

**INSULIN-RELATED
METABOLIC AND ENDOCRINE
EFFECTS OF VALPROATE IN
PATIENTS WITH EPILEPSY**

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AND ENDOCRINE EFFECTS OF
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Academic Dissertation to be presented with the assent of the Faculty of Medicine, University of Oulu, for public discussion in the Auditorium 8 of Oulu University Hospital, on September 2nd, 2005, at 12 noon.

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Abstract

The purpose of this study was to elucidate the background of valproate-related weight gain and hyperinsulinaemia both in men and women by studying markers of insulin resistance and metabolic syndrome. In addition, the role of leptin, a messenger between adipose tissue and the central nervous system was studied.

Valproate has a broad spectrum of antiepileptic activity and is widely used for the treatment of epilepsy. It has been the drug of choice for generalised epilepsy, such as juvenile myoclonic epilepsy, and it is also effective for treatment of partial seizures. In addition, valproate is used to treat other diseases, such as bipolar psychiatric disorders and migraine.

The results show that valproate-treated patients have higher serum insulin levels in relation to body mass index than control subjects. This indicates that the high serum insulin levels are not a consequence of increased body mass, especially, as the body mass index did not differ between the VPA treated patients and the control groups. Valproate therapy started at a young age may more often result in elevated serum insulin levels and associated other untoward metabolic changes. Furthermore, according to the present data, high serum insulin levels are a consequence of compromised metabolism of insulin in the liver, rather than reflecting reduced insulin sensitivity. However, the valproate-treated patients cluster risk factors for cardiovascular diseases, although the occurrence of metabolic syndrome is not more common in valproate-treated patients than in control subjects. Leptin does not play an independent role in valproate-related weight gain.

Keywords: epilepsy, insulin, lipids, metabolic syndrome x, valproic acid

To my mother

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Virpi Pylvänen

Abbreviations

AED	antiepileptic drug
BMI	body mass index
BP	blood pressure
CBZ	carbamazepine
CNS	central nervous system
CoA	Co enzyme A
CP	C-peptide
DHEAS	dehydroepiandrosterone sulphate
EEG	electroencephalography
FFA	free fatty acid
GABA	gamma-amino-butyric acid
HDL	high density lipoprotein cholesterol
HI	hyperinsulinaemia
HYCZ	10,11,-dihydro-10-hydroxycarbazepine
IGFBP-1	insulin like growth factor binding protein-1
ILAE	International League Against Epilepsy
IR	insulin resistance
LDL	low density lipoprotein cholesterol
LTG	lamotrigine
MRI	magnetic resonance imagining
MBS	metabolic syndrome
NMDA	N-methyl-D-aspartate
OXC	oxcarbazepine
PCO	polycystic ovary
PCOS	polycystic ovary syndrome
PI	proinsulin
SHBG	sex hormone binding globulin
T4	thyroxine
T3	triiodothyronine
TG	triglycerides
TRH	thyrotropin

TSH	thyroid stimulating hormone
VLDL	very low density lipoprotein
VPA	valproate
WHR	waist-hip ratio

List of original articles

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I Pylvänen V, Knip M, Pakarinen A, Kotila M, Turkka J & Isojärvi JI (2002). Serum insulin and leptin levels in VPA-associated weight gain. *Epilepsia*. 43(5):514–7.
- II Pylvänen V, Knip M, Pakarinen AJ, Turkka J, Kotila M, Rättyä J, Myllylä VV & Isojärvi JI (2003). Fasting serum insulin and lipid levels in men with epilepsy. *Neurology*. 60(4):571–4.
- III Pylvänen V, Pakarinen A., Knip M & Isojärvi JI. Characterization of insulin secretion in VPA-treated patients with epilepsy. Submitted.
- IV Pylvänen V, Pakarinen A, Knip M & Isojärvi JI. Insulin related metabolic changes during treatment with valproate. Submitted.

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1 Introduction

More than a century ago, Hughlings Jackson defined epileptic seizures as the result of an occasional, sudden and excessive discharge of gray matter (Jackson 1873). Over the years these discharges have been studied and classified (Commission on Classification and Terminology of the International League Against Epilepsy 1981). Seizures are still important signals of the possibility of underlying brain disorders that need to be identified and treated, i.e. seizures are symptoms of abnormal brain function (Dodson 2004).

Today the prevalence of epilepsy is 0.7–0.8% around the world (Hauser *et al.* 1991). In Finland there are more than 37000 patients receiving antiepileptic medication. Most of the patients can be treated with antiepileptic drugs (AEDs), although some types of epilepsies need lifelong medication. Depending upon the type of epilepsy, approximately 50–90% of the patients can be satisfactorily controlled, which means that the balance between seizures and adverse effects is tolerable to the patients (Rogvi-Hansen & Gram 1995). On the other hand, more than half of the patients treated with established AEDs in monotherapy will experience adverse effects. As differences in efficacy are marginal, the adverse effects may be a major consideration in the choice of the AED.

Valproate (VPA) is a branched fatty acid, which is structurally unrelated to any other AED. It has a broad spectrum of antiepileptic efficacy in the prevention of both partial and generalised seizures (Davis *et al.* 1994). Today VPA is also used in other indications such as bipolar psychiatric disorders and migraine (Soares 2000; Silberstein & Collins 1999; Licht 1998; Klapper 1997; Post *et al.* 2000). However, the use of VPA may be associated with adverse effects; one of the most common being weight gain (Dinesen *et al.* 1984; Biton *et al.* 2001; Egger & Brett 1981). In women with epilepsy, VPA-related weight gain is associated with hyperinsulinaemia (HI) and low serum concentrations of insulin-like growth factor-binding-protein-1 (IGFBP-1), which may promote hyperandrogenism and the development of polycystic ovaries (PCO) (Isojärvi *et al.* 1996; Isojärvi *et al.* 1993a). There is also evidence that epilepsy itself may affect the hormonal balance (Herzog *et al.* 1986). HI in general is known to be associated with weight gain, dyslipidemia and insulin resistance (IR). These conditions are risk factors for type 2 diabetes, hypertension and eventually coronary heart disease (Conaway & Jacobs 1993). Furthermore, HI is related to the PCO-syndrome in obese women (Moller & Flier 1991).

The pathogenetic mechanisms leading to weight gain during VPA medication are still poorly defined. In addition, it is also important to assess metabolic changes associated with VPA-related weight gain, and to evaluate in more detail the possible risks of cardiovascular co-morbidity in patients taking VPA.

2 Review of the literature

2.1 Epilepsy

2.1.1 General aspects

An epileptic seizure is a symptom of abnormal brain function resulting from excessive, synchronous, abnormal firing patterns of neurons that are located predominantly in the cerebral cortex. Epilepsy is present when seizures are recurrent and are not due to easily reversed, transient metabolic or toxic disorders. Although a causative brain disease can sometimes be identified, in the majority of cases, the cause is not known and the best diagnosis possible is descriptive. An important goal of epilepsy research is to identify the aetiology and pathology of the symptoms. (Waltimo 1983; Dodson 2004).

