechanism of action is not yet known. Possible mechanisms of action for valproate have been described in the literature and will be discussed in the next section. The effect of valproate on fatty acid metabolism will also be discussed.

2.2.1 Mechanisms of action of valproate

Gamma-aminobutyric acid (GABA)

The mechanism of the antiepileptic action of valproate involves the regional changes in the concentration of the neurotransmitter gamma-aminobutyric acid (GABA). GABA is an amino acid that functions as an inhibitory neurotransmitter in the brain and spinal cord. The increase of GABAergic activity results in anticonvulsive effects, because GABA can bind to the GABA receptor and exert an anticonvulsive effect.

The effects on the GABAergic mechanism within substantia nigra are thought to be important for the anticonvulsant activity of valproate. Miller *et al.* (1988) showed that valproate inhibited GABA turnover, thus causing a higher concentration of GABA and Löscher (1981) showed that GABA might increase because of a valproate induced increase in the activity of glutamic acid decarboxylase (GAD).

Valproate increased GABA synthesis in the substantia nigra (Löscher, 1989), cortex (Miller *et al.,* 1988), striatum, hippocampus and cerebellum of rats (Chapman *et al.,* 1982). These are all evidence that valproate increases GABA in different brain areas. Valproate may affect various enzymes and intermediates involved in the synthesis and degradation of GABA and GABA-metabolites, including α-ketoglutarate, GAD, GABA-transport (GAT-I), GABA transaminase (GABA-T).

Figure 2.1 depicts that GAD activity increases parallel to elevation of brain GABA levels (Nau and Löscher, 1982). This activation of GAD was maximal at low dosages of valproate. GABA-T is the enzyme involved in GABA degradation. According to Löscher (1981), the possibility exists that valproate acts as an inhibitor of GABA-T, thus causing the concentration of GABA to increase. Valproate therefore seems to effect the GABA concentration through its modulating effect on the enzymes, GAD and GABA-T.

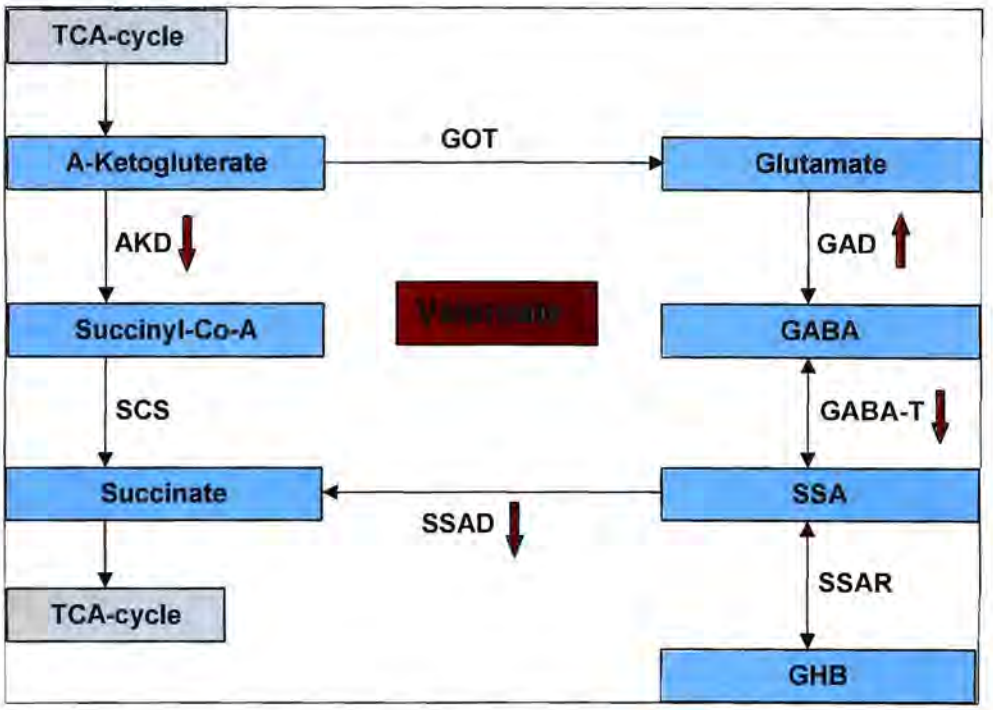


Figure 2.1 The synthesis and degradation of GABA and the effect of valproate on the related enzymes of GABA (Van der Laan, 1979; Löscher, 1999; Johannessen, 2000). GOT = glutamic-oxaloacetice transaminase; GAD = Glutamate decarboxylase; GABA = γ-aminobutyric acid; GABA-T = γ-aminobutyric acid transaminase; SSA = succinate semi-aldehyde reductase; GHB = γ-hydroxybutyrate; SSAD = Succinate semi-aldehyde dehydrogenase; SSAR = Succinate semi-aldehyde reductase; SCS = Succinyl CoA synthetase; AKD= α-ketoglurate dehydrogenase.

Succinate semi-aldehyde (SSA) is formed after the transamination of GABA by the enzyme GABA-T. SSAD is the enzyme responsible for degradation of SSA to succinic acid. Van der Laan *et al.* (1979) and Anlezark *et al.* (1976) suggested that valproate could inhibit SSAD and thereby increases GABA concentration.

SSA is also metabolised to gamma-hydroxy-butyrate (GHB) by succinate semi-aldehyde reductase (SSAR). According to Snead *et al.* (1980), valproate increases GHB significantly in the whole brain and that the increase in GHB concentration was for a short time. They confirmed that acute valproate treatment resulted in as significant increase of GHB brain levels, while chronic valproate treatment showed no increase of GHB concentration.

Luder *et al.* (1990) suggested that the proposed mechanism of valproate action might be based on the inhibition and/or inactivation of alfa-ketoglutarate dehydrogenase (AKD), because this would reduce citric acid cycle flux and increase flux into GABA synthesis. They also

demonstrated that AKD from mammalian brain was inactivated and inhibited by valproate metabolites.

Whether the effect of valproate is due to the activation of GAD, the enzyme responsible for GABA synthesis, or to inhibition of the catabolic enzymes succinic semialdehyde dehydrogenase (SSAD) and GABA transaminase (GABA-T), or to a combination of these effects, remains unclear (Silva *et al.,* 2008).

Effect on sodium channels

Willow *et al.* (1985) showed that valproate proportionately reduced Na^+-current membrane potentials and that the reversal potential of the Na^+-current was unaffected. They concluded that valproate had a significant inhibitory action on Na^+-channels at therapeutic concentrations during steady state. Valproate rapidly binds to the Na^+-channels in the inactivated state, stabilising those channels in an inactive form and prevent them from returning to the closed state.

McLean and MacDonald (1986a) suggested that the effect of valproate would be a use-dependent reduction of inward sodium current. They showed that the effects on Na^+-channels interfered indirectly from changes in the maximal rate of increase of Na^+-dependent action potentials. Van den Berg *et al.* (1993) showed that valproate strongly delayed the recovery from inactivation of sodium channels. They also indicated that valproate did have a direct inhibitory effect on voltage-sensitive sodium channels.

Valproate seems to have an effect on the sodium-channels, which may contribute to the drug's antiepileptic effect. However, evidence from other studies showed that valproate produced no effect on the sodium current in hippocampal neurons of the rat (Takahashi *et al.*, 1992; Albus and Williamson, 1998). In cultured hippocampal neurons, on the other hand, the drug inhibited sodium currents (Van den Berg *et al.,* 1993). These conflicting results may be explained by differences in the origin and function of neurons as well as differences in applied experimental conditions and techniques (Otoom and Alkadhi, 2000).

Effect of neurotransmitters

Another mechanism, which would also result in lowering seizures, would be to inhibit the excitatory neurotransmission.

Glutamate

Ion glutamate receptors are present in the brain at high concentrations, and can be divided into three groups: kainate (K), α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA) and N-

methyl-D-aspartate (NMDA) receptors. NMDA is an excitatory amino acid and an increase in NMDA levels could provoke a seizure (Nicoll, 2001).

Ko *et al.* (1997) suggested that the anticonvulsive effect of valproate might be ascribed to its direct effect on NMDA receptors. Zeise *et al.* (1991) suggested that valproate was not a specific blocker of the NMDA receptor, but had a strong effect on NMDA-induced firing. Blockage of NMDA-mediated processes plays an important role in the anticonvulsant action of valproate.

Aspartate

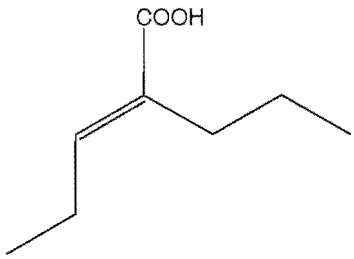
According to Chapman *et al.* (1984), there was an inverse dose dependent relationship between valproate and aspartate brain levels. Löscher and Hörstermann (1994) also showed that valproate significantly reduced aspartate levels in most brain regions and concluded that this could be relevant to the anticonvulsant effect of valproate.

Contradictory to the above results, Slevin and Ferrara (1985) found no effect on aspartate or glutamate uptake or binding activity in the cortex and hippocampus after chronic valproate treatment in rats. Glutamate and aspartate uptake in astroglial cultures was investigated and found to be decreased after acute, but not after five days of chronic, exposure to valproate (Nilsson *et al.,* 1992).

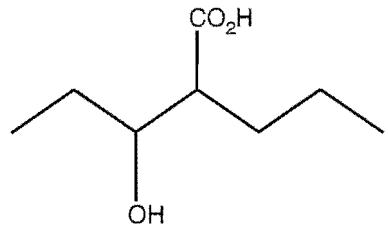
Valproate also exerts an effect on other neurotransmitters. An increase in levels of noradrenalin, dopamine, and serotonin in several brain regions of rats, after chronic treatment with valproate, was demonstrated, while a decrease in noradrenalin and serotonin was seen in the hypothalamus (Baf *et al.,* 1994). From microdialysis studies in rats, an elevation in the metabolites of serotonin and dopamine, (5-hydroxyindolacetic acid and homovanilic acid and dihydroxyphenylacetic acid), has been demonstrated with valproate treatment (Horton *et al.,* 1977). The effect of valproate on neurotransmitters could possibly be secondary effects to the changes in GABAergic neurotransmission.

Antiepileptic mechanisms of valproate metabolites

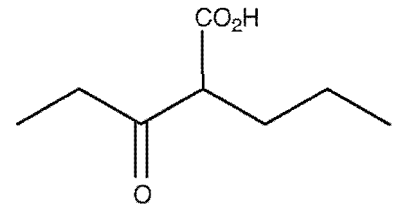
According to the review of Löscher (1999), one of the major metabolites of valproate is the trans isomer of 2-en-valproate: E-2-en-valproate. He concluded that although many of the metabolites shown below exert anticonvulsant activity in animal models, it is unlikely that these metabolites contribute to the anticonvulsant effect of valproate, because of low concentrations in the brain compared to the parent drug.



E-Δ^2-valproate



3-Hydroxy-valproate



3-Keto-valproate

2.2.2 Effect of valproate on fatty acid metabolism

Valproate and Carnitine

Carnitine deficiency is a known side effect in patients receiving valproate treatment, probably because of the formation of valproylcarnitine. Valproate combines with carnitine within the mitochondria via carnitine-acyltransferases, resulting in valproylcarnitine ester, which is then transported out of the mitochondria and is excreted in the urine. Valproylcarnitine is not reabsorbed in the kidney, resulting in the decease of L-carnitine concentrations (Okamura *et al.*, 2006).

Carnitine depletion has several consequences, for instance: impaired transport of long chain fatty acids into the mitochondrial matrix, resulting in decreased β-oxidation, acetyl-CoA, and ATP production. Impairment of β-oxidation could shift the metabolism of valproate towards predominantly peroxisomal ω-oxidation and as a result, ω-oxidation products may accumulate in the system (Lheureux *et al.*, 2005).

Carnitine deficiency has been associated with clinical symptoms of lethargy, hypotonia or weakness, and hepatoxicity (Coulter, 1991). Administration of carnitine corrected above-mentioned clinical symptoms (Raskind and El-Chaar, 2000).

Valproate and β-oxidation

Inhibition of fatty acid β-oxidation and induced hepatotoxicity may occur with valproate treatment. Impaired mitochondrial fatty acid β-oxidation may have important metabolic consequences such as lower levels of ketone bodies, induction of ω- and (ω–1)-oxidation and formation of acetyl-conjugates, leading to secondary carnitine insufficiency. The toxic effects of valproate and valproate metabolites may be explained by these biochemical abnormalities (Silva *et al.*, 2001a).

Valproate may enter the mitochondria via two pathways (Silva *et al.*, 2008). Firstly, it has been assumed that valproate predominantly crosses the mitochondrial membrane using a carnitine-independent process. Once inside the mitochondria it is converted to an active intermediate, valproyl-CoA, in order to gain access to the β-oxidation system. The activation of valproate has not yet been defined, but medium-chain acyl-CoA synthetase is thought to be the major catalyst. Once activated, valproyl-CoA enters the β-oxidation system resulting in 2-en-valproyl-CoA, 3-hydroxyvalproyl-CoA and 3-ketovalproyl-CoA as acyl-CoA metabolites. Valproate and its metabolites might impair mitochondrial β-oxidation by the direct inhibition of fatty acid oxidation enzymes: acyl-CoA dehydrogenase and 2-enoyl-CoA hydratase. Ito and co-workers (1990) showed that valproyl-CoA inhibited human short and medium-chain acyl-CoA dehydrogenase, while valproate did not significantly affect the activity of acyl-CoA dehydrogenase.

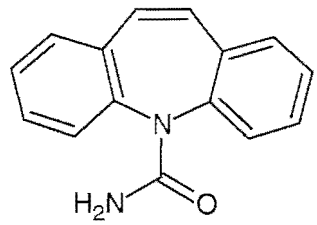
Secondly, cytosolic acyl-CoA synthetase (Aires *et al.*, 2007) may activate valproate in the extramitochonrial compartment to valproyl-CoA. Valproyl-CoA formed in the cytosol may enter the mitochondria via the carnitine shuttle (carnitine palmitoyltransferase, CPT-I). The hypothesis that valproylcarnitine interferes with mitochondrial carnitine-shuttle proteins is currently studied by Aires and his colleagues (Silva *et al.*, 2008). They provided evidence that valproyl-CoA is a competitive inhibitor of CPT-I activity *in vitro*. This inhibition may account for the decreased rate of long-chain fatty acid oxidation reported by (Silva *et al.*, 2001b).

Van den Braden and Roels (1985) showed that valproate had no effect on peroxisomal β-oxidation. The inhibitory effects of valproate were restricted to the mitochondrial system. It is likely that peroxisomal β-oxidation compensates for impaired mitochondrial β-oxidation at low valproate concentrations.

Valproate and glycine conjugate formation

Conjugation with glycine (an amino acid) and fatty acid acyl-CoA may occur in the mitochondria via glycine-*N*-acylase. This seems to be the least significant secondary metabolic pathway of valproate in the rat (Granneman *et al.*, 1984). Abbott and Anari (1999), identified valproyl-glycine as a urinary metabolite of valproate in humans.

2.3 Carbamazepine



Carbamazepine is chemically related to tricyclic antidepressants and is widely used as an anti-epilepticum (Brodie and French, 2000). Possible mechanisms of action for carbamazepine have been described in the literature and will be discussed in the next section. The effect of carbamazepine on fatty acid metabolism will also be considered.

2.3.1 Mechanisms of action of carbamazepine

Effect on sodium channels

The primary mode of action of carbamazepine is well known and is based primarily on its effects on voltage-gated sodium channels (Willow *et al.,* 1985; Kuo *et al.,* 1997). Courtney and Etter (1983) showed that carbamazepine selectively blocks the inactive form of closed sodium-channels and demonstrated pronounced frequency-dependent blockage of sodium-channels. The drug blockage therefore appeared to be selective for the inactive closed sodium-channels. Sitges and co-workers speculated that the mechanism of action of carbamazepine could involve the down modulation of presynaptic sodium-channels (2007).

The fundamental basis of carbamazepine action could be summarized as 1) its binding to the inactivated state of the sodium-channel, 2) to stabilise sodium-channels in an inactive form and 3) to prevent them from returning to the closed state.

Effect on Calcium-channels

Although inhibition of sodium channel activity has been considered the major pharmacological effect of carbamazepine explaining its antiepileptic property, Schirrmacher and co-workers confirmed that carbamazepine has calcium-antagonistic properties as well (1993). Ambrósio *et al.* (1999) suggested that high concentrations carbamazepine might act on L-type calcium-channels, causing calcium antagonist activity which was confirmed by Schirrmacher *et al.* (1993).

In contrast, Schumacher *et al.* (1998) reported that therapeutic dosages of carbamazepine did not affect the calcium-currents. They also reported that carbamazepine demonstrated a

reversible concentration dependent inhibition of high-voltage-activated calcium-currents, without affecting voltage dependent activation.

N-methyl-D-aspartate (NMDA)

Glutamate-mediated excitation appears to play a major role in the initiation and spread of seizures. Drugs that block ionotropic glutamate receptors have anticonvulsant properties and neuroprotective effects (Suzuki *et al.*, 2005).

NMDA receptor antagonists have a broad spectrum of anticonvulsive activity (Rogawski, 1993). Hough *et al.* (1996) showed that carbamazepine rapidly and reversibly inhibits NMDA receptor responses within the therapeutic range to prevent seizures.

Gamma-aminobutyric acid (GABA)

It has been shown that the postsynaptic GABA-responses were not affected by carbamazepine (McLean and MacDonald, 1986b; Bonnet and Bingmann, 1998), however Granger *et al.* (1995) demonstrated that carbamazepine acts as a positive allosteric modulator of $GABA_A$ receptors in cultured cortical neurons, with the GABA-induced current being reversibly increased by carbamazepine in single-cell recordings.

Serotonin

Carbamazepine is a tricyclic antidepressant (Brodie and French, 2000), and it is likely that carbamazepine should show properties of the tricyclic antidepressant class. Okada *et al.* (1998) showed that the therapeutic concentration of carbamazepine increased hippocampal serotonin and serotonin metabolites in rats. Two studies reported that the antiepileptic drug carbamazepine causes increases in extracellular serotonin in genetically epilepsy-prone rats (GEPRs) (Yan *et al.*, 1992), and in non-epileptic Sprague–Dawley rats (Daily *et al.*, 1997).

Dailey *et al.* (1997) provided results supporting the hypothesis that release of serotonin by carbamazepine is an important part of the pharmacodynamic action by which this drug suppresses seizures.

Adenosine

Marangos *et al.* (1983) reported that carbamazepine had specific and potent effects on adenosine receptors *in vitro*. The finding of Klitgaard and co-workers (1993) showed that adenosine A_1 and A_2 receptor agonists were pro-convulsive with respect to induction of seizures.

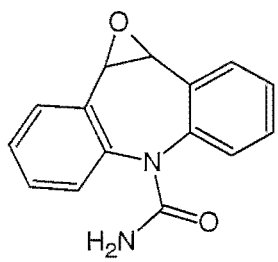
Adenylate cyclase

Carbamazepine directly inhibited adenylate cyclase and as a result, inhibited cAMP production at the therapeutic concentration of carbamazepine (Chen *et al.*, 1996). Ludvig *et al.* (1992)

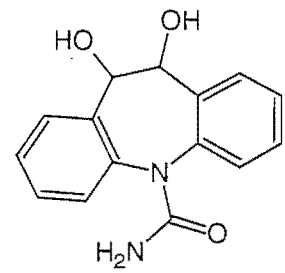
suggested that one of the cAMP second messenger system functions might include an epileptogenic pathway. Thus, carbamazepine might exert an antiepileptic effect by inhibiting this enzyme and attenuating cAMP mediated signalling.

Metabolites of carbamazepine – mechanism of action

The 10,11-epoxide derivative of carbamazepine, but not the 10,11-diol derivative, might contribute to anticonvulsant efficacy by the same mechanism, as carbamazepine. The epoxide might add to the anticonvulsant action of carbamazepine by a shared cellular mechanism. Much higher concentrations of the diol derivative were required to encounter a therapeutic effect. It is concluded that with therapeutic carbamazepine doses, the 10,11-epoxy derivative of carbamazepine, but not the 10,11-diol, may contribute to anticonvulsant efficacy (Mclean and MacDonald, 1986b).



Carbamazepine-10,11-epoxide



Carbamazepine-10,11-diol

2.3.2 Effect of carbamazepine on fatty acid metabolism

Carnitine

According to Kurul *et al.* (2003), there were no differences in the serum concentrations of total and free carnitine in patients treated with carbamazepine compared to the control, thus suggesting that carbamazepine as monotherapy does not cause carnitine deficiency.

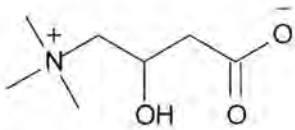
Effects on fatty acids

According to König *et al.* (2003), the change in serum fatty acid concentrations with the use of carbamazepine was minimal, while Yuen *et al.* (2008) showed that carbamazepine treatment was associated with lower levels of docosahexaenoic acid (DHA) and long-chain omega-3 fatty acids in plasma. Yuen *et al.* (2008) emphasized that this study required confirmation in subjects taking carbamazepine as monotherapy.

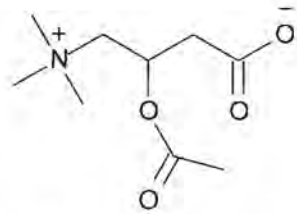
According to Bazinet *et al.* (2006), chronic carbamazepine therapy did not significantly change the plasma concentration of unlabeled unesterified arachidonic acid (AA), DHA, or other fatty acids, compared with control values. However chronically administered carbamazepine

selectively decreased the rate of incorporation of arachidonoyl-CoA and decreased the turnover of AA (but not of DHA) in brain phospholipids of the unanesthetized rats.

2.4 Acetyl-L-carnitine



L-carnitine



Acetyl-L-carnitine

Carnitine (3-hydroxy-4-N-trimethylammoniobutanoate) is a naturally occurring quaternary ammonium compound. Carnitine is present in biological cells and tissues at relatively high concentrations as free carnitine, acylcarnitine, or acetyl-L-carnitine.

Acetyl-L-carnitine is the most widely distributed short-chain ester of L-carnitine in the body. Acetyl-L-carnitine is present in relatively high levels in brain (Shug *et al.*, 1982), and is particularly high in the hypothalamus (Bresolin *et al.*, 1982).

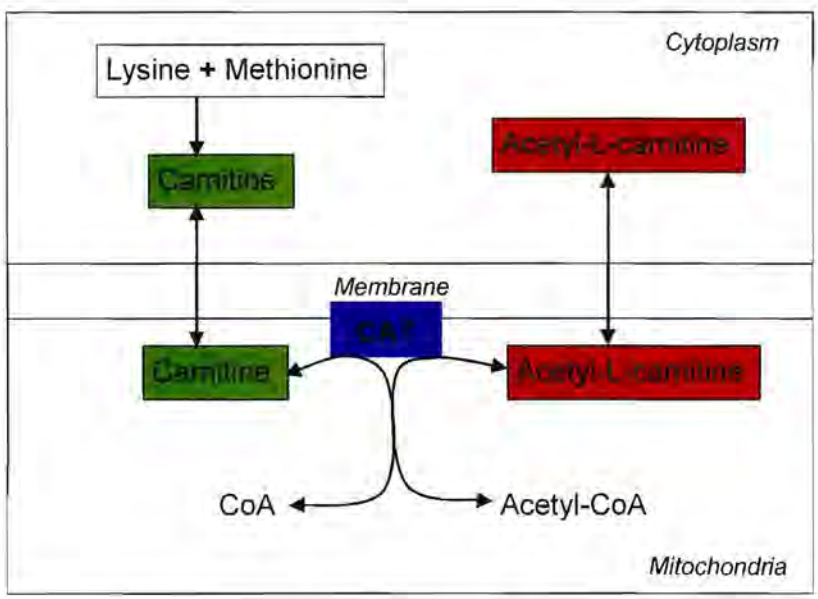


Figure 2.2 Schematic summery of the formation of carnitine in the mitochondria (Pettegrew *et al.*, 2000). CAT = carnitine acetyltransferase

Carnitine supplies are present in two forms: nutritional and biosynthetic. According to Lennon *et al.* (1986), approximately 75% of total body carnitine originates from food sources (red meat and diary products): carnitine, lysine, and methionine. In addition, carnitine intake correlates with the plasma carnitine concentration. Krajcovicova-Kudlackova *et al.* (2000) showed that amino

acids; lysine and methionine were related to carnitine biosynthesis; and Rebouche *et al.* (1989) demonstrated that carnitine synthesis was regulated by the availability of trimethyl-lysine, (figure 2.2).

2.4.1 Importance in cell regulations

Although 99% of carnitine occurs intracellularly, the relationship between serum acetyl-L-carnitine and free carnitine concentration was highly sensitive to intra-mitochondrial metabolic alterations. Such alterations occur in both normal and abnormal situations. Fasting, for instance causes a reduction in plasma-free carnitine and a corresponding increase in acetyl-L-carnitine (Gatti *et al.,* 1998).

Carnitine performs important cellular functions, especially in mitochondrial and peroxisomal metabolism. The biochemical and physiological roles of carnitine have been reviewed by Tein (2002) and Virmani and Binienda (2004). Carnitine is responsible to transport fatty acid acyl-CoA across the inner mitochondrial membrane for β-oxidation, (figure 2.8). Secondly, carnitine facilitates the oxidation of pyruvate and branched-chain keto-acids, by preventing their accumulation, and contributes to the protection of cells from potentially membrane-destabilising acyl-CoAs. Thirdly, carnitine is involved in the regulation of fatty acid metabolism and ketogenesis; carnitine is utilised in the oxidation of branched-chain amino acids as carnitine shuttles acetyl moieties shortened by the peroxisomal β-oxidation system from peroxisomes to mitochondria for further oxidation, and interacts with membranes to change their physiochemical properties.

According to Stanley (1987), carnitine acts as cofactor for mitochondrial fatty acid oxidation by transferring long-chain fatty acids as acylcarnitine esters across the inner mitochondrial membrane. Carnitine facilitates branched-chain α-keto-acid oxidation, transports acyl-CoA products of peroxisomal β-oxidation into the mitochondrial matrix in the liver, modulates the acyl-CoA to CoA ratio in mammalian cells, and esterifies potentially toxic acyl-CoA metabolites that impair the citric acid cycle, urea cycle, gluconeogenesis, and fatty acid oxidation during acute clinical crises.

According to Beal (2003), the transport of fatty acids into cellular mitochondria for their conversion, via β-oxidation, into energy, is the main role of the carnitine system. In addition, carnitine participates to regulate the mitochondrial acyl-CoA/CoA ratio, peroxisomal oxidation of fatty acids, and the production of ketone bodies. Due to these essential interactions with the bio-energetic processes, carnitine deficiency may play an important role in mitochondrial-related disorders. A carnitine deficiency has major deleterious effects on the central nerve system.

2.4.2 Importance of carnitine in epilepsy

According to Rebounch and Engel (1980), seizures might be the presenting symptoms of a metabolic inborn error, such as a mitochondrial encephal-O-myopathy, or a defect in fatty acid oxidation, which might also be associated with carnitine deficiency.

Secondary carnitine deficiency is usually genetically determined, as in β-oxidation disorders, amino acid disorders, or acquired medical conditions. Iatrogenic factors such as pivampicillin and valproate might cause carnitine deficiency, especially when the diet is poor in carnitine supplementation (Virmani and Binienda, 2004).

Carnitine deficiency is common amongst patients with epilepsy, especially since some anticonvulsants decrease carnitine concentration. Several studies demonstrated that total plasma carnitine concentrations were remarkably lower in patients taking only valproate or multiple antiepileptic drugs. Thus, carnitine deficiency was mainly linked to valproate usage (Coppola *et al.,* 2006).

According to Steiber *et al.* (2004), there is renewed interest in carnitine as medicine, both as a supplement and as a therapeutic agent.

2.4.3 Acetyl-L-carnitine as detoxification agent

According to Moreno *et al.* (2005), carnitine acts as a carrier for fatty acids across the inner mitochondrial membrane, in order to present fatty acids to β-oxidation. Carnitine is also responsible for the removal of potentially toxic metabolites from the inner mitochondrion, as acylcarnitines.

Yokoi *et al.* (2007) studied patients with medium-chain acyl-CoA dehydrogenase deficiency (MCADD) and showed removal of toxic metabolites form the mitochondria and the excretion of these metabolites as acylcarnitines in the urine. They concluded that carnitine supplementation might be useful to enhance urinary excretions of toxic acyl-CoAs as corresponding acylcarnitines.

2.4.4 Acetyl-L-carnitine and energy metabolism

According to Joseph (2003), there is little evidence that supplemental L-carnitine improves energy status, increases athletic performance, or inhibits obesity. According to Pettegrew *et al.* (2000), acetyl-L-carnitine had a favourable role in restoring cerebral energy.

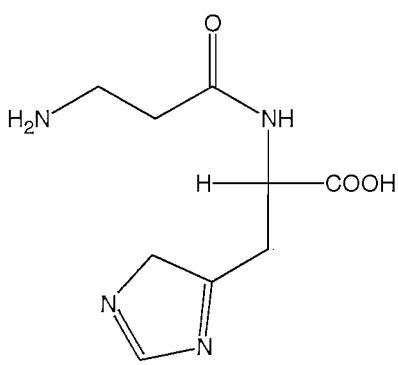
2.5 Carnosine

A number of studies have suggested that the histaminergic neuron system plays an important role in the pathogenesis of seizure disorders. Histamine seems to be involved in mechanisms regulating seizure susceptibility, and the role of histamine as a possible anticonvulsant has been well-documented (Scherkl *et al.,* 1991; Kamei *et al.,* 1998; Zhang *et al.,* 2003).

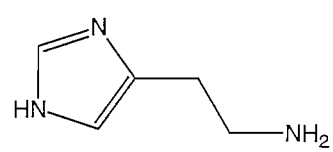
Carnosine, also known, as β-alanine-L-histidine, is a dipeptide first discovered by Gulewitsch and Amiradzibi (1900) in a meat extract. It has been characterized as a putative neurotransmitter in olfactory receptor neurons (Bonfanti *et al.,* 1999). However, the understanding about the role of carnosine in the brain is still poorly understood.

Histamine (decarboxylated L-histidine) and carnosine (β-alanine-L-histidine) are structurally related, both containing an imidazole ring. According to Schwartz *et al.* (1991), histamine could not cross the blood brain barrier, while according to Crush (1970) carnosine is a naturally occurring dipeptide, and could easily enter the central nervous system.

Jin *et al.* (2005) studied the effects of carnosine on amygdaloid-kindled seizures and found that carnosine protected against these seizures in rats. In 2006, Wu and co-workers showed that carnosine could protect against pentylenetetrazol (PTZ)-induced seizures in rats. The findings of Kozan and colleagues in 2008 indicated that carnosine had an anticonvulsant effect on penicillin-induced epilepsy in rats. Thus, all of the above data support the hypothesis that carnosine may be a potential anticonvulsant drug for clinical therapy of epilepsy in the future.



Carnosine



Histamine

2.5.1 Mechanism of action

The mechanism of protection of carnosine may involve at least two pathways (Jin *et al.,* 2005). Firstly, by directly acting on the H_1-receptors and provoking a histamine-like response and secondly, by activating H_1-receptors after metabolic transformation into histamine through the carnosine-histidine-histamine pathway (figure 2.3). Shen *et al.* (2007a) showed that the

protective effect of carnosine was antagonised by the H_1-receptor antagonist pyrilamine, but not by the H_2-receptor antagonist cimetidine, confirming that its protection may in part be due to the activation of the postsynaptic histamine H_1-receptor.

Figure 2.3 The suggested metabolically pathway of carnosine to histamine.

Since histamine cannot cross the blood brain barrier, it has been speculated that carnosine could be metabolically transformed into histamine in the brain by the carnosinase and histidine decarboxylase (HDC) enzymes (Kasziba *et al.*, 1988; Fitzpatrick *et al.*, 1991).

Zhu *et al.* (2007) also suggested that the protective effect of carnosine was mainly through the carnosine-histidine-histamine pathway in the brain.

Shen *et al.* (2007b) found that carnosine induced a significant increase in intracellular and extracellular histidine. Carnosine also increased histamine levels in a time-dependent manner. Kamei *et al.* (1998) showed that histidine caused a dose-dependent inhibition of amygdaloid kindled seizures, and confirmed that histidine increased brain histamine content.

Zhu *et al.* (2007) showed that histidine significantly inhibited PTZ-induced seizures in mice but not in histidine decarboxylase knockout (HDC-KO) mice. Carnosine showed no significant anti-seizure effect on HDC-KO mice. HDC is the key enzyme for the synthesis of histamine from histidine.

The carnosine-histidine-histamine pathway may not be the only mechanism contributing to the protective effect of carnosine, and other mechanisms may exist (Shen *et al.*, 2007b).

2.5.2 Effects of carnosine on fatty acids metabolism

Shen *et al.* (2008) investigated the *in vivo* effects of L-carnosine on the sympathetic nerve activity innervating white adipose tissue and lipolysis. Intraperitoneal administration of 100 ng and 10 µg of L-carnosine increased and decreased the levels of plasma free fatty acids, respectively.

2.6 The Ketogenic diet

The ketogenic diet is a high fat, low protein and low carbohydrate diet. The diet mimics the fasting state when fat is metabolised for energy, thus the brain uses ketone bodies for energy instead of glucose. The ketogenic diet is useful as an anticonvulsant in a wide range of

epileptic conditions, especially refractory epilepsy. The mechanisms by which the ketogenic diet affects epilepsy are still controversial.

Bough *et al.* (2000) demonstrated a positive correlation between seizure threshold and ketonemia exists. Ketonemia is a result of a high fat diet and indicates the success of the diet. There was no indication whether certain levels of ketonemia were required for seizure protection, or whether protection was a continuous function of ketone levels. According to Noh *et al.* (2003), the ketogenic diet had a neuroprotective effect against the kainic acid-induced excitotoxicity.

The anticonvulsive effects of the ketogenic diet are disputed by several other researchers. Otani *et al.* reported that the ketogenic diet had no anticonvulsive effects (as indicated by Thavendiranathan *et al.,* 2003) while Thavendiranathan *et al.* (2000) found that the ketogenic diet actually had a proconvulsant effect.

2.6.1 Importance of carnitine

According Rutledge *et al.* (1989) the ketogenic diet could deplete carnitine stores by several mechanisms. The diet might decrease carnitine intake due to the moderately low protein content, increase the demand for carnitine use in the oxidation of fatty acids, and/or increase urinary acetyl-carnitine excretion. Supplementation with L-carnitine should facilitate ketogenesis and thereby contribute to the anticonvulsive action of the diet. According to Hack *et al.* (2006), there was an increase in the acetylcarnitine to free carnitine ratio with microdialysis in humans on the ketogenic diet.

Berry-Kravis *et al.* (2001) reported mild carnitine depletion in patients during the early stages of ketogenic diet treatment, but carnitine levels stabilised to normal with long-term treatment. Thus, no carnitine supplementation should be required during the treatment with the ketogenic diet.

2.6.2 Potential mechanism of seizure prevention by the ketogenic diet

The mechanism of action of the ketogenic diet is unknown, but several hypotheses have been put forward. The mechanisms discussed in this study are according to articles written by Schwartzkroin (1999), Sheth *et al.* (2005), Hartman *et al.* (2007) and Bough and Rho (2007). Figure 2.4 illustrates the hypothetical pathways for the anticonvulsive effects of the ketogenic diet.

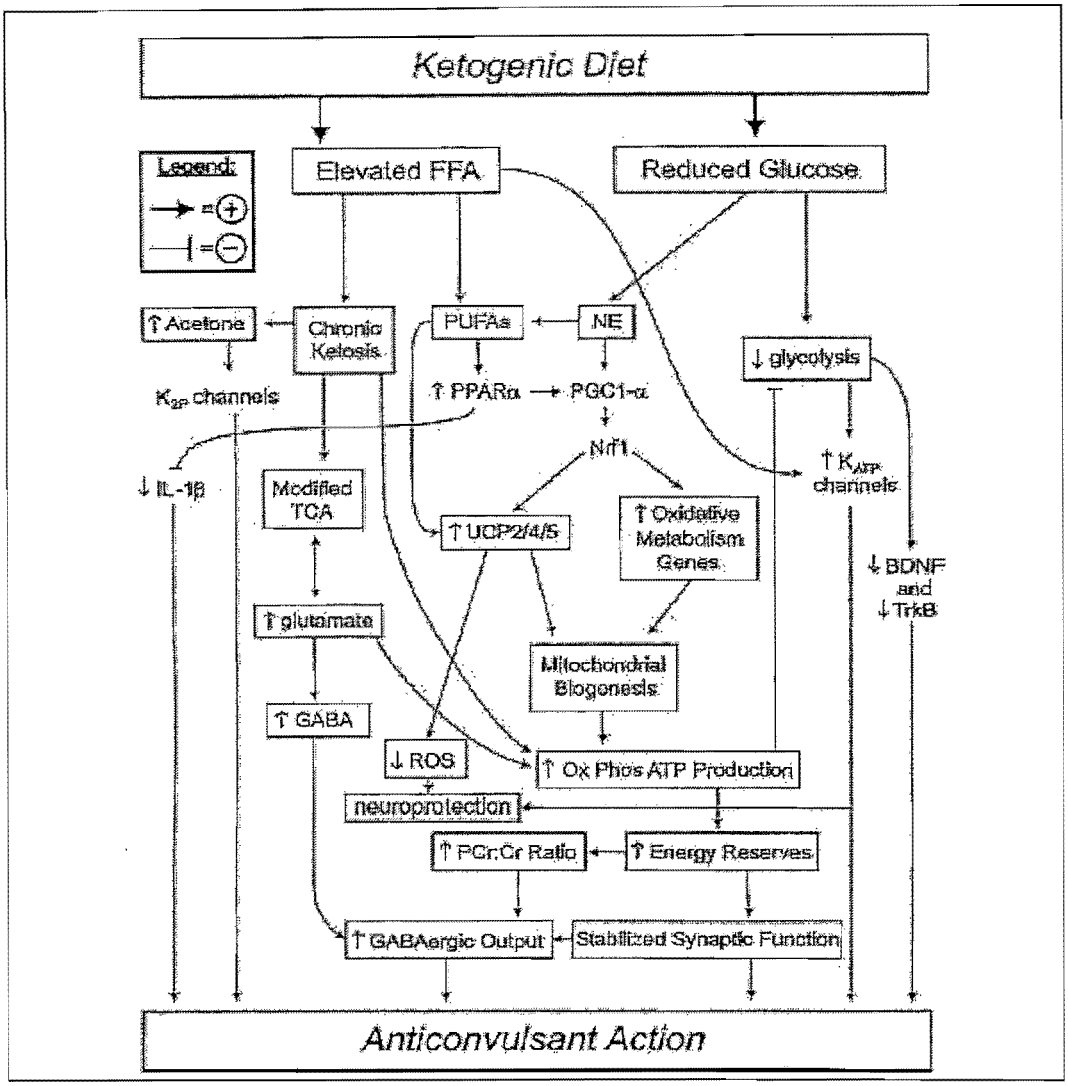


Figure 2.4 The hypothetical pathways for the anticonvulsive effects of the ketogenic diet. (FFA = free fatty acids; PUFAs = polyunsaturated fatty acids; TCA = tricarboxylic acid; UCP = uncoupling proteins; ROS = reactive oxygen species; PPARα = peroxisome proliferator-activated receptor-α; PCr:Cr = phosphor-creatine:creatine; BDNF = brain-derived neurotrophic factor (adapted from Bough and Rho, 2007).

Glucose levels are low during fasting and the use of the ketogenic diet. When insufficient glucose is available to the brain, the brain will use ketones for energy. Fatty acids are oxidised in the liver to ketones, i.e. β-hydroxybutyric acid (BHB), aminoacetic acid (ACA), and acetone. The brain extracts and breaks down ketones, which are subsequently funneled into the tricarboxylic acid cycle and the electron transport chain, with energy release as a result (Sheth *et al.,* 2005).

The anticonvulsive effects of the ketogenic diet can be direct or indirect. The following possible mechanisms of action of the ketogenic diet include: the effect of ketone bodies, glucose restriction, role of fatty acids, GABAergic hypothesis, noradrenergic hypothesis and changes in

energy metabolism in the brain. These generally accepted mechanisms of action will be described in detail. Other potential mechanisms include a change in pH, dehydration, membrane fluidity, and the effect in hormonal changes in insulin, changes in lipid metabolism, effects on the excitatory amino acid system and changes in cellular properties, but will not be discussed any further (Bough and Rho, 2007).

Ketone bodies

Ketone bodies are products of lipid pyruvate metabolism and are produced from acetyl–CoA. The two main ketone bodies are β-hydroxybutyric acid (BHB) and aminoacetic acid (ACA) (Anderson, 2002:954). Figure 2.5 shows the metabolic pathway of ketone body synthesis. The ketogenic diet causes a several fold increase serum and urine levels of ketone bodies. Ketone bodies are utilized as source of energy by the brain (Hartman *et al.,* 2007).

Animals on the ketogenic diet were ketotic (Hori *et al.,* 1997) and showed higher levels of BHB in the blood during treatment (Bough *et al.,* 1999; Thavendiranathan *et al.,* 2000). The ketogenic diet resulted in an increased seizure threshold and seizure threshold was found to correspond to the level of ketonemia. Although BHB is not essential to prevents seizures it plays an important part in seizure protection (Bough *et al.,* 1999).

Donevan *et al.* (2003) and Likhodii *et al.* (2003) provided evidence that ACA and acetone also showed anticonvulsant activity. Donevan *et al.* (2003) found that BHB exerted a concentration- and voltage-dependent block on the NMDA-evoked current. They found that in cultured neocortical neurons, neither BHB nor ACA directly interact with either $GABA_A$ or ionotropic glutamate receptors and that neither isomer of BHB nor ACA changed the amplitude of whole-cell currents evoked by GABA.

Thio *et al.* (2000) indicated that ketone bodies did not change either excitatory or inhibitory synaptic transmission in the hippocampus. They hypothesised that the ketogenic diet reduced seizures, because of the ketone bodies (BHB and ACA) anticonvulsive properties. Possible mechanisms include a direct increase of inhibitory neurotransmission, or inhibition of excitatory neurotransmission. BHB or ACA showed no direct effect on ion channel currents, mediated by these postsynaptic receptors in cultured hippocampal neurons. They concluded that ketone bodies did not directly alter $GABA_A$, AMPA, NMDA, kainate, or glycine receptor function.

Bought and Rho (2007) concluded in their review that, although ketone bodies were shown to possess anticonvulsant properties *in vivo*, there was no evidence that they mediate these effects directly. In addition, a certain amount of sustained ketosis was required for clinical efficacy. Efficacy was maximised over a period of weeks, despite a rapid onset of ketosis.

According to Prasad and Stafstrom (1998), ketosis was necessary but not sufficient to explain the anticonvulsive mechanism of the ketogenic diet.

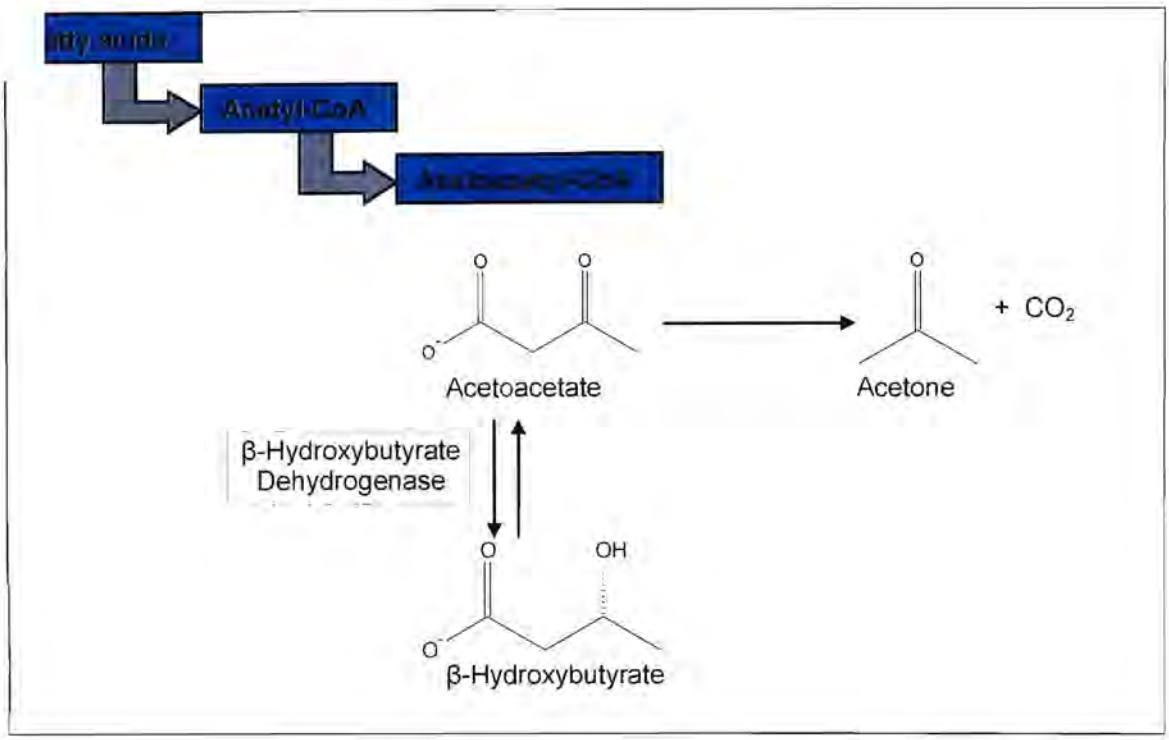


Figure 2.5 The metabolic pathways of the production of ketone bodies from fatty acids. Beta-hydroxybutyrate levels are commonly measured in blood as clinical indicator for successful ketogenic diet treatment (Illustration by Likhodii and Burnham, 2004; in Bough and Rho 2007).

The GABAergic hypothesis

The anticonvulsive effect mediated by GABA is one of the most widely accepted mechanisms of action. The ketogenic diet could affect GABA directly or indirectly.

The ketogenic diet had no significant effect on Wistar rats' cerebral GABA levels. However, these finding do not rule out a GABA-mediated mechanism, because an increase in GABA concentration at the synapses might not be reflected by an increased total GABA concentration in the cerebral cortex (Al-Mudallal *et al.,* 1996). Thus, brain ketosis might exert a GABA agonist effect. Yudkoff *et al.* (1997) indicated that the amino group of GABA was incorporated into glutamine, and that this process was inhibited by ACA or BHB. The ketone bodies might interfere with glial GABA metabolism, thereby favouring an expression of the GABA pool.

Yudokoff *et al.* (2004) hypothesised that the transamination of glutamate to aspartate, (the major route of brain glutamate disposition), was decreased during ketosis. Thus, more glutamate became available for the synthesis of both GABA and glutamine (an important GABA

precursor). During ketosis, glial glutamine metabolism to GABA increased because the brain imported and produced more acetate. Increased acetate metabolism during ketosis probably indicated an increase in acetate uptake into the brain. A metabolic adaptation that favours the synthesis of GABA from glutamate would be expected to cause an antiepileptic effect. Acetate alone may inhibit glutamine synthesis and glutamate uptake Yudkoff *et al.* (1997).

Cheng *et al.* (2004) showed that calorie restriction increased brain GAD (glutamic acid decarboxylase) expression in several brain regions, independent of the ketogenic effect. This explains why caloric restriction improved the efficacy of the ketogenic diet during epilepsy treatment. The activity of the enzymes responsible for brain amino acid synthesis may change during treatment with the ketogenic diet. The ketogenic diet and calorie restriction increased this GAD in several brain regions.

Changes in several cerebrospinal amino acids occurred during treatment with the ketogenic diet. The amino acids of importance are those that increase inhibition and decrease excitation. The changes in certain brain amino acid levels were a clear indication that the diet could influence excitability in the central nervous system. This could be important to explain the mechanism of action of the ketogenic diet to achieve seizure control (Dahlin *et al.,* 2005).

If ketosis reduces the flow of glutamate through aspartate aminotransferase, and favours the flow through glutamate decarboxylase, improved seizure control may be achieved. In addition, an antiepileptic effect could result from a reduction of aspartate synthesis.

The noradrenergic hypothesis

According to Yan *et al.* (1993) noradrenalin epinephrine re-uptake inhibitors could prevent seizure activity in GEPR; and according to Weinshenker and Szot (2002) pharmacological noradrenalin agonists were generally anticonvulsant. They also assumed that animals were more prone to seizures when treated with reserpine (deplete monoamine neurotransmitters chemically).

In addition, Weinshenker and Szot (2002) reported an approximate two-fold increase in noradrenalin levels in the hippocampus of animals with the ketogenic diet. This suggests that the ketogenic diet increases basal release of noradrenalin. Thus, the ketogenic diet may show an anticonvulsant effect by increasing the catecholamine, noradrenalin.

Glucose restriction

The ketogenic diet is also known as a calorie-restricted diet. Glucose restriction may influence the anticonvulsive mechanism of the ketogenic diet in several ways. Glucose restriction also plays an important role in the mechanism of the ketogenic diet. For instance, animals fed a

calorie-restricted normal diet exhibited an increased resistance to pentylenetetrazole (PTZ)-induced seizures (Bough *et al.,* 1999). Glucose restriction may activate ATP-sensitive potassium channels (K_{ATP}-channels), causing an anticonvulsive effect (Vamecq *et al.,* 2005).

Greene *et al.* (2001) showed that the seizure-protective effect correlated with the degree of calorie-restriction. This indicated that calorie restriction delayed epileptogenesis and that the antiepileptogenic effect of calorie-restriction was proportional to the degree of calorie-restriction. Their findings indicated that plasma glucose levels were predictive for seizure susceptibility.

According to Greene *et al.* (2003), ketone metabolism slowly reduced neuronal excitability through effects on neurotransmitter levels and membrane potential. Thus, a restriction of caloric energy intake should enhance the anticonvulsive effects of the ketogenic diet. They suggested that calorie restriction might underlie the anticonvulsant effect of the ketogenic diet. Optimal epilepsy management was achieved by a ketogenic diet combined with restricted food or caloric intake.

Role of fatty acids

The ketogenic diet affected serum free fatty acids concentrations and particularly, polyunsaturated fatty acids (PUFAs), like docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA), were increased in the plasma of rats treated with the ketogenic diet (Cunnane *et al.,* 2002). Furthermore, it was found that omega-3 PUFA supplementation improved the symptoms of epilepsy in humans Schlanger *et al.* (2002). Figure 2.6 illustrates the potential pathways by which polyunsaturated fatty acids might limit hyper-excitability in the brain.

Xiao and Li (1999) showed that free long-chain omega-3 PUFAs modify the membrane excitability of hippocampal neurons. Sodium influx through opening sodium-channels was responsible for initiation and propagation of action potentials. They found that omega-3 PUFA reduced the frequency of action potentials and increased the action potential threshold. In addition, they suggested a suppressant effect on ion channels of hippocampal neurons. EPA inhibited both sodium and calcium-channels in neuronal cells, and another important finding was that EPA blocks the glutamate-activated inward current.

According to Vamecq *et al.* (2005), PUFA in combination with ketone bodies, might influence the K_{2P} potassium channels and Wu *et al.* (2004) showed that the altered lipid composition of all membranes induced by PUFA could affect the molecular activity of the membrane proteins. According to Xu *et al.* (2008), the ketogenic diet could prevent seizures by causing PUFA-induced opening of voltage-gated potassium-channels.

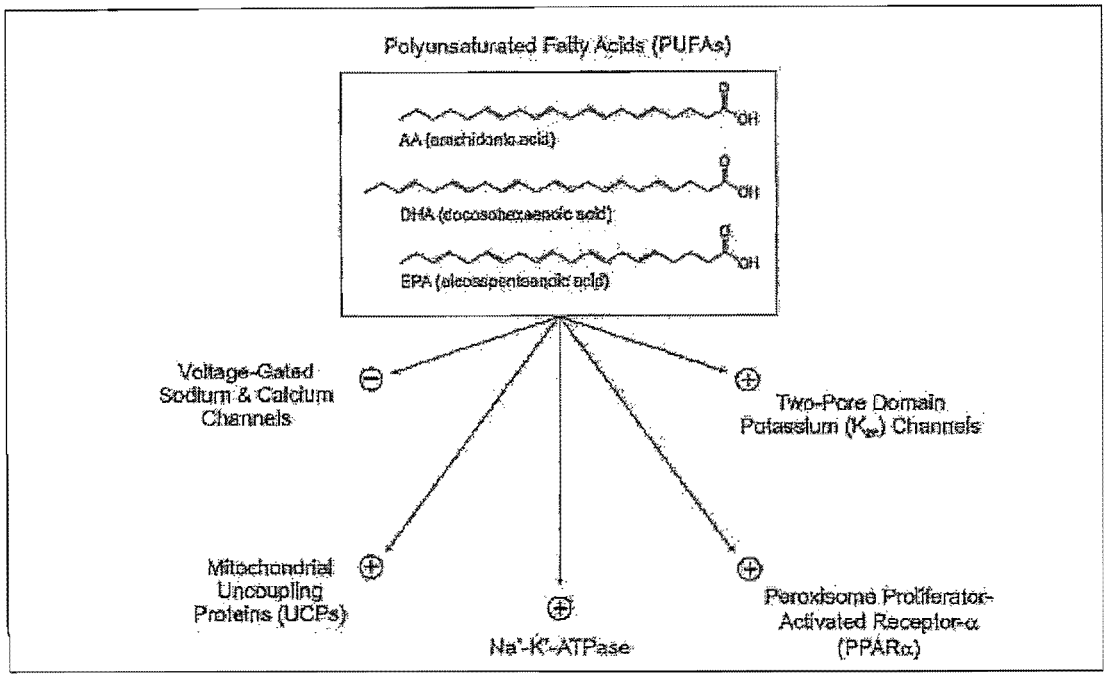


Figure 2.6 The potential direct and indirect pathways by which PUFA might limit hyper-excitability in the brain (reproduced from Bough and Rho, 2007). 1) Inhibited voltage-gated Na^+ and Ca^{2+} channels; 2) activated K_{2P} potassium channels; 3) improved the activity of the Na^+/K^+-ATPase to reduce seizure activity; 4) PUFAs might reduce neuronal dysfunction and induce a neuroprotective effect. Finally, PUFAs induce an up-regulation of energy transcripts leading to enhanced energy reserves, stabilised synaptic function and limited hyper-excitability.

Changes in energy metabolism in the brain

The increased availability of fatty acids and the relative scarcity of glucose activate a non-glycolytic pathway, which is normally induced under conditions of significant caloric restriction.

According to DeVivo (1978), there was improved energy storage in the brain of rats, because of the ketogenic diet and successive ketosis. This investigation showed that there was a higher ATP to ADP ratio and an increase in creatine levels in the brain. Thio *et al.* (2006) showed that juvenile rodents fed a ketogenic diet had a slower weight gain than those fed a standard diet. Schwartzkroin (1999) assumed that more information about the impact of the ketogenic diet on energy pathways and mitochondrial function was required. The metabolic changes are still a key factor for the investigation of the mechanisms of the ketogenic diet's anticonvulsive effect.

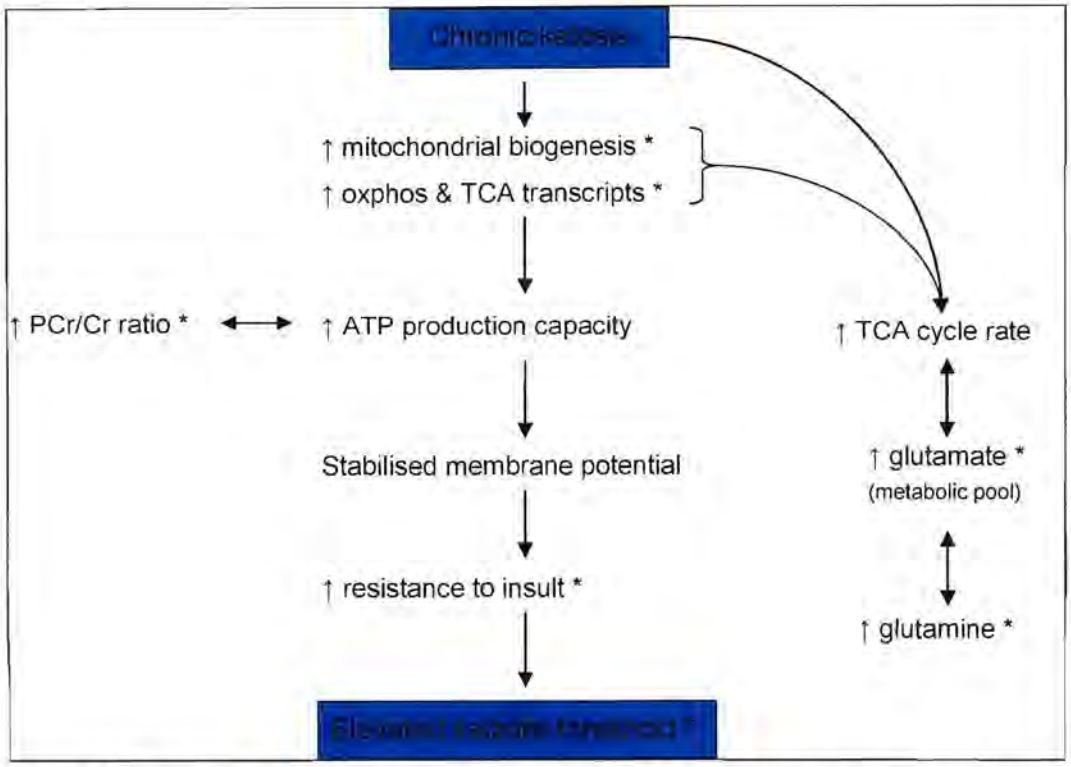


Figure 2.7 The possible anticonvulsive effect of the ketogenic diet (adapted from Bough *et al.*, 2006). Chronic ketosis in the brain triggers mitochondria biogenesis and associated with the tricarboxylic acid (TCA) pathways. Mitochondrial biogenesis increases ATP production capacity. Ketones serve as a substrate for glutamate synthesis via the TCA cycle and some glutamate reversibly converted to glutamine. The enhanced resistance of hippocampal tissue to metabolic stress associated hyper-excitability results in an elevated seizure threshold. The * indicates results observed in their study.

Energy production

Bough *et al.* (2006) proposed that a chronic, but not an acute ketosis, activates a genetic programme, resulting in mitochondrial biogenesis in the hippocampus, thus achieving enhanced energy stores (Figure 2.7). They suggested that mitochondrial biogenesis increased ATP production capacity, with excess high-energy phosphates stored as creatine phosphate (PCr). Glutamate and glutamine formed by the ketone-boosted TCA cycle provide an important second energy store that, in concert with PCr, could be drawn upon to sustain ATP levels in times of need. The ability to maintain ATP levels during metabolic or physiological stress should allow neurons to fuel Na^+/K^+-ATPase; and other transporters that stabilise membrane potential in neurons, and thus maintain ionic homeostasis for longer periods. Under conditions of low glucose availability, transmitter release from perforated path terminals could be maintained about 60% longer in hippocampal slices taken from a ketogenic diet compared with control-fed rats, a likely consequence of enhanced energy stores.

Bough *et al.* (2006) concluded that the ketogenic diet could significantly affect neuronal function within the hippocampus. In reaction to a high fat, calorie-restricted diet, the hippocampus responds by inducing mitochondrial biogenesis, enhancing metabolic gene expression, and increasing energy reserves. Their findings support an energy preservation hypothesis for the anticonvulsant effects of the ketogenic diet, which might be particularly important for more metabolically active GABAergic interneurons.

Boero *et al.* (2003) concluded that PCr and energy levels were especially critical to the maintenance of GABAergic inhibitory output. A ketogenic diet induced increased energy reserves, and might enhance GABAergic function in particular, causing improved seizure control. Kunz *et al.* (2000) found metabolic dysfunction and hyper-excitability in the human brain, which was associated with several epileptic conditions. Impairment of mitochondrial function was observed in the seizure foci of both humans and experimental epilepsies.

Schwartzkroin (1999) originally hypothesised that a ketogenic diet induces elevations in ATP concentrations, and may enhance or prolong the activation of the Na^+/K^+-ATPase. According to Hulbert and Else (2000), ATP was primarily used to maintain ionic gradients, especially through actions of the transmembrane sodium pump.

Bough and Rho (2007) concluded that despite progressive research on the anticonvulsive effects of the ketogenic diet, the mechanism of action remains unproven. It seems impossible that only one mechanism will explain the entire protection against seizures associated with the ketogenic diet. Presumably, several mechanisms are responsible for this broadly efficacious treatment of epilepsy; the challenge remains to find the key variables for this mechanism of action.

2.7 Fatty acid metabolism

Since it is apparent that antiepileptic treatments may interfere in fatty acid metabolism, a short overview of the physiology of normal fatty acid metabolism is presented here.

Fatty acids are the largest reserve of energy in the body. The body derives energy from fatty acids by the process of β-oxidation. Fatty acids are classified as short chain fatty acids (C_4-C_6), medium chain fatty acids (C_8-C_{12}) and long chain fatty acids (C_{14}-C_{20}).

2.7.1 Carnitine transport

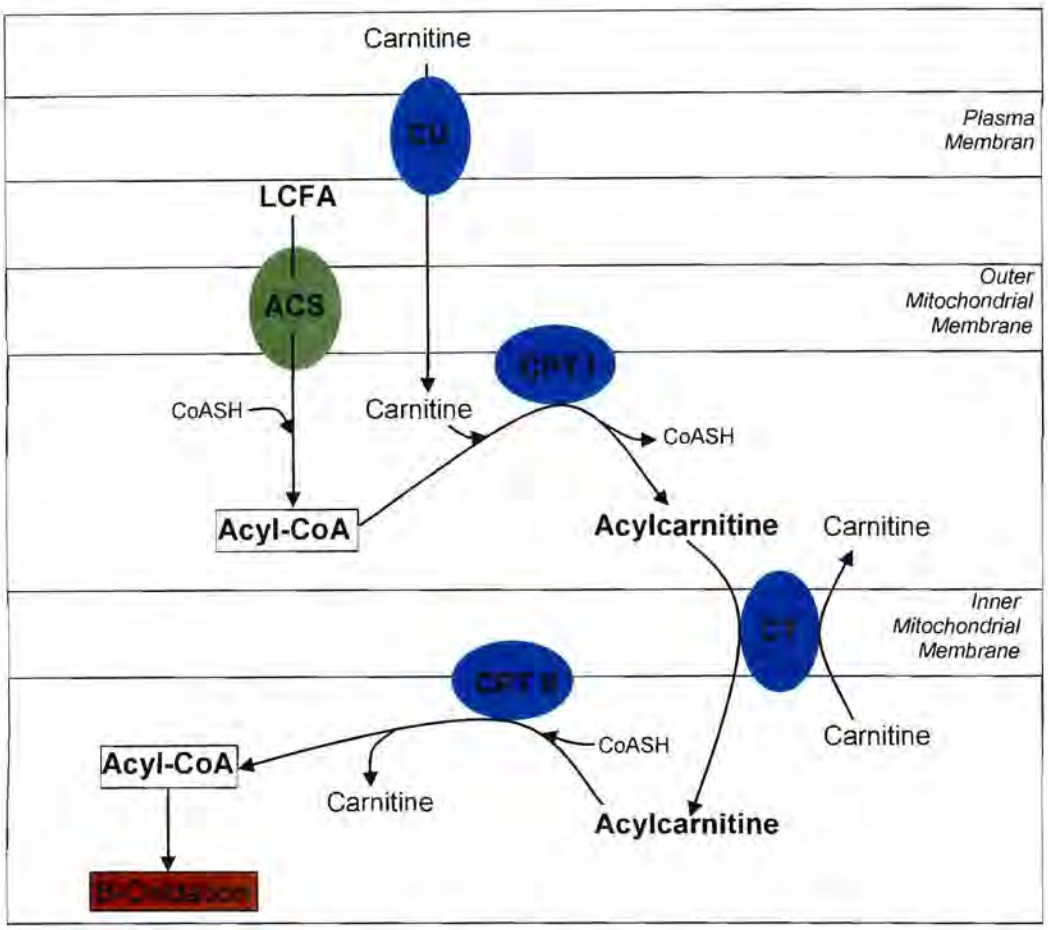


Figure 2.8 The carnitine cycle. ACS = acyl-CoA synthetase; CoASH = coenzyme A; CPT = carnitine palmitoyl-transferase; CT = carnitine/acylcarnitine translocase; CU = carnitine uptake; LCFA = long chain fatty acids (Roe and Ding, 2001).

According to Moreno *et al.* (2005), carnitine acts as a carrier for fatty acids across the inner mitochondrial membrane, for subsequent β-oxidation. Carnitine is also responsible for the removal of potentially toxic metabolites from the inner mitochondrion as acyl-CoA and acetyl-carnitine.

The short and medium chain fatty acids diffuse freely across the mitochondrial membrane into the mitochondrial matrix and do not require a carnitine shuttle system. The activation of short-chain and medium-chain fatty acids is also catalysed by acyl-CoA synthetases in the mitochondria (Schulz, 1991; Fromenty and Pessayre, 1995).

Long chain fatty acids (LCFA) are transported across the mitochondrial membrane by the carnitine shuttle mechanism. The carnitine shuttle mechanism involves carnitine palmitoyltransferase I (CPT-I), carnitine palmitoyltransferase II (CPT-II) and carnitine acylcarnitine translocase (CACT), (Figure 2.8).

Long chain fatty acids are activated to their coenzyme A (CoA) derivatives by long chain acyl-CoA synthetase before being transported into the mitochondria. Long chain fatty acids cannot freely cross the inner mitochondrial membrane and require a specific transport system. Transport into the mitochondrion occurs by the carnitine transacylation pathway (Watkins, 1997). These long chain acyl-CoA synthetases are membrane-bound enzymes and are located in the mitochondrial outer membrane (Schulz, 1991; Fromenty and Pessayre, 1995).

Acyl-CoA is converted to the corresponding acylcarnitine by a transesterification reaction, catalyzed by CPT-I (Kerner and Hoppel, 2000). CPT-I, which is found in the mitochondrial outer membrane, plays a prominent role in the transport system, where it balances the fatty acid oxidation and synthesis, and drives the LCFA and VLCFA towards oxidation, instead of esterification into triglycerides (Fromenty and Pessayre, 1995).

Long-chain acylcarnitine is transported into the mitochondrial matrix in a transmembrane exchange reaction, where acylcarnitine is exchanged for carnitine. This reaction is mediated by CACT (Kerner and Hoppel, 2000). Within the inner surface of the mitochondrial inner membrane, CPT-II reconverts the acylcarnitines to their corresponding acyl-CoAs, which are substrates for the β-oxidation process (Kerner and Hoppel, 2000).

2.7.2 Mitochondrial β-oxidation

The majority (90%) of β-oxidation reactions take place in the mitochondria, whereas peroxisomal β-oxidation most likely accounts for approximately 10% of the β-oxidation flux, even in tissues where peroxisomes are abundant, such as the liver. Peroxisomal β-oxidation is only capable to chain-shorten and not to degrade fatty acids to completion, whereas mitochondrial β-oxidation degrades fatty acids to completion (Wanders, 2004).

During each cycle of the β-oxidation of fatty acids, energy is released, acetyl CoA is produced, and the fatty acid chain is shortened by a two carbon unit (Hoppel, 2003) (Figure 2.9). The released acetyl-CoA could follow one of two pathways. It may condense into ketone bodies (acetoacetate and β-hydroxybutyrate) or it could enter the Krebs cycle (tricarboxylic acid cycle) where it is oxidised to carbon dioxide and water (Fromenty and Pessayre, 1995; Roe and Ding, 2001:2299).

Within the mitochondria, the fatty acyl-CoA esters undergo repeat cycles of four sequential enzyme catalysed reactions. These reactions are catalysed by acyl-CoA dehydrogenase, 2-enoyl-CoA hydratase, L-3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase (Roe and Ding, 2001:2299).

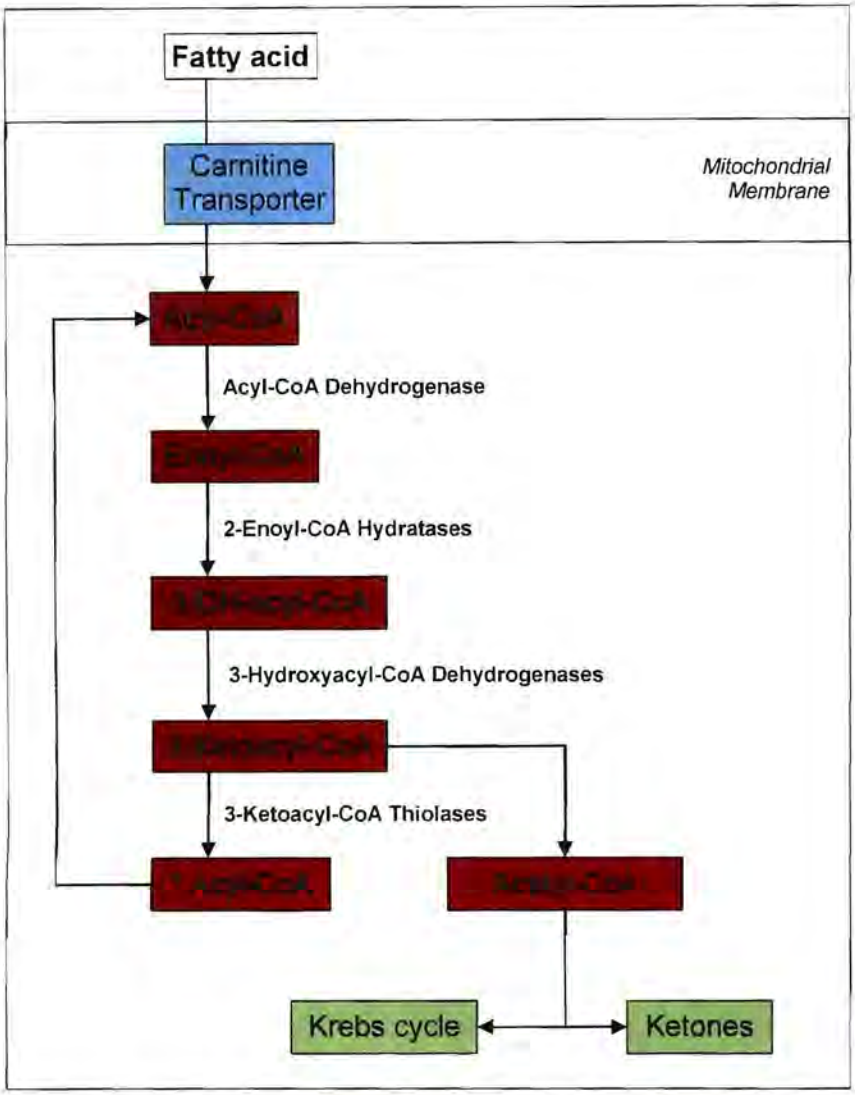


Figure 2.9 Mitochondrial β-oxidation. *Acyl-CoA shortened by two carbons (Sim *et al.*, 2002)

2.7.3 ω-oxidation in the cytoplasm

Accumulated free fatty acids in the cytoplasm may undergo ω- and ω-1- oxidation by dicarboxylic and hydroxyl-dicarboxylic acids (Sim *et al.*, 2002).

According to Sanders *et al.* (2008), ω-oxidation converts fatty acids to dicarboxylic acids. This ω-oxidation normally takes place in the endoplasmic reticulum. This ω-oxidation is particularly prominent in the brain (Alexander *et al.*, 1998).

2.7.4 Peroxisomal β-oxidation

According to Wanders (2004), peroxisomes are capable to catalyse fatty acid by the same β-oxidation mechanism as in the mitochondria. No carnitine is required for the transport of fatty acyl-CoA into the peroxisomes (Wanders and Tager, 1998; Roe and Ding, 2001). However,

carnitine transport seems to export acyl-CoA out of the peroxisome to the cytosol (Wanders, 2004).

Peroxisomal β-oxidation is exclusively responsible for the metabolism of very long chain fatty acids (Mannaerts and Veldhoven, 1996; Wanders, 2004). These very long chain fatty acids are shortened by peroxisomal β-oxidation to optimal length before being transported into the mitochondria for further oxidation (Leighton *et al.,* 1989; Reddy and Hashimoto, 2001; Wanders, 2004). According to Ferdinandusse *et al.* (2001), polyunsaturated fatty acids are formed by peroxisomal β-oxidation.

2.7.5 Conjugate formation

Accumulation of free fatty acid and toxic acyl-CoA intermediates by inhibiting enzymes in the β-oxidation enzymatic pathway, leads to the formation of dicarboxylic and hydroxy-dicarboxylic acids from fatty acids via ω- and ω-1 oxidation; and changes of acyl-CoA esters to the corresponding acylglycines and acylcarnitines (Sim *et al.,* 2002).

Intracellular accumulation of acylcarnitine leads to export of the compound to the plasma, from where acylcarnitine can be transported to other tissues for further metabolism, or to the kidneys for excretion in the urine (Brass, 2002).