2.1.2 Epidemiology

Epilepsy is a relatively common neurological disorder. However, it is often difficult to compare the incidence and prevalence rates across studies because of differences in the definitions of seizure disorders. According to Hauser, several studies show incidence rates from 30.9 to 56.8 cases per 100,000 or about 70,003–128,717 new cases per year in the United States. By the age of 80, 1.3–3.1% of the population will have had, or will continue to have epilepsy (Hauser *et al.* 1993; Hauser *et al.* 1991). In Finland there were 37,000 patients receiving antiepileptic medication at the end of 2004 according to the Social Insurance Institution.

2.1.3 Aetiology and classification

Epilepsy is a heterogeneous group of disorders with multiple causes and manifestations. In the adult population the most important causes of epilepsies are trauma, brain tumors and vascular diseases of the brain, as only a small proportion of epilepsy is inherited (Lehesjoki & Pitkänen 1999; Shorvon 2000). In children, genetic diseases, metabolic defects, congenital malformations and infections are the most important causes of epileptic disorders. (Luhdorf *et al.* 1989; Nelson & Ellenberg 1987).

Epilepsies can be classified according to the aetiology of epilepsy. Epilepsy with known aetiology is called symptomatic, and if the presumed pathologic process of the brain is not detected with the methods used, cryptogenic. Epilepsy with unknown aetiology, especially in children with epileptic syndromes, is called idiopathic. Furthermore, epilepsy is classified as partial or generalised seizures. (Commission on Classification and Terminology of the International League Against Epilepsy 1981 & 1989). In 2001, “A Task Force on Classification and Terminology” was appointed by ILAE, which proposed a diagnostic scheme that makes use of standardized terminology and concepts to describe individual patients (Engel 2001).

2.1.4 Treatment

The primary objective of treatment is complete seizure control. This, however, should not be achieved at all costs. AEDs may cause severe side effects, particularly when they are administered at high doses or in combination. The patient is not to suffer more from the side effects of treatment than from the symptoms of the underlying disease.

A correct diagnosis should be formulated before rational treatment can be started. Seizure type and syndrome should be identified, because these are important in determining drug selection and prognosis (Perucca 1996). Drug therapy should generally be initiated when a second unprovoked seizure occurs. Treatment may be considered already after a single seizure if specific prognostic factors indicate a high risk of recurrence, in particular when the EEG shows interictal epileptiform abnormalities, and/or there is an identified persisting cause for the seizures, such as a MRI-documented lesion or when it is felt that the physical or psychosocial consequences of a seizure recurrence outweigh the risks associated with drug treatment (Hirtz *et al.* 2003).

When specific triggers precipitate seizures, avoidance of the trigger may be sufficient to prevent seizure recurrence (Perucca 1996). Continuous treatment is not indicated in most children with febrile seizures (Hirtz *et al.* 2003).

The overall risk of seizure recurrence after a first unexplained seizure is 30–40% in adults, and, after a second seizure, the risk increases to 80–90% (Shinnar *et al.* 1990). Early intervention also seems to prevent the epileptic process from becoming chronic (Scheuer & Bedley 1990).

In many patients with epilepsy, monotherapy is a possible and preferred treatment. Monotherapy has the advantage of avoiding drug-drug interactions and provides a simpler regimen that may improve compliance. However, if the patient has a more severe form of epilepsy, polypharmacy is needed. As many as 30–50% of the patients with

epilepsy may not be adequately treated with a single AED, and two or more drugs may be needed (Ferrendelli 1987; Mattson *et al.* 1985; Kwan & Brodie 2000). About 25% of the patients fail to respond to drug treatment, which may be a consequence of recurrent seizure precipitants, such as alcohol withdrawal or sleep deprivation, false diagnosis, or a combination of epileptic and non epileptic seizures or intractable epilepsy (Keränen & Kälviäinen 1997). Up to 68% will enter remission in five years of follow up when treated adequately with AEDs (Cockerell *et al.* 1997).

To avoid medical over treatment, surgical treatment should be explored in any patient who has not become seizure free with three adequate AEDs in a period of 3–4 years. Successful surgery results in complete seizure control in 60–70% of the patients (Busch *et al.* 1993).

It is possible that long-term anticonvulsant therapy may have long-term side effects that may be associated with potential co-morbidity. However, the discontinuation of therapy should always be balanced against the risks related to possible seizure recurrence. The impact of duration of seizure freedom as a result of successful drug therapy on reducing the risk of seizure recurrence after drug discontinuation has not been established. Discontinuation of medication after seizure control for 2–4 years has similar risks of recurrence. Two-thirds of patients remain seizure free after AED withdrawal. Risk factors for recurrence have been identified, such as abnormal findings in neurological or developmental evaluation, remote symptomatic epilepsy, older age, EEG abnormality, and certain generalised seizure disorders. Long duration of epilepsy and a high number of seizures are other recognized risk factors for seizure recurrence. Withdrawal of medication should be done slowly, but the optimal rate has not been established (Gross-Tsur & Shinnar 1993; Keränen & Kälviäinen 1997).

2.1.5 Antiepileptic medication

In general there are no established significant differences in the efficacy of the standard AEDs phenytoin, carbamazepine (CBZ), or VPA and the new AEDs, oxcarbazepine (OXC), lamotrigine (LTG), topiramate, vigabatrin, gabapentin, levetiracetam, tiagabine (French *et al.* 2004), or pregabalin (French *et al.* 2003) when used according to recommendations on each seizure type. There are no studies comparing the new drugs with each other.

The drug recommendations for localization related epilepsy are CBZ, LTG, OXC, phenytoin and VPA, and as adjunctive therapy gabapentin, levetiracetam, LTG, tiagabine and topiramate (listed in alphabetical order). For primarily generalised epilepsies VPA and LTG are recommended as first choice drugs and LTG and topiramate as adjunctive therapy. Ethosuximide is used for absence seizures. (Keränen & Kälviäinen 1997; Kälviäinen 2001; Perucca 1996; French *et al.* 2004 a; French *et al.* 2004 b).

2.1.5.1 Valproate

Although VPA was already synthesised in 1882 and used as an organic solvent, its anticonvulsive effect was not discovered until 1963, when it was used as a solvent for other potential AEDs in drug testing. Its mechanism of action is probably multifactorial, influencing neurotransmitters such as gamma-amino-butyric acid (GABA), as well as stabilizing neuronal membranes. VPA also affects sodium channels (McLean & McDonald 1986; Van den Berg *et al.* 1993) and glutamate transporters (Hassel *et al.* 2001). It has been found to be an effective AED in many types of epilepsy, but is a drug of choice in primarily (primary??) generalised epilepsies (DeSilva *et al.* 1996; Heller *et al.* 1995; Davis *et al.* 1994). The plasma protein binding is 90%, and it is degraded into several active metabolites. A curvilinear relationship of dose and serum levels exists due to a concentration-dependent binding (Gram *et al.* 1979). Unlike other older AEDs VPA is not an inducer of the hepatic P450 enzyme system. CBZ, PHT, phenobarbital (PB) and ethosuximide (ESM) decrease VPA blood levels, whereas salicylates and primidone increase VPA blood levels (Levy & Koch 1982). Tremor is a common central nervous system (CNS) adverse effect of VPA (Karas *et al.* 1982). Haematological (May & Sunder 1993) and liver adverse effects have also been noted, and fulminant hepatitis has been reported with an incidence of 1 in 20,000–40,000 patients.

VPA is associated with a dose dependent risk for congenital malformations; this risk may increase on polytherapy. (Holmes *et al.* 2001; Lindhout & Schmidt 1986; Artama *et al.* 2005). Retrospective studies suggest that impaired cognitive development may be associated with maternal drug therapy, particularly VPA (Barrett & Richens, 2003). Gaily *et al.* found out that exposure to VPA polytherapy during pregnancy were associated with significantly reduced verbal intelligence (Gaily *et al.* 2004). Stimulation of appetite and weight gain has been reported in approximately half of the VPA-treated patients (Egger & Brett 1981; Dinesen *et al.* 1984; Biton 2001). VPA is frequently associated with reproductive endocrine disorders in women taking VPA for epilepsy (Isojärvi *et al.* 1993a; Isojärvi *et al.* 1996; Isojärvi *et al.*, 2001b). Weight gain and metabolic and endocrine problems related to the use of VPA are discussed in detail in Chapter 2.8.

2.1.5.2 Carbamazepine

CBZ is chemically related to the tricyclic antidepressants (Ferrendelli 1987). It is a sodium channel blocker, which accounts for most of its anticonvulsive action, but it also blocks an n-methyl-D-aspartate (NMDA)-receptor (MacDonald 2002). It has limited water solubility and 75% is bound to plasma-proteins in the peripheral circulation. As CBZ induces its own metabolism, the half-life may range from 5–26 hours. As a consequence of this autoinduction, higher doses are needed to maintain plasma concentrations over time (Pitlick *et al.* 1976).

Pharmacokinetic interactions with other drugs are common (Basiewicz 1986) as CBZ accelerates the metabolism of endogenous and exogenous hormones, and increases the elimination of ESM, VPA and benzodiazepines, whereas e.g. erythromycin inhibits the

metabolism of CBZ and may increase serum CBZ concentrations to toxic levels in a brief period of time (Dam & Christiansen 1977; Wong *et al.* 1983).

CNS side effects are fairly common when the serum concentration is high. These include nystagmus, drowsiness, blurred vision and ataxia (Pellock 1987). Rash is seen in 2–4% of the patients (Ramsay *et al.* 1983). Also decreased plasma sodium and cognitive adverse effects are seen (Andrewes *et al.* 1986; Aldenkamp *et al.* 1994). Serious adverse events such as aplastic anaemia, dermatitis and eosinophilia are rare.

The risk of congenital malformations in offspring of mothers treated with CBZ was not significantly higher than that of untreated patients. No excess risk was related to CBZ monotherapy (Artama *et al.* 2005; Kaaja *et al.* 2003; Gaily *et al.* 2004).

CBZ is the drug of choice for the treatment of partial seizures with or without generalisation (Shorvon 2000).

2.1.5.3 Oxcarbazepine

OXC is a keto-derivative of CBZ and a prodrug. Its anticonvulsive action is probably mediated mainly by interaction with sodium channels but also with potassium channels (Schmutz *et al.* 1994). It is rapidly metabolised to the active compound, 10,11-dihydro-10-hydroxy-carbamazepine (HYCZ), and the binding to plasma proteins is about 40%, with a half-life of 8–14 hours. The enzyme inducing potential of OXC is low, since it is not metabolised by cytochrome P450-dependent enzymes (Larkin *et al.* 1991).

OXC has fewer pharmacokinetic interactions than CBZ. Elimination of HYCZ is increased by CBZ, PHT, and PB by induction of hepatic enzymes (Tartara *et al.* 1993), and OXC may increase the metabolism of some oral contraceptives (Klosterkov *et al.* 1992; Patsalos *et al.* 1990).

The efficacy of OXC in a 50% higher dose is the same as that of CBZ (Houtkooper *et al.* 1987; Reinikainen *et al.* 1987; Dam *et al.* 1989), but adverse effects are less frequent. The theoretical reason for the lower toxicity is that OXC is not metabolised to any active epoxide metabolite. The most common adverse effects are drowsiness, dizziness, and fatigue occurring during initiation of treatment and these side effects may need dose adjustment. Rash is not seen as often as with CBZ. The cognitive adverse effects of OXC have been suggested to be similar to those of PHT (Äikiä *et al.* 1992). The potential to reduce serum sodium levels appear to be more pronounced when compared to CBZ (Houtkooper *et al.* 1987; Isojärvi *et al.* 2001).

OXC has not been shown to be teratogenic in animal studies. However, the clinical experience of possible teratogenicity of OXC is limited (Friis *et al.* 1993; Artama *et al.* 2005).

OXC is the drug of first choice in the treatment of partial seizures with or without generalisation (Perucca 1996b).

2.2 Serum proinsulin, C-peptide and insulin

2.2.1 Proinsulin

Pancreatic beta cells synthesise proinsulin (PI) that is cleaved into insulin and C-peptide (CP) in the secretory granules (Figure 1). Only small amounts of proinsulin are released into the peripheral circulation and it has less than 5% of the bioactivity of insulin (Granner 1988). The metabolism of proinsulin is slower than that of insulin although proinsulin is degraded by the kidneys to a greater extent than insulin (Polonsky & Rubenstein 1989). The concentrations of circulating proinsulin are elevated in patients with type 2 diabetes, and the magnitude of the elevation may be related to the degree of metabolic control (Reaven *et al.* 1991). An increase in the proinsulin concentrations may occur in the peripheral circulation in subjects with insulinoma, familial hyperproinsulinaemia, chronic renal failure or thyreotoxicosis (Polonsky & Rubenstein 1989).

High fasting proinsulin concentrations as well as high proinsulin to insulin ratios are associated with the development of type 2 diabetes within 2–5 years (Khan & Flier 2000; Mykkänen *et al.* 1995).