Glycine conjugation is an important detoxification system of the human body. Glycine-N-acylase is responsible for glycine conjugation of exogenous compounds in the liver mitochondria. These glycine conjugates are then excreted in the urine (Wilcox, 1999). Analysis of acylglycine excretion in the urine is important to determine mitochondrial β-oxidation (Bonafé, 2000).

2.7.6 Summary of fatty acid metabolism

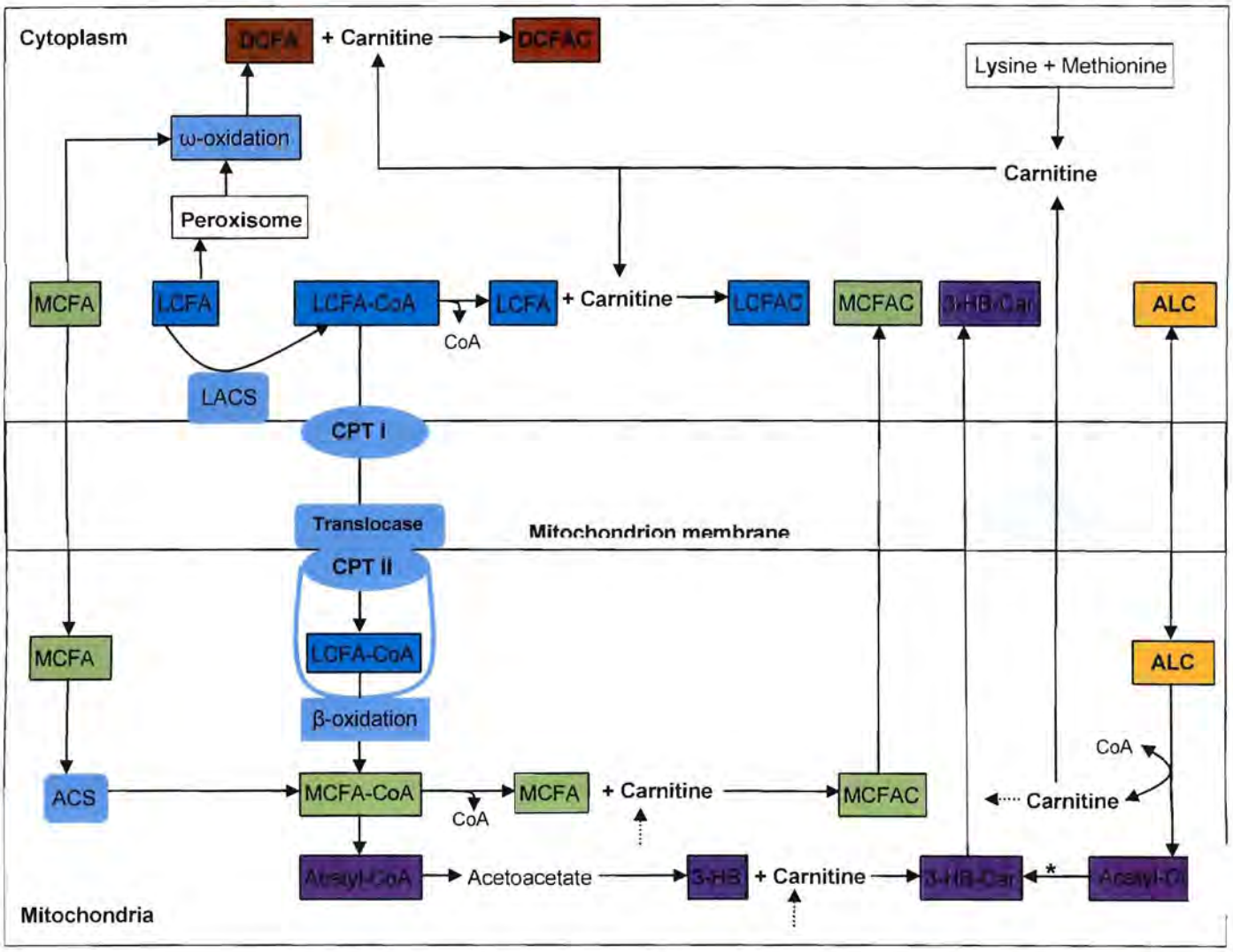


Figure 2.10 **A summary of fatty acid metabolism describe above. DCFA = dicarboxylic fatty acids; DCFAC = dicarboxylic fatty acid acylcarnitine; LCFA = long chain fatty acid; LCFAC = long chain fatty acylcarnitine; MCFA = medium chain fatty acid; MCFAC = medium chain fatty acylcarnitine; 3-HB = 3-hydroxybutyrate; 3-HB-CAR = 3-hydroxybutrate-carnitine; CPT = Carnitine pamtoyltransferase; ALC = acetylcarnitine; VPA = valproate; ACS = acyl-CoA synthetase; LACS = long chain acyl-CoA synthetase. The * indicates the synthetase of 3-HB-CAR.**

2.8 Aim of this study

From the literature discussed above, it is clear that both drug and dietary treatments may be effective in the treatment of epilepsy. This offers the exciting possibility that dietary treatment may potentiate drug treatment. Furthermore, it is also possible that drugs and dietary treatment may have similar metabolic effects, resulting in antiepileptic activity. For example, Krüger (2006) reported an increase in serum omega-3 fatty acids, especially DHA, as well as an increase in serum levels of various acylcarnitines in epileptic children treated with valproate or carbamazepine. However, the study of Krüger could not differentiate whether epilepsy per sé, or drug treatment for epilepsy, caused the altered fatty/acylcarnitine patterns and concentrations in serum.

Based on these observations the purpose of this study was two-fold:

1. to characterize the effects of a ketogenic diet, valproate, carbamazepine, acetyl-L-carnitine and carnosine on acylcarnitines and glycine conjugates excretion patterns in urine,

2. to determine whether valproate, carbamazepine, acetyl-L-carnitine and carnosine could mimic the changes in acylcarnitine profiles characteristic of a ketogenic diet.

Chapter 3

3 Experimental procedures

The development and undertaking of this project were done in accordance with the guidelines stipulated by the Ethics committee for Use of Experimental Animals at the North-West University, Potchefstroom (approved number 07D02).

3.1 Animals

Male Sprague Dawley rats were chosen as animal model and were housed at the *Animal* Research Centre of the North-West University, Potchefstroom, under constant conditions of temperature (25 °C) and humidity (50 $\pm$ 10 %) and with a 12:12 hour light-dark cycle. Sixty rats were housed individually in metabolic cages with free access to food (except group 6) and water and were marked individually to distinguish between them.

The 60 rats were divided into 6 groups and were treated **daily** for 28 days as follows (see table 3.2 for more information):

Group 1: Control group treated with 0.5 ml saline p.o.
Group 2: Valproate 187.5 mg/kg in 0.5 ml saline p.o.
Group 3: Carbamazepine 125 mg/kg in 0.5 ml DMSO p.o.
Group 4: Acetyl-L-carnitine 100 mg/kg in 0.5 ml saline p.o.
Group 5: Carnosine 500 mg/kg in 0.5 ml saline p.o.
Group 6: Ketogenic diet and 0.5 ml saline p.o.

Dosages were administered orally using a 0.5 ml volume syringe. The rats were weighed weekly in order to calculate the dosage correctly (see appendix A for dosages). Fresh solutions of treatments were prepared daily. Urine was collected every day till day 28 and frozen at -20 ºC. After 28 days, rats were decapitated and blood collected with EDTA as anti-coagulant. Blood was centrifuged (5000 rpm, 18 ºC) and the plasma frozen at -20 ºC.

3.2 Dosages

The animal dosages were determined to correlate with conventional human dosage using body surface area (BSA). BSA-based dose calculation is the most appropriate method and is far more superior than simply transfusion based on body weight (Hahn, 2005; Reagan-Shaw *et al.*, 2008).

Determination of the conversion coefficient

K_m = body weight (Kg) / BSA (m^2)

The K_m factor is determined by dividing the body mass by BSA (see table 3.1):

Table 3.1 Determination of equivalent conversion coefficient

	Average body mass	Average BSA	K_m factor	Equivalent conversion coefficient
Human	60 kg	1.6 m^2	37.5	**6.25**
Rat	0.15 kg	0.025 m^2	6	0.16

Conversion coefficient to determine rat equivalent dose to human dose

= Human K_m / Rat K_m

= 37.5 / 6

= **6.25**

Formula for dose translation based on BSA
Animal dose (mg/kg) = HED (mg/kg) x $\frac{\text{Human } K_m}{\text{Animal } K_m}$

*HED = Human equivalent dosage

This formula was used to determine the animal dosage, equivalent to the human dosage.

Table 3.2 Summery of treatments used

Control	Group 1
Dosage	0.5 ml saline daily
Valproate	Group 2
Solubility	Valproate is soluble in water, 50 mg/ml (Sigma)
Stability	Stability in solution for 8-24 h (Lund *et al.*, 1994:1046)
Human dosage	30 mg/kg (Gibbon *et al.*, 2005:408)
Animal dosage	187.5 mg/kg (30 mg/kg x 6.25)
Solution	A fresh solution of valproate in saline was prepared every day

Carbamazepine	Group 3
Solubility	Insoluble in water but soluble in ethanol (Axxora, 2004) Ethanol has anticonvulsive and antieptogenic effects (Fischer and Kittner, 1998; Fischer, 2004), thus unsuitable for use DMSO had only negligible effects on fatty acid synthesis (Guo *et al.*, 2003), thus DMSO could be used as a vehicle for carbamazepine
Stability	Stable is solution at room temperature for up to 8 weeks (Lund *et al.*, 1994:774)
Human dosage	20 mg/kg (Gibbon *et al.*, 2005:406)
Animal dosage	125 mg/kg (20 mg/kg x 6.25)
Solution	A fresh solution of carbamazepine in DMSO was prepared every week and protected from light.
Acetyl-L-carnitine	Group 4
Solubility	Water solubility of 1 g / 3.1 ml in 25 °C (Budavari *et al.*, 1989:1857)
Stability	Stable for up to 24 h at 25 °C; protected from light, (Levocarnitine, 2008)
Dosage	100 mg/kg (Levine *et al.*, 2005; Traina *et al.*, 2006)
Solution	A fresh solution of acetyl-L-carnitine in saline was prepared every day
Carnosine	Group 5
Solubility	Hygroscopic and soluble in water, (Budavari *et al.*, 1989:1857). Insoluble in DMSO
Dosage	Effective dosages to treat epileptiform activity are 500 mg/kg, (Kozan *et al.*, 2008; Zhu *et al.*, 2007)
Solution	A fresh solution of carnosine in saline was prepared every week
Ketogenic diet	Group 6
Type of diet	KetoCal diet, (Zhou *et al.*, 2007)
Preparation	Preparing a fresh pasta every day, KetoCal diet : water, 1:1
Dosage	Started at 4 g per rat and increased with 1 g every week

3.3 Analysis of acylcarnitines in urine

3.3.1 Introduction

A technique based on isotope-dilution tandem mass spectrometry for quantifying acylcarnitines in small volumes of plasma, whole blood and urine was used. This method quantifies amino acids and various acylcarnitines in one analysis, and is a standardised method in use at the laboratory for metabolic diseases, school for Physical and Chemical Science (subjected group Biochemistry) at the North West University (Potchefstroom Campus).

Isotopically labeled acylcarnitines were added to the samples. Ions of the derivatives of compounds were produced and entered the mass spectrometer. Precursor ions were fragmented by colliding them with argon gas in the collision cell, which was then analysed in a second mass analyser according to their mass. The relation of endogenous metabolites to the internal standards was determined by measuring the virtual intensities of the mass spectra of the peaks attained. Acylcarnitines were identified by scanning precursor ions that yield a common mass fragment of 85 Da.

3.3.2 Material and preparation of stock solution

Preparation of butanolic hydrochloride: Butanol (50 ml) was cooled in a beaker on ice for 5 minutes. Acetylchloride (12.5 ml) was added dropwise (this is important for this step generates heat), while stirring frequently. The solution was covered with parafilm and left on ice for 20 minutes. The solution remains stable for one week.

Acetonitrile : Water 50 % (v/v). 500 ml of acetonitrile was slowly added to 500 ml of water in a reagent bottle, followed by sonification for at least 20 minutes to remove air bubbles.

Acylcarnitine isotope preparation were done according to the volumes indicated in table 3.3

Table 3.3 Preparation of isotope stock solution en dilutions.

Isotope	Stock solution (mmol/l)	Dilution: take x ml of stock solution, to volume of 200 ml	Final concentration (mmol/ml)
Free carnitine	0.32317	1.000 ml	1.6159
Acetylcarnitine	0.20534	0.500 ml	0.5134
Propionylcarnitine	0.19417	0.130 ml	0.1262
Isovalerylcarnitine	0.17153	0.130 ml	0.1115
Octanoylcarnitine	0.15408	0.130 ml	0.1002
Palmitoylcarnitine	0.11377	0.500 ml	0.2844

3.3.3 Creatinine determinations

Creatinine determinations were preformed on urine samples before extractions, to normalise results. The creatinine values were expressed in µmol/l and were determined spectrophotometrically by a standard procedure using the UV 3.0 Spectro, Biotech, Roche.

Normal creatinine values range between 4300-9300 µmol/l and 62-132 µmol/l in urine and plasma respectively. Creatinine concentrations obtained from urine and plasma values for these samples are summarized in appendix B.

3.3.4 Sample preparation for the determination of acylcarnitine in urine

100 µl of the sample was centrifuged for 30 minutes to rid the urine of crystals. 10 µl of the urine sample was added to 400 µl of the carnitine isotopes mixture. The sample was centrifuged for 20 minutes and the supernatant was transferred to a new tube.

The supernatant was dried under a flow of nitrogen at 65 ºC for 30 minutes. After drying, the caps on the microtube (in which the sample was present) were closed, 200 µl 3 N butanolic HCl was added and the sample was incubated at 65 ºC for 20 minutes. Again, the solvent was dried under nitrogen for 35-40 minutes. The residue was reconstituted in acetonitrile: water and 1 % formic acid (80:20). The sample was analysed mass-spectrometric.

3.3.5 LC-MS/MS analysis

This procedure is based on electrospray ionization (ESI). It is a "soft ionization" technique where little fragmentation of the ions occurs before they reach the detector. Acylcarnitines are detected using a "precursors of 85" scan.

Table 3.4 Setting for LC-MS/MS

Tuning paramters: ES+	
Capillary	3.50 kVolts
HV Lens	0.50 kVolts
Cone	35 Volts
Skimmer offset	5 Volts
Skimmer	1.5 Volts
RF lens	0.2 Volts
Source temperature	75 ºC
Mass spectrometer 1	
Ion energy	1.0 Volts
Ion energy ramp	0.0 Volts

LM resolution	15.0
HM resolution	15.0
Lens 5	100 Volts
Lens 6	5 Volts
Multiplier	750 Volts
Mass Spectrometer 2	
Ion energy	1.0 Volts
Ion energy ramp	0.0 Volts
LM resolution	13.0
HM resolution	13.0
Lens 7	250 Volts
Lens 8	40 Volts
Lens 9	5 Volts
Multiplier	750 Volts
Pressure	
Analyzer vacuum	2.3e-5 mBar
Gas cell	1.4 e-3 mBar
Acylcarnitine analysis functions	
Scans in functions	1
Cycle time	3.510 secs
Scan duration	3.47 secs
Interscan delay	0.04 secs
Retention window	0.600 to 2.000 mins
Ionization mode	ES+
Data type	Accurate mass
Function type	Parents of 85.20
Mass range	210 to 650
Cone voltage	35
Collision energy	25.0

3.4 Organic acid analysis in the urine

3.4.1 Introduction

The analyses of organic acids were done using gas chromatography/mass spectrometry (GC/MS). This method is a standardised method in use at the Laboratory for metabolic diseases, school for Physical and Chemical Science (subjected group Biochemistry) at the North West University (Potchefstroom Campus).

Organic acids were isolated from physiological fluids with ethyl acetate and diethyl ether extractions. The most important steps of this method are the isolation of the organic acids from the physiological fluids, formation of volatile derivatives and GC-MS analysis.

3.4.2 Reagent preparations

Internal standard: 26.25 mg of 3-phenyl-butyric acid were dissolved in a few drops of NaOH, and made up to 50 ml with distilled water. The internal standard solution was stored at 4 ºC.

The 5 M HCl were prepared by adding 50 ml of 32 % HCl to 100 ml of distilled water and was subsequently stored at 4 ºC.

External standard: C_{24} 52.2 mg of tetracosanoic acid was added to 100 ml of ethyl acetate and stored at -20 ºC and used at room temperature.

Bis(trimethylsilyl)-trifluoracetamid (BSTFA); Sigma T1506 and trimethylchlorosilane (TMCS); Sigma T4252, were stored in the fridge.

Procedure notes:

- Hamilton syringes were rinsed with pyridine, acetone or sonificated if dirty.
- Hamilton syringes were well rinsed with hexane between and after use (5x), and the plunger was removed when not in use.
- If silylation crystallize, derivative again (BSTFA & TMCS)
- If water condensed with the sample during evaporation, a few drops hexane was added and the sample was allowed to dry by evaporation.

3.4.3 Experimental procedures for organic acid extraction from urine samples

The volume of urine used from each sample, was determined according to their creatinine values:

- Creatinine > 100 mg% 0,5 ml urine was used
- Creatinine < 100 mg% 1 ml urine was used
- Creatinine < 5 mg% 2 ml urine was used

- Creatinine < 2 mg% 3 ml urine was used

Six drops of a 5 M HCl solution were added to the urine to adjust the pH to 1. Internal standard (IS), (volume of IS in µl = 5 x creatinine mg %) and ethyl acetate (6 ml) was added; the samples were shaken for 30 minutes (Roto-torque) and then centrifuged for approximately 3 minutes. The organic (top) phase was aspirated into a clean tube and 3 ml of diethylether was added to the aqueous (lower) phase, shaken for 10 minutes and then centrifuged for 3 minutes. Again, the organic phase was aspirated and added to the ethyl acetate phase. Two spatulas of powdered Na_2SO_4 (anhydrous) were added after which the samples were thoroughly mixed using a vortex mixer.

Thereafter, the samples were centrifuged again and the organic phase poured into clean, kimax tubes. The samples were dried under a flow of nitrogen at 40 ºC for 1 hour. After drying, BSTFA (volume in µl = 2 x creatinine mg%) was added, along with TMCS (volume in µl = 0.4 x creatinine mg%). The samples were then incubated at 60 ºC for 1 hour and subsequently analysed by GC-MS.

3.4.4 GC/MS analysis

The analyses were done using GC/MS (Hewlett Packard 5880 and mass spectrometer (MS), Hewlett Packard 5988A).

A Macherey-Nagel (MN 30962-52) column and flame ionization detector was used. The inlet for the GC was splitless while the MS was GC dependent splitless.

The following settings applied to the GC. The carrier gas was hydrogen at 2.5 x 100 kpa and 1 ml/min. The makeup gas was nitrogen at 30 ml/min. The final oven temperature was 280 ºC. The carrier gas in MS was helium.

3.5 Analysis of glycine in urine

3.5.1 Introduction

For the determination of glycine (an amino acid), concentration in urine a standardized method employing GC/MS for free amino acids (EZ: Faast amino acid kit: Phenomenex) was used (Husek *et al.,* 2002). This method may be used to assay > 60 aliphatic and aromatic amino acids, but in this study, only glycine was determined.

3.5.2 Material and preparation of stock solution

Table 3.5 Different reagents

Reagent	Ingredients	Volume
A Internal standard solution	Norvaline 0.2mM & N-propanol 10 %	50 ml
B Washing solution	N-propanol	90 ml
C Eluting medium compound I	Sodium Hydroxide	60 ml
D Eluting medium compound II	N-propanol	40 ml
E Organic solution I	Chloroform	24 ml
F Organic solution II	Iso-octane	50 ml
G Re-dissolution solvent	Iso-octane (80 %) & Chloroform (20 %)	50 ml

Eluting Medium

A fresh eluant solution was prepared daily. Three parts of reagent **C** were mixed with two parts of reagent **D**. The solution was stored at room temperature during the experiments.

The sorbent tip

When using the sorbent tip the following caution should be taken. The piston should not been pulled back quickly. Take at least one minute to pass low viscosity sample through the sorbent tip, although urine passes relatively fast through the sorbent bed. The syringe should be capable of drawing the sample and subsequent wash reagent into the barrel. The liquid accumulates inside the syringe barrel and move the piston only as the accumulation liquids slows down. When you run out of piston range, detach the sorbent tip, expel the solution from the syringe barrel, then reattach the sorbent tip, and proceed with sample preparation.

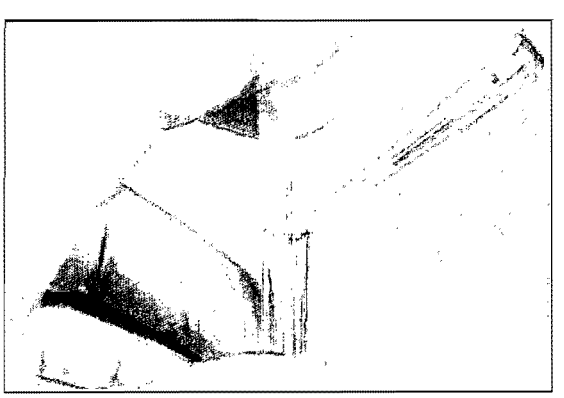


Figure 3.1 The sorbent tip in the sample preparation vial

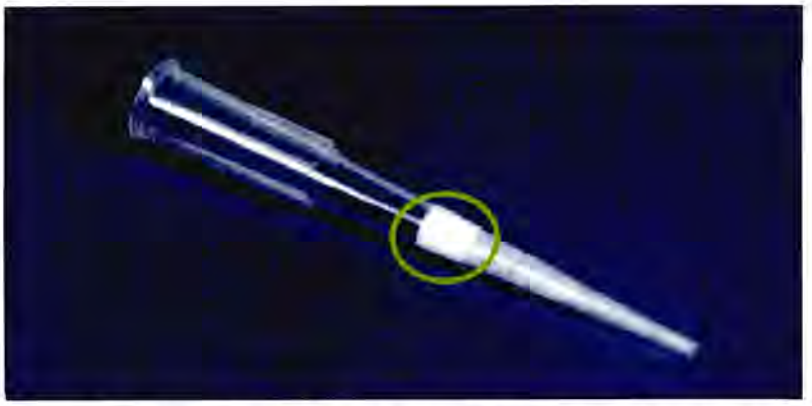


Figure 3.2 The sorbent tip. The filter is indicated by the green circle.

Wet the sorbent with Eluting Medium (**C** en **D**) and stop before it gets to the filter, and then expel all the liquid and sorbent particles out of the tip. Only the filter disk should remain in the empty tip.

3.5.3 Solid phase extraction of glycine

100 µL of the urine sample were added to 100 µl of the internal standard solution (**A**) in a vial. Attach a sorbent tip to a 1.5 ml syringe and loosen the syringe piston (figure 3.1). Passed the solution in the sample preparation vial through the sorbent tip by slowly pulled back the syringe piston. 200 µl washing solution (**B**) was pipette into the sample preparation vial. The solution was slowly passed through the sorbent tip, and into syringe barrel. 200 µl eluting medium (**C** en **D**) was added to the sample preparation vial, and the piston of a 0.6 ml syringe was halfway up the barrel and attached to the sorbent tip. The sorbent was wetted with eluting medium, stop when the liquid reached the filter in the sorbent tip (figure 3.2). Expel all the liquid and sorbent particles out of the tip. The liquid and sorbent were collected in the sample preparation vial.

A Drummond Dialamatic Microdispenser was used to add 50 µL chloroform (**E**) to the sample preparation. The liquid was emulsified by repeatedly vortexing it for a few seconds. One minute was allowed for the reaction to proceed. The emulsion separated gradually into two layers. The solution was vortexed again for a few seconds to re-emulsify the content of the vial. The reaction proceeded for one more minute. 100 µL iso-octane (**F**) was added, using the microdispenser, and the vial was vortexed for five seconds, and one minute was allowed for reaction to proceed. The organic upper layer was transferred to an autosampler vial using a Pasteur pipette.

The solvent was slowly concentrated until almost dry under a stream of nitrogen (maximum period of 10 minutes). Amino acid derivatives were redissolved in 100 µL iso-octane (80 %) and chloroform (20 %), (**G**). The reconstituted sample was transferred to an insert in the same auto sampler vial and subsequent analysed by GC/MS as described below.

3.5.4 GC/MS analysis

The analyses were done by GC-MS (GC: Hewlett Packard 5880 and MS: Hewlett Packard 5988A). The instrumental settings for the GC were 1.5-2 µl and the samples were injected at split 1:15 at 250 ºC. The constant flow for the helium carrier gas was 1.1 ml/min. The oven program was set for 30 ºC/rnin from 110 ºC to 320 ºC. The instrumental settings for the MS were: MS source 240 ºC, MS quad 180 ºC, auxiliary 310 ºC, scan range 45-450 m/z and sampling rate 2^2 (3.5 scans/s). A Zebron ZB-AAA column supplied with the kit, was used.

3.6 Quantification of data

The results were analyzed to quantify the raw data by AMDIS. AMDIS is an Automated Mass Spectral Deconvolution and Identification System, for the identification of the concentration of glycine conjugates. The concentration of glycine in urine was determined by w-search software.

The concentration of organic acids was determined as follows:

Organic acids (µmol/l) = Area of specific organic acid / Area of IS x 262.5

The concentration of amino acids were determined as follows:

Amino acid (µmol/l) = Area of specific amino acid / Area of IS x RF x 200

3.7 Statistics

Statistical analyses were carried out at the Department of Statistical Services at the North-West University (Potchefstroom Campus) using Statistica 8® software. The Tukey HSD test was used to compare the different acylcarnitines and ratios in all the groups, while the unequal N HSD test (a generalization of Tukey's multiple comparisons test) was used to compare the body weight in all the groups. Differences were regarded as significant if $p \leq 0.05$. The Kruskall-Wallis test (H (5,N=60,59,55,57) was used for the glycine conjugate data (multiple comparisons p-values (2-tailed) H (2, N = 38). See appendix H for statistical analyses.

Chapter 4

4 Results and discussion

Biochemical testing of blood and urine for carnitine, acylcarnitines, acylglycines, and organic acids is used as a diagnostic investigation for disorders of fatty acid oxidation and also to establish the influence of drug treatment on fatty acid oxidation. Excessive acyl-CoA, which accumulates proximal to a metabolic block, may be converted into acylcarnitines by chain-length specific carnitine acyltransferases. These acylcarnitines are then transported out of the mitochondria and out of the cell to be filtered by the kidneys.

4.1 Carnitine conjugates

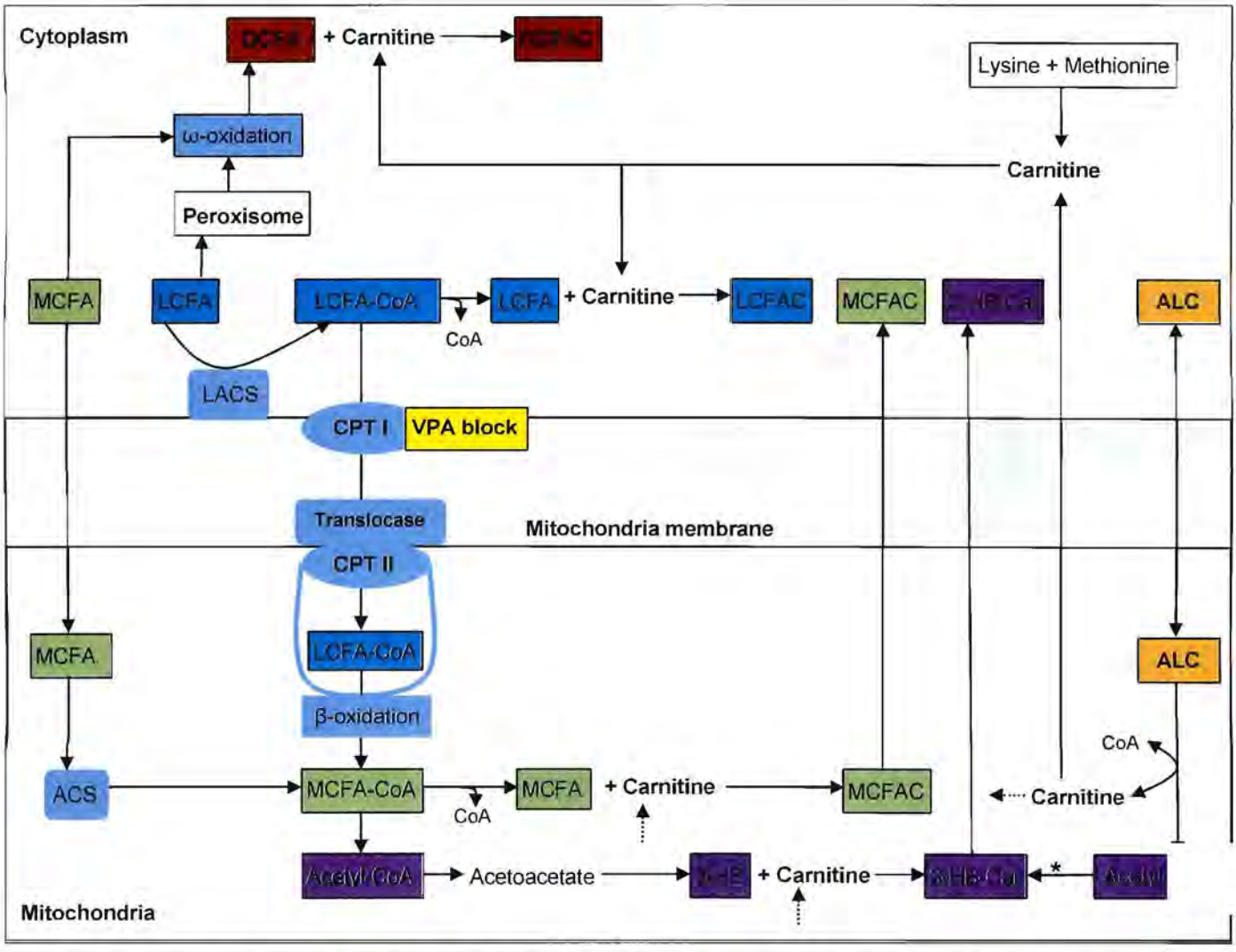


Figure 4.1 Illustration of fatty acid metabolism and conjugation. DCFA = dicarboxylic fatty acids; DCFAC = dicarboxylic fatty acid acylcarnitine; LCFA = long chain fatty acid; LCFAC = long chain fatty acylcarnitine; MCFA = medium chain fatty acid; MCFAC = medium chain fatty acylcarnitine; 3-HB = 3-hydroxybutyrate; 3-HB-CAR = 3-hydroxybutrate-carnitine; CPT = Carnitine pamtoyltransferase; ALC = acetylcarnitine; VPA = valproate; ACS = acyl-CoA synthetase; LACS = long chain acyl-CoA synthetase. The * indicates the synthesis of 3-HB-CAR.

Figure 4.1 illustrates the metabolism of fatty acids. In the cytoplasm, long chain acyl-CoA synthetase (LACS) activates the long chain fatty acids (LCFA) to LCFA-CoA. This LCFA-CoA enters the mitochondria through the carnitine shuttle for subsequent β-oxidation to medium chain fatty acyl-CoA (MCFA-CoA). LCFA-CoA in the cytoplasm binds to carnitine to form long chain fatty acylcarnitines (LCFAC) that are excreted in the urine (indicated in blue in figure 4.1).

Medium chain fatty acids (MCFA) enter the mitochondria without a transport mechanism and are activated by acyl-CoA synthetase (ACS) to MCFA-CoA to undergo further β-oxidation. MCFA-CoA in the mitochondria binds to carnitine to form medium chain fatty acylcarnitines (MCFAC), which are exported out of the mitochondria, and excreted in the urine (indicated in green in figure 4.1).

Acetyl-L-carnitine (ALC) moves freely over the mitochondrial membrane. In the mitochondria, ALC is converted to acetyl-CoA with the release of carnitine (indicated in orange in figure 4.1). This carnitine is available for conjugation of MCFA and 3-hydroxybutyrate (3-HB) to form MCFAC and 3-hydroxybutyrylcarnitine (3-HB-CAR), which in turn can be exported out of the mitochondria and excreted in the urine (indicated in purple in figure 4.1).

The acetyl-CoA in the mitochondria, released from either the β-oxidation pathway or when ALC is converted to carnitine, forms acetoacetate, which is then converted to 3-HB. 3-HB binds to carnitine to form 3-HB-CAR, which in turn is exported out of the mitochondria and excreted in the urine (indicated in purple in figure 4.1).

Although the ω-hydroxylation pathway is a minor pathway in the metabolism of fatty acids (4–15%), its importance is dramatically increased during starvation, by ethanol, hypolipidemic drugs, peroxisome proliferators, and in different metabolic diseases. In the cytoplasm, LCFA and MCFA enter the peroxisomes to produce shorter chain fatty acids by several cycles of β-oxidation, where after ω-oxidation takes place to form dicarboxylic fatty acids (DCFA). These DCFA bind to carnitine to form dicarboxylic fatty acylcarnitines (DCFAC) that are excreted in the urine (indicated in red in figure 4.1). The carnitine in the cytoplasm originates from lysine and methionine, or carnitine that is exported out of the mitochondria.

In the following paragraphs the results obtained in the various groups of rats receiving valproate, carbamazepine, acetyl-L-carnitine, carnosine and the ketogenic diet are reported. (See appendix C for the raw data of the urine carnitine analyses and appendix I for a summery).

Analyses were performed on both urine and blood samples, however, only results from urine samples are reported and discussed. Because blood data only measures a metabolite at the specific time the sample is taken, and because individual subjects differ regarding their rate of

metabolism, huge differences were found for individuals when blood data are compared. Urine samples, on the other hand, measure biological markers excreted over a period of time, resulting in integrated values over time and thus diminishing intersubject variation. Blood samples were analysed for various groups and are given in appendix D, but did neither support nor contradict our urinary data.

4.1.1 Total acylcarnitines (MCFAC, LCFAC, DCFAC)

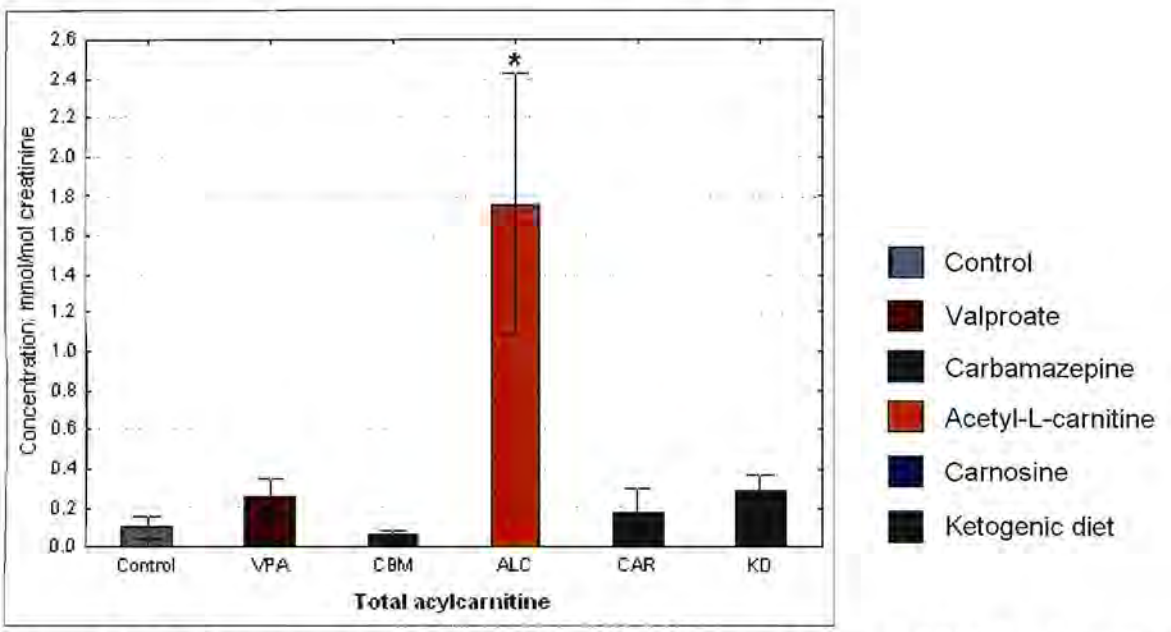


Figure 4.2 Total acylcarnitine is the sum of MCFAC, LCFAC, and DCFAC. * indicates a significant difference ($p < 0.001$) between ALC and the control. Error bars indicate a 95% confidence interval.

The group of rats that received acetyl-L-carnitine showed a statistically significant ($p < 0.001$) increase of mean urinary total acylcarnitine excretion, mainly because of a significant increase in MCFAC (See par 4.1.3) and a slight increase in DCFAC (see par 4.1.4) excretion.