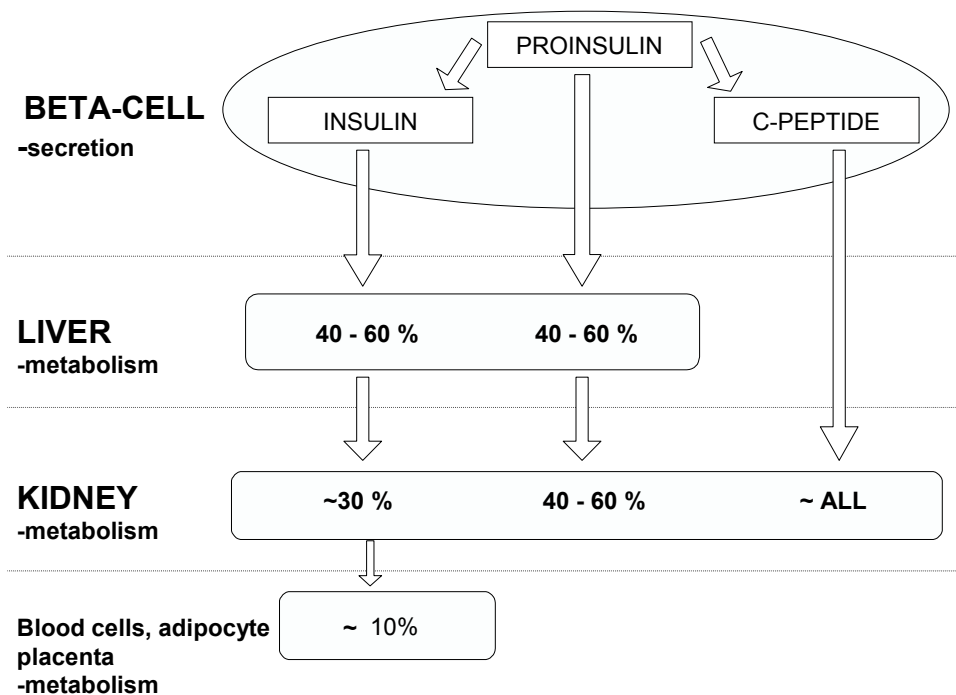


Fig. 1. Excretion and metabolism of insulin, C-peptide, and proinsulin.

2.2.2 C-peptide

CP is secreted from the beta cells in equimolar amounts with insulin (Figure 1). The CP is a peripheral marker of insulin secretion from the beta cells whereas the peripheral insulin concentrations reflect the post hepatic delivery rather than the pancreatic secretion of insulin (Wojcikowski *et al.* 1990). Circulating CP concentrations are generally higher in obese subjects. However, patients with either impaired glucose tolerance or type 2 diabetes have significantly higher CP levels than individuals with normal glucose tolerance. The levels are similarly high in obese and non-obese subjects (Reaven *et al.* 1991). Hepatic extraction of CP is negligible, and the peptide is eliminated through renal excretion (Polonsky & Rubenstein 1989). (Figure 1).

2.2.3 Insulin and glucose metabolism

Insulin is a polypeptide that consists of two chains, A and B, and it is secreted by the beta cells in the Langerhans islets dispersed throughout the pancreas. A number of mediators have been implicated in the regulation of insulin release. Glucose is the crucial metabolite stimulating insulin secretion, whereas glucagon, somatostatin and protein ingestion are other important regulators (Granner 1988).

Insulin is metabolised in the liver, about 50% of the insulin secreted by the beta cells is removed in the first pass through the liver by insulin specific protease and glutathione-insulin transhydrogenase enzymes (Granner 1988). Plasma glucose homeostasis is maintained by an insulin-regulated balance between the rate of glucose appearance and the rate of glucose utilisation. While fasting, glucose is derived from endogenous hepatic glucose production (Owen *et al.* 1969) that equals the glucose utilisation rate. This is mainly non-insulin mediated. In healthy subjects there are also low rates of nonoxidative glucose metabolism in skeletal muscle (Mandarino *et al.* 1987). After a meal, glucose is derived from glucose absorbed by the intestine and the glucose produced by the liver. An adequate amount of insulin is then secreted to maintain euglycemia and suppress endogenous glucose production and increase glucose uptake in skeletal muscle (DeFronzo *et al.* 1981). The physiological effects of insulin are listed in table 1.

The first step in glucose uptake by the muscle cell is initiated by the binding of insulin to the alpha-subunit of the insulin receptor, which leads to stimulation of the tyrosine kinase activity in the beta subunit. The insulin signal is mediated through phosphorylation/dephosphorylation reactions and the first intracellular protein activated is the insulin receptor substrate-1, which serves as a docking protein. After glucose has been transported into the muscle cell, it is rapidly phosphorylated by hexokinase 2 and routed either to the glycolytic or glycogen synthesis pathway. Glycolysis leads to production of pyruvate, which can be metabolised to lactate and alanine (anaerobic glycolysis) or acetyl-CoA, which is either oxidized in the Krebs cycle (oxidative metabolism) or used for de novo lipogenesis.

Table 1. The physiological effects of insulin according to Granner D. K. (Granner 1988).

Rate of glucose transport
Glucose utilisation→conversion of glucose to energy (glycolysis), to fat, and to glycogen
Glucose production→inhibition of gluconeogenesis
Glucose metabolism →decrease the blood glucose level
Lipid metabolism →lipogenic action, inhibition of lipolysis
Anabolic effect on protein metabolism
Stimulation of cell proliferation

2.2.4 Hyperinsulinaemia

HI is generally regarded as a risk factor for arteriosclerosis and coronary heart disease (CHD) (Pyörälä 1979; Welborne & Wearne 1979; Zavaroni *et al.* 1989) including myocardial infarction (Ducimitiere *et al.* 1980), although these views have also been challenged by some investigators (Savage & Saad 1993). Pyörälä *et al.* noted that although HI predicts risk for CHD, its predictive value diminishes along with increasing follow-up time (Pyörälä *et al.* 1998). Increased insulin secretion leads to weight gain (Bray 2004) but also vice versa, weight gain leads to HI (Nakae & Accilli 1999; Khan & Flier 2000). HI is said to correlate with the degree of IR (Bonadonna *et al.* 1990). Relative glucose intolerance, high concentrations of serum TG and uric acid, low HDL serum concentration and high blood pressure (BP), irrespective of the degree of weight gain, are among the clinical and biochemical findings that have commonly been associated with high serum insulin levels (Zavaroni *et al.* 1989; Zavaroni *et al.* 1994). Furthermore, individuals with a family history of either type 2 diabetes or hypertension have been shown to be relatively hyperinsulinaemic (Laws *et al.* 1989).

The mechanism by which high insulin levels would induce high BP may be due to the vasoactive capabilities of insulin, including increased sympathetic activity, renal sodium retention, and vascular smooth muscle cell proliferation (Goalstone *et al.* 1998; Kawasaki *et al.* 2000).