The ketogenic diet group showed a slight increase in mean total acylcarnitine urinary excretion, because of the significant increase in LCFAC (see par 4.1.2) and DCFAC (see par 4.1.4) excretion. The increase in urinary total acylcarnitine excretion of the ketogenic diet was not statistically significant.

The valproate group showed a slight increase in mean total acylcarnitines excretion, because of formation of valproylcarnitine that has the same molecular weight as medium chain fatty acylcarnitines (MCFAC). This increase, however, was not statistically significant.

The carbamazepine and carnosine groups showed no significant increases in mean total acylcarnitines excretion in comparison with the control group.

4.1.2 Total long chain fatty acylcarnitine (LCFAC)

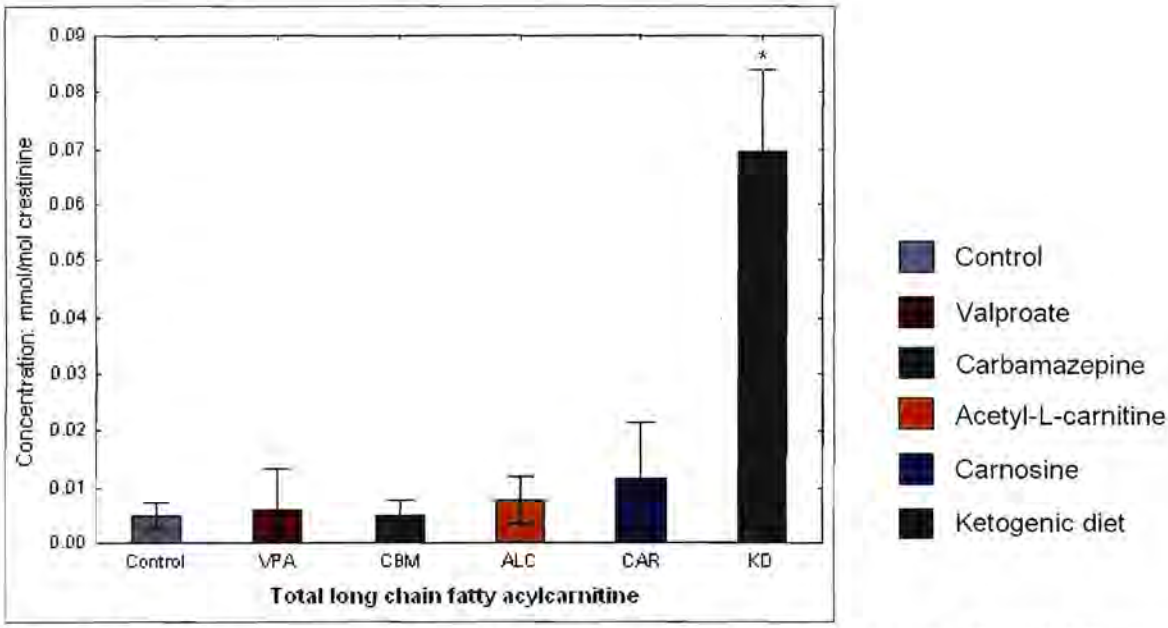


Figure 4.3 Total long chain fatty acylcarnitine (LCFAC). * indicates a significant difference ($p < 0.001$) between KD and the control. Error bars indicate a 95% confidence interval.

The LCFAC group consists of lauroylcarnitine, myristoylcarnitine, palmitoylcarnitine, stearylcarnitine, and adipylcarnitine.

The ketogenic diet group exhibited significantly ($p < 0.001$) higher urinary excretion of mean LCFAC, which could be attributed to this diet consisting of several long chain fatty acids. These long chain fatty acids do not all enter the mitochondria for β-oxidation, but accumulate in the cytoplasm and bind to carnitine to form LCFAC, which is excreted in the urine.

The acetyl-L-carnitine group showed a slight increase in mean LCFAC, possibly due to a higher release of carnitine when ALC is converted to acetyl-CoA in the mitochondria. This carnitine is exported out of the mitochondria and binds to LCFA to form LCFAC which is excreted in the urine. This increase did not significant differ from the control group.

There was no significant difference between the control, valproate, carbamazepine and carnosine groups, with respect to urinary mean LCFAC excretion.

4.1.3 Total medium chain fatty acylcarnitine (MCFAC)

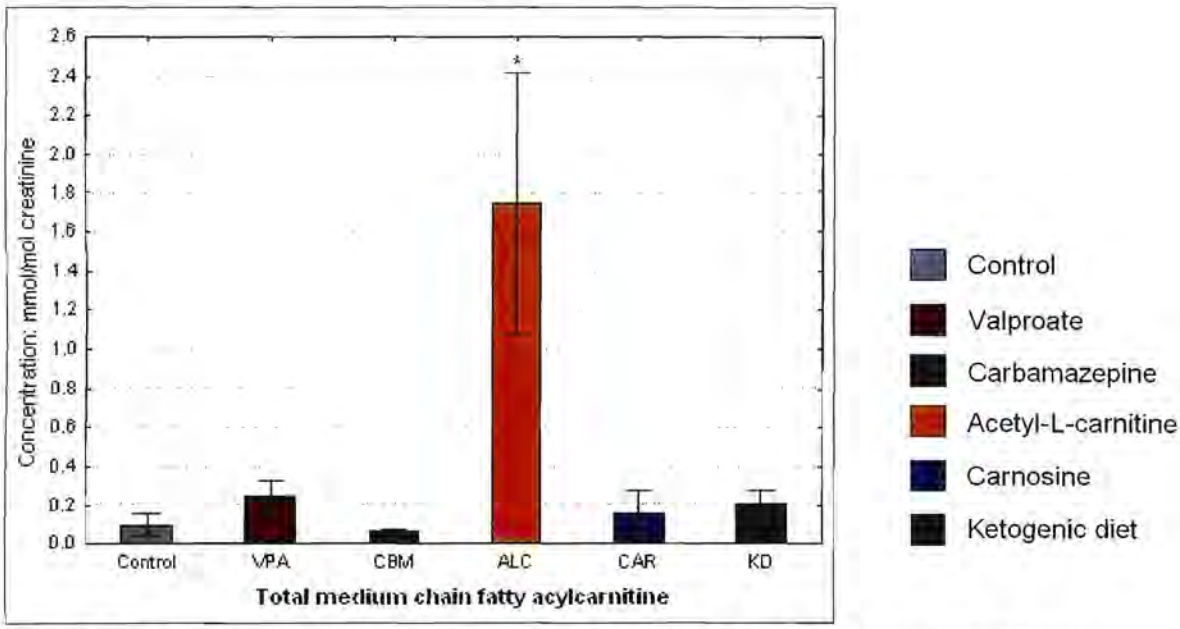


Figure 4.4 Total medium chain fatty acylcarnitine (MCFAC). * indicates a significant difference ($p < 0.001$) between ALC and the control. Error bars indicate a 95% confidence interval.

The MCFAC group consists of butanoylcarnitine, hexanoylcarnitine, octanoylcarnitine, and decanoylcarnitine.

The acetyl-L-carnitine group showed a statistically significant ($p < 0.001$) increase in mean urinary MCFAC excretion. This may be attributed to a high import of acetyl-L-carnitine into the mitochondria, where it is converted to acetyl-CoA with the release of carnitine. The carnitine binds to the available medium chain acyl-CoA in the mitochondria to form MCFAC, which are exported out of the mitochondria and excreted in the urine. This high excretion of medium chain fatty acylcarnitines is in agreement with Moreno *et al.* (2005) and Yokoi *et al.* (2007) who showed that toxic metabolites in the mitochondria are excreted as acylcarnitines.

The ketogenic diet group showed a slight, but not significant ($p > 0.1$) increase in mean MCFAC, which may be explained by the higher content of LCFA in the diet which are subsequently oxidised to MCFA. Carnitine binds to the MCFA and the MCFAC is exported out of the mitochondria and excreted in the urine.

Valproate is structurally related to medium chain fatty acids and this group also showed a slight, but non-significant, increase in mean MCFAC excretion, which may be explained by valproylcarnitine that is formed and exported out of the mitochondria.

The carbamazepine and carnosine groups showed no statistically significant difference in mean MCFAC excretion in comparison with the control group.

4.1.4 Total dicarboxylic fatty acylcarnitines (DCFAC)

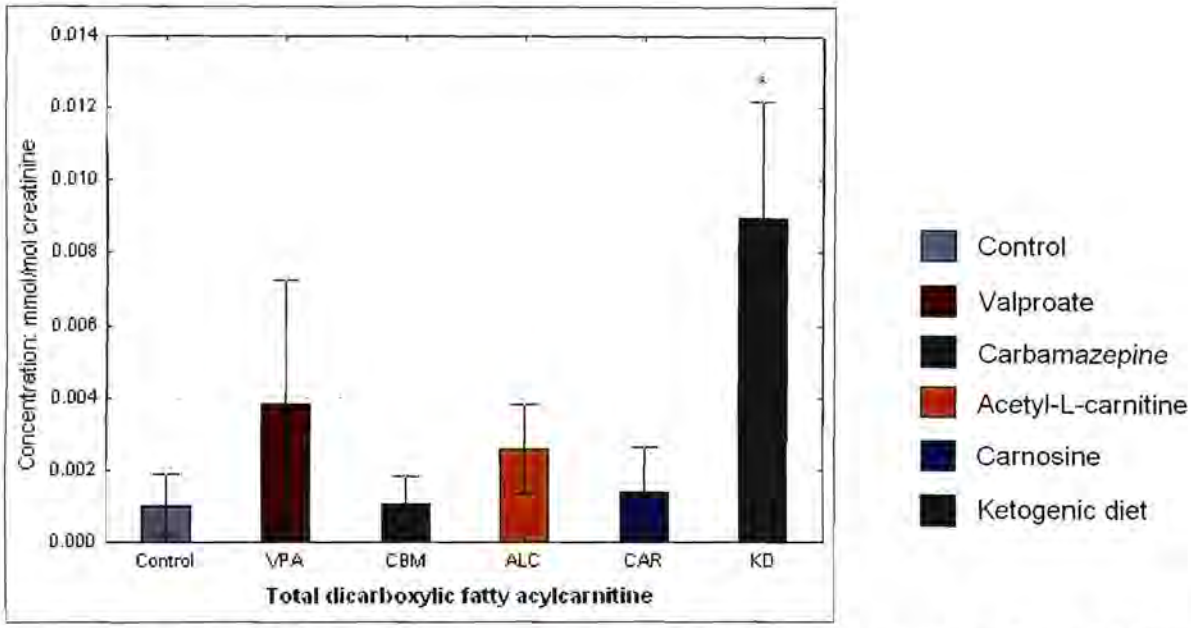


Figure 4.5 Total dicarboxylic fatty acylcarnitine (DCFAC). * indicates a significant difference between ($p < 0.001$) KD and the control. Error bars indicate a 95% confidence interval.

The DCFAC group consists of suberylcarnitine, CC10-458, CC12-486, CC14-514, and CC16-542.

The ketogenic diet showed a statistically significant increase in mean urinary DCFAC excretion. (KD vs. control, CBM, ALC, and CAR $p < 0.001$, and KD vs. VPA $p < 0.01$). The ketogenic diet mimics a state of starvation, which increases intracellular levels of free fatty acids, which become available as substrates for ω-oxidation, causing dicarboxylic fatty acids to be produced. DCFA can either be β-oxidized in the mitochondria or peroxisomes or excreted into the urine as DCFAC.

Compared to controls the mean DCFAC excretion of the valproate group showed a considerable increase but this increase was not statistically significant due to large variation in the data. It is possible that the higher DCFAC excretion is explained by valproate blockage of CPT-I, leading to less β-oxidation, the induction of ω-oxidation and thus the production of DCFA that is excreted as carnitine conjugates in the urine.

The acetyl-L-carnitine group also showed a non-significant increase in mean urinary DCFAC excretion, possibly due to a higher release of carnitine when ALC is converted to acetyl-CoA in the mitochondria. This carnitine is exported out of the mitochondria and binds to DCFA to form DCFAC, which is excreted in the urine.

The carbamazepine and carnosine groups showed no effect in mean urinary DCFAC excretion.

4.1.5 Acetylcarnitine

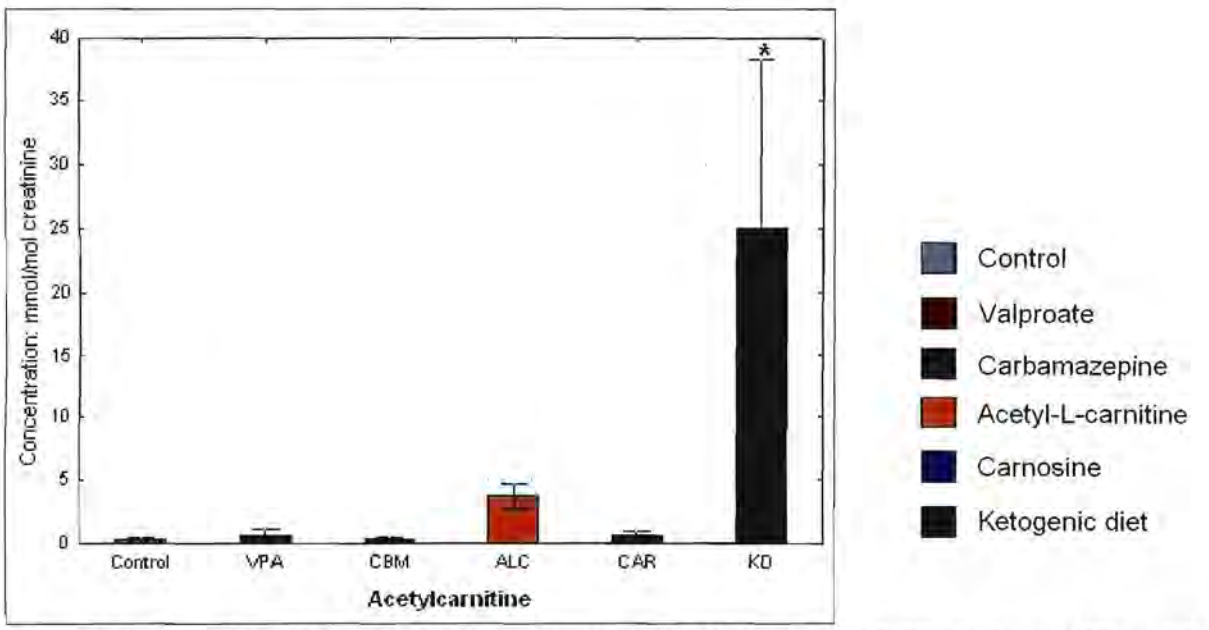


Figure 4.6 Acetylcarnitine. * indicates a significant difference ($p < 0.001$) between KD and the control. Error bars indicate a 95% confidence interval.

Compared to the control, the ketogenic diet significantly increased ($p < 0.001$) mean urinary acetylcarnitine excretion. This is potentially due to the induction of β-oxidation by the ketogenic diet, leading to a higher production of acetyl-CoA, and therefore indicates that the ketogenic diet stimulates acetylcarnitine production. Acetylcarnitine excretion in the other groups did not differ significantly from the control group.

4.1.6 Free Carnitine

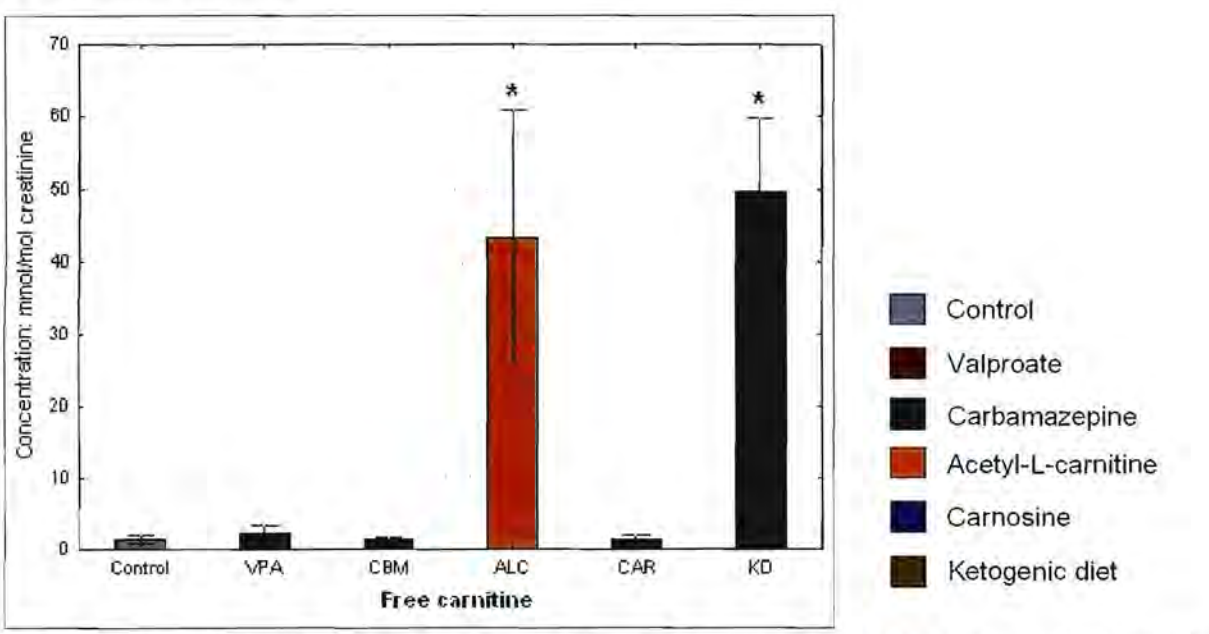


Figure 4.7 Free carnitine. * indicate a significant difference ($p < 0.001$) between ALC and KD and the control. Error bars indicate a 95% confidence interval.

The acetyl-L-carnitine and the ketogenic diet groups demonstrated statistically significant increases in mean urinary free carnitine excretion ($p < 0.001$). This result is quite obvious for the acetyl-L-carnitine group, because acetyl-L-carnitine is administered. In the case of the ketogenic group, the enhanced mean free carnitine excretion is probably caused by a stimulation of carnitine biosynthesis.

4.1.7 3-Hydroxybutyryl-carnitine (3-HB-CAR)

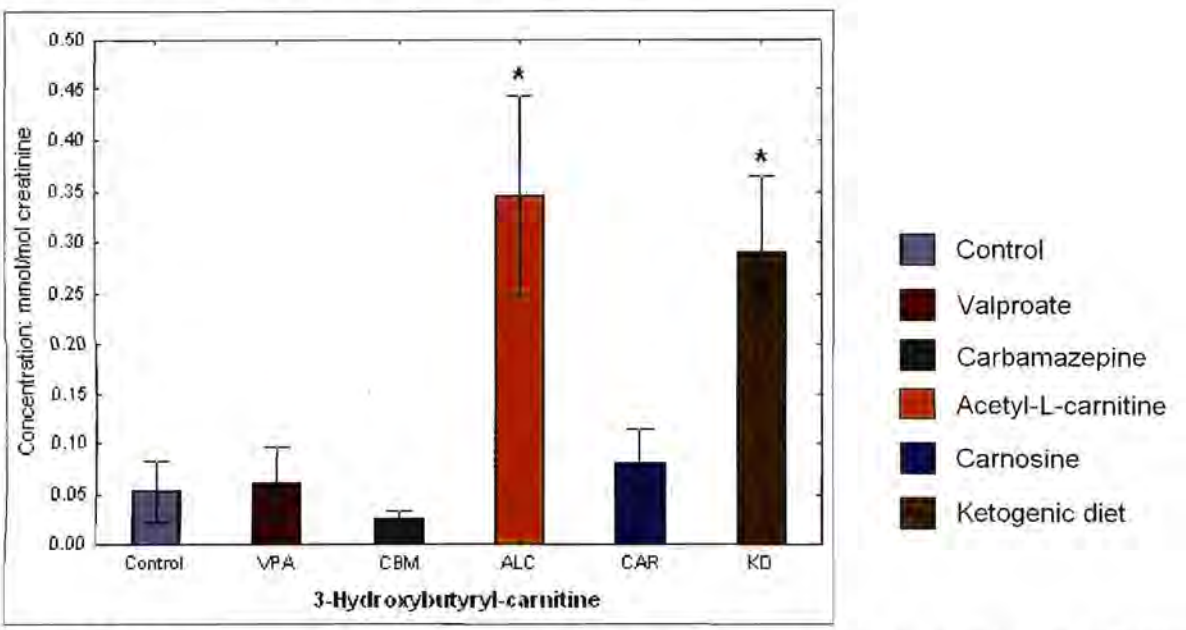


Figure 4.8 3-Hydroxybutyryl-carnitine (3-HB-CAR). * indicates a significant difference between ($p < 0.001$) ALC and KD and the control. Error bars indicate a 95% confidence interval.

Both the acetyl-L-carnitine and the ketogenic diet groups significantly increased the mean urinary 3-hydroxybutyrylcarntine excretion ($p < 0.001$). The ketogenic diet increases β-oxidation, causing high levels of acetyl-CoA to be generated, leading to the synthesis, primarily in the liver, of the three ketone bodies β-hydroxybutyrate, acetoacetate, and acetone. Because of the high rate of β-oxidation, the Krebs cycle cannot manage the flux, and excess acetyl-CoA is shunted to the production of ketone bodies. Ketone bodies spill into the circulation, causing serum and urine levels to rise several fold, and then are utilized as an energy source in the brain (Hartman *et al.*, 2007). This could play an important role in the treatment of epilepsy with a ketogenic diet.

Acetyl-L-carnitine is an ester of L-carnitine (trimethylamino-β-hydroxybutyrate). ALC is converted to acetyl-CoA in the mitochondria with the release of carnitine. Acetyl-CoA is then used for the synthesis of 3-hydroxybutyrate.

4.1.8 Total acylcarnitine : free carnitine ratio

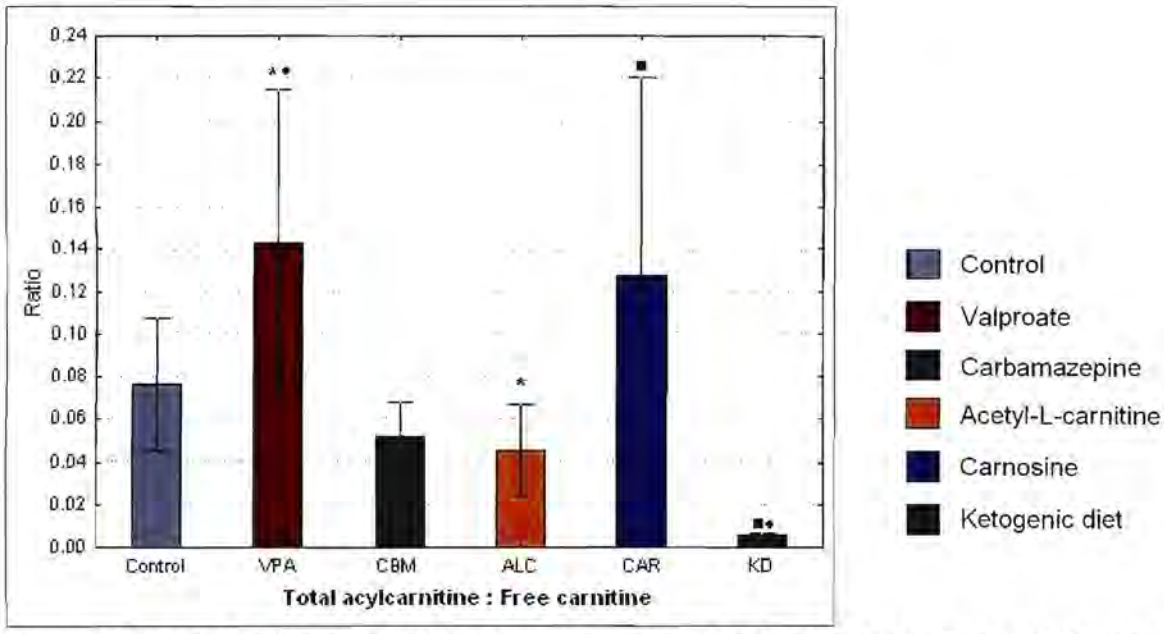


Figure 4.9 Total acylcarnitine : Free carnitine ratio. * indicates a significant difference ($p < 0.05$) between VPA and ALC. • indicates a significant difference ($p < 0.01$) between VPA and KD. ▪ indicates a significant difference ($p < 0.01$) between CAR and KD. Error bars indicate a 95% confidence interval.

Pathological conditions with abnormal acyl-bound carnitine versus free carnitine ratios are exhibited in several inborn errors of metabolism, such as organic acidurias; cirrhosis or chronic renal failure; iatrogenic complications, such as treatment with valproate; all types of diabetes, heart failure and Alzheimer's disease (Rubio *et al.*, 1998). Under normal conditions, about 80% of serum carnitine is free carnitine, and the normal acyl-conjugated/free carnitine ratio is 0.25. Ratios > 0.4 are considered abnormal and indicative of carnitine insufficiency (Deufel, 1990; Berry-Kravis *et al.*, 2001).

There was a statistically significant difference in the total acylcarnitine-to-free carnitine ratio between VPA and ALC, $p < 0.05$; VPA and KD, $p < 0.01$; CAR and KD, $p < 0.01$. There were no statistical differences when results were compared with the control group. The total acylcarnitine-to-free carnitine ratio for the ketogenic diet was low, indicating that the ketogenic diet did not cause a carnitine deficiency (Berry-Kravis *et al.*, 2001).

The total acylcarnitine-to-free carnitine ratio for valproate was high, indicating a carnitine insufficiency, which is in agreement with the literature (Coppola *et al.*, 2006). Valproate binds to carnitine to form valproylcarnitine, which explains the loss of carnitine and therefore the high ratio.

The total acylcarnitine-to-free carnitine ratio for carnosine was high, indicating a carnitine insufficiency with this treatment.

4.1.9 Acetylcarnitine : free carnitine ratio

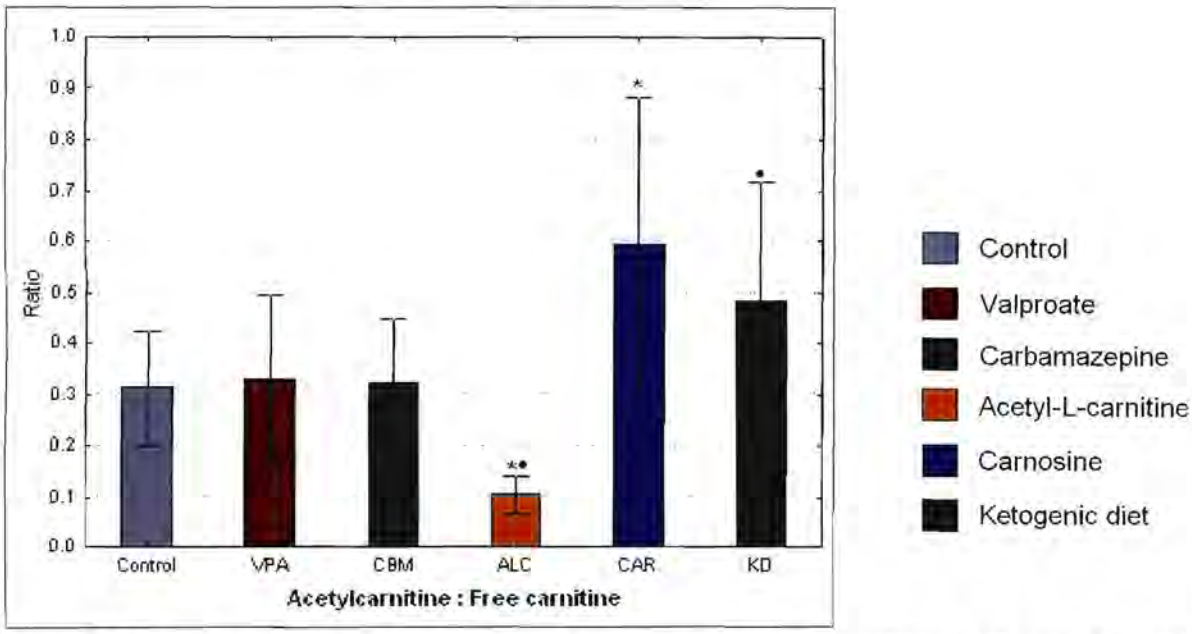


Figure 4.10 Acetylcarnitine : Free carnitine ratio. * indicates a significant difference ($p < 0.01$) between CAR and ALC. • indicates a significant difference ($p < 0.05$) between ALC and KD. Error bars indicate a 95% confidence interval.

The ALC group differed significantly from CAR ($p < 0.01$) and the KD ($p < 0.05$) groups.

The acetylcarnitine-to-free carnitine ratio for the acetyl-L-carnitine group was decreased, whereas the ketogenic diet and carnosine groups showed an increased ratio. The marked increase in the acetylcarnitine to free carnitine ratio for the ketogenic diet is in agreement with the study of Hack *et al.* (2006) where patients on the ketogenic diet showed a marked increase in their acetylcarnitine-to-free carnitine ratios.

4.1.10 Acetylcarnitine : 3-HB-CAR ratio

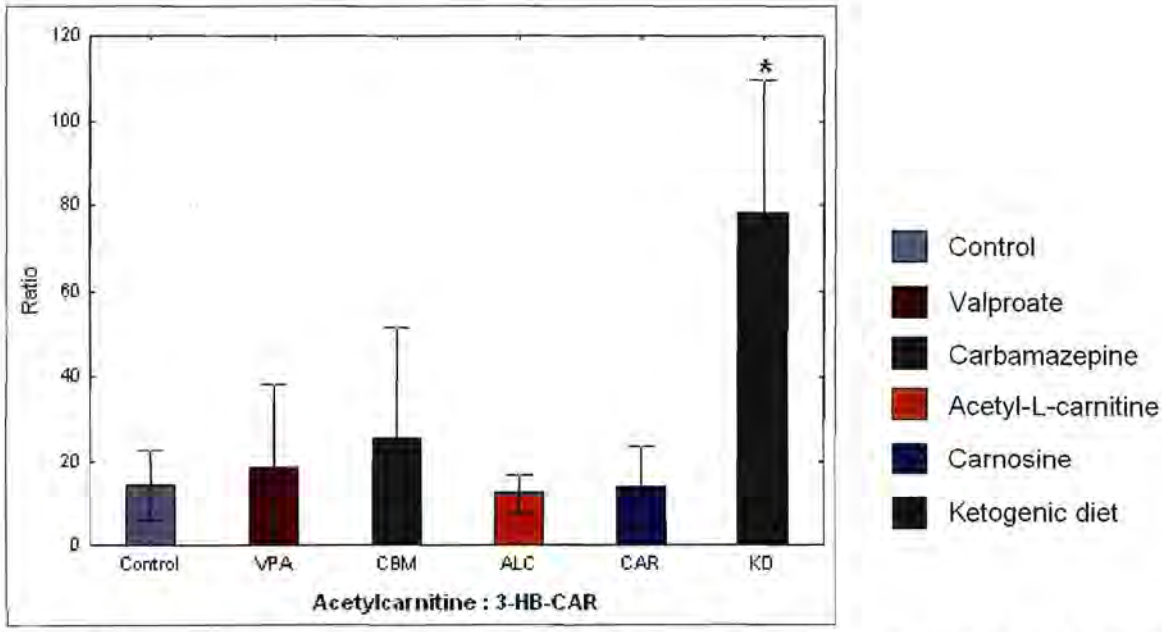


Figure 4.11 Acetylcarnitine : 3-hydroxybutytyl-carnitine ratio. * indicate a significant difference ($p < 0.001$) between KD and control. Error bars indicate a 95% confidence interval.

Compared to controls, the acetylcarnitine-to-3-HB-CAR ratio increased significantly in the ketogenic group ($p < 0.001$). These results imply that the ketogenic diet might stimulate acetylcarnitine production as was indicted in par 4.1.5 (figure 4.6).

Although the acetyl-L-carnitine group showed a higher mean urinary acetylcarnitine excretion (figure 4.6), the excretion of mean 3-HB-CAR was also much higher (figure 4.8), thus lowering the ratio.

4.1.11 Total acylcarnitines : 3-HB-CAR ratio

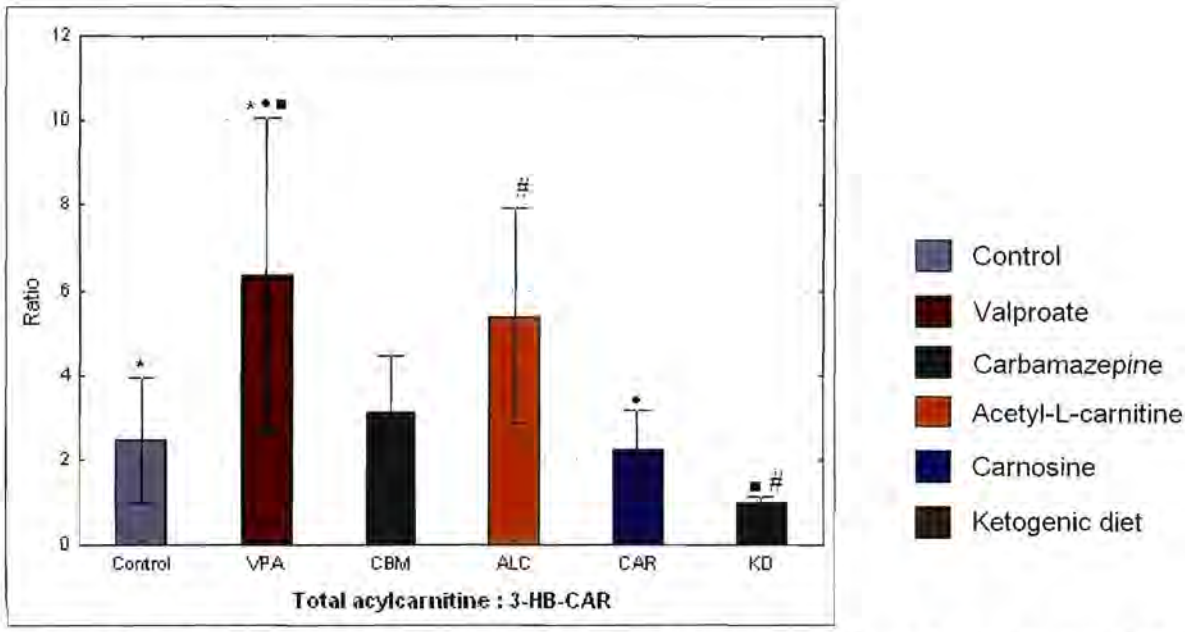


Figure 4.12 Total acylcarnitines : 3-hydroxybutytyl-carnitine ratio. * indicates a significant difference ($p < 0.05$) between VPA and control. • indicates a significant difference ($p < 0.05$) between VPA and CAR. ▪ indicates a significant difference ($p < 0.01$) between VPA and KD. # indicates a significant difference ($p < 0.05$) between ALC and KD. Error bars indicate a 95% confidence interval.

The total acylcarnitine-to-3-HB-CAR ratio for the valproate group differed significantly from the control group ($p < 0.05$). The higher total acylcarnitine excretion can be attributed to the formation of valproylcarnitine.

The total acylcarnitine-to-3-HB-CAR ratio for the acetyl-L-carnitine group was statistically higher than for the ketogenic diet ($p < 0.05$). Although the 3-HB-CAR in figure 4.8 is higher for the acetyl-L-carnitine group, the total acylcarnitines are also higher for the acetyl-L-carnitine group (figure 4.2). This ratio implies that there is a significant loss of carnitine in the form of total acylcarnitines (figure 4.2) in the acetyl-L-carnitine group and this could imply a loss in available potential energy.

The ratio of total acylcarnitine to 3-HB-CAR of the ketogenic diet is very low in comparison with the other groups. The minimum loss of acylcarnitines in figure 4.2 in relation to the high production of 3-HB-CAR in figure 4.8 implies that there is no loss of fatty acids and an increase in potential available energy.

The carbamazepine and carnosine groups showed no significant changes in their total acylcarnitine-to-3-HB-CAR ratios compared to controls.