2.2.5 Mechanism of insulin resistance

The ability of insulin to stimulate glucose uptake varies widely among individuals. IR is defined as relative impairment of insulin-mediated uptake of glucose by tissues, mainly muscle (Reaven 1988). The pancreatic beta cell attempts to secrete the required amount of insulin to maintain the plasma glucose concentration within the normal range. The more resistant a normal individual is to insulin-mediated glucose disposal, the greater will be the degree of compensatory HI. Once the plasma glucose begins to increase and the plasma insulin to decrease, a series of other events takes place in liver and adipose tissue that further increases the degree of fasting hyperglycaemia. Furthermore, continuous HI, for any reason (insulinoma, IR etc.) increases the likelihood for

development of hypertension, dyslipidemia and changes in fibrinolytic function increasing the risk of CHD (Reaven 1995).

The concentrations of serum triglycerides (TG) and uric acid are higher in hyperinsulinaemic persons compared to subjects with normal insulin levels. This is the case among both obese and non-obese subjects. Similarly BP is higher and high-density lipoprotein-cholesterol (HDL) levels are lower in hyperinsulinaemic persons. HI seems to be relatively independent of other variables known to modulate insulin mediated glucose uptake and the insulin response to oral glucose does not always correlate with weight gain (Zavaroni *et al.* 1994).

The mechanism linking weight gain and IR are not known. One study compared glucose and plasma insulin levels in lean and obese individuals following an oral glucose load (Felber *et al.* 1993). Plasma glucose levels were similar in the lean and obese subjects, but serum insulin concentrations were higher in the obese group. Accordingly it has been proposed that HI may contribute to the development of IR in obese subjects by down-regulating insulin receptors.

The glucose-fatty acid cycle seems to play an important role in the development of IR during weight gain. The source of long-chain fatty acids (FFA) is either de novo synthesis from acetyl-CoA derived from carbohydrate or from dietary lipids. In the tissues fatty acids may be oxidized or esterified to acylglycerols. Fatty acids are transported in the circulation bound to albumin. They are transported into the cell through the cell membrane and through the cytosol bound to specific fatty acid binding proteins. Before further transportation, the fatty acids associate with coenzyme A in the cytosol. An oxidative breakdown, i.e. beta-oxidation occurs in the mitochondrion matrix, which is further oxidized in the Krebs's cycle. Acetyl-CoA has several important fates. Firstly, when derived from carbohydrate it is oxidized completely in the Krebs's cycle yielding energy. Secondly, it is a source of the carbon atoms in cholesterol. Thirdly, in the liver it forms acetoacetate, the parent ketone body, which becomes an important source of energy under certain conditions (Mayes 1988).

Rates of glucose oxidation and uptake have been shown to decrease in response to increased plasma FFA levels. Studies in healthy volunteers showed that increased circulating insulin levels stimulated glucose uptake and decreased hepatic insulin sensitivity (Ferrannini *et al.* 1983). These features are seen in weight gain, suggesting that increased FFA levels may be involved in the development of IR.

2.2.6 Hyperinsulinaemia and hormones

HI and IR are associated with PCO and hyperandrogenism in women (Möller & Flier 1991), and may increase the risk for cardiovascular diseases, type 2 diabetes and hypertension among women with PCOS (Conaway & Jacobs 1993). The relationship between IR and hyperandrogenism is complex, as there has been controversy as to which is the primary defect. IR contributes more to hyperandrogenism than vice versa as suppression of hyperandrogenism does not restore insulin sensitivity (Dunaif *et al.* 1990) but reducing HI or IR may improve hyperandrogenism (Dunaif *et al.* 1996; Nestler *et al.* 1995).

Men tend to show an inverse relationship between androgen and insulin concentrations and insulin sensitivity appears to improve with greater androgenization (Haffner *et al.* 1994).

Other hormones that exert a stimulatory role on insulin secretion include growth hormone (Felig *et al.* 1971), glucocorticoids (Kalhan & Adam 1975), thyroid hormone excess (Foss *et al.* 1990), PTH (stimulatory at low doses) (Kim *et al.* 1971), prolactin (Landgraf *et al.* 1977) and placental lactogen (Beck & Daughaday 1967). Norepinephrine (Lembo *et al.* 1994) and epinephrine (Marangou *et al.* 1988) decrease insulin-mediated glucose uptake both in diabetic (Walters *et al.* 1992) and non-diabetic subjects (Capaldo *et al.* 1992).

2.3 Leptin

Leptin is a product of the OB-gene, which was first detected in mice. Mutations in this gene lead to weight gain in rodents. Leptin is considered to be a signal factor that regulates body weight and energy expenditure (Zhang *et al.* 1994; Halaas *et al.* 1995; Campfield *et al.* 1996). In rats leptin has been shown to reduce food intake and increase energy expenditure (Halaas *et al.* 1995). It is likely that leptin regulates body weight via neuropeptide Y that stimulates food intake and decreases thermogenesis in the hypothalamus (Smith *et al.* 1996). A strong correlation has been observed between serum leptin concentrations, body mass index (BMI) and body fat mass in man (Considine *et al.* 1996). This suggests that weight gain may be associated with a decreased sensitivity to leptin. On the other hand, lean as well as obese human subjects with IR have high concentrations of serum leptin. However, a similar degree of IR is associated with higher levels of circulating leptin in obese than in lean subjects (Segal *et al.* 1996).

2.4 Thyroid hormones

The thyroid gland secretes thyroxine (T4) and small amounts of triiodothyronine (T3). The principal role of these substances is to regulate tissue metabolism (Larsen 1988; Granner 1988). Thyroid hormones are metabolised largely by deiodination in peripheral tissues, liver and kidney, and to a lesser degree by the oxidative deamination and decarboxylation of the alanine chain. A portion of the thyroid hormones is excreted in the bile and undergoes enterohepatic circulation. The thyroid hormones circulate largely bound by plasma proteins (Baxter 1988).

Thyroid function is under hypothalamic pituitary control. Thyrotropin (TRH), which stimulates synthesis and release of the thyroid stimulating hormone (TSH), is secreted by hypothalamic cells. TSH in turn stimulates all of the steps involved in thyroid hormone synthesis and release (Larsen 1988).

2.5 Lipids

Fat absorbed from the diet and lipid synthesised by the liver and adipose tissue must be transported between the various tissues and organs for utilisation and storage. In man excess calories are ingested in the anabolic phase of the feeding cycle, followed by a period of negative calorie balance when the organism draws upon its carbohydrate and fat stores. Lipoproteins mediate this cycle by transporting lipids from the intestines as chylomicrons, and from the liver as very low-density lipoproteins (VLDL), to most tissues for oxidation and to adipose tissue for storage. Lipid is mobilized from adipose tissue as FFA attached to serum albumin. Two other major groups of lipoproteins have been identified that are important physiologically and in clinical diagnostics, low-density lipoproteins (LDL), which is formed from VLDL, and HDL which is synthesised and secreted from both the liver and intestine (Mayes 1988).

2.6 Uric acid

Uric acid is a product of purine metabolism. It is degraded in most mammals by the hepatic enzyme urate oxidase to allantoin, which is freely excreted in the urine. Uric acid levels also vary significantly between individuals in humans as the result of factors that increase its generation or decrease its excretion. Serum uric acid is frequently elevated in subjects at cardiovascular risk (Toryn *et al.* 1998). In subjects with weight gain, IR, and dyslipidemia or metabolic syndrome (MBS), hyperuricemia frequently occurs because insulin stimulates sodium and urate reabsorption in the proximal tubule (Galvan *et al.* 1995). Hyperuricemia predicts future development of hypertension (Selby *et al.* 1990).

2.7 Weight gain and type 2 diabetes

Obesity is a chronic disease in the same sense as hypertension and arteriosclerosis. The aetiology or cause of weight gain is an imbalance between the energy ingested in food and the energy utilised. Excess energy is stored in fat cells that enlarge and/or increase in number. Enlarged fat cells induce the clinical problems associated with weight gain either because of the weight or mass of the extra fat (Gortmaker *et al.* 1993) or because of the increased secretion of FFAs and numerous peptides from enlarged fat cells. These changes in fat cell mass and function may lead to other diseases such as e.g. type 2 diabetes and cardiovascular diseases (Bray 2004). The distribution of fat in the body influences the degree of IR that develops with weight gain. Patients with central weight gain have a greater degree of peripheral IR compared with patients with lower body weight gain (Kissebah 1991).

Type 2 diabetes is characterized by IR, which appears to be independent of the effects of weight gain. A study of lean patients with type 2 diabetes showed decreased insulin-stimulated glucose uptake and decreased hepatic insulin sensitivity. A reduction in the suppression of lipolysis by insulin was also observed in these patients (Groop *et al.*

1989). Studies have shown that non-diabetic relatives of patients with type 2 diabetes have an increased risk of developing type 2 diabetes suggesting that the IR associated with this form of diabetes may be caused by an inherited abnormality (Vauhkonen *et al.* 1998).

At least a third of the patients affected by type 2 diabetes are obese with a BMI greater than 30 kg/m². A study showed that obese patients had a further decrease in hepatic insulin sensitivity and glucose oxidation rates compared with lean patients (Groop *et al.* 1991). The main consequence of weight gain in patients with type 2 diabetes is increased hepatic IR. There is some indirect evidence that weight loss is associated with a reduced IR (Golay *et al.* 1985) and reduction of risk factors reduces the development of cardiovascular diseases (Sowers 2003).

2.8 Metabolic and endocrine effects of valproate

2.8.1 Body weight

Shortly after VPA was introduced as an effective AED, its side effect, weight gain was noticed in a six-month trial in children with epilepsy (Sherard *et al.* 1980). These findings were confirmed later: out of 100 children treated with VPA 44 gained weight during treatment and after drug withdrawal there was a rapid weight loss (Egger & Brett 1981). Dinesen *et al.* examined 63 VPA-treated patients therapy with 36 patients gaining weight more than 4 kg during the treatment. No differences were seen in weight gain with regard to age, sex, pre-treatment overweight, and duration of treatment, dosage or serum levels of VPA. Also the impression that weight gain is difficult to reverse by dietary restrictions was mentioned (Dinesen *et al.* 1984). In a prospective and randomised study Biton *et al.* showed an increase in BMI over 8 months of follow up in VPA treated patients, where as the LTG treated patients did not gain weight during the study. (Biton *et al.* 2001). The pathogenetic mechanisms behind the weight gain have been studied. VPA is known to affect the concentrations of carnitine in humans and it is hypothesized that a possible VPA induced deficiency of the beta-oxidation of fatty acids is important for the development of weight gain in epileptic patients on long term treatment with VPA, but changes in catecholamines or other hormones might also be of importance (Breum *et al.* 1992). Gidal also suggested according to his studies on resting energy expenditure that beta-oxidation is significantly reduced in those VPA-treated patients who gain weight (Gidal *et al.* 1996). As VPA is a fatty acid and competes in binding to human serum albumin (Brodersen *et al.* 1990) it has been proposed that weight gain may result from increased availability of long chain fatty acids due to competitive binding of VPA to serum albumin (Vorum *et al.* 1993). According to Ponchaut and Veitch, the influence of VPA on mitochondrial metabolism is extremely complex including sequestration of free CoA by the drug and some of its metabolites, the inactivation of mitochondrial enzymes and the interference with substrate carriers (Ponchaut & Veitch 1993). After replacing VPA with LTG in women with epilepsy, weight reduction occurred gradually and progressively during a 12-month follow up, whereas the insulin concentrations and

unfavourable lipid profile returned to normal within 2 months (Isojärvi *et al.* 1998), suggesting that the weight gain is a consequence of HI.

2.8.2 Hyperinsulinaemia

In female patients on VPA, weight gain is associated with HI and low IGFBP-1 levels, which can promote hyperandrogenism and the PCO-syndrome. These hormonal disturbances can cause irregular menstruation and infertility in female patients taking VPA. Due to HI, these women may have higher risk for cardiovascular diseases. (Isojärvi *et al.* 1993a; Isojärvi *et al.* 1998; Isojärvi *et al.* 1996).

Isojärvi *et al.* demonstrated that in women serum insulin levels became normal within 2 months after VPA was changed to LTG, whereas the weight loss after discontinuing VPA was considerably slower, suggesting that increased insulin secretion precedes weight gain in women with epilepsy (Isojärvi *et al.* 1998). Luef *et al.*, found in an experimental study based on pancreatic islet cell culture that VPA may stimulate insulin secretion from the pancreatic beta cells resulting in higher levels of fasting serum insulin (Luef *et al.* 2003). Furthermore, he and his co-workers have reported that the occurrence of non-alcoholic fatty liver disease is more common among VPA-treated patients than among CBZ-treated patients (Luef *et al.* 2004), although the fasting insulin levels were not significantly higher in the former group than in the CBZ-treated patients. In a short prospective study no significant changes were observed in serum insulin levels during VPA treatment (Breum *et al.* 1992).

2.8.3 Hormones

Epilepsy itself may be associated with an increased frequency of reproductive endocrine disorders, including PCOS, and hyperandrogenism, hypothalamic amenorrhoea and functional hyperprolactinemia (Herzog *et al.*, 1986). Isojärvi *et al.* have documented in a series of studies that female VPA-treated patients have menstrual irregularities, PCOS or raised serum testosterone concentrations more often than control subjects or patients taking other AEDs (Isojärvi *et al.* 1993a; Isojärvi *et al.* 2001a; Isojärvi *et al.* 1996; Luef *et al.* 2002a). Discontinuation of VPA resulted in normalised insulin, androgen and lipid levels as well as a decrease in the number of patients with PCO (Isojärvi *et al.* 1998).