4.2 Glycine conjugates

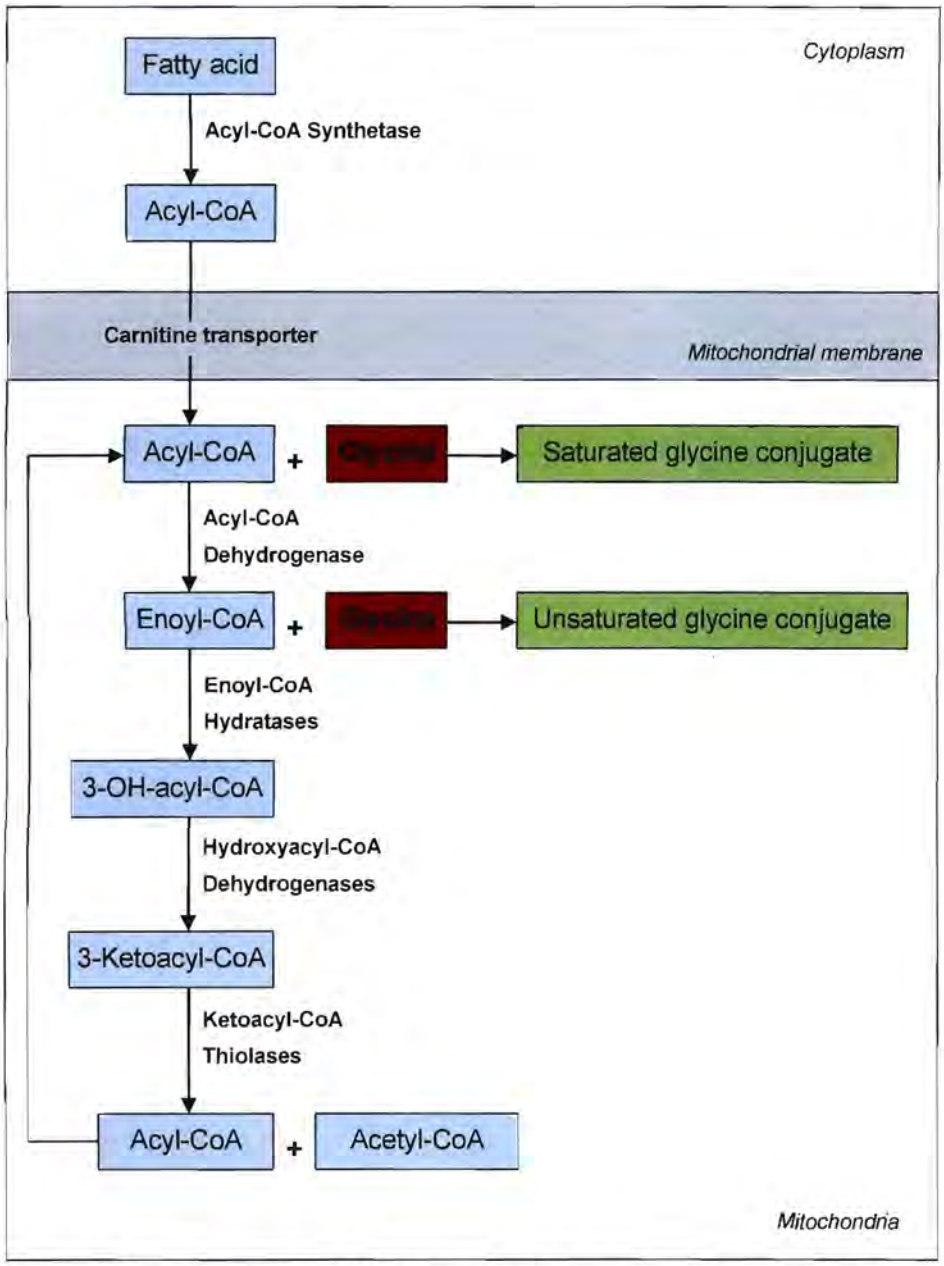


Figure 4.13 Illustration of the formation of glycine conjugates during β-oxidation.

Glycine conjugates can be divided into two groups - saturated and unsaturated glycine conjugates. Glycine conjugates form when fatty acid metabolites of the β-oxidation cycle bind with the amino acid, glycine. An increase in the saturated glycine conjugates can result from inhibition of the acyl-CoA dehydrogenase enzyme in the β-oxidation cycle. This will lead to an accumulation of saturated glycine conjugates. The accumulation of unsaturated glycine conjugates is caused by an inhibition of the enoyl-CoA hydratase enzyme of β-oxidation cycle.

4.2.1 Total glycine conjugates

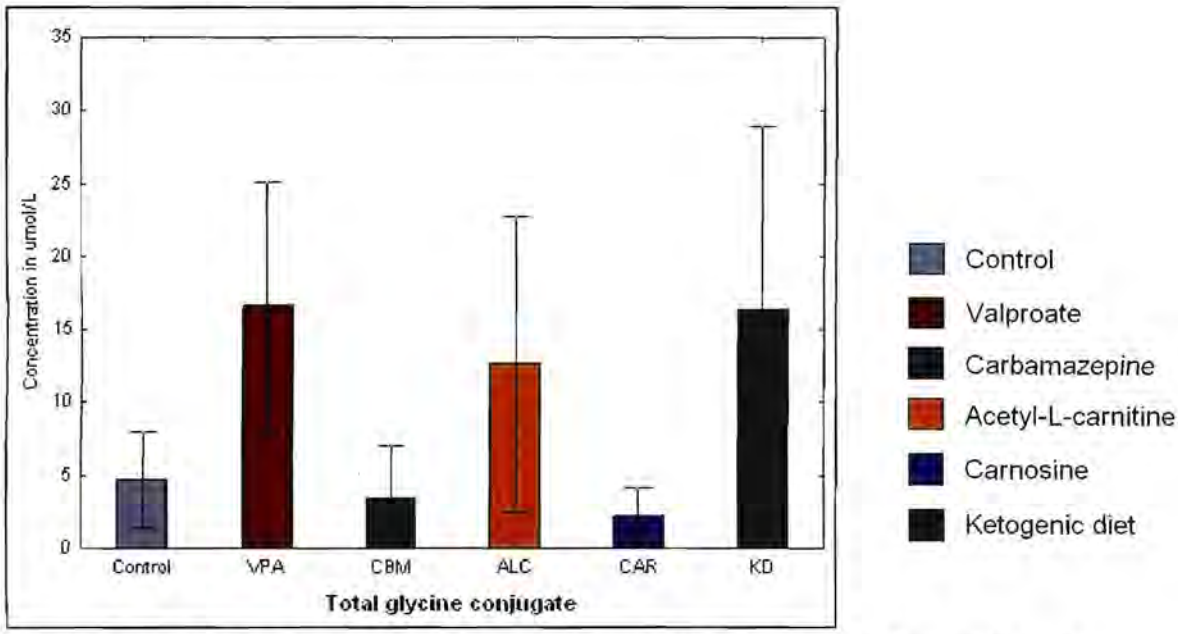


Figure 4.14 Total glycine conjugates. Error bars indicate a 95% confidence interval.

No statically significant differences were found between the groups, although valproate, acetyl-L-carnitine and the ketogenic diet showed an increase in their mean total glycine conjugates urinary excretion.

The increase in the valproate group could be explained by its inhibition of β-oxidation enzymes in the mitochondria (Ito *et al.*, 1990). The increase of the acetyl-L-carnitine group may be due to the higher ability of acyl-CoA to enter the mitochondria. The increase of the ketogenic diet is likely caused by the high concentration of fatty acids available. This high concentration of fatty acids seems to slow down its movement through the β-oxidation system to produce acetyl-CoA.

We divided the glycine conjugates for furtherer analysis into a saturated and unsaturated group as well as the individual conjugates. See appendix F for the raw data of the glycine conjugates and appendix I for a summery (organic acid analyses).

If the treatment inhibits acyl-CoA dehydrogenase, a marked increase of saturated glycine conjugates will result (see figure 4.13). In the case of an inhibitory effect of enoyl-CoA hydratase, an increase of unsaturated glycine conjugates is expected to occur.

4.2.2 Saturated glycine conjugates

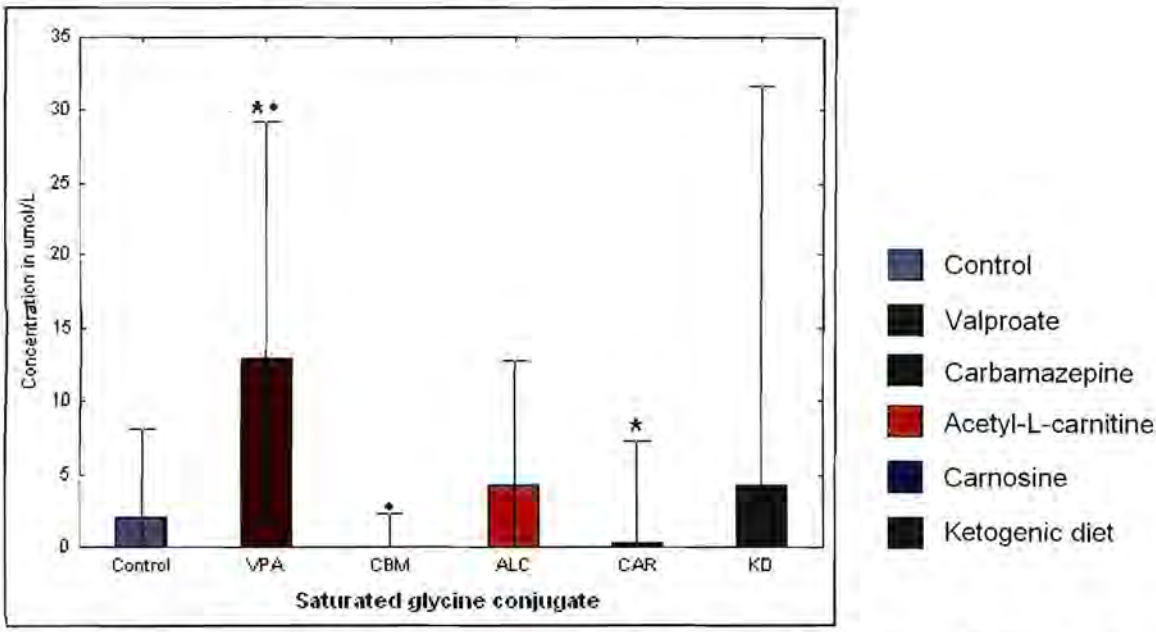


Figure 4.15 Saturated glycine conjugates. * indicates a significant difference ($p < 0.05$) between VPA and CAR. • indicates a significant difference ($p < 0.01$) between VPA and CBM. Error bars indicate a 95% confidence interval.

The saturated glycine conjugates include butyrylglycine, isobutyrylglycine, isovalerylglycine and isovanilylglycine.

There were significant differences between the VPA and CBM group ($p < 0.01$) and VPA and CAR group ($p < 0.05$). At this stage, the increase in the mean saturated glycines observed in the valproate group was thought to be the result of its inhibition of the acyl-CoA dehydrogenase enzyme in the β-oxidation cycle.

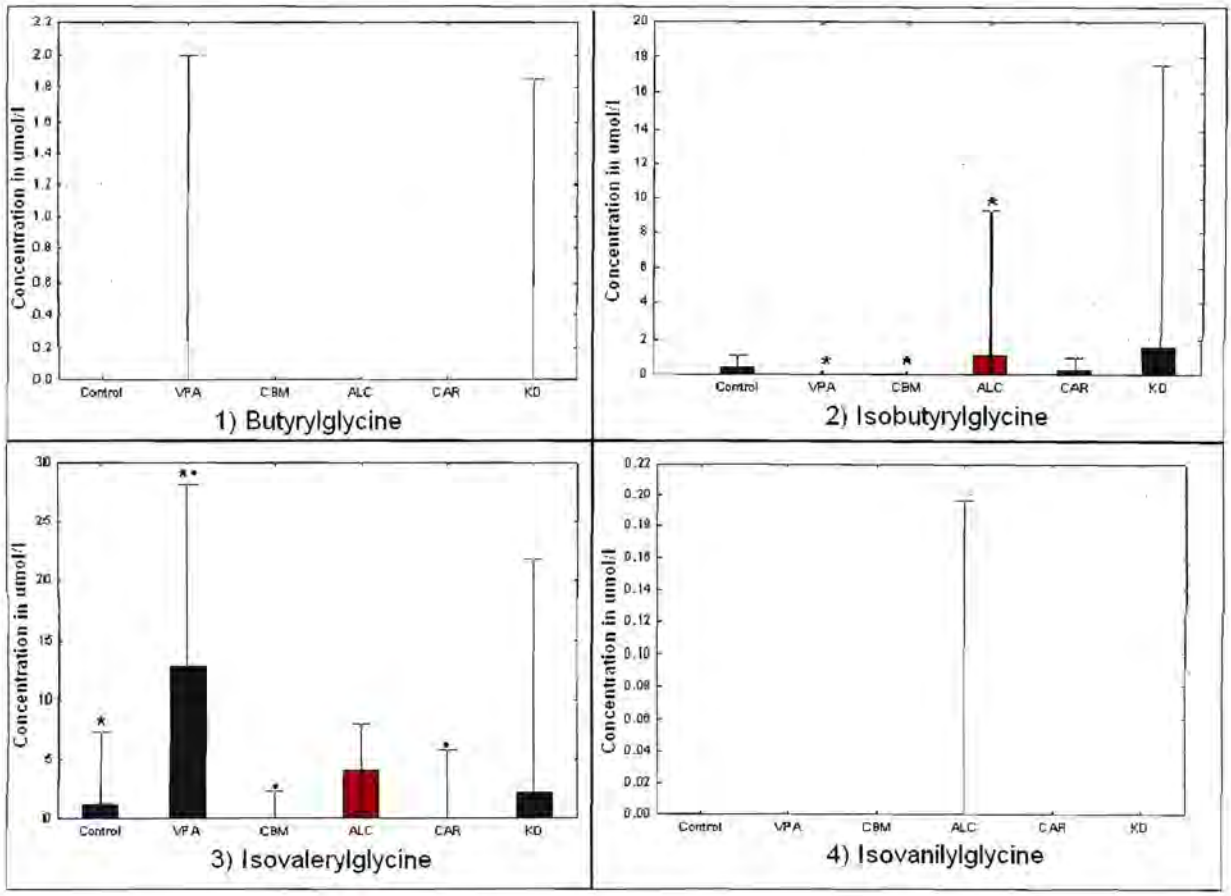


Figure 4.16 Individual saturated glycine conjugates. 1) Butyrylglycine. 2) Isobutyrylglycine: * indicates a significant difference ($p < 0.01$) between ALC and VPA and CBM. 3) Isovalerylglycine: * indicates a significant difference ($p < 0.05$) between VPA and control, • indicates a significant difference ($p < 0.01$) between VPA and CAR and CBM. 4) Isovanilylglycine. Error bars indicate a 95% confidence interval.

No significant increase was found for the mean butyrylglycine conjugate, contrary to our previous deduction, indicating that valproate did not inhibit acyl-CoA dehydrogenase in the β-oxidation cycle. The mean isobutyrylglycine conjugate as well as the mean isovalerylglycine conjugate were elevated but these conjugates do not originate from the β-oxidation cycle but are the products of the leucine and valine metabolism respectively. Isovanilylglycine originates from bacteria.

4.2.3 Unsaturated glycine conjugates

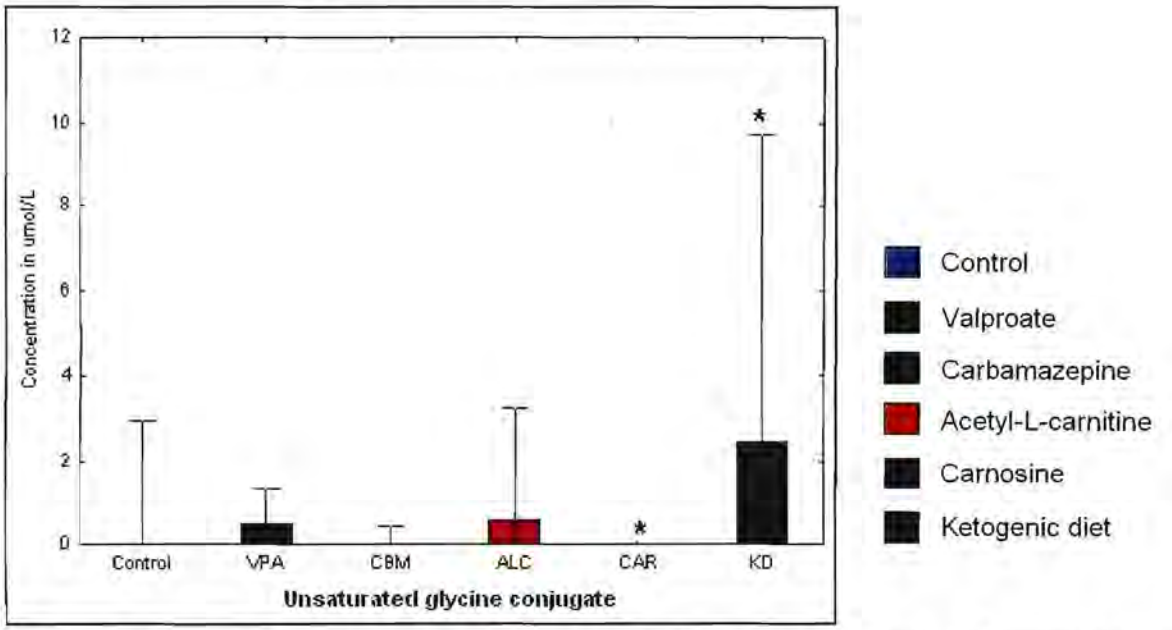


Figure 4.17 Unsaturated glycine conjugate. * indicates a significant difference ($p < 0.05$) between KD and CAR. Error bars indicate a 95% confidence interval.

The unsaturated glycine conjugates consists of crotonylglycine, phenylacetylglycine, tiglylglycine, and 2-methylbutrylglycine.

There was a statistically significant difference between the KD and CAR group ($p < 0.05$).

Individual unsaturated glycine conjugates:

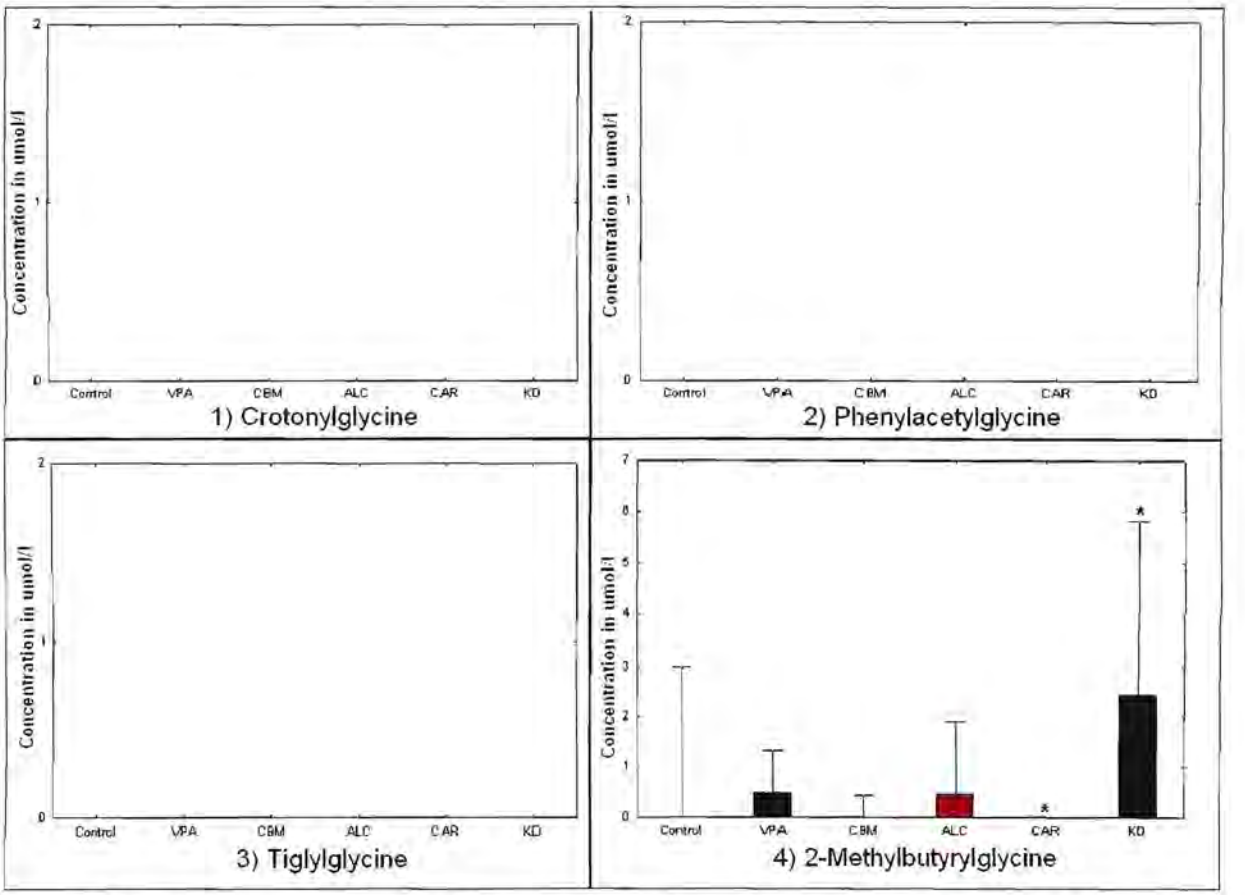


Figure 4.18 Individual unsaturated glycine conjugates. 1) Crotonylglycine. 2) Phenylacetylglycine. 3) Tiglylglycine. 4) 2-Methylbutyrylglycine: * indicates a significant difference ($p < 0.05$) between CAR and KD. Error bars indicate a 95% confidence interval.

Crotonylglycine originates from the fatty acid metabolism. Because no statistically significant differences were observed, it may be concluded that there was no inhibition of the enoyl-CoA hydratase enzyme by any of the interventions.

Tiglylglycine and 2-methylbutyrylglycine are not formed during β-oxidation but both originate from the isoleucine metabolism, while phenylacetylglycine forms during the phenylalanine metabolism.

4.3 Glycine

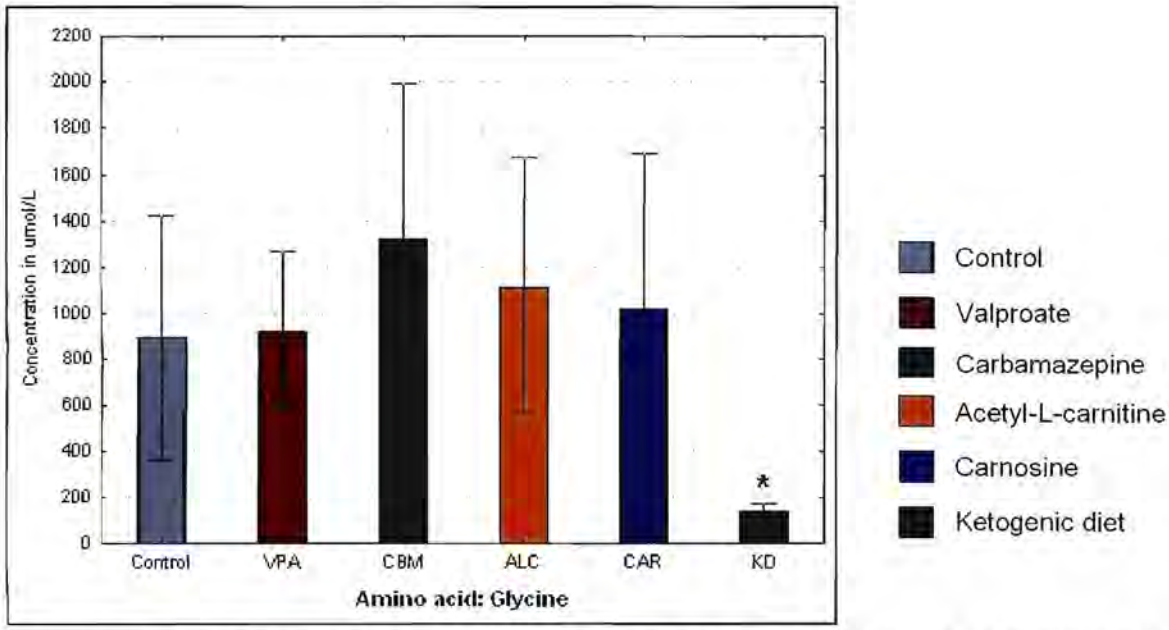


Figure 4.19 Amino acid: glycine. * indicates a significant difference ($p < 0.05$) between KD and control. Error bars indicate a 95% confidence interval.

The ketogenic diet group showed a statistically significant lower concentration of mean glycine urinary excretion compared to the other groups (to the control $p < 0.05$). The low concentration of mean glycine in the urine of the ketogenic diet group suggests that the ketogenic diet does not cause formation of toxic by-products.

See appendix E for the raw data of glycine and appendix I for a summery (amino acid analyses).

4.4 Body weight

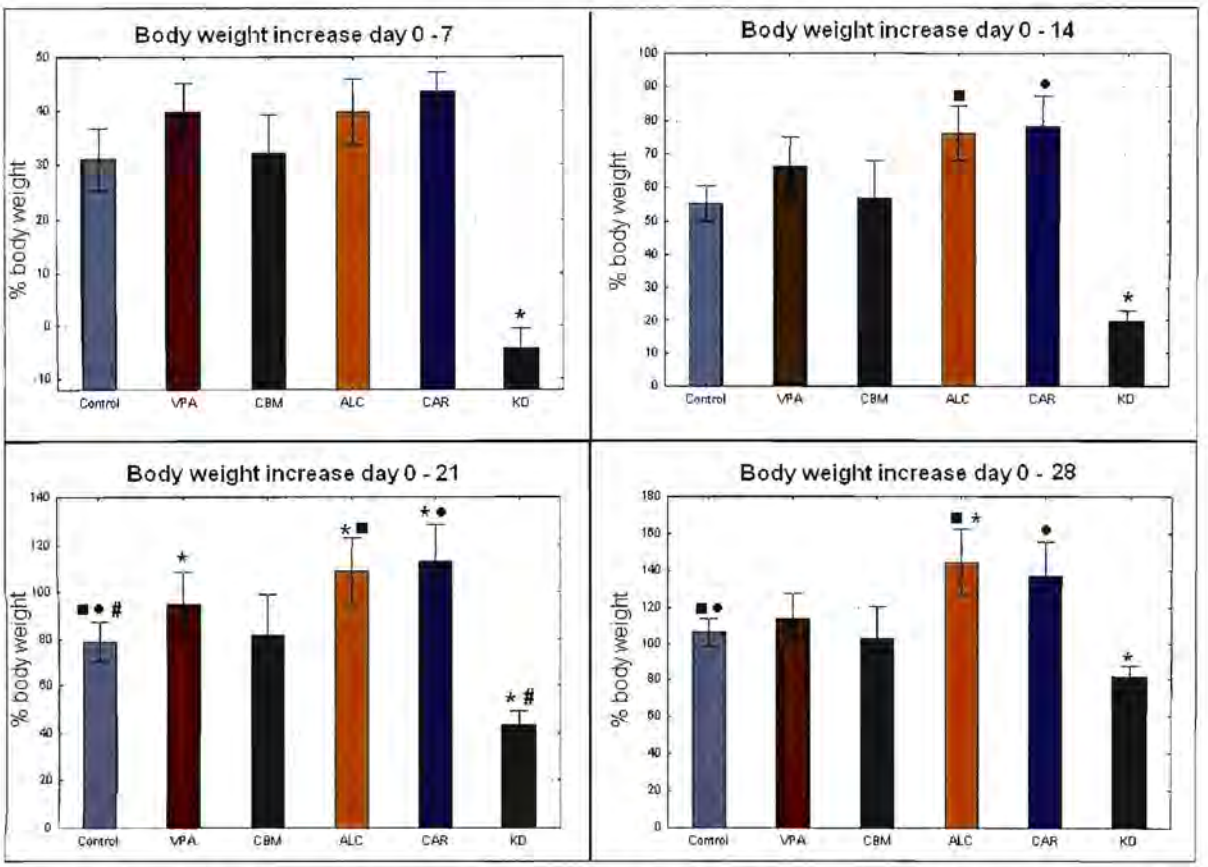


Figure 4.20 Body mass in percentage. 1) Day 0-7: * indicates a significant difference ($p < 0.001$) between KD and control. 2) Day 0-14: * indicates a significant difference ($p < 0.001$) between KD and control, ▪ between ALC and control ($p < 0.01$), and • between CAR and control ($p < 0.001$). 3) Day 0-21: * indicates a significant ($p < 0.001$) difference between VPA, ALC, CAR and control, • between CAR and control ($p < 0.001$), ▪ between ALC and control ($p < 0.01$), # between KD and control ($p < 0.001$). 4) Day 0-28: * indicates a significant difference ($p < 0.001$) between ALC and KD, • between CAR and control ($p < 0.01$), ▪ between ALC and control ($p < 0.01$). Error bars indicate a 95% confidence interval.

Above results indicate that the ketogenic diet contributed to a slower weight gain over the 28 day period of treatment in relation to the other treatments. Thio and co-workers (2006) found that juvenile rodents on a ketogenic diet had slower weight gain than those on a standard diet. Rats on a ketogenic diet had higher serum leptin levels, and it appeared that a functioning leptin signaling system is necessary for the ketogenic diet to slow weight gain. They also speculated that the increase in leptin might contribute to the anticonvulsant effects of the ketogenic diet. See appendix G for raw data and appendix I for a summery.

Chapter 5

5 Conclusion

Valproic acid, a branched chain dicarboxylic acid, with a chemical structure very similar to medium chain fatty acids, is a broad-spectrum antiepileptic drug that has been used for many types of epileptic seizures. It has been shown that valproate treatment may induce hepatotoxicity and although the exact mechanism of this toxicity is unknown, various theories have been proposed. Valproate is a medium-chain fatty acid that requires carnitine for oxidation. One theory, relating to its toxicity, is that valproate combines with carnitine within the mitochondria via carnitine-acyltransferases, resulting in valproylcarnitine ester, which is then transported out of the mitochondria and is eliminated in the urine, thus depleting body carnitine stores. Another mechanism involves valproate's inhibition of mitochondrial oxidation of long-chain fatty acids by the formation of valproyl CoA, which sequesters unbound CoA and decreases its availability. Valproate may also cause hepatotoxicity by inhibiting the enzymes involved in β-oxidation by valproate metabolites, mainly 4-en-valproic acid, which may be directly hepatotoxic (Silva *et al.*, 2008).

In this study, the higher total acylcarnitine-to-free carnitine ratio confirmed the carnitine loss associated with valproate treatment (figure 4.9, paragraph 4.1.8), which is also in agreement with a study done by Coppola *et al.* (2006) that linked carnitine deficiency to valproate treatment. Another theory supported by Lheureux *et al.* (2005) was that carnitine depletion impaired transport of long chain fatty acids into the mitochondrial matrix, with a subsequent decrease in β-oxidation, acetyl-CoA, and ATP production.

Valproate also caused a slight increase in dicarboxylic fatty acylcarnitines, which are formed via ω-oxidation, thus showing that valproate is potentially an inhibitor of CPT-I, resulting in a smaller amount of fatty acids being able to enter the mitochondria. These fatty acids then undergo ω-oxidation, which is in agreement with the hypothesis of Aires *et al.* (2007), that valproyl-CoA is a competitive inhibitor of CPT-I activity *in vitro.* It should be noted that in this study, therapeutic valproate doses were administrated, and that CPT-I inhibition may require higher valproate doses. It is also possible that the relatively small group size, as well as the large variation in data, masked an inhibitory effect which could have been present (type-II statistical error). Based on the data reported here, it may be useful to plan a follow-up study using larger groups of rats to which therapeutic and high doses of valproate are administrated in order to investigate a possible role of valproate as CPT-I inhibitor.

Results from the glycine conjugate determinations showed that valproate, at therapeutic concentrations, does not inhibit β-oxidation enzymes, acyl-CoA dehydrogenase and enoyl-CoA

hydratases, suggesting that inhibition of fatty acid β-oxidation with valproate might be the result of carnitine transport (CPT-I) inhibition.

According to Beal (2003), the primary role of the carnitine system is the transport of fatty acids into cellular mitochondria for their conversion, via β-oxidation, into energy. Acetyl-L-carnitine moves freely across the mitochondrial membrane and is converted to acetyl-CoA with the release of carnitine in the mitochondria.

Treatment with acetyl-L-carnitine caused a high excretion of total acylcarnitines especially medium chain fatty acylcarnitines (figure 4.4, paragraph 4.1.3), indicating that acetyl-L-carnitine is a promising detoxifying agent. This high excretion of medium chain acylcarnitines is in agreement with Moreno *et al.* (2005) and Yokoi *et al.* (2007) who showed that toxic metabolites in the mitochondria are excreted as acylcarnitine.

The ketogenic diet contains several LCFA, which is confirmed by the increased excretion of the long chain fatty acylcarnitines and dicarboxylic fatty acylcarnitine. Due to the subsequent high concentration of fatty acids in the cytoplasm, these fatty acids do not enter the mitochondria fast enough for β-oxidation and is either conjugated with carnitine or metabolised via ω-oxidation to form dicarboxylic fatty acids.

The low total acylcarnitine-to-free carnitine ratio for the ketogenic diet (figure 4.9, paragraph 4.1.8) implied no carnitine deficiency, while the high acetylcarnitine-to 3-HB-CAR (figure 4.11, paragraph 4.1.10) implicated that the ketogenic diet might stimulate acetylcarnitine production – a theory which is supported by the results illustrated in figure 4.6 (paragraph 4.1.5).

The total acylcarnitine to 3-HB-CAR ratio in the ketogenic diet was very low implying that there was an increase in potential available energy. The ketogenic diet is also characterised by low glucose levels, thus the higher production of ketone bodies (3-hydroxybutyrate) (figure 4.8, paragraph 4.1.7). The brain uses ketone bodies for energy. Our results show high concentrations of the ketone body, 3-HB-CAR, which correlate with the findings of Hack *et al.* (2006), who demonstrated that high 3-hydroxybutyrate levels in plasma provide an additional parameter for the carnitine reserve of the body and reflect an optimal ketonic energy supply. The low concentrations of the amino acid, glycine, in the urine also indicate that the ketogenic diet is a safe treatment that is non-toxic. Our results also showed that the ketogenic diet contributed to a slower weight gain over the 28 day period of treatment in relation to the other treatments, which is in agreement with Thio *et al.* (2006) who showed that juvenile rodents, on a ketogenic diet, had slower weight gain than those on a standard diet.

The carbamazepine and carnosine groups had no effect on the β-oxidation of fatty acids, but the carnosine group had a high total acylcarnitine-to-free carnitine ratio, indicating a possible carnitine insufficiency with carnosine treatment.

None of the other treatments used in the study mimicked the acylcarnitine urinary excretion profiles of the ketogenic diet in Sprague-Dawley rats. It therefore seems unlikely that the antiepileptic drugs studied exert their anticonvulsant effects by similar mechanisms than the ketogenic diet.

To summarise: the ketogenic diet is a safe, non-toxic epileptic treatment that showed the stimulation of acetylcarnitine with no carnitine deficiency after 28 days of treatment, which is in agreement with Berry-Kravis *et al.* (2001) who concluded that carnitine deficiency in patients on the ketogenic diet is uncommon. When all results are taken into account, it does indeed seem that the ketogenic diet is the most appropriate treatment for refractory epilepsy with the added benefit of lesser weight gain. This study showed that the ketogenic diet induces profound changes in fatty acid energy metabolism. An interesting issue that remains is whether the epileptic mitochondrion would respond in the same manner to the ketogenic diet as the normal mitochondrion. Mitochondria from the epileptic rat may be functionally impaired (Kunz, 2002). Would these "epileptic" mitochondria have the same changes in energy metabolism or perhaps an even more robust response? The answer is unknown and calls for further studies.