A study on 21 VPA-treated men showed that serum levels of androstendione (ADION) were high in VPA-treated men, and half of the patients had circulating concentrations of testosterone, ADION or DHEAS above the normal range (Rättyä *et al.* 2001). Similar findings were reported in women (Isojärvi *et al.* 1993a; Isojärvi *et al.* 1998) and young girls taking VPA for epilepsy (Vainionpää *et al.* 1999). On the contrary, Verrotti *et al.*, 2000, reported that VPA-treated men showed no significant differences in free testosterone and DHEAS concentrations when compared to CBZ-treated men. (Verrotti *et al.* 2000).

The findings on thyroid hormones in VPA-treated patients are controversial. In some studies patients on VPA monotherapy had higher concentrations of free T4, T3 and TSH

(Isojärvi *et al.* 1990; Isojärvi *et al.* 1992; Ericsson *et al.* 1985), but also decreased (Fischer & Knopfle 1978) and unchanged serum levels of thyroid hormones and TSH (Liewendahl *et al.* 1978; Larkin *et al.* 1989; Isojärvi *et al.* 2001b) have been reported in patients taking VPA.

2.8.4 Leptin

VPA-related weight gain may be associated with elevated serum leptin concentrations in women with epilepsy. Verrotti *et al.*, found that those female patients who gained weight while taking VPA had higher serum leptin and insulin levels than patients who did not gain weight and suggested that these changes are related to the increase in BMI. (Verrotti *et al.* 1999). As female VPA-treated patients seem to have higher serum insulin levels than control subjects (Isojärvi *et al.* 1998; Isojärvi *et al.* 1996), it would be reasonable to assume that they may also have elevated serum leptin levels. It is well known that HI is associated with increased leptin levels (Considine *et al.* 1996).

2.8.5 Fasting plasma glucose levels and serum lipid levels

Breum *et al.* did not find any differences in a short-term study on glucose levels in VPA-treated patients (Breum *et al.* 1992). In another study, patients on VPA and CBZ were compared and the glucose concentrations were higher in the VPA-treated patients than in the CBZ treated patients (Luef *et al.* 2002b).

The total cholesterol/HDL ratio has been seen to be lower in VPA-treated patients (Calandre *et al.* 1991) than in the control subjects. In female patients in VPA the total cholesterol/HDL ratio and HDL concentration was lower than in the female control subjects (Isojärvi *et al.* 2001).

2.9 Carbamazepine and oxcarbazepine and endocrine function

CBZ is a known inducer of the cytochrome P450 enzyme system in the liver (Perucca *et al.* 1984). Therefore, serum concentrations of hormones metabolised via this enzyme system change during CBZ medication. The hepatic synthesis of hormone binding proteins may also be induced (Connell *et al.* 1984).

A majority of previous studies have shown that serum free T4 and T4 decrease whereas TSH levels remain unchanged in CBZ treated patients (Dana-Haeri & Richens 1981; Strandjord *et al.* 1981; Bentsen *et al.* 1983; Isojärvi *et al.* 1989; Isojärvi 1990; Isojärvi *et al.* 1992; Verrotti *et al.* 2000;). In children receiving CBZ treatment, the circulating levels of T3, and free T3 were not affected and T4 and free T4 were lower than in the control subjects (Verrotti *et al.* 2001). The effects of CBZ on thyroid function appear to be too complex to be attributed to a single action of the drug. Apart from a direct effect on the thyroid gland (De-Luca *et al.* 1986), it has been suggested that the

serum T4 and FT4 levels are low in epileptic patients receiving CBZ because of the accelerated metabolism of thyroid hormones in the liver (Isojärvi *et al.* 1990; Isojärvi *et al.* 1993b). As CBZ is an inducer of the microsomal enzyme system in the liver (Perucca *et al.* 1984), increased degradation of thyroid hormones may be the main reason for the decreased T4 and free T4 serum levels (Eravci *et al.* 2000).

The use of CBZ is also associated with changes in serum sex hormone concentrations as well as in levels of the sex hormone binding globulin (SHBG) (Dana-Haeri & Richens 1981; Isojärvi *et al.* 1988; Isojärvi *et al.* 1990; Isojärvi *et al.* 1991; Isojärvi *et al.* 1995a).

Studies on the effects of OXC on the liver P450 enzyme system indicated that OXC is a weaker inducer of liver enzymes than CBZ, and this may result in less endocrine side effects. It has been reported that substituting CBZ for OXC resulted in normalisation of the T4, free T4, TSH, DHEAS and SHBG concentrations in the peripheral circulation (Isojärvi *et al.* 1995b; Isojärvi *et al.* 1994). However, OXC may also induce liver enzymes at high doses (Patsalos *et al.* 1990) and it is known to decrease the bioavailability of oral contraceptives (ethinylestradiol and levonorgestrol) by increasing their metabolism (Fattore *et al.* 1999; Felson *et al.* 1988). Data on the effects of OXC on endocrine function are limited.

2.10 Carbamazepine, and oxcarbazepine and lipids

Long-term treatment with AEDs is associated with increased total cholesterol, LDL and TG levels in previous studies in children (Eiris *et al.* 2000; Aynaci *et al.* 2001) as well as in adult patients (Berlit *et al.* 1982). Serum lipid levels change during CBZ medication; increases in serum concentrations of total cholesterol and HDL have been seen (Bramswig *et al.* 2002) as well as increases in serum LDL and TG levels after starting treatment with CBZ (Isojärvi *et al.* 1993c). Furthermore, the total cholesterol/HDL ratio was significantly lower in patients receiving CBZ (Zeitlhofer *et al.* 1993; Chalandre *et al.* 1991). Similar findings have been observed in children on CBZ medication (Sozuer *et al.* 1997; Demircioglu *et al.* 2000, Aynaci *et al.* 2001). OXC does not seem to affect the serum lipid levels. Replacement of CBZ by OXC resulted in a decrease in serum total cholesterol and LDL levels with HDL-cholesterol and TG remaining unchanged (Isojärvi *et al.* 1994).

2.11 Carbamazepine, and oxcarbazepine and glucose levels

The glucose concentrations were higher in the VPA-treated patients than in the CBZ treated patients according to Luef *et al.* (Luef *et al.* 2002b). Overall, the data on glucose metabolism in patients treated with CBZ, or OXC are limited.

3 Purpose of the present study

The purpose of the present study was to evaluate the effects of valproate on the insulin-related metabolic and endocrine systems in patients with epilepsy. More precisely defined, this study aimed at:

1. evaluating whether the occurrence or risk of the MBS is increased among VPA-treated patients with epilepsy and assessing several metabolic factors in association with VPA treatment, especially in relation to weight gain;
2. assessing serum insulin levels and define the underlying pathophysiology of HI associated with VPA treatment;
3. assessing serum lipid levels in patients with epilepsy.