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Group 1: 6-12 February 2008

Valproate

		mass
Dosage valproate:	187.5 mg/kg	114 g
Rats avg. mass:	114 g	118 g
Dosage per rat:	21.38 mg	115 g
Amount for 6 rats:	128.25 mg	109 g
		114 g

0.5ml	128.25 mg/3ml	213.75 mg/5ml	4.28%

Carnosine

		mass
Dosage carnosine:	500 mg/kg	108 g
Rats avg. mass:	108 g	108 g
Dosage per rat:	53.9 mg	107 g
Amount for 6 rats:	323.4 mg	108 g
		108 g

0.5ml	323.4 mg/3ml	1078 mg/10ml	21.56 %

Carbamazepine

		mass
Dosage carbamazepine:	125 mg/kg	100 g
Rats avg. mass:	100 g	98 g
Dosage per rat:	12.55 mg	102 g
Amount for 6 rats:	75.3 mg	100 g
		102 g

0.5ml	75.3 mg/3ml	125.5 mg/5ml	2.51%

Week

0.5ml	527.1 mg/21ml	627.5 mg/25ml	2.51%

Carnitine

		mass
Dosage carnitine:	100 mg/kg	105 g
Rats avg. mass:	105 g	103 g
Dosage per rat:	10.5 mg	105 g
Amount for 6 rats:	63 mg	107 g
		105 g

0.5ml	63 mg/3ml	105 mg/5ml	2.10 %

Week

0.5ml	441 mg/21ml	525 mg/25ml	2.10 %

Group 1: 13-19 February 2008

Valproate				mass
Dosage valproate:	187.5mg/kg			168 g
Rats avg. mass:	166g			175 g
Dosage per rat:	31.09mg			174 g
Amount for 6 rats:	186.53mg			155 g
				157 g
0.5ml	186.53mg/3ml		310.88mg/5ml	6.22%

Carnosine				mass
Dosage carnosine:	500 mg/kg			152 g
Rats avg. mass:	156 g			152 g
Dosage per rat:	77.8 mg			163 g
Amount for 6 rats:	466.8 mg			155 g
				156 g
0.5ml	466.8mg/3ml		1556mg/10ml	31.12%

Carbamazepine				mass
Dosage carbamazepine:	125 mg/kg			145 g
Rats avg. mass:	138 g			124 g
Dosage per rat:	17.3 mg			137 g
Amount for 6 rats:	103.8 mg			134 g
				152 g
0.5ml	103.8mg/3ml		173mg/5ml	3.46%
Week				
0.5ml	726.6mg/21ml		865mg/25ml	3.46%

Carnitine				mass
Dosage carnitine:	100 mg/kg			161 g
Rats avg. mass:	143 g			138 g
Dosage per rat:	14.34 mg			144 g
Amount for 6 rats:	86.04 mg			129 g
				145 g
0.5ml	86.04mg/3ml		143.4mg/5ml	2.87 %
Week				
0.5ml	602.28mg/21ml		717mg/25ml	2.87 %

Group1: 20-26 February 2008

Valproate

		mass
Dosage valproate:	187.5mg/kg	177 g
Rats avg. mass:	195 g	206 g
Dosage per rat:	36.64 mg	199 g
Amount for 6 rats:	219.83 mg	199 g
		196 g

0.5ml	219.83mg/3ml	366.38mg/5ml	7.33%

Carnosine

		mass
Dosage carnosine:	500.mg/kg	188 g
Rats avg. mass:	185g	165 g
Dosage per rat:	92.4mg	190 g
Amount for 6 rats:	554.4mg	194 g
		187 g

0.5ml	554.4mg/3ml	1848mg/10ml	36.96%

Carbamazepine

		mass
Dosage carbamazepine:	125 mg/kg	185 g
Rats avg. mass:	165 g	148 g
Dosage per rat:	20.68 mg	148 g
Amount for 6:	124.05 mg	167 g
		179 g

0.5ml	124.05mg/3ml	206.75mg/5ml	4.14%

Week

0.5ml	868.35mg/21ml	1033.75mg/25ml	4.14%

Carnitine

		mass
Dosage carnitine:	100 mg/kg	175 g
Rats avg. mass:	176 g	175 g
Dosage per rat:	17.62 mg	189 g
Amount for 6 rats:	105.72 mg	160 g
		182 g

0.5ml	105.72mg/3ml	176.2mg/5ml	3.52%

Week

0.5ml	740.04mg/21ml	881mg/25ml	3.52%

Group 1: 27 February - 6 March

Valproate

		mass
Dosage valproate:	187.5 mg/kg	214 g
Rats avg. mass:	231 g	249 g
Dosage per rat:	43.35 mg	219 g
Amount for 6 rats:	260.1 mg	242 g
		232 g

0.5ml	260.1mg/3ml	433.5mg/5ml	8.67%

Carnosine

		mass
Dosage carnosine:	500 mg/kg	220 g
Rats avg. mass:	211 g	192 g
Dosage per rat:	105.7 mg	225 g
Amount for 6 rats:	634.2 mg	214 g
		206 g

0.5ml	634.2mg/3ml	2114mg/10ml	42.28%

Carbamazepine

		mass
Dosage carbamazepine:	125 mg/kg	229 g
Rats avg. mass:	198 g	172 g
Dosage per rat:	24.75 mg	189 g
Amount for 6 rats:	148.5 mg	210 g
		190 g

0.5ml	148.5mg/3ml	247.5mg/5ml	4.95%

Week

0.5ml	1039.5mg/21ml	1237.5mg/25ml	4.95%

Carnitine

		mass
Dosage carnitine:	100 mg/kg	205 g
Rats avg. mass:	202 g	193 g
Dosage per rat:	20.22 mg	207 g
Amount for 6 rats:	121.32 mg	189 g
		217 g

0.5ml	121.32mg/3ml	202.2mg/5ml	4.04%

Week

0.5ml	849.24mg/21ml	1011mg/25ml	4.04%

Group 2: 26 March - 1 April

Valproate

		mass
Dosage valproate:	187.5mg/kg	92 g
Rats avg. mass:	102 g	104 g
Dosage per rat:	19.13 mg	107 g
Amount for 6 rats:	114.75 mg	103 g
		104 g

0.5ml	114.75mg/3ml	191.25mg/5ml	3.83%

Carnosine

		mass
Dosage carnosine:	500 mg/kg	82 g
Rats avg. mass:	81 g	82 g
Dosage per rat:	40.6 mg	83 g
Amount for 6 rats:	243.6 mg	81 g
		78 g

0.5ml	243.6mg/3ml	812mg/10ml	16.24%

Carbamazepine

		mass
Dosage carbamazepine:	125 mg/kg	121 g
Rats avg. mass:	119 g	118 g
Dosage per rat:	14.83 mg	119 g
Amount for 6 rats:	88.95 mg	118 g
		117 g

0.5ml	88.95mg/3ml	148.25mg/5ml	2.97 %

Week

0.5ml	622.65mg/21ml	741.25mg/25ml	2.97 %

Carnitine

		mass
Dosage carnitine:	100 mg/kg	90 g
Rats avg. mass:	86 g	85 g
Dosage per rat:	8.62 mg	86 g
Amount for 6 rats:	51.72 mg	84 g
		86 g

0.5ml	51.72mg/3ml	86.2mg/5ml	1.72 %

Week

0.5ml	362.04mg/21ml	431.mg/25ml	1.72 %

Group 2: 2 April - 8 April

Valproate

		mass
Dosage valproate:	187.5mg/kg	132 g
Rats avg. mass:	137 g	133 g
Dosage per rat:	25.76 mg	145 g
Amount for 6 rats:	154.58 mg	140 g
		137 g

0.5ml	154.58mg/3ml	257.63mg/5ml	5.15%

Carnosine

		mass
Dosage carnosine:	500 mg/kg	116 g
Rats avg. mass:	116 g	112 g
Dosage per rat:	57.9 mg	124 g
Amount for 6 rats:	347.4 mg	111 g
		116 g

0.5ml	347.4mg/3ml	1158mg/10ml	23.16%

Carbamazepine

		mass
Dosage carbamazepine:	125 mg/kg	155 g
Rats avg. mass:	150 g	140 g
Dosage per rat:	18.7 mg	154 g
Amount for 6 rats:	112.2 mg	158 g
		141 g

0.5ml	112.2mg/3ml	187mg/5ml	3.74%

Week

0.5ml	785.4mg/21ml	935mg/25ml	3.74%

Carnitine

		mass
Dosage carnitine:	100 mg/kg	132 g
Rats avg. mass:	123 g	122 g
Dosage per rat:	12.3 mg	122 g
Amount for 6 rats:	73.8 mg	123 g
		116 g

0.5ml	73.8mg/3ml	123mg/5ml	2.46%

Week

0.5ml	516.6mg/21ml	615mg/25ml	2.46%

Group 2: 9 April - 15 April

Valproate

		mass
Dosage valproate:	187.5mg/kg	170 g
Rats avg. mass:	164 g	160 g
Dosage per rat:	30.68 mg	170 g
Amount for 6 rats:	184.05 mg	155 g
		163 g

0.5ml	184.05mg/3ml	306.75mg/5ml	6.14%

Carnosine

		mass
Dosage carnosine:	500.mg/kg	149 g
Rats avg. mass:	150g	144 g
Dosage per rat:	75.2mg	161 g
Amount for 6 rats:	451.2mg	142 g
		156 g

0.5ml	451.2mg/3ml	1504mg/10ml	30.08%

Carbamazepine

		mass
Dosage carbamazepine:	125 mg/kg	172 g
Rats avg. mass:	175 g	166 g
Dosage per rat:	21.93 mg	184 g
Amount for 6 rats:	131.55 mg	187 g
		168 g

0.5ml	131.55mg/3ml	219.25mg/5ml	4.39 %

Week

0.5ml	920.85mg/21ml	1096.25mg/25ml	4.39 %

Carnitine

		mass
Dosage carnitine:	100 mg/kg	167 g
Rats avg. mass:	158 g	156 g
Dosage per rat:	15.82 mg	160 g
Amount for 6 rats:	94.92 mg	160 g
		148 g

0.5ml	94.92mg/3ml	158.2mg/5ml	3.16 %

Week

0.5ml	664.44mg/21ml	791mg/25ml	3.16 %

Group 2: 16 April - 22 April

Valproate

		mass
Dosage valproate:	187.5mg/kg	206 g
Rats avg. mass:	191 g	186 g
Dosage per rat:	35.74 mg	195 g
Amount for 6 rats:	214.43 mg	182 g
		184 g

0.5ml	214.43mg/3ml	357.38mg/5ml	7.15%

Carnosine

		mass
Dosage carnosine:	500 mg/kg	189 g
Rats avg. mass:	186 g	171 g
Dosage per rat:	93.2 mg	203 g
Amount for 6 rats:	559.2 mg	176 g
		193 g

0.5ml	559.2mg/3ml	1864mg/10ml	37.28%

Carbamazepine

		mass
Dosage carbamazepine:	125 mg/kg	186 g
Rats avg. mass:	196 g	187 g
Dosage per rat:	24.48 mg	220 g
Amount for 6 rats:	146.85 mg	201 g
		185 g

0.5ml	146.85mg/3ml	244.75 mg/5ml	4.90 %

Week

0.5ml	1027.95mg/21ml	1223.75mg/25ml	4.90 %

Carnitine

		mass
Dosage carnitine:	100 mg/kg	197 g
Rats avg. mass:	193 g	193 g
Dosage per rat:	19.32 mg	202 g
Amount for 6 rats:	115.92 mg	197 g
		177 g

0.5ml	115.92mg/3ml	193.2mg/5ml	3.86%

Week

0.5ml	811.44mg/21ml	966mg/25ml	3.86%

Sample	Creatinine Value	Sample	Creatinine Value	Sample	Creatinine Value
K-101	11	CBM-111	5	CAR-121	6
K-102	8	CBM112	3	CAR-122	4
K-103	6	CBM-113	5	CAR-123	6
K-104	10	CBM-114	3	CAR-124	7
K-105	10	CBM-115	4	CAR-125	7
K-231	6	CBM-241	3	CAR-251	18
K-232	7	CBM-242	6	CAR-252	8
K-233	8	CBM-243	5	CAR-253	12
K-234	4	CBM-244	3	CAR-254	10
K-235	5	CBM-245	6	CAR-255	6
VPA-106	8	ALC-116	7	KD-126	4
VPA-107	9	ALC-117	5	KD-127	3
VPA-108	7	ALC-118	7	KD-128	2
VPA-109	4	ALC-119	5	KD-129	3
VPA-110	8	ALC-120	4	KD-130	3
VPA-236	19	ALC-246	14	KD-256	4
VPA-237	8	ALC-247	13	KD-257	5
VPA-238	16	ALC-248	8	KD-258	3
VPA-239	13	ALC-249	9	KD-259	3
VPA-240	7	ALC-250	17	KD-260	4

	Carnitine	Acetylcarnitine	Propionyl-carnitine	Butanoyl-carnitine	3-Methylcrotonyl-carnitine	Isovaleryl-carnitine	3-Hydroxybutyryl-carnitine	Hexanoyl-carnitine
Control								
K-101	1.6934	0.4078	0.0088	0.1143	0	0.0059	0.0152	0.0003
K-102	0.7622	0.1362	0.0015	0.0204	0	0.0023	0.0072	0
K-103	1.892	0.2935	0.0123	0.0569	0.0002	0.0201	0.0451	0.001
K-104	0.8322	0.373	0.0021	0.062	0.0049	0.0114	0.0327	0
K-105	1.479	0.5312	0.0136	0.0701	0.0013	0.0044	0.0563	0.0025
K-231	0	0.44	0.0068	0.0124	0	0.0052	0.0112	0.0002
K-232	1.1565	0.7063	0.0073	0.0204	0.0048	0.0051	0.0382	0.0002
K-233	2.8215	0.5846	0.0139	0.2648	0.0002	0.0348	0.1317	0.0006
K-234	1.1421	0.3084	0.0057	0.1481	0.0008	0.0115	0.1029	0.0021
K-235	1.0986	0.3773	0.0126	0.1342	0.0039	0.0178	0.0867	0.0011
Valproate								
VPA-106	1.4192	0.3607	0.0041	0.1081	0.0008	0.0194	0.1027	0.0036
VPA-107	3.6137	0.4902	0.0108	0.2069	0.002	0.0087	0.048	0.0006
VPA-108	1.6714	1.0591	0.0533	0.3071	0.0004	0.0196	0.0503	0
VPA-109	1.2244	0.2488	0.001	0.0196	0	0.0055	0.0192	0.001
VPA-110	1.5824	0.3618	0.0015	0.1275	0.0011	0.0113	0.0339	0.0039
VPA-236	3.889	2.407	0.007	0.1983	0	0.0022	0.0251	0
VPA-237	0.8291	0.1164	0.0012	0.2021	0.0002	0.0156	0.0219	0.0025
VPA-238	5.0939	0.5607	0.0163	0.2377	0.0002	0.0122	0.0806	0.0032
VPA-239	2.6559	0.8194	0.0045	0.1556	0.0037	0	0.182	0.0017
VPA-240	0.8413	0.5753	0.0006	0.1967	0	0.0057	0.041	0.0018
Carbamazepine								
CBM-111	1.3327	0.4368	0.011	0.0997	0	0.0179	0.0468	0.0001
CBM-112	1.7897	0.5166	0.0037	0.0241	0	0.007	0.0041	0.0011
CBM-113	1.1071	0.1665	0.0038	0.0457	0.0009	0.0097	0.0164	0.0002
CBM-114	1.4031	0.172	0.0057	0.0632	0	0.0064	0.033	0
CBM-115	2.1476	0.3535	0.0112	0.0932	0.0004	0.021	0.0305	0.0013
CBM-241	1.7712	0.3502	0.0037	0.0348	0.0013	0.0078	0.0147	0.0001
CBM-242	0.6506	0.3005	0.0026	0.0267	0.0018	0.004	0.0221	0.0001
CBM-243	0.8105	0.5262	0.0035	0.0616	0.0005	0.0046	0.0248	0.0002
CBM-244	1.4752	0.7616	0.0035	0.0403	0.0017	0.0122	0.0362	0.0002
CBM-245	0.9781	0.3221	0.0214	0.0595	0.0021	0.0154	0.0224	0.0004

	Carnitine	Acetylcarnitine	Propionyl-carnitine	Butanoyl-carnitine	3-Methylcrotonyl-carnitine	Isovaleryl-carnitine	3-Hydroxybutyryl-carnitine	Hexanoyl-carnitine
Acetyl-L-carnitine								
ALC-116	58.8015	2.683	0.1463	1.5536	0.0047	0.2187	0.3656	0.0032
ALC-117	15.6741	1.6945	0.0434	0.4942	0.0076	0.1311	0.1842	0
ALC-118	26.7459	2.5643	0.1961	3.3608	0.0113	0.115	0.2333	0.0017
ALC-119	36.0435	5.6725	0.0923	1.7088	0.0099	0.2996	0.2806	0.0025
ALC-120	17.4912	3.4045	0.0337	0.474	0.0096	0.0722	0.1485	0.0026
ALC-246	90.6963	6.2562	0.1669	2.2306	0.0199	0.2099	0.3179	0.0116
ALC-247	61.1284	3.9764	0.0956	1.7157	0.0206	0.3132	0.5362	0.0206
ALC-248	61.201	3.5154	0.1585	2.0727	0.0031	0.3555	0.4815	0.008
ALC-249	20.5028	3.3004	0.1118	0.8934	0.0117	0.2375	0.3976	0.0086
ALC-250	46.8703	4.4568	0.2282	2.727	0.0083	0.3658	0.5115	0.0065
Carnosine								
CAR-121	1.1759	0.4703	0.0065	0.2317	0.0009	0.0238	0.1405	0.0007
CAR-122	1.8555	0.4496	0.0012	0.0662	0.0009	0.0043	0.0211	0
CAR-123	1.5872	0.5316	0.0058	0.0508	0.0001	0.0192	0.0416	0.0001
CAR-124	2.9801	0.6624	0.0081	0.176	0.0015	0.0218	0.054	0
CAR-125	1.4879	0.6709	0	0.071	0	0.0001	0.105	0.0001
CAR-251	1.9844	0.6799	0.0119	0.0534	0.0034	0.0206	0.1171	0.0015
CAR-252	1.393	1.5935	0.0996	0.5612	0.0006	0.0398	0.1471	0.0028
CAR-253	0.4622	0.4983	0.0031	0.0531	0.0002	0.0161	0.0858	0.0014
CAR-254	0.8741	1.1256	0.0163	0.0576	0.0002	0.0061	0.0225	0.0002
CAR-255	1.4892	0.6029	0.0086	0.1618	0.0007	0.0292	0.0761	0.0007
Ketogenic diet								
KD-126	77.1155	31.4256	0.208	0.2071	0.0037	0.2308	0.2979	0.0097
KD-127	56.2882	44.1603	0.3198	0.266	0.0035	0.4457	0.4352	0.0077
KD-128	54.5363	51.2938	0.1	0.1296	0.0058	0.1883	0.3587	0.0023
KD-129	30.8759	19.559	0.0947	0.1007	0.0072	0.1204	0.1754	0.0045
KD-130	53.8408	51.5717	0.155	0.1941	0.0086	0.2843	0.4247	0.0068
KD-256	40.3434	6.0564	0.0226	0.0378	0.0024	0.1248	0.1953	0
KD-257	41.5228	15.9567	0.0819	0.1072	0.0023	0.1565	0.2566	0.0028
KD-258	29.5252	3.9464	0.0157	0.0307	0.003	0.07	0.1374	0.001
KD-259	55.9209	4.6083	0.0104	0.1039	0.0031	0.1165	0.2879	0.0001
KD-260	56.7648	21.085	0.145	0.1274	0.0044	0.2482	0.3443	0.0005

	3-Hydroxyisovaleryl-carnitine	Octanoylcarnitine	Decanoylcarnitine	Methylmalonyl-carnitine	Glutaconyl-carnitine	Glutaryl-carnitine	Lauroylcarnitine	Myristoyl-carnitine
Control								
K-101	0.0052	0.0002	0.0002	0.0232	0.0043	0.0008	0	0.0001
K-102	0.0029	0.0014	0.0005	0.0092	0.0006	0.0007	0.0002	0.0005
K-103	0.0106	0.0054	0.0028	0.0349	0.0018	0.0071	0.0005	0.0033
K-104	0.0194	0.0029	0.0007	0.0172	0.0027	0.0012	0	0
K-105	0.0272	0.0101	0.0022	0.0371	0	0.0006	0.0033	0
K-231	0.0075	0.0016	0.0003	0.016	0.0001	0.0033	0.0011	0.0002
K-232	0.0242	0.0003	0.0003	0.0497	0.0002	0.0038	0.0003	0
K-233	0.0558	0.0047	0.0017	0.0193	0	0.0008	0.0016	0.0001
K-234	0.0195	0.0039	0.0005	0.0534	0.0005	0.0026	0.0014	0.0003
K-235	0.0298	0.0003	0.0021	0.0324	0.0056	0.0013	0.0002	0.0002
Valproate								
VPA-106	0.0336	0.0341	0.0008	0.0246	0.0019	0.0088	0	0
VPA-107	0.0212	0.0786	0.0018	0.0468	0.0144	0.0091	0.0001	0
VPA-108	0.0239	0.0719	0.005	0.0291	0.0126	0.0129	0.0036	0.0001
VPA-109	0.0054	0.0102	0.0008	0.0091	0.001	0.0034	0.0008	0
VPA-110	0.0166	0.0377	0	0.026	0.0059	0	0	0
VPA-236	0.006	0.2408	0.0006	0.1313	0.0011	0.0129	0.0011	0.0011
VPA-237	0.0062	0.0389	0.0009	0.0238	0.0047	0.0001	0.0001	0.0003
VPA-238	0.0256	0.0715	0.0005	0.0245	0.0027	0.0005	0.0005	0.0005
VPA-239	0.0247	0.0339	0.0023	0.0231	0.008	0.0025	0.0002	0.0004
VPA-240	0.0054	0.0383	0.0005	0.0214	0.0002	0.0023	0.0002	0.0002
Carbamazepine								
CBM-111	0.0114	0.0034	0	0.0216	0.0047	0.0025	0.0049	0
CBM-112	0.0031	0.0033	0	0.0124	0.0002	0.0033	0.0019	0
CBM-113	0.0058	0.0025	0.0012	0.0153	0.0022	0.002	0.0011	0.0007
CBM-114	0.0099	0.0022	0.0008	0.0138	0.0007	0.0024	0.0008	0.0001
CBM-115	0.0074	0.004	0	0.0341	0.0025	0.005	0.0026	0.0004
CBM-241	0.0079	0.0016	0.0001	0.0445	0.0001	0	0.0003	0.0001
CBM-242	0.005	0.0009	0.0001	0.0122	0.001	0.0001	0.0002	0.0004
CBM-243	0.0109	0.0002	0.0003	0.0193	0.0002	0.0042	0.0018	0.0003
CBM-244	0.0087	0.0059	0.0002	0.0291	0.0023	0.0034	0.002	0.0002
CBM-245	0.0201	0.0001	0.0055	0.0225	0.0003	0.0004	0.0001	0.0001

	3-Hydroxyisovaleryl-carnitine	Octanoylcarnitine	Decanoylcarnitine	Methylmalonyl-carnitine	Glutaconyl-carnitine	Glutaryl-carnitine	Lauroylcarnitine	Myristoyl-carnitine
Acetyl-L-carnitine								
ALC-116	0.089	0.0147	0.0023	0.0477	0.0008	0.0105	0.0091	0.0032
ALC-117	0.0415	0.0124	0.005	0.036	0.002	0.0017	0.0023	0.0001
ALC-118	0.1371	0.0012	0.0059	0.0323	0.0017	0.0088	0	0.0001
ALC-119	0.0786	0.0076	0.0024	0.0447	0.0004	0.0026	0	0
ALC-120	0.058	0.0026	0.0017	0.0727	0.0004	0.0107	0	0.0011
ALC-246	0.0847	0.0332	0.0142	0.0895	0.0012	0.0006	0.0083	0.0006
ALC-247	0.1311	0.0124	0.0069	0.0411	0.0147	0.0029	0.0046	0.0007
ALC-248	0.0989	0.038	0.0047	0.0674	0.0071	0.0076	0.0009	0.0009
ALC-249	0.0976	0.0089	0.0005	0.0301	0.0014	0.0024	0.0005	0.0002
ALC-250	0.0906	0.0081	0.0014	0.0394	0.0003	0.0009	0.0023	0.0003
Carnosine								
CAR-121	0.0252	0.0007	0.0048	0.0316	0.0011	0.0081	0.0006	0.0031
CAR-122	0.0073	0.0036	0.0003	0.0389	0.0001	0.0102	0.0003	0.0013
CAR-123	0.0063	0.0052	0	0.0396	0.0001	0.0015	0.0001	0.0001
CAR-124	0.0152	0.0011	0.0012	0.034	0.0023	0.0019	0	0
CAR-125	0.0203	0.0082	0.0044	0.0546	0	0.0033	0.0046	0
CAR-251	0.0177	0.0047	0.0019	0.0342	0.0006	0.006	0.0004	0.0032
CAR-252	0.0454	0.0159	0.0079	0.1165	0.0026	0.0035	0.0406	0.0018
CAR-253	0.0172	0.0052	0.0063	0.0275	0.0018	0.0005	0.0005	0.0003
CAR-254	0.0074	0.001	0.0023	0.0751	0.011	0.0047	0.0017	0.0007
CAR-255	0.0082	0.01	0.0015	0.0196	0.0005	0.0005	0.0044	0.0005
Ketogenic diet								
KD-126	0.034	0.0415	0.0451	0.1083	0.0636	0.0123	0.0214	0.0398
KD-127	0.0463	0.0479	0.054	0.0781	0.0747	0.0153	0.0178	0.0302
KD-128	0.0375	0.023	0.041	0.0901	0.0474	0.0241	0.0207	0.0193
KD-129	0.021	0.0331	0.0264	0.0502	0.0292	0.0064	0.0178	0.0157
KD-130	0.0366	0.0361	0.0358	0.0607	0.0469	0.0121	0.0307	0.0372
KD-256	0.0313	0.0288	0.0195	0.0469	0.025	0.0047	0.0074	0.0043
KD-257	0.0225	0.0383	0.0391	0.0498	0.0233	0.0072	0.0151	0.0065
KD-258	0.0255	0.0185	0.0205	0.0305	0.0171	0.0076	0.0087	0.0073
KD-259	0.0375	0.0372	0.029	0.0643	0.032	0.0087	0.015	0.0145
KD-260	0.0271	0.0693	0.039	0.0641	0.033	0.0065	0.0154	0.0228

	Palmitoyl-carnitine	Stearylcarnitine	Adipylcarnitine	Suberyl-carnitine	CC10-458	CC12-486	CC14-514	CC16-542	Total	Free carnitine
Control										
K-101	0.0001	0.0001	0.0057	0.0001	0.0001	0	0.0001	0	2.29	1.35
K-102	0.0002	0.0006	0.0008	0.0008	0.0004	0	0	0	0.95	1.24
K-103	0.0016	0	0.0042	0.0025	0	0	0	0.0014	2.4	1.27
K-104	0	0.0005	0.0018	0	0	0	0	0	1.36	1.64
K-105	0	0	0.0088	0	0	0	0	0	2.25	1.52
K-231	0.0022	0	0.0002	0.0001	0.0001	0.0001	0	0.0001	0.51	0
K-232	0.0002	0.0002	0.0036	0.0007	0.0002	0.0004	0.0002	0.0004	2.02	1.75
K-233	0.0002	0	0	0.0002	0.0002	0.0002	0.0002	0	3.94	1.4
K-234	0.0002	0.0002	0.0008	0.0002	0	0	0.0002	0	1.8	1.58
K-235	0.0003	0.0002	0.0025	0.0003	0.0002	0.0003	0.0002	0.0003	1.81	1.65
Valproate										
VPA-106	0	0	0.0003	0.0072	0	0.0001	0	0	2.13	1.5
VPA-107	0.0002	0.0001	0	0.0021	0.0001	0.0001	0	0	4.56	1.26
VPA-108	0.0001	0.003	0.0289	0.0156	0.0002	0	0	0.0001	3.37	2.02
VPA-109	0	0	0.0002	0.003	0.0015	0	0	0	2.22	1.27
VPA-110	0	0	0.0035	0.0016	0.0011	0	0	0	2.2158	1.4
VPA-236	0	0.0003	0.004	0	0.0001	0	0.0001	0	6.93	1.78
VPA-237	0.0001	0.0001	0.0005	0.0027	0.0001	0.0001	0.0001	0.0001	1.27	1.53
VPA-238	0.0001	0.0003	0.0034	0.0004	0.0001	0.0001	0.0003	0.0003	6.14	1.2
VPA-239	0	0.0001	0.003	0.0001	0.0001	0.0001	0.0001	0	3.92	1.48
VPA-240	0.0002	0.0001	0.0021	0.0003	0.0001	0.0002	0.0001	0	1.73	2.06
Carbamazepine										
CBM-111	0	0.0009	0.0026	0	0	0	0	0	2	1.5
CBM-112	0.0005	0	0.0023	0	0	0	0	0.0004	2.37	1.33
CBM-113	0	0	0.0016	0	0.0007	0	0	0	1.38	1.25
CBM-114	0	0	0.0021	0	0.0002	0	0	0	1.72	1.22
CBM-115	0.0001	0.0006	0.0028	0	0	0.0001	0	0	2.72	1.27
CBM-241	0.0002	0.0005	0.0002	0.0002	0.0002	0.0005	0	0.0002	2.24	1.26
CBM-242	0.0015	0.0004	0.0005	0.0001	0.0003	0.0003	0.0003	0.0003	1.03	1.59
CBM-243	0.0004	0.0004	0.0002	0.0004	0.0002	0.0004	0.0004	0.002	1.47	1.82
CBM-244	0.0067	0.0005	0.0057	0.0005	0.0003	0.0005	0.0005	0.0005	2.4	1.63
CBM-245	0.0002	0.0002	0.0001	0.0002	0.0001	0.0002	0.0002	0.0002	1.47	1.5

	Palmitoyl-carnitine	Stearylcarnitine	Adipylcarnitine	Suberyl-carnitine	CC10-458	CC12-486	CC14-514	CC16-542	Total	Free carnitine
Acetyl-L-carnitine										
ALC-116	0.0011	0.0001	0.0082	0.0001	0.002	0.0002	0	0	63.97	1.09
ALC-117	0.0001	0	0.0082	0.0031	0.0012	0	0	0	18.43	1.17
ALC-118	0.0001	0	0.0045	0	0.0062	0	0	0	33.42	1.25
ALC-119	0.0009	0	0.0045	0.0009	0	0	0	0	44.25	1.23
ALC-120	0.0013	0.0017	0.0073	0.0003	0	0	0	0	21.79	1.25
ALC-246	0.0006	0.0003	0.0006	0.0006	0.0006	0.0006	0.0006	0.0003	100.15	1.1
ALC-247	0.0005	0	0.0002	0.0002	0	0.0002	0.0002	0.0002	68.02	1.11
ALC-248	0.0008	0.0005	0.0008	0.0005	0.0008	0.0008	0.0003	0.0008	68.03	1.11
ALC-249	0.0002	0.0002	0.0004	0.0021	0.0002	0.0004	0.0004	0.0002	25.61	1.25
ALC-250	0.0006	0.0003	0	0.0006	0.0003	0.0003	0.0003	0.0003	55.32	1.18
Carnosine										
CAR-121	0	0	0.0057	0	0.0001	0	0	0	2.13	1.81
CAR-122	0.001	0	0.0008	0	0	0	0	0	2.46	1.33
CAR-123	0.0003	0.0016	0.0022	0	0.0001	0	0	0.0001	2.29	1.44
CAR-124	0	0.0013	0.0019	0	0	0	0	0	3.96	1.33
CAR-125	0	0.0001	0.0093	0.0001	0	0	0	0	2.44	1.64
CAR-251	0.0007	0.0004	0.0148	0.0007	0.0004	0.0007	0.0007	0	2.96	1.49
CAR-252	0.0008	0.0017	0.0025	0	0.0017	0.0017	0.0008	0.0008	4.08	2.93
CAR-253	0.0001	0.0003	0.0001	0.0027	0.0001	0.0001	0.0003	0.0001	1.18	2.56
CAR-254	0	0.0043	0.0004	0.0003	0.0003	0.0001	0	0.0001	2.21	2.53
CAR-255	0.0005	0.0005	0.0005	0.0005	0.0002	0.0005	0.0002	0.0005	2.42	1.62
Ketogenic diet										
KD-126	0.0108	0.0043	0.0222	0.0074	0.0033	0.0009	0.0002	0	109.91	1.43
KD-127	0.016	0	0.0207	0.0085	0.0037	0.0005	0	0	102.34	1.82
KD-128	0.0148	0.0005	0.0216	0.0015	0.0028	0	0	0	106.96	1.96
KD-129	0.0058	0.0019	0.0126	0.0029	0.0021	0.0009	0	0	51.61	1.66
KD-130	0.0061	0.0002	0.0161	0.0096	0.0048	0	0	0	106.82	1.98
KD-256	0.0099	0.0003	0.0246	0.0052	0.0006	0.0003	0.0003	0.0003	46.99	1.16
KD-257	0.0085	0.0024	0.0309	0.0047	0.006	0.0002	0.0002	0.0002	58.34	1.41
KD-258	0.004	0	0.0139	0.0004	0.0001	0.0001	0.0001	0.0001	33.88	1.15
KD-259	0.0093	0.0007	0.0326	0.0115	0.0007	0.0005	0.0005	0.0004	61.35	1.1
KD-260	0.0066	0.0007	0.0289	0.007	0.0005	0.0001	0.0001	0.0003	79.04	1.39

	Total medium chain fatty acylcarnitine	Total long chain fatty acylcarnitine		Total acylcarnitine	Carnitine	Acetylcarnitine	3-Hydroxybutyryl-carnitine
Control							
K-101	0.115	0.006	0.0003	0.1213	1.6934	0.4078	0.0152
K-102	0.0223	0.0023	0.0012	0.0258	0.7622	0.1362	0.0072
K-103	0.0661	0.0096	0.0039	0.0796	1.892	0.2935	0.0451
K-104	0.0656	0.0023	0	0.0679	0.8322	0.373	0.0327
K-105	0.0849	0.0121	0	0.097	1.479	0.5312	0.0563
K-231	0.0145	0.0037	0.0004	0.0186	0	0.44	0.0112
K-232	0.0212	0.0043	0.0019	0.0274	1.1565	0.7063	0.0382
K-233	0.2718	0.0019	0.0008	0.2745	2.8215	0.5846	0.1317
K-234	0.1546	0.0029	0.0004	0.1579	1.1421	0.3084	0.1029
K-235	0.1377	0.0034	0.0013	0.1424	1.0986	0.3773	0.0867
Valproate							
VPA-106	0.1466	0.0003	0.0073	0.1542	1.4192	0.3607	0.1027
VPA-107	0.2879	0.0004	0.0023	0.2906	3.6137	0.4902	0.048
VPA-108	0.384	0.0357	0.0159	0.4356	1.6714	1.0591	0.0503
VPA-109	0.0316	0.001	0.0045	0.0371	1.2244	0.2488	0.0192
VPA-110	0.1691	0.0035	0.0027	0.1753	1.5824	0.3618	0.0339
VPA-236	0.4397	0.0065	0.0002	0.4464	3.889	2.407	0.0251
VPA-237	0.2444	0.0011	0.0031	0.2486	0.8291	0.1164	0.0219
VPA-238	0.3129	0.0048	0.0012	0.3189	5.0939	0.5607	0.0806
VPA-239	0.1935	0.0037	0.0004	0.1976	2.6559	0.8194	0.182
VPA-240	0.2373	0.0028	0.0007	0.2408	0.8413	0.5753	0.041
Carbamazepine							
CBM-111	0.1032	0.0084	0	0.1116	1.3327	0.4368	0.0468
CBM-112	0.0285	0.0047	0.0004	0.0336	1.7897	0.5166	0.0041
CBM-113	0.0496	0.0034	0.0007	0.0537	1.1071	0.1665	0.0164
CBM-114	0.0662	0.003	0.0002	0.0694	1.4031	0.172	0.033
CBM-115	0.0985	0.0065	0.0001	0.1051	2.1476	0.3535	0.0305
CBM-241	0.0366	0.0013	0.0011	0.039	1.7712	0.3502	0.0147
CBM-242	0.0278	0.003	0.0013	0.0321	0.6506	0.3005	0.0221
CBM-243	0.0623	0.0031	0.0034	0.0688	0.8105	0.5262	0.0248
CBM-244	0.0466	0.0151	0.0023	0.064	1.4752	0.7616	0.0362
CBM-245	0.0655	0.0007	0.0009	0.0671	0.9781	0.3221	0.0224

	Total medium chain fatty acylcarnitine	Total long chain fatty acylcarnitine	[illegible]	Total acylcarnitine	Carnitine	Acetylcarnitine	3-Hydroxybutyryl-carnitine
Acetyl-L-carnitine							
ALC-116	1.5738	0.0217	0.0023	1.5978	58.8015	2.683	0.3656
ALC-117	0.5116	0.0107	0.0043	0.5266	15.6741	1.6945	0.1842
ALC-118	3.3696	0.0047	0.0062	3.3805	26.7459	2.5643	0.2333
ALC-119	1.7213	0.0054	0.0009	1.7276	36.0435	5.6725	0.2806
ALC-120	0.4809	0.0114	0.0003	0.4926	17.4912	3.4045	0.1485
ALC-246	2.2896	0.0104	0.0027	2.3027	90.6963	6.2562	0.3179
ALC-247	1.7556	0.006	0.0008	1.7624	61.1284	3.9764	0.5362
ALC-248	2.1234	0.0039	0.0032	2.1305	61.201	3.5154	0.4815
ALC-249	0.9114	0.0015	0.0033	0.9162	20.5028	3.3004	0.3976
ALC-250	2.743	0.0035	0.0018	2.7483	46.8703	4.4568	0.5115
Carnosine							
CAR-121	0.2379	0.0094	0.0001	0.2474	1.1759	0.4703	0.1405
CAR-122	0.0701	0.0034	0	0.0735	1.8555	0.4496	0.0211
CAR-123	0.0561	0.0043	0.0002	0.0606	1.5872	0.5316	0.0416
CAR-124	0.1783	0.0032	0	0.1815	2.9801	0.6624	0.054
CAR-125	0.0837	0.014	0.0001	0.0978	1.4879	0.6709	0.105
CAR-251	0.0615	0.0195	0.0025	0.0835	1.9844	0.6799	0.1171
CAR-252	0.5878	0.0474	0.005	0.6402	1.393	1.5935	0.1471
CAR-253	0.066	0.0013	0.0033	0.0706	0.4622	0.4983	0.0858
CAR-254	0.0611	0.0071	0.0008	0.069	0.8741	1.1256	0.0225
CAR-255	0.174	0.0064	0.0019	0.1823	1.4892	0.6029	0.0761
Ketogenic diet							
KD-126	0.3034	0.0985	0.0118	0.4137	77.1155	31.4256	0.2979
KD-127	0.3756	0.0847	0.0127	0.473	56.2882	44.1603	0.4352
KD-128	0.1959	0.0769	0.0043	0.2771	54.5363	51.2938	0.3587
KD-129	0.1647	0.0538	0.0059	0.2244	30.8759	19.559	0.1754
KD-130	0.2728	0.0903	0.0144	0.3775	53.8408	51.5717	0.4247
KD-256	0.0861	0.0465	0.0067	0.1393	40.3434	6.0564	0.1953
KD-257	0.1874	0.0634	0.0113	0.2621	41.5228	15.9567	0.2566
KD-258	0.0707	0.0339	0.0008	0.1054	29.5252	3.9464	0.1374
KD-259	0.1702	0.0721	0.0136	0.2559	55.9209	4.6083	0.2879
KD-260	0.2362	0.0744	0.008	0.3186	56.7648	21.085	0.3443

	Total acylcarnitine	Carnitine	Acetylcarnitine	Carnitine	Acetylcarnitine	Total Acylcarnitine	3-HB-CAR
Control							
K-101		0.0716	0.2408		26.8289		7.9803
K-102		0.0338	0.1787		18.9167		3.5833
K-103		0.0421	0.1551		6.5078		1.7650
K-104		0.0816	0.4482		11.4067		2.0765
K-105		0.0656	0.3592		9.4352		1.7229
K-231					39.2857		1.6607
K-232		0.0237	0.6107		18.4895		0.7173
K-233		0.0973	0.2072		4.4389		2.0843
K-234		0.1383	0.2700		2.9971		1.5345
K-235		0.1296	0.3434		4.3518		1.6424
Valproate							
VPA-106		0.1087	0.2542		3.5122		1.5015
VPA-107		0.0804	0.1357		10.2125		6.0542
VPA-108		0.2606	0.6337		21.0557		8.6600
VPA-109		0.0303	0.2032		12.9583		1.9323
VPA-110		0.1108	0.2286		10.6726		5.1711
VPA-236		0.1148	0.6189		95.8964		17.7849
VPA-237		0.2998	0.1404		5.3151		11.3516
VPA-238		0.0626	0.1101		6.9566		3.9566
VPA-239		0.0744	0.3085		4.5022		1.0857
VPA-240		0.2862	0.6838		14.0317		5.8732
Carbamazepine							
CBM-111		0.0837	0.3278		9.3333		2.3846
CBM-112		0.0188	0.2887		126.0000		8.1951
CBM-113		0.0485	0.1504		10.1524		3.2744
CBM-114		0.0495	0.1226		5.2121		2.1030
CBM-115		0.0489	0.1646		11.5902		3.4459
CBM-241		0.0220	0.1977		23.8231		2.6531
CBM-242		0.0493	0.4619		13.5973		1.4525
CBM-243		0.0849	0.6492		21.2177		2.7742
CBM-244		0.0434	0.5163		21.0387		1.7680
CBM-245		0.0686	0.3293		14.3795		2.9955

	Total acylcarnitine : Carnitine	Acetylcarnitine : Carnitine	Acetylcarnitine : 3-HB-CAR	Total Acylcarnitine : 3-HB-CAR
Acetyl-L-carnitine				
ALC-116	0.0272	0.0456	7.3386	4.3704
ALC-117	0.0336	0.1081	9.1992	2.8588
ALC-118	0.1264	0.0959	10.9914	14.4899
ALC-119	0.0479	0.1574	20.2156	6.1568
ALC-120	0.0282	0.1946	22.9259	3.3172
ALC-246	0.0254	0.0690	19.6798	7.2435
ALC-247	0.0288	0.0650	7.4159	3.2868
ALC-248	0.0348	0.0574	7.3009	4.4247
ALC-249	0.0447	0.1610	8.3008	2.3043
ALC-250	0.0586	0.0951	8.7132	5.3730
Carnosine				
CAR-121	0.2104	0.3999	3.3473	1.7609
CAR-122	0.0396	0.2423	21.3081	3.4834
CAR-123	0.0382	0.3349	12.7788	1.4567
CAR-124	0.0609	0.2223	12.2667	3.3611
CAR-125	0.0657	0.4509	6.3895	0.9314
CAR-251	0.0421	0.3426	5.8061	0.7131
CAR-252	0.4596	1.1439	10.8328	4.3521
CAR-253	0.1527	1.0781	5.8077	0.8228
CAR-254	0.0789	1.2877	50.0267	3.0667
CAR-255	0.1224	0.4048	7.9225	2.3955
Ketogenic diet				
KD-126	0.0054	0.4075	105.4904	1.3887
KD-127	0.0084	0.7845	101.4713	1.0869
KD-128	0.0051	0.9405	142.9992	0.7725
KD-129	0.0073	0.6335	111.5108	1.2794
KD-130	0.0070	0.9579	121.4309	0.8889
KD-256	0.0035	0.1501	31.0108	0.7133
KD-257	0.0063	0.3843	62.1851	1.0214
KD-258	0.0036	0.1337	28.7220	0.7671
KD-259	0.0046	0.0824	16.0066	0.8889
KD-260	0.0056	0.3714	61.2402	0.9254

	Carnitine	Acetylcarnitine	Propionyl-carnitine	Butanoyl-carnitine	3-Methylcrotonyl-carnitine	Isovaleryl-carnitine	3-Hydroxybutyryl-carnitine	Hexanoyl-carnitine
Control								
K-101-P	12.9153	3.1041	0.0605	0.108	0	0.0026	0	0
K-102-P	15.6459	3.5996	0.0652	0.0711	0	0.012	0	0
K-103-P	9.0062	1.9068	0.0356	0.022	0	0.0055	0	0
K-104-P	9.048	2.9053	0.1652	0.0415	0	0.0068	0.0072	0
K-105-P	12.2847	5.223	0.0885	0.0537	0	0.005	0	0
K-231-P	31.1983	20.4491	0.024	0	0	0	0	0
K-232-P	10.1288	4.3919	0.1991	0	0	0	0	0.001
K-233-P	12.7217	3.9402	0.699	0.5954	0.0019	0	0.0038	0.0019
K-234-P	21.6116	9.9171	0.0361	0.649	0	0.0426	0	0
K-235-P	24.3426	8.4026	0.0219	0.406	0	0.0082	0	0
Valproate								
VPA-106-P	12.69	4.0877	0.0478	0.0686	0	0	0	0
VPA-107-P	34.3929	14.7194	0.074	0.3141	0	0.0201	0	0
VPA-108-P	33.922	16.0469	0.0191	0.1412	0	0.0183	0.0075	0
VPA-109-P	25.2593	6.3704	0.0816	0.1603	0	0.0169	0.0005	0.0144
VPA-110-P	10.2199	2.1859	0.032	0.039	0	0	0.0018	0
VPA-236-P	14.0988	6.6136	0	0.0224	0	0	0	0
VPA-237-P	18.2773	6.651	0.2832	0.197	0	0.0012	0.0012	0
VPA-238-P	21.0282	4.9159	0.1018	0.2121	0	0.0071	0	0
VPA-239-P	26.1523	6.6498	0.1491	0	0	0	0	0
VPA-240-P	17.7629	8.8435	0	0.0594	0	0	0	0
Carbamazepine								
CBM-111-P	12.7963	3.3661	0.007	0.0558	0.0007	0.0033	0	0
CBM-112-P	9.1545	0.8114	0.2269	0	0	0	0	0
CBM-113-P	11.5852	3.8581	0.0165	0.0412	0	0.0107	0	0.0054
CBM-114-P	15.0628	6.7962	0.1109	0.1026	0	0.0182	0	0
CBM-115-P	11.5944	4.6386	0.0959	0.047	0.0004	0.016	0	0.0097
CBM-241-P	15.5482	14.4856	0.0918	0.0918	0.0075	0.0075	0.0075	0.0075
CBM-242-P	13.5929	6.228	0.0435	0.0696	0	0	0	0
CBM-243-P	20.3566	8.7662	0.0092	0	0	0	0	0.0023
CBM-244-P	21.0795	12.5151	0.3684	0.3274	0	0.1102	0	0
CBM-245-P	34.5993	25.39	0	0.021	0	0	0	0

	Carnitine	Acetylcarnitine	Propionyl-carnitine	Butanoyl-carnitine	3-Methylcrotonyl-carnitine	Isovaleryl-carnitine	3-Hydroxybutyryl-carnitine	Hexanoyl-carnitine
Acetyl-L-carnitine								
ALC-116-P	11.2642	2.3222	0.0503	0.0717	0	0.0079	0	0
ALC-117-P	15.1456	4.4958	0.0783	0.0575	0	0.0185	0.0008	0
ALC-118-P	25.0375	9.2763	0.1837	0.1788	0	0.0238	0	0.0005
ALC-119-P	55.1818	7.7295	0	0.0939	0	0	0	0
ALC-120-P	11.723	3.0277	0.0503	0.3094	0	0.0347	0	0
ALC-246-P	66.9722	19.7324	2.0753	0.5048	0	0.0055	0	0
ALC-247-P	21.5387	11.4372	0.8974	0	0	0	0	0
ALC-248-P	27.5355	10.4611	0.331	0.0414	0	0	0	0
ALC-249-P	20.7226	5.1731	0.0393	0.0328	0.0016	0.0016	0	0.0016
ALC-250-P	13.6858	4.8598	0.2712	0.2637	0.0029	0.0015	0.0015	0.0015
Carnosine								
CAR-121-P	13.1505	7.062	0.0947	0.0606	0	0.0005	0.0005	0
CAR-122-P	5.5148	4.1363	0.0193	0.0044	0	0	0	0
CAR-123-P	34.7495	15.1975	0.2766	0.0998	0	0.0478	0	0
CAR-124-P	19.2646	8.4104	0.1351	0.1742	0	0	0	0
CAR-125-P	32.8045	18.8791	0.3354	0.3872	0	0	0	0.0026
CAR-251-P	8.8671	4.3733	0	0	0	0	0	0
CAR-252-P	24.1258	22.0887	0	0.3996	0	0	0	0
CAR-253-P	18.4277	4.3155	0	0.0553	0	0.0035	0.0035	0.0035
CAR-254-P	14.3411	7.7892	0	0.0369	0	0	0	0
CAR-255-P	14.8682	15.7001	0	0.1343	0	0	0	0
Ketogenic diet								
KD-126-P	25.4244	13.1082	0.0387	0.0018	0	0.0053	0.0124	0
KD-127-P	20.0903	17.5242	0	0.0548	0	0	0.0286	0
KD-128-P	7.0204	4.0676	0.0152	0.0319	0	0	0	0
KD-129-P	23.8493	19.1547	0.3182		0	0	0	0
KD-130-P	18.3896	25.3827	0.0307	0.0967	0	0.0015	0.009	0.0008
KD-256-P	13.6254	17.6969	0.0229	0.4819	0	0	0	0
KD-257-P	16.4896	71.7782	0	0.0988	0	0	0	0
KD-258-P	22.9635	28.528	0.3512	0	0	0	0.005	0
KD-259-P	34.408	41.0102	0	0	0	0	0	0
KD-260-P	12.6212	24.3104	0.1882	0.0046	0	0	0.1467	0

	3-Hydroxyisovaleryl-carnitine	Octanoylcarnitine	Decanoyl-carnitine	Methylmalonyl-carnitine	Glutaconyl-carnitine	Glutaryl-carnitine	Lauroylcarnitine	Myristoyl-carnitine
Control								
K-101-P	0	0	0	0	0	0	0.0019	0
K-102-P	0.0034	0.0039	0	0	0	0	0	0
K-103-P	0	0.0022	0	0	0	0	0	0.0007
K-104-P	0	0	0	0	0	0	0	0
K-105-P	0	0	0	0	0	0	0	0
K-231-P	0	0	0	0	0	0	0	0
K-232-P	0	0.0008	0	0	0	0	0	0
K-233-P	0.0057	0.0009	0	0	0	0	0	0.0009
K-234-P	0.0033	0	0.0013	0.0013	0.0013	0.0013	0.0013	0.0013
K-235-P	0	0	0.0013	0	0	0	0	0
Valproate								
VPA-106-P	0	0	0	0	0	0	0	0.012
VPA-107-P	0	0	0	0	0	0	0	0
VPA-108-P	0	0	0	0	0	0	0	0
VPA-109-P	0	0	0	0	0	0	0	0.0158
VPA-110-P	0.0022	0	0	0	0	0	0	0
VPA-236-P	0	0	0	0	0	0	0	0
VPA-237-P	0	0	0	0	0	0	0	0
VPA-238-P	0.0071	0	0	0	0	0	0	0
VPA-239-P	0	0.0018	0.0018	0	0	0	0	0.0018
VPA-240-P	0	0	0	0	0	0	0	0
Carbamazepine								
CBM-111-P	0	0	0	0	0	0	0	0
CBM-112-P	0	0	0	0	0	0	0	0
CBM-113-P	0.0008	0	0	0	0	0	0	0
CBM-114-P	0	0	0	0	0	0	0	0.0104
CBM-115-P	0	0	0	0	0	0	0	0
CBM-241-P	0.0075	0.003	0.0015	0.003	0.003	0.0015	0.0044	0.003
CBM-242-P	0	0	0.0042	0	0	0	0	0
CBM-243-P	0.0023	0	0	0.0009	0.0009	0	0.0009	0
CBM-244-P	0.005	0	0	0	0	0	0	0
CBM-245-P	0	0	0.0066	0.0066	0	0	0	0

	3-Hydroxyisovaleryl-carnitine	Octanoylcarnitine	Decanoyl-carnitine	Methylmalonyl-carnitine	Glutaconyl-carnitine	Glutaryl-carnitine	Lauroylcarnitine	Myristoyl-carnitine
Acetyl-L-carnitine								
ALC-116-P	0	0	0	0	0	0	0	0
ALC-117-P	0.0004	0.0003	0	0	0	0	0	0
ALC-118-P	0	0	0.0005	0	0	0.0005	0	0
ALC-119-P	0	0	0	0	0	0	0	0
ALC-120-P	0	0.0004	0	0	0	0	0	0
ALC-246-P	0	0.0066	0	0	0	0	0	0.0066
ALC-247-P	0.0049	0	0	0.0016		0	0	0
ALC-248-P	0.0029	0	0	0	0.0248	0	0	0
ALC-249-P	0.0016	0.0015	0	0	0.0015	0.0015	0	0
ALC-250-P	0.0015	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016	0.0016
Carnosine								
CAR-121-P	0	0	0	0	0	0	0	0
CAR-122-P	0	0	0	0	0	0	0	0
CAR-123-P	0.0142	0	0	0	0	0	0	0
CAR-124-P	0	0.0043	0	0	0	0	0	0
CAR-125-P	0	0	0	0	0	0	0	0
CAR-251-P	0	0	0	0	0	0	0	0.0011
CAR-252-P	0.008	0	0	0	0	0	0	0
CAR-253-P	0	0	0.0018	0.0018	0	0.0018	0	0.0018
CAR-254-P	0	0	0	0	0	0	0	0
CAR-255-P	0	0	0	0	0	0	0	0
Ketogenic diet								
KD-126-P	0	0	0	0	0	0	0	0.0222
KD-127-P	0.0119	0	0	0	0	0	0	0
KD-128-P	0	0	0	0	0	0	0	0
KD-129-P	0	0	0	0	0	0	0	0
KD-130-P	0	0	0	0	0	0	0.0078	0
KD-256-P	0	0	0	0	0	0	0	0
KD-257-P	0	0	0	0	0	0	0	0
KD-258-P	0	0	0	0	0	0	0	0
KD-259-P	0	0	0	0	0	0	0	0
KD-260-P	0	0	0	0	0	0	0	0

	Palmitoyl-carnitine	Stearyl-carnitine	Adipyl-carnitine	Suberyl-carnitine	CC10-458	CC12-486	CC14-514	CC16-542	Total	Free carnitine
Control										
K-101-P	0.0009	0	0	0	0	0	0	0	16.1933	1.41
K-102-P	0	0	0	0	0	0	0	0	19.4011	1.24
K-103-P	0	0	0	0	0	0	0	0	10.979	1.22
K-104-P	0	0	0	0	0	0	0	0	12.174	1.35
K-105-P	0.0177	0	0	0	0	0	0	0	17.6726	1.44
K-231-P	0	0	0	0	0	0	0	0	51.6714	1.66
K-232-P	0.0194	0	0	0	0	0	0	0	14.741	1.46
K-233-P	0.0004	0	0.0004	0	0.0004	0	0	0	17.9726	1.41
K-234-P	0	0.0009	0	0	0	0	0.0009	0	32.2693	1.49
K-235-P	0	0.0007	0.0007	0	0	0	0	0.0007	33.1847	1.36
Valproate										
VPA-106-P	0	0	0	0	0	0	0	0	16.9061	1.33
VPA-107-P	0.0075	0	0	0	0	0	0	0	49.528	1.44
VPA-108-P	0.0146	0	0	0	0	0	0	0	50.1696	1.48
VPA-109-P	0.0107	0	0	0	0	0	0	0	31.9299	1.26
VPA-110-P	0.0087	0	0	0	0	0	0	0	12.4895	1.22
VPA-236-P	0	0	0	0	0	0	0	0	20.7348	1.47
VPA-237-P	0.004	0	0	0	0	0	0	0	25.4149	1.39
VPA-238-P	0.0006	0	0	0	0	0	0	0	26.2728	1.25
VPA-239-P	0	0.0037	0.0009	0	0.036	0.0009	0	0	32.9981	1.26
VPA-240-P	0	0	0	0	0	0	0	0	26.6658	1.5
Carbamazepine										
CBM-111-P	0.018	0	0	0	0	0	0	0	16.2472	1.27
CBM-112-P	0	0	0	0	0	0	0	0	10.1928	-
CBM-113-P	0.0195	0	0	0	0	0	0	0	15.5374	1.34
CBM-114-P	0.0207	0	0	0	0	0	0	0	22.1218	1.24
CBM-115-P	0	0	0	0	0	0	0	0	16.402	1.41
CBM-241-P	0.0008	0.0015	0.0023	0.0015	0.0015	0.0015	0.0015	0.0015	30.2864	1.95
CBM-242-P	0	0	0	0	0	0	0.0023	0	19.9405	1.47
CBM-243-P	0	0	0.0011	0	0	0	0	0	29.1404	1.43
CBM-244-P	0.0011	0	0	0	0	0	0.0011	0	34.4078	1.63
CBM-245-P	0.0058	0.0015	0	0	0	0	0	0	60.0308	-

	Palmitoyl-carnitine	Stearyl-carnitine	Adipyl-carnitine	Suberyl-carnitine	CC10-458	CC12-486	CC14-514	CC16-542	Total	Free carnitine
Acetyl-L-carnitine										
ALC-116-P	0	0	0	0	0.0015	0	0	0	13.7178	1.22
ALC-117-P	0.0169	0	0	0	0	0	0	0	19.8141	1.31
ALC-118-P	0.0165	0.0144	0.0005	0	0.0005	0.0005	0.0005	0	34.7345	1.39
ALC-119-P	0.0027	0	0	0	0	0	0	0	63.0079	1.14
ALC-120-P	0	0	0	0	0	0	0	0.0022	15.1477	1.29
ALC-246-P	0.0137	0	0	0	0.0023	0	0.0023	0	89.3217	1.33
ALC-247-P	0	0	0	0	0.0007	0.0007	0	0.0007	33.8819	1.57
ALC-248-P	0.0081	0	0	0	0	0	0	0	38.4048	1.39
ALC-249-P	0	0.0006	0.0006	0.0006	0	0.0006	0	0	25.9811	1.25
ALC-250-P	0	0.0007	0.0007	0.0007	0	0.0007	0.0007	0	19.1041	1.4
Carnosine										
CAR-121-P	0.0087	0	0	0	0	0	0	0	20.3775	1.55
CAR-122-P	0	0	0	0	0	0	0	0	9.6748	1.75
CAR-123-P	0.0049	0	0	0	0	0	0	0	50.3903	1.45
CAR-124-P	0.0008	0	0	0	0	0	0	0	27.9894	1.45
CAR-125-P	0.0109	0.0023	0	0	0	0	0	0	52.422	1.6
CAR-251-P	0.0004	0	0	0	0	0	0	0	13.2419	1.49
CAR-252-P	0	0	0	0	0	0	0	0	46.6221	1.93
CAR-253-P	0.0006	0.0006	0	0	0	0.0006	0	0	22.818	1.24
CAR-254-P	0	0	0	0	0	0	0	0	22.1672	1.55
CAR-255-P	0	0	0	0	0	0	0	0	30.7026	2.06
Ketogenic diet										
KD-126-P	0.0208	0.0154	0	0	0	0	0	0	38.6492	1.52
KD-127-P	0.01	0	0	0	0	0	0	0	37.7198	1.88
KD-128-P	0.0161	0.0043	0	0	0	0	0	0	11.1555	1.59
KD-129-P	0.0028	0.0009	0	0	0.0056	0	0	0	43.3315	1.82
KD-130-P	0.0149	0.0195	0	0	0	0	0	0	43.9532	2.39
KD-256-P	0	0	0	0	0	0	0	0	31.8271	2.34
KD-257-P	0.034	0.0134	0	0	0	0	0	0	88.414	5.36
KD-258-P	0	0	0	0	0.0031	0	0	0	51.8508	2.26
KD-259-P	0	0.0567	0	0	0	0	0	0	75.4749	2.19
KD-260-P	0	0.0244	0	0	0	0	0	0	37.2955	2.95

	Total medium chain fatty acylcarnitine	Total long chain fatty acylcarnitine		Total acylcarnitine	Carnitine	Acetylcarnitine	3-Hydroxybutyryl-carnitine
Control							
K-101-P	0.108	0.0028	0	0.1108	1.6934	0.4078	0.0152
K-102-P	0.075	0	0	0.075	0.7622	0.1362	0.0072
K-103-P	0.0242	0.0007	0	0.0249	1.892	0.2935	0.0451
K-104-P	0.0415	0	0	0.0415	0.8322	0.373	0.0327
K-105-P	0.0537	0.0177	0	0.0714	1.479	0.5312	0.0563
K-231-P	0	0	0	0	0	0.44	0.0112
K-232-P	0.0018	0.0194	0	0.0212	1.1565	0.7063	0.0382
K-233-P	0.5982	0.0017	0.0004	0.6003	2.8215	0.5846	0.1317
K-234-P	0.6503	0.0035	0.0009	0.6547	1.1421	0.3084	0.1029
K-235-P	0.4073	0.0014	0.0007	0.4094	1.0986	0.3773	0.0867
Valproate							
VPA-106-P	0.0686	0.012	0	0.0806	1.4192	0.3607	0.1027
VPA-107-P	0.3141	0.0075	0	0.3216	3.6137	0.4902	0.048
VPA-108-P	0.1417	0.0146	0	0.1563	1.6714	1.0591	0.0503
VPA-109-P	0.1621	0.0265	0	0.1886	1.2244	0.2488	0.0192
VPA-110-P	0.039	0.0087	0	0.0477	1.5824	0.3618	0.0339
VPA-236-P	0.0224	0	0	0.0224	3.889	2.407	0.0251
VPA-237-P	0.197	0.004	0	0.201	0.8291	0.1164	0.0219
VPA-238-P	0.2121	0.0006	0	0.2127	5.0939	0.5607	0.0806
VPA-239-P	0.0036	0.0064	0.0369	0.0469	2.6559	0.8194	0.182
VPA-240-P	0.0594	0	0	0.0594	0.8413	0.5753	0.041
Carbamazepine							
CBM-111-P	0.0558	0.018	0	0.0738	1.3327	0.4368	0.0468
CBM-112-P	0	0	0	0	1.7897	0.5166	0.0041
CBM-113-P	0.0466	0.0195	0	0.0661	1.1071	0.1665	0.0164
CBM-114-P	0.1026	0.0311	0	0.1337	1.4031	0.172	0.033
CBM-115-P	0.0567	0	0	0.0567	2.1476	0.3535	0.0305
CBM-241-P	0.1038	0.012	0.0075	0.1233	1.7712	0.3502	0.0147
CBM-242-P	0.0738	0	0.0023	0.0761	0.6506	0.3005	0.0221
CBM-243-P	0.0023	0.002	0	0.0043	0.8105	0.5262	0.0248
CBM-244-P	0.3274	0.0011	0.0011	0.3296	1.4752	0.7616	0.0362
CBM-245-P	0.0276	0.0073	0	0.0349	0.9781	0.3221	0.0224

	Total medium chain fatty acylcarnitine	Total long chain fatty acylcarnitine		Total acylcarnitine	Carnitine	Acetylcarnitine	3-Hydroxybutyryl-carnitine
Acetyl-L-carnitine							
ALC-116-P	0.0717	0	0.0015	0.0732	58.8015	2.683	0.3656
ALC-117-P	0.0578	0.0169	0	0.0747	15.6741	1.6945	0.1842
ALC-118-P	0.1798	0.0314	0.0015	0.2127	26.7459	2.5643	0.2333
ALC-119-P	0.0939	0.0027	0	0.0966	36.0435	5.6725	0.2806
ALC-120-P	0.3098	0	0.0022	0.312	17.4912	3.4045	0.1485
ALC-246-P	0.5114	0.0203	0.0046	0.5363	90.6963	6.2562	0.3179
ALC-247-P	0.0016	0	0.0021	0.0037	61.1284	3.9764	0.5362
ALC-248-P	0.0414	0.0081	0	0.0495	61.201	3.5154	0.4815
ALC-249-P	0.0359	0.0012	0.0012	0.0383	20.5028	3.3004	0.3976
ALC-250-P	0.2684	0.0046	0.0021	0.2751	46.8703	4.4568	0.5115
Carnosine							
CAR-121-P	0.0606	0.0087	0	0.0693	1.1759	0.4703	0.1405
CAR-122-P	0.0044	0	0	0.0044	1.8555	0.4496	0.0211
CAR-123-P	0.0998	0.0049	0	0.1047	1.5872	0.5316	0.0416
CAR-124-P	0.1785	0.0008	0	0.1793	2.9801	0.6624	0.054
CAR-125-P	0.3898	0.0132	0	0.403	1.4879	0.6709	0.105
CAR-251-P	0	0.0015	0	0.0015	1.9844	0.6799	0.1171
CAR-252-P	0.3996	0	0	0.3996	1.393	1.5935	0.1471
CAR-253-P	0.0606	0.003	0.0006	0.0642	0.4622	0.4983	0.0858
CAR-254-P	0.0369	0	0	0.0369	0.8741	1.1256	0.0225
CAR-255-P	0.1343	0	0	0.1343	1.4892	0.6029	0.0761
Ketogenic diet							
KD-126-P	0.0018	0.0584	0	0.0602	77.1155	31.4256	0.2979
KD-127-P	0.0548	0.01	0	0.0648	56.2882	44.1603	0.4352
KD-128-P	0.0319	0.0204	0	0.0523	54.5363	51.2938	0.3587
KD-129-P	0	0.0037	0.0056	0.0093	30.8759	19.559	0.1754
KD-130-P	0.0975	0.0422	0	0.1397	53.8408	51.5717	0.4247
KD-256-P	0.4819	0	0	0.4819	40.3434	6.0564	0.1953
KD-257-P	0.0988	0.0474	0	0.1462	41.5228	15.9567	0.2566
KD-258-P	0	0	0.0031	0.0031	29.5252	3.9464	0.1374
KD-259-P	0	0.0567	0	0.0567	55.9209	4.6083	0.2879
KD-260-P	0.0046	0.0244	0	0.029	56.7648	21.085	0.3443

	Total acylcarnitine : Carnitine	Acylcarnitine : Carnitine	Acylcarnitine : 3-HS-FAR	Total Acylcarnitine : 3-HS-CNT
Control				
K-101-P	0.06543	0.24082	26.82895	7.28947
K-102-P	0.09840	0.17869	18.91667	10.41667
K-103-P	0.01316	0.15513	6.50776	0.55211
K-104-P	0.04987	0.44821	11.40673	1.26911
K-105-P	0.04828	0.35916	9.43517	1.26821
K-231-P	-	-	39.28571	0.00000
K-232-P	0.01833	0.61072	18.48953	0.55497
K-233-P	0.21276	0.20719	4.43888	4.55809
K-234-P	0.57324	0.27003	2.99708	6.36249
K-235-P	0.37266	0.34344	4.35179	4.72203
Valproate				
VPA-106-P	0.05679	0.25416	3.51217	0.78481
VPA-107-P	0.08899	0.13565	10.21250	6.70000
VPA-108-P	0.09351	0.63366	21.05567	3.10736
VPA-109-P	0.15403	0.20320	12.95833	9.82292
VPA-110-P	0.03014	0.22864	10.67257	1.40708
VPA-236-P	0.00576	0.61893	95.89641	0.89243
VPA-237-P	0.24243	0.14039	5.31507	9.17808
VPA-238-P	0.04176	0.11007	6.95658	2.63896
VPA-239-P	0.01766	0.30852	4.50220	0.25769
VPA-240-P	0.07061	0.68382	14.03171	1.44878
Carbamazepine				
CBM-111-P	0.05538	0.32776	9.33333	1.57692
CBM-112-P	0.00000	0.28865	126.00000	0.00000
CBM-113-P	0.05971	0.15039	10.15244	4.03049
CBM-114-P	0.09529	0.12259	5.21212	4.05152
CBM-115-P	0.02640	0.16460	11.59016	1.85902
CBM-241-P	0.06961	0.19772	23.82313	8.38776
CBM-242-P	0.11697	0.46188	13.59729	3.44344
CBM-243-P	0.00531	0.64923	21.21774	0.17339
CBM-244-P	0.22343	0.51627	21.03867	9.10497
CBM-245-P	0.03568	0.32931	14.37946	1.55804

	Total Acylcarnitine / Carnitine	Acetylcarnitine / Carnitine	Acetylcarnitine / 3-HB-CAR	Total Acylcarnitine / 3-HB-CAR
Acetyl-L-carnitine				
ALC-116-P	0.00124	0.04563	7.33862	0.20022
ALC-117-P	0.00477	0.10811	9.19924	0.40554
ALC-118-P	0.00795	0.09588	10.99143	0.91170
ALC-119-P	0.00268	0.15738	20.21561	0.34426
ALC-120-P	0.01784	0.19464	22.92593	2.10101
ALC-246-P	0.00591	0.06898	19.67977	1.68701
ALC-247-P	0.00006	0.06505	7.41589	0.00690
ALC-248-P	0.00081	0.05744	7.30093	0.10280
ALC-249-P	0.00187	0.16097	8.30080	0.09633
ALC-250-P	0.00587	0.09509	8.71320	0.53783
Carnosine				
CAR-121-P	0.05893	0.39995	3.34733	0.49324
CAR-122-P	0.00237	0.24231	21.30806	0.20853
CAR-123-P	0.06597	0.33493	12.77885	2.51683
CAR-124-P	0.06017	0.22227	12.26667	3.32037
CAR-125-P	0.27085	0.45090	6.38952	3.83810
CAR-251-P	0.00076	0.34262	5.80615	0.01281
CAR-252-P	0.28686	1.14393	10.83277	2.71652
CAR-253-P	0.13890	1.07810	5.80769	0.74825
CAR-254-P	0.04221	1.28772	50.02667	1.64000
CAR-255-P	0.09018	0.40485	7.92247	1.76478
Ketogenic diet				
KD-126-P	0.00078	0.40751	105.49043	0.20208
KD-127-P	0.00115	0.78454	101.47128	0.14890
KD-128-P	0.00096	0.94054	142.99916	0.14580
KD-129-P	0.00030	0.63347	111.51083	0.05302
KD-130-P	0.00259	0.95786	121.43089	0.32894
KD-256-P	0.01194	0.15012	31.01075	2.46749
KD-257-P	0.00352	0.38429	62.18511	0.56976
KD-258-P	0.00010	0.13366	28.72198	0.02256
KD-259-P	0.00101	0.08241	16.00660	0.19694
KD-260-P	0.00051	0.37144	61.24020	0.08423

	AA-glycine		AA-glycine
Control		**Acetyl-L-carnitine**	
K-101	1637.963	ALC-116	2205.428
K-102	412.030	ALC-117	2718.816
K-103	208.474	ALC-118	1062.715
K-104	1043.439	ALC-119	936.263
K-105	2556.132	ALC-120	784.871
K-231	376.300	ALC-246	-
K-232	723.276	ALC-247	1049.384
K-233	-	ALC-248	609.972
K-234	1015.177	ALC-249	685.953
K-235	883.615	ALC-250	1007.704
Valproate		**Carnosine**	
VPA-106	1248.374	CAR-121	1172.871
VPA-107	665.590	CAR-122	1815.840
VPA-108	1694.061	CAR-123	1709.495
VPA-109	1297.344	CAR-124	1193.843
VPA-110	988.522	CAR-125	2880.644
VPA-236	1249.530	CAR-251	62.117
VPA-237	402.284	CAR-252	165.198
VPA-238	-	CAR-253	129.596
VPA-239	959.885	CAR-254	144.764
VPA-240	668.811	CAR-255	921.875
Carbamazepine		**Ketogenic diet**	
CBM-111	1965.120	KD-126	114.250
CBM-112	2647.543	KD-127	171.273
CBM-113	1043.347	KD-128	160.491
CBM-114	2130.724	KD-129	200.647
CBM-115	2706.849	KD-130	178.919
CBM-241	297.505	KD-256	94.756
CBM-242	455.193	KD-257	80.665
CBM-243	591.120	KD-258	202.647
CBM-244	761.805	KD-259	61.594
CBM-245	570.059	KD-260	88.613

	Butyryl-glycine	Isobutyryl-glycine	Isovaleryl-glycine	Isovanilyl-glycine	Crotonyl-glycine	Phenylacetyl-glycine	Tiglylglycine	2-Methylbutyryl-glycine
Control								
K-101	0	0	3.42088	0	0	0	0	0
K-102	0	0.35756	0.35282	1.06768	0	0	0	0
K-103	0	0.47484	0	0	0	0	0	2.98776
K-104	0	0.40847	2.04380	0	0	0	0	1.25030
K-105	0	1.07282	7.02610	0	0	0	0	1.48855
K-231	0	0	0	0	0	0	0	0
K-232	0	0	0	0	0	0	0	0
K-233	0	0.39242	2.18261	0	0	0	0	0
K-234	0	0	0	0	0	0	0	0
K-235	0.67952	0.68124	7.31635	0	0	0	0	0
Valproate								
VPA-106	0	0	8.79615	0	0	0	0	0.98187
VPA-107	0	0	9.79424	0	0	0	0	0
VPA-108	0	0	1.71730	0	0	0	0	0
VPA-109	0	0	3.56390	0	0	0	0	1.27524
VPA-110	0	0	21.30133	0.16978	0	0	0	0
VPA-236	2.00046	0	24.72997	0	0	0	0	4.99223
VPA-237	1.07233	0	28.23312	0	0	118.67895	1.25024	4.99169
VPA-238	3.40722	0	21.28463	0	0	0	0	0
VPA-239	0	0	5.20782	0	0	0	0	1.31973
VPA-240	0	0	15.91900	0	0	0	0	0
Carbamazepine								
CBM-111	0	0	0	0	0	0	0	0
CBM-112	0	0	2.24057	0	0	0	0	5.82448
CBM-113	0	0	0	0	0	0	0	3.79319
CBM-114	0	0	0	0	0	0	0	0
CBM-115	0	0	0	0	0	0	0	0
CBM-241	0	0	12.63290	0	0	0	0	0.44495
CBM-242	0	0	2.35699	0	0	0	0	0
CBM-243	0	0	0	0	0	0	0	0
CBM-244	0	0	0	0	0	0	0	0
CBM-245	0	0	0	0	0	0	0	0

	Butyryl-glycine	Isobutyryl-glycine	Isovaleryl-glycine	Isovanilyl-glycine	Crotonyl-glycine	Phenylacetyl-glycine	Tiglylglycine	2-Methylbutyryl-glycine
Acetyl-L-carnitine								
ALC-116	0	0.98880	0.59418	0.15787	0	0	0	0
ALC-117	0	0		0	0	0	0	0.52049
ALC-118	0	0.35477	0.62589	0.11329	0	0	0	0
ALC-119	0	9.25891	20.27771	0	0	0	0	1.45279
ALC-120	1.20464	13.78331	19.26760	0.19646	0	0	0.83389	1.92480
ALC-246	0	0.82008	4.65310	0	0	7.66398	0.30281	1.29372
ALC-247	0	1.25576	2.77063	0	0	0	0	0.66586
ALC-248	0	0.38387	4.09254	0	0	0	0	0
ALC-249	0	4.75607	8.05761	0	0	0	0	0
ALC-250	0	2.06184	1.40438	0	0	2.79772	0	0.44818
Carnosine								
CAR-121	0	0.28425	0	0	0	0	0	0
CAR-122	0	0.23853	0	0	0	0	0	0.94925
CAR-123	0	0.28850	0	0.09345	0	0	0	0
CAR-124	0	1.46918	5.76085	0	0	0	0	0
CAR-125	0	0.18445	0	0	0	0	0	0
CAR-251	0	0.43350	2.93952	0	0	0	0	0.40686
CAR-252	0	0	0	0	0	0	0	0
CAR-253	0.50806	0.96513	4.04078	0	0	0	0	0
CAR-254	0	0	0	0	0	0	0	0
CAR-255	0	0	0	0	0	0	0	0
Ketogenic diet								
KD-126	0.77999	16.01377	12.55844	0	0	0.68092	0	3.98580
KD-127	1.31745	2.76391	2.26888	0	0	0	0	3.15525
KD-128	1.85759	17.58025	12.24829	0	0	0	0	9.69977
KD-129	0	9.83497	21.71164	0	0	0.43680	0	3.67289
KD-130	0	7.12215	10.58306	0	0	0	0	5.81214
KD-256	0	0	2.12550	0	0	0	0	0.24819
KD-257	0	0	0.52823	0	0	0	0	0
KD-258	0	0.34486	0.79573	0	0	0	0	0.64662
KD-259	0	0	0	0	0	0	0	1.74194
KD-260	0	0	0.68441	0	0	0	0	1.31868

	3-Methyl-cortonylglycine	2-Furoylglycine	4-Hydrocyphenyl-acetylglycine	Acetylglycine	[redacted]		Total glycine
Control							
K-101	0	0	4.57148	0	3.42088	0	7.99236
K-102	0	0	0.73378	0	1.77806	0	2.51184
K-103	0	0	0.89134	0	0.47484	2.98776	4.35394
K-104	0	0	2.02582	0	2.45227	1.25030	5.72838
K-105	0	0	3.45374	0	8.09892	1.48855	13.04121
K-231	0	0	0	0	0	0	0
K-232	0	0	0	0	0	0	0
K-233	0	0	0.60280	0	2.57503	0	3.17783
K-234	0	0	0	0	0	0	0
K-235	0	0	1.86597	0	8.67711	0	10.54308
		0	0				
Valproate							
VPA-106	0	0	0.29626	0	8.79615	0.98187	10.07429
VPA-107	0	0	0.40542	0	9.79424	0	10.19966
VPA-108	0	0	0	0	1.71730	0	1.71730
VPA-109	0	0	0.33261	0	3.56390	1.27524	5.17175
VPA-110	0	0	0	0	21.47111	0	21.47111
VPA-236	0.48027	0	0.68230	0	26.73043	4.99223	32.88523
VPA-237	0	0	0.49548	0	29.30544	124.92088	154.72180
VPA-238	0.40248	0	0.73850	0	24.69184	0	25.83282
VPA-239	0	0	0.22936	0	5.20782	1.31973	6.75691
VPA-240	0	0	0	0	15.91900	0	15.91900
Carbamazepine							
CBM-111	0	0	0.77728	0	0	0	0.77728
CBM-112	0	0	3.53375	0	2.24057	5.82448	11.59880
CBM-113	0	0	1.52429	0	0	3.79319	5.31748
CBM-114	0	0	0.81176	0	0	0	0.81176
CBM-115	0	0	0.57938	0	0	0	0.57938
CBM-241	0	0	0	0	12.63290	0.44495	13.07786
CBM-242	0	0	0	0	2.35699	0	2.35699
CBM-243	0	0	0	0	0	0	0
CBM-244	0	0	0	0	0	0	0
CBM-245	0	0	0	0	0	0	0

	3-Methyl-cortonylglycine	2-Furoylglycine	4-Hydrocyphenyl-acetylglycine	Acetylglycine	[redacted]		Total glycine
Acetyl-L-carnitine							
ALC-116	0	0	0.71732	0	1.74085	0	2.45817
ALC-117	0	0	0	0	0	0.52049	0.52049
ALC-118	0	0	0.71797	0	1.09395	0	1.81192
ALC-119	0	0	1.18453	0	29.53663	1.45279	32.17395
ALC-120	0	0	0.05801	5.24476	34.45201	2.75869	42.51347
ALC-246	0.29737	0	1.25929	0	5.47317	9.26050	16.29034
ALC-247	0	0	0.29706	0	4.02638	0.66586	4.98931
ALC-248	0	0	0.73636	0	4.47641	0	5.21277
ALC-249	0	0	0	0	12.81368	0	12.81368
ALC-250	0.14427	0	0.74393	0	3.46622	3.24591	7.60033
Carnosine							
CAR-121	0	0	0.92927	0	0.28425	0	1.21352
CAR-122	0	0	0.37729	0	0.23853	0.94925	1.56508
CAR-123	0	0	0.33005	0	0.38195	0	0.71201
CAR-124	0	0	0	0	7.23003	0	7.23003
CAR-125	0	0	0.75229	0	0.18445	0	0.93674
CAR-251	0	0	0.56118	0	3.37302	0.40686	4.34106
CAR-252	0	0	0	0	0	0	0
CAR-253	0	0	0.29069	0	5.51397	0	5.80466
CAR-254	0	0	0	0.19200	0	0	0.19200
CAR-255	0	0	0	0	0	0	0
Ketogenic diet							
KD-126	0.21006	0	1.80297	0	29.35220	4.66671	36.03194
KD-127	0	0	1.31017	0	6.35023	3.15525	10.81565
KD-128	0	0	2.37276	0	31.68613	9.69977	43.75866
KD-129	0	0	2.88283	0	31.54661	4.10969	38.53914
KD-130	0.08492	0	1.61778	0.06201	17.70521	5.81214	25.28206
KD-256	0	0	0	0	2.12550	0.24819	2.37369
KD-257	0	0	0	0	0.52823	0	0.52823
KD-258	0	0	0.13973	0	1.14060	0.64662	1.92695
KD-259	0	0	0	0	0	1.74194	1.74194
KD-260	0	0	0	0	0.68441	1.31868	2.00309

	K-101	K-102	K-103	K-104	K-105	K-231	K-232	K-233	K-234	K-235
0	120.2	138.2	130.7	128.7	130.4	111	112	112	114	115
7	175	176	176	176	173	146	136	145	133	154
14	192	224	199	214	202	180	168	163	164	178
21	230	256	240	255	242	196	182	190	185	202
28	258	296	267	293	271	234	212	227	220	229

	VPA-106	VPA-107	VPA-108	VPA-109	VPA-110	VPA-236	VPA-237	VPA-238	VPA-239	VPA-240
0	114	118	115	109	114	92	104	107	103	104
7	168	175	171	155	157	132	133	145	140	137
14	177	206	199	199	196	170	160	170	155	163
21	214	249	219	242	232	206	186	195	182	184
28	244	265	243	259	251	227	204	210	204	207

	CBM-111	CBM-112	CBM-113	CBM-114	CBM-115	CBM-241	CBM-242	CBM-243	CBM-244	CBM-245
0	99	97	102	100	102	121	118	119	118	117
7	145	124	137	134	152	155	140	154	158	141
14	185	148	148	167	179	172	166	184	187	168
21	229	172	189	210	190	186	187	220	201	185
28	247	196	210	226	230	211	210	238	222	214

	ALC-116	ALC-117	ALC-118	ALC-119	ALC-120	ALC-246	ALC-247	ALC-248	ALC-249	ALC-250
0	105	103	105	107	105	90	85	86	84	86
7	161	138	144	129	145	132	122	122	123	116
14	175	175	189	160	182	167	156	160	160	148
21	205	193	207	189	217	197	193	202	197	177
28	243	248	254	219	214	224	228	241	228	209

	CAR-121	CAR-122	CAR-123	CAR-124	CAR-125	CAR-251	CAR-252	CAR-253	CAR-254	CAR-255
0	108	108	107	108	108	82	82	83	81	78
7	152	152	163	155	156	116	112	124	111	116
14	188	165	190	194	187	149	144	161	142	156
21	220	192	225	214	206	187	171	203	176	193
28	235	227	251	243	214	209	198	227	193	216

	KD-126	KD-127	KD-128	KD-129	KD-130	KD-256	KD-257	KD-258	KD-259	KD-260
0	97	94	91	88	89	93	89	94	89	87
7	94	81	90	87	92	92	86	82	86	81
14	113	104	111	107	113	112	111	107	108	103
21	130	118	127	127	128	137	138	131	134	131
28	163	157	163	160	164	171	175	166	166	164

Acylcarnitine

Total acylcarnitine

Groep	Tukey HSD test; Variable: Total acyl carnitine (Ubbink_urien.sta) Marked differences are significant at p < .05000					
	{1} M=.10124	{2} M=.25451	{3} M=.06444	{4} M=1.7585	{5} M=.17064	{6} M=.28470
Controle {1}		0.953553	0.999949	0.000138	0.998804	0.904539
Valproate {2}	0.953553		0.890805	0.000138	0.996962	0.999981
Carbamazepine {3}	0.999949	0.890805		0.000138	0.990775	0.814820
Acetyl-L-carnitine {4}	0.000138	0.000138	0.000138		0.000138	0.000138
Carnosine {5}	0.998804	0.996962	0.990775	0.000138		0.987229
Ketogenic diet {6}	0.904539	0.999981	0.814820	0.000138	0.987229	

Total long chain fatty acylcarnitine

Groep	Tukey HSD test; Variable: Total long chain fatty acids carnitine (Ubbink_urien.sta) Marked differences are significant at p < .05000					
	{1} M=.00485	{2} M=.00598	{3} M=.00492	{4} M=.00792	{5} M=.01160	{6} M=.06945
Controle {1}		0.999928	1.000000	0.990346	0.768010	0.000138
Valproate {2}	0.999928		0.999947	0.998933	0.876748	0.000138
Carbamazepine {3}	1.000000	0.999947		0.991314	0.775654	0.000138
Acetyl-L-carnitine {4}	0.990346	0.998933	0.991314		0.978149	0.000138
Carnosine {5}	0.768010	0.876748	0.775654	0.978149		0.000138
Ketogenic diet {6}	0.000138	0.000138	0.000138	0.000138	0.000138	

Total medium chain fatty acylcarnitine

Groep	Tukey HSD test; Variable: Total medium chain fatty acids carnitine (Ubbink_urien.sta) Marked differences are significant at p < .05000					
	{1} M=.09537	{2} M=.24470	{3} M=.05848	{4} M=1.7480	{5} M=.15765	{6} M=.20630
Controle {1}		0.957554	0.999947	0.000138	0.999297	0.988511
Valproate {2}	0.957554		0.897207	0.000138	0.996301	0.999936
Carbamazepine {3}	0.999947	0.897207		0.000138	0.993146	0.959341
Acetyl-L-carnitine {4}	0.000138	0.000138	0.000138		0.000138	0.000138
Carnosine {5}	0.999297	0.996301	0.993146	0.000138		0.999794
Ketogenic diet {6}	0.988511	0.999936	0.959341	0.000138	0.999794	

Total dicarboxylic fatty acylcarintine

Groep	Tukey HSD test; Variable: Total dicarboxylic fatty acid carnitine (Ubbink_urien.sta) Marked differences are significant at p < .05000					
	{1} M=.00102	{2} M=.00383	{3} M=.00104	{4} M=.00258	{5} M=.00139	{6} M=.00895
Controle {1}		0.282801	1.000000	0.840550	0.999769	0.000139
Valproate {2}	0.282801		0.290255	0.930788	0.438327	0.003580
Carbamazepine {3}	1.000000	0.290255		0.847640	0.999824	0.000139
Acetyl-L-carnitine {4}	0.840550	0.930788	0.847640		0.943253	0.000274
Carnosine {5}	0.999769	0.438327	0.999824	0.943253		0.000142
Ketogenic diet {6}	0.000139	0.003580	0.000139	0.000274	0.000142	

Acetylcarnitine

Groep	Tukey HSD test; Variable: Acetylcarnitine (Ubbink_urien.sta) Marked differences are significant at p < .05000					
	{1} M=.41583	{2} M=.69994	{3} M=.39060	{4} M=3.7524	{5} M=.72850	{6} M=24.966
Controle {1}		0.999999	1.000000	0.924497	0.999999	0.000138
Valproate {2}	0.999999		0.999999	0.947277	1.000000	0.000138
Carbamazepine {3}	1.000000	0.999999		0.922219	0.999999	0.000138
Acetyl-L-carnitine {4}	0.924497	0.947277	0.922219		0.949278	0.000139
Carnosine {5}	0.999999	1.000000	0.999999	0.949278		0.000138
Ketogenic diet {6}	0.000138	0.000138	0.000138	0.000139	0.000138	

Free carnitine

Groep	Tukey HSD test; Variable: Carnitine (Ubbink_urien.sta) Marked differences are significant at p < .05000					
	{1} M=1.2878	{2} M=2.2820	{3} M=1.3466	{4} M=43.515	{5} M=1.5289	{6} M=49.673
Controle {1}		0.999966	1.000000	0.000138	1.000000	0.000138
Valproate {2}	0.999966		0.999975	0.000138	0.999991	0.000138
Carbamazepine {3}	1.000000	0.999975		0.000138	1.000000	0.000138
Acetyl-L-carnitine {4}	0.000138	0.000138	0.000138		0.000138	0.841800
Carnosine {5}	1.000000	0.999991	1.000000	0.000138		0.000138
Ketogenic diet {6}	0.000138	0.000138	0.000138	0.841800	0.000138	

3-Hydroxybutyryl-carnitine

Groep	Tukey HSD test; Variable: 3-Hydroxybutyryl-carnitine (Ubbink_urien.sta) Marked differences are significant at p < .05000					
	{1} M=.05272	{2} M=.06047	{3} M=.02510	{4} M=.34569	{5} M=.08108	{6} M=.29134
Controle {1}		0.999924	0.966020	0.000138	0.961980	0.000138
Valproate {2}	0.999924		0.906913	0.000138	0.990774	0.000138
Carbamazepine {3}	0.966020	0.906913		0.000138	0.586021	0.000138
Acetyl-L-carnitine {4}	0.000138	0.000138	0.000138		0.000138	0.616420
Carnosine {5}	0.961980	0.990774	0.586021	0.000138		0.000139
Ketogenic diet {6}	0.000138	0.000138	0.000138	0.616420	0.000139	

Total acylcarintine : free carnitine ratio

Groep	Tukey HSD test; Variable: Total acyl-carnitine : Carnitine (Ubbink_urien.sta) Marked differences are significant at p < .05000 {1} M=.07595	{2} M=.14286	{3} M=.05176	{4} M=.04556	{5} M=.12706	{6} M=.00567
Controle {1}		0.328124	0.975718	0.936452	0.623336	0.275908
Valproate {2}	0.328124		0.061947	0.038028	0.996106	0.001037
Carbamazepine {3}	0.975718	0.061947		0.999962	0.184914	0.695313
Acetyl-L-carnitine {4}	0.936452	0.038028	0.999962		0.123654	0.806591
Carnosine {5}	0.623336	0.996106	0.184914	0.123654		0.004547
Ketogenic diet {6}	0.275908	0.001037	0.695313	0.806591	0.004547	

Acetylcarnitine : free carnitine ratio

Groep	Tukey HSD test; Variable: Acetyl carnitine : Carnitine (Ubbink_urien.sta) Marked differences are significant at p < .05000 {1} M=.31260	{2} M=.33170	{3} M=.32084	{4} M=.10492	{5} M=.59076	{6} M=.48458
Controle {1}		0.999984	1.000000	0.483916	0.178188	0.679337
Valproate {2}	0.999984		0.999999	0.354673	0.217361	0.755890
Carbamazepine {3}	1.000000	0.999999		0.409592	0.180759	0.699186
Acetyl-L-carnitine {4}	0.483916	0.354673	0.409592		0.001136	0.017697
Carnosine {5}	0.178188	0.217361	0.180759	0.001136		0.934885
Ketogenic diet {6}	0.679337	0.755890	0.699186	0.017697	0.934885	

Acetylcarnitine : 3-Hydroxybutyryl-carnitine ratio

Groep	Tukey HSD test; Variable: AcetylCarnitine : 3-HB-CAR (Ubbink_u Marked differences are significant at p < .05000 {1} M=14.26	{2} M=18.51	{3} M=25.63	{4} M=12.20	{5} M=13.64	{6} M=78.20
Controle {1}		0.99930	0.93344	0.99998	1.00000	0.00016
Valproate {2}	0.99930		0.99129	0.99510	0.99861	0.00023
Carbamazepine {3}	0.93344	0.99129		0.87371	0.91792	0.00091
Acetyl-L-carnitine {4}	0.99998	0.99510	0.87371		0.99999	0.00015
Carnosine {5}	1.00000	0.99861	0.91792	0.99999		0.00015
Ketogenic diet {6}	0.00016	0.00023	0.00091	0.00015	0.00015	

Total acylcarnitine : 3-Hydroxybutyryl-carnitine ratio

Groep	Tukey HSD test; Variable: Total Acyl-carnitine : 3-HB-CAR (Ubbink_urien.sta) Marked differences are significant at p < .05000 {1} M=2.4767	{2} M=6.3371	{3} M=3.1046	{4} M=5.3825	{5} M=2.2344	{6} M=.97323
Controle {1}		0.041559	0.996302	0.220344	0.999967	0.844662
Valproate {2}	0.041559		0.131975	0.974706	0.025291	0.001420
Carbamazepine {3}	0.996302	0.131975		0.482567	0.983198	0.555790
Acetyl-L-carnitine {4}	0.220344	0.974706	0.482567		0.151584	0.013049
Carnosine {5}	0.999967	0.025291	0.983198	0.151584		0.919433
Ketogenic diet {6}	0.844662	0.001420	0.555790	0.013049	0.919433	

Glycine conjugates

Total glycine conjugates

Groep	Unequal N HSD; Variable: TOTAL (Ubbink_urien2.sta) Marked differences are significant at p < .05000 {1} M=4.7349	{2} M=28.729	{3} M=3.4520	{4} M=12.555	{5} M=2.1995	{6} M=16.300
Controle {1}		0.123589	0.999993	0.959349	0.999808	0.816758
Valproate {2}	0.123589		0.091158	0.518617	0.066650	0.767538
Carbamazepine {3}	0.999993	0.091158		0.924341	0.999994	0.741973
Acetyl-L-carnitine {4}	0.959349	0.518617	0.924341		0.876151	0.998656
Carnosine {5}	0.999808	0.066650	0.999994	0.876151		0.660606
Ketogenic diet {6}	0.816758	0.767538	0.741973	0.998656	0.660606	

Saturated glycine conjugates

Depend.: Saturated glycine	Multiple Comparisons p values (2-tailed); Saturated glycine (Ubbink_urien2.sta) Independent (grouping) variable: Groep Kruskal-Wallis test: H (5, N= 60) =21.02485 p =.0008 Controle R:25.700	Valproate R:45.700	Carbamazepine R:16.300	Acetyl-L-carnitine R:37.300	Carnosine R:21.100	Ketogenic diet R:36.900
Controle		0.15667	1.00000	1.00000	1.00000	1.00000
Valproate	0.15667		0.00250	1.00000	0.02451	1.00000
Carbamazepine	1.00000	0.00250		0.10757	1.00000	0.12525
Acetyl-L-carnitine	1.00000	1.00000	0.10757		0.57091	1.00000
Carnosine	1.00000	0.02451	1.00000	0.57091		0.64612
Ketogenic diet	1.00000	1.00000	0.12525	1.00000	0.64612	

Butyrylglycine

Depend.: BUTYRYLGLYCINE	Multiple Comparisons p values (2-tailed); BUTYRYLGLYCINE (Ubbink_urien2.sta) Independent (grouping) variable: Groep Kruskal-Wallis test: H (5, N= 60) =6.500619 p =.2605 Controle R:28.700	Valproate R:35.600	Carbamazepine R:26.000	Acetyl-L-carnitine R:29.000	Carnosine R:28.600	Ketogenic diet R:35.100
Controle		1.00000	1.00000	1.00000	1.00000	1.00000
Valproate	1.00000		1.00000	1.00000	1.00000	1.00000
Carbamazepine	1.00000	1.00000		1.00000	1.00000	1.00000
Acetyl-L-carnitine	1.00000	1.00000	1.00000		1.00000	1.00000
Carnosine	1.00000	1.00000	1.00000	1.00000		1.00000
Ketogenic diet	1.00000	1.00000	1.00000	1.00000	1.00000	

Isobutyrylglycine

Depend.: ISOBUTYRYLGLYCINE	Multiple Comparisons p values (2-tailed); ISOBUTYRYLGLYCINE (Ubbink_urien2.sta) Independent (grouping) variable: Groep Kruskal-Wallis test: H (5, N= 60) =27.23712 p =.0001 Controle R:32.600	Valproate R:16.500	Carbamazepine R:16.500	Acetyl-L-carnitine R:45.850	Carnosine R:32.850	Ketogenic diet R:38.700
Controle		0.588982	0.588982	1.000000	1.000000	1.000000
Valproate	0.588982		1.000000	0.002570	0.544698	0.067159
Carbamazepine	0.588982	1.000000		0.002570	0.544698	0.067159
Acetyl-L-carnitine	1.000000	0.002570	0.002570		1.000000	1.000000
Carnosine	1.000000	0.544698	0.544698	1.000000		1.000000
Ketogenic diet	1.000000	0.067159	0.067159	1.000000	1.000000	

Isovalerylglycine

Depend.: ISOVALERYLGLYCINE	Multiple Comparisons p values (2-tailed); ISOVALERYLGLYCINE (Ubbink_urien2.sta) Independent (grouping) variable: Groep Kruskal-Wallis test: H (5, N= 59) =24.01354 p =.0002					
	Controle R:24.100	Valproate R:47.800	Carbamazepine R:18.500	Acetyl-L-carnitine R:37.333	Carnosine R:18.500	Ketogenic diet R:34.500
Controle		0.030484	1.000000	1.000000	1.000000	1.000000
Valproate	0.030484		0.002047	1.000000	0.002047	1.000000
Carbamazepine	1.000000	0.002047		0.255147	1.000000	0.558737
Acetyl-L-carnitine	1.000000	1.000000	0.255147		0.255147	1.000000
Carnosine	1.000000	0.002047	1.000000	0.255147		0.558737
Ketogenic diet	1.000000	1.000000	0.558737	1.000000	0.558737	

Isovanilylglycine

Depend.: ISOVANILYLGLYCINE	Multiple Comparisons p values (2-tailed); ISOVANILYLGLYCINE (Ubbink_urien2.sta) Independent (grouping) variable: Groep Kruskal-Wallis test: H (5, N= 60) =6.475182 p =.2627					
	Controle R:30.750	Valproate R:30.550	Carbamazepine R:27.500	Acetyl-L-carnitine R:36.450	Carnosine R:30.250	Ketogenic diet R:27.500
Controle		1.000000	1.000000	1.000000	1.000000	1.000000
Valproate	1.000000		1.000000	1.000000	1.000000	1.000000
Carbamazepine	1.000000	1.000000		1.000000	1.000000	1.000000
Acetyl-L-carnitine	1.000000	1.000000	1.000000		1.000000	1.000000
Carnosine	1.000000	1.000000	1.000000	1.000000		1.000000
Ketogenic diet	1.000000	1.000000	1.000000	1.000000	1.000000	

Unsaturated glycine conjugates

Depend.: Unsaterated glyci	Multiple Comparisons p values (2-tailed); Unsaterated glycine (Ubbink_urien2.s Independent (grouping) variable: Groep Kruskal-Wallis test: H (5, N= 60) =15.03007 p =.0102					
	Controle R:24.600	Valproate R:35.000	Carbamazepine R:25.500	Acetyl-L-carnitine R:33.600	Carnosine R:19.900	Ketogenic diet R:44.400
Controle		1.00000	1.00000	1.00000	1.00000	0.16860
Valproate	1.00000		1.00000	1.00000	0.79788	1.00000
Carbamazepine	1.00000	1.00000		1.00000	1.00000	0.23287
Acetyl-L-carnitine	1.00000	1.00000	1.00000		1.00000	1.00000
Carnosine	1.00000	0.79788	1.00000	1.00000		0.02561
Ketogenic diet	0.16860	1.00000	0.23287	1.00000	0.02561	

Crotonylglycine

Depend.: CROTONYLGLYCINE	Multiple Comparisons p values (2-tailed); CROTONYLGLYCINE (Ubbink_urien2.sta) Independent (grouping) variable: Groep Kruskal-Wallis test: H (5, N= 55) =0.000000 p =1.000					
	Controle R:28.000	Valproate R:28.000	Carbamazepine R:28.000	Acetyl-L-carnitine R:28.000	Carnosine R:28.000	Ketogenic diet R:28.000
Controle		1.000000	1.000000	1.000000	1.000000	1.000000
Valproate	1.000000		1.000000	1.000000	1.000000	1.000000
Carbamazepine	1.000000	1.000000		1.000000	1.000000	1.000000
Acetyl-L-carnitine	1.000000	1.000000	1.000000		1.000000	1.000000
Carnosine	1.000000	1.000000	1.000000	1.000000		1.000000
Ketogenic diet	1.000000	1.000000	1.000000	1.000000	1.000000	

Phenylacetylglycine

Depend.: PHENYLACETYLGLYCI	Multiple Comparisons p values (2-tailed); PHENYLACETYLGLYCINE (Ubbink_ur Independent (grouping) variable: Groep Kruskal-Wallis test: H (5, N= 55) =10.03836 p =.0742 Controle R:25.50(	Valproate R:28.450	Carbamazepine R:25.500	Acetyl-L-carnitine R:36.700	Carnosine R:25.500	Ketogenic diet R:30.700
Controle		1.00000	1.00000	1.00000	1.00000	1.00000
Valproate	1.00000		1.00000	1.00000	1.00000	1.00000
Carbamazepine	1.00000	1.00000		1.00000	1.00000	1.00000
Acetyl-L-carnitine	1.00000	1.00000	1.00000		1.00000	1.00000
Carnosine	1.00000	1.00000	1.00000	1.00000		1.00000
Ketogenic diet	1.00000	1.00000	1.00000	1.00000	1.00000	

Tiglylglycine

Depend.: TIGLYLGLYCINE	Multiple Comparisons p values (2-tailed); TIGLYLGLYCINE (Ubbink_urien2.sta) Independent (grouping) variable: Groep Kruskal-Wallis test: H (5, N= 55) =6.266667 p =.2811 Controle R:27.00(	Valproate R:29.800	Carbamazepine R:27.000	Acetyl-L-carnitine R:32.400	Carnosine R:27.000	Ketogenic diet R:27.000
Controle		1.00000	1.00000	1.00000	1.00000	1.00000
Valproate	1.00000		1.00000	1.00000	1.00000	1.00000
Carbamazepine	1.00000	1.00000		1.00000	1.00000	1.00000
Acetyl-L-carnitine	1.00000	1.00000	1.00000		1.00000	1.00000
Carnosine	1.00000	1.00000	1.00000	1.00000		1.00000
Ketogenic diet	1.00000	1.00000	1.00000	1.00000	1.00000	

2-Methylbutyrylglycine

Depend.: 2-METHYLBUTYRYLGLYCINE	Multiple Comparisons p values (2-tailed); 2-METHYLBUTYRYLGLYCINE (Ubbink_urien2.sta) Independent (grouping) variable: Groep Kruskal-Wallis test: H (5, N= 60) =14.82517 p =.0111 Controle R:25.650	Valproate R:32.550	Carbamazepine R:26.350	Acetyl-L-carnitine R:31.900	Carnosine R:20.600	Ketogenic diet R:45.950
Controle		1.000000	1.000000	1.000000	1.000000	0.140183
Valproate	1.000000		1.000000	1.000000	1.000000	1.000000
Carbamazepine	1.000000	1.000000		1.000000	1.000000	0.181342
Acetyl-L-carnitine	1.000000	1.000000	1.000000		1.000000	1.000000
Carnosine	1.000000	1.000000	1.000000	1.000000		0.017572
Ketogenic diet	0.140183	1.000000	0.181342	1.000000	0.017572	

Glycine

Depend.: AA-glycine	Multiple Comparisons p values (2-tailed); AA-glycine (Ubbink_urien2.sta) Independent (grouping) variable: Groep Kruskal-Wallis test: H (5, N= 57) =21.09437 p =.0008					
	Controle R:31.222	Valproate R:34.778	Carbamazepine R:35.400	Acetyl-L-carnitine R:36.889	Carnosine R:29.500	Ketogenic diet R:7.8000
Controle		1.000000	1.000000	1.000000	1.000000	0.031977
Valproate	1.000000		1.000000	1.000000	1.000000	0.006060
Carbamazepine	1.000000	1.000000		1.000000	1.000000	0.003010
Acetyl-L-carnitine	1.000000	1.000000	1.000000		1.000000	0.002049
Carnosine	1.000000	1.000000	1.000000	1.000000		0.051940
Ketogenic diet	0.031977	0.006060	0.003010	0.002049	0.051940	

Body weight

Day 7

Groep		Unequal N HSD; Variable: Day 7 (adrienne-massas) Marked differences are significant at p < .05000					
		{1} M=31.003	{2} M=39.882	{3} M=32.220	{4} M=39.792	{5} M=43.498	{6} M=-4.327
K	{1}		0.110535	0.999220	0.117151	0.006817	0.000138
VPA	{2}	0.110535		0.228996	1.000000	0.892601	0.000138
CBM	{3}	0.999220	0.228996		0.240389	0.018946	0.000138
ALC	{4}	0.117151	1.000000	0.240389		0.882260	0.000138
CAR	{5}	0.006817	0.892601	0.018946	0.882260		0.000138
KD	{6}	0.000138	0.000138	0.000138	0.000138	0.000138	

Day 14

Groep		Unequal N HSD; Variable: Day 14 (adrienne-massas) Marked differences are significant at p < .05000					
		{1} M=55.160	{2} M=66.211	{3} M=56.655	{4} M=76.060	{5} M=78.380	{6} M=19.639
K	{1}		0.261426	0.999708	0.001770	0.000495	0.000138
VPA	{2}	0.261426		0.419418	0.385265	0.172222	0.000138
CBM	{3}	0.999708	0.419418		0.004307	0.001095	0.000138
ALC	{4}	0.001770	0.385265	0.004307		0.997358	0.000138
CAR	{5}	0.000495	0.172222	0.001095	0.997358		0.000138
KD	{6}	0.000138	0.000138	0.000138	0.000138	0.000138	

Day 21

Groep		Unequal N HSD; Variable: Day 21 (adrienne-massas) Marked differences are significant at p < .05000					
		{1} M=79.058	{2} M=95.332	{3} M=81.573	{4} M=108.96	{5} M=112.65	{6} M=43.012
K	{1}		0.364083	0.999650	0.007596	0.001978	0.000823
VPA	{2}	0.364083		0.551568	0.561942	0.296219	0.000138
CBM	{3}	0.999650	0.551568		0.018123	0.004943	0.000376
ALC	{4}	0.007596	0.561942	0.018123		0.997574	0.000138
CAR	{5}	0.001978	0.296219	0.004943	0.997574		0.000138
KD	{6}	0.000823	0.000138	0.000376	0.000138	0.000138	

Day 28

Groep		Unequal N HSD; Variable: Day 28 (adrienne-massas) Marked differences are significant at p < .05000					
		{1} M=106.35	{2} M=114.40	{3} M=103.23	{4} M=144.17	{5} M=137.05	{6} M=81.239
K	{1}		0.944810	0.999333	0.001325	0.013963	0.071379
VPA	{2}	0.944810		0.810738	0.018628	0.132295	0.006329
CBM	{3}	0.999333	0.810738		0.000510	0.005064	0.154547
ALC	{4}	0.001325	0.018628	0.000510		0.966986	0.000138
CAR	{5}	0.013963	0.132295	0.005064	0.966986		0.000139
KD	{6}	0.071379	0.006329	0.154547	0.000138	0.000139	

Group	AVG	± SD
Total acylcarnitines		
Control	0.101	0.078
Valproate	0.255	0.126
Carbamazepine	0.064	0.027
Acetyl-L-Carnitine	1.759	0.937
Carnosine	0.171	0.177
Ketogenic diet	0.285	0.116
Total long chain fatty acylcarnitines		
Control	0.005	0.003
Valproate	0.006	0.011
Carbamazepine	0.005	0.004
Acetyl-L-Carnitine	0.008	0.006
Carnosine	0.012	0.014
Ketogenic diet	0.069	0.020
Total medium chain fatty acylcarnitines		
Control	0.095	0.079
Valproate	0.245	0.119
Carbamazepine	0.058	0.026
Acetyl-L-Carnitine	1.748	0.938
Carnosine	0.158	0.164
Ketogenic diet	0.206	0.094
Total dicarboxylic fatty acylcarnitines		
Control	0.001	0.001
Valproate	0.004	0.005
Carbamazepine	0.001	0.001
Acetyl-L-Carnitine	0.003	0.002
Carnosine	0.001	0.002
Ketogenic diet	0.009	0.005

Group	AVG	± SD
Acetylcarnitine		
Control	0.416	0.162
Valproate	0.700	0.659
Carbamazepine	0.391	0.179
Acetyl-L-Carnitine	3.752	1.401
Carnosine	0.729	0.360
Ketogenic diet	24.966	18.710
Free carnitine		
Control	1.288	0.755
Valproate	2.282	1.465
Carbamazepine	1.347	0.472
Acetyl-L-Carnitine	43.516	24.524
Carnosine	1.529	0.678
Ketogenic diet	49.673	14.698
3-Hydroxybutyryl-carnitine		
Control	0.053	0.042
Valproate	0.060	0.050
Carbamazepine	0.025	0.012
Acetyl-L-Carnitine	0.346	0.137
Carnosine	0.081	0.046
Ketogenic diet	0.291	0.102
Total acylcarnitine : Free carnitine ratio		
Control	0.076	0.040
Valproate	0.143	0.100
Carbamazepine	0.052	0.022
Acetyl-L-Carnitine	0.046	0.030
Carnosine	0.127	0.130
Ketogenic diet	0.006	0.002

Group	AVG	± SD
[redacted]		
Control	0.313	0.146
Valproate	0.332	0.225
Carbamazepine	0.321	0.175
Acetyl-L-Carnitine	0.105	0.050
Carnosine	0.591	0.409
Ketogenic diet	0.485	0.328
[redacted]		
Control	14.266	11.723
Valproate	18.511	27.699
Carbamazepine	25.634	35.759
Acetyl-L-Carnitine	12.208	6.179
Carnosine	13.649	13.773
Ketogenic diet	78.207	44.160
[redacted]		
Control	4.735	4.584
Valproate	28.475	45.430
Carbamazepine	3.452	4.965
Acetyl-L-Carnitine	12.638	14.120
Carnosine	2.200	2.620
Ketogenic diet	16.300	17.683
[redacted]		
Control	2.748	3.217
Valproate	14.720	10.257
Carbamazepine	1.723	3.951
Acetyl-L-Carnitine	9.708	12.309
Carnosine	1.721	2.683
Ketogenic diet	12.112	13.961

Group	AVG	± SD
Unsaturated glycine conjugates		
Control	0.573	1.023
Valproate	13.349	39.232
Carbamazepine	1.006	2.065
Acetyl-L-Carnitine	1.790	2.880
Carnosine	0.136	0.313
Ketogenic diet	3.140	3.040
Valine		
Control	984.0	731.4
Valproate	1019.4	398.6
Carbamazepine	1316.9	944.8
Acetyl-L-Carnitine	1229.0	728.3
Carnosine	1019.6	933.4
Ketogenic diet	135.4	53.0
Body weight increase: day 0-28		
Control	250.7	30.4
Valproate	231.4	23.9
Carbamazepine	220.4	15.2
Acetyl-L-Carnitine	230.8	15.1
Carnosine	221.3	18.8
Ketogenic diet	164.9	5.1