4 Subjects and methods

4.1 Subjects

The patients participating the study were primarily from the Outpatient clinic of neurology, Oulu University Hospital in Finland, which is the primary referral centre for all adult patients with epilepsy from a source adult population of approximately 260 000. During the years 1996–1997 1386 patients were seen in the Department of Neurology for epilepsy (Ranua *et al.* 2004). One hundred and twenty-four were on VPA monotherapy. The hospital records of these patients were reviewed, 74 had no exclusion criteria and were invited and 51 eventually agreed to participate in the study (III and IV). An additional 183 patients having VPA, CBZ or OXC monotherapy for epilepsy from the Outpatient clinic of neurology, Oulu University Hospital and six men with VPA monotherapy from the Outpatient clinic of Neurology, Helsinki University Hospital, were recruited to participate the study (I and II). 128 healthy volunteers served as control subjects. They consisted of subjects from the Occupational Health Service of the State Railways, Population Register Centre, Outpatient Clinic of Neurology, and hospital staff. A description of the subjects in the different studies are shown in Table 2

The cross-sectional study was carried out during the years 1997–2000 in the Outpatient Departments of Neurology, University of Oulu, Oulu, Finland with the approval of the local Ethics Committee according to the principles of the Declaration of Helsinki. The Department of Clinical Chemistry, University of Oulu, the Department of Neurology, Helsinki University Central Hospital and the Hospital for Children and Adolescents, University of Helsinki, were also involved in the study. Written informed consent was obtained from all the patients and control subjects. The antiepileptic medication used was chosen according to accepted European guidelines; CBZ or OXC were the drugs used particularly in partial seizures, while VPA was primarily used in primarily generalised seizures. One of these drugs was used as monotherapy for at least six months.

All the patients and control subjects were examined once. Data on weight before VPA medication was started, was collected retrospectively from the hospital records (study III). Some of the patients took part in more than one of the substudies, but they were

examined and interviewed for each substudy separately, and the blood samples were taken for each substudy as well. The medical history was obtained by interviewing the patients by the author and by reviewing the hospital records. Those who had no other long-term diseases besides epilepsy, no medications, or oral contraceptives, were not alcohol abusers, or were not pregnant or lactating were accepted to the study. All the patients and control subjects were Caucasian, Finnish speaking and they had normal mental status.

Table 2. Description of the subjects in the different studies.

Study	No. of subjects by sex		Age (yrs) mean (range)	No of patients by the type of epilepsy		Duration of medication (yrs) mean (range)
	male	female		G	L	
Study I						
VPA	46	35	29.6(17–68)	58	23	6.5 (1–23)
Controls	24	27	37.1(18–57)			
Study II						
VPA	37		28.7 (18–43)	26	11	5.2 (0.5–15)
OXC	27		29.7(18–50)	8	19	2.5(0.7–8.9)
CBZ	38		34.5(19–50)	4	34	9.0(0.9–33)
Controls	32		35.0(32.0–45.6)			
Studies III&IV						
VPA	31	20	31.4(18–68)	40	11	6.4±5.0(1–22)
Controls	23	22	30.9(16–57)			

* Monohydroxy-carbamazepine, the active metabolite of OXC. G = primarily generalised; L = localization related; VPA=valproate; OXC=oxcarbazepine; CBZ = carbamazepine; yrs=years.

4.2 Methods

4.2.1 Clinical examination

All participants were clinically examined and interviewed (a structured interview with special emphasis on side effects, especially drug-related weight gain). The data on the medical history were collected from the hospital records. The epilepsy type was classified according to the recommendations of the ILAE (Commission on Classification and Terminology of the International League Against Epilepsy 1989) Computed tomography of the brain or MRI had been performed on each patient, as well as an EEG.

The height and weight were measured along with hip and waist circumferences measured to the nearest 0.5 cm and 0.5 kg. The BMI was calculated: weight in kilograms divided by the square of the height in metres. Body fat distribution was determined by calculating the waist hip ratio (WHR): waist circumference in centimetres divided by the hip circumference in centimetres.

4.2.2 Biochemical evaluations

Blood samples were drawn at 8.00 am after a 12 hour fast for the analysis of plasma glucose, serum insulin, C-peptide, proinsulin, TSH, free T4, HDL, LDL, total cholesterol, and uric acid.

The following indices were calculated: The homeostasis model assessment (HOMA) = insulin (uU/m x [glucose(mmol/l)/22.5], the C-peptide (pmol/L) to insulin (pmol/L) ratio, (CP/I), the proinsulin (pmol/L)to insulin (pmol/L) ratio (PI/I), and insulin to BMI ratio.

4.2.3 Laboratory assays

The description of the different assays performed in each of the studies I–IV are shown in Table 3.

Table 3. Characteristics of the assays used in different studies

Assay	Method, Manufacturer	Typical sensitivity values	Typical coefficient of intraassay variation (%) in the reference interval	Typical coefficient of interassay variation (%) in the reference interval
Insulin	Immunological chemiluminescence Bayer ADVIA Centaur analyser, Tarrytown, NY	0.1 mU/L	2.9	4.7
T4	Immunological chemiluminescence Bayer ADVIA Centaur analyser, Tarrytown, NY	2.0 pmol/L	3.9	4.9
TSH	Two-site fluoroimmunoassay methods Wallac Ltd, Turku, Finland	>0.03 mU/L	2.0	3.5
C-peptide	Radioimmunoassay Diagnostic products Co. Los Angeles, CA	0.07 nmol/L	3.4	7.2
VPA	Fluorescence polarization immunoassay system AxSYM analyser, Abbot diagnostic Division, Irving, Tx	4.1 µmol/L	1.8	2.7
CBZ	Fluorescence polarization immunoassay system AxSYM analyser, Abbot diagnostic Division, Irving, Tx	2.1 µmol/L	1.2	3.2
OXC	High-pressure liquid chromatography Enzymatic photometric method	0.1 µmol/L	<5	<5
Free fatty acid	Immunoassay method using reagent kits from Dako Ltd, Ely, UK			
Proinsulin	Enzymatic colorimetric methods of Roche Diagnostic (Rotkreuz, Switzerland) using Cobas Integra 700 analyzer (F. Hoffmann La Roche Ltd., Basel, Switzerland)			
Plasma glucose, serum				
urate, TG, total cholesterol, HDL, LDL				

T4 = thyroxine; TSH = thyroid stimulating hormone; VPA = valproate; CBZ = carbamazepine; OXC = oxcarbazepine; TG = triglycerides; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; HOMA = homeostasis model assessment.

4.2.4 Definitions

Obesity was defined as BMI exceeding 25 kg/m². Alcohol consumption exceeding 40g/day in men and 20 g/day in women was considered as abuse (English *et al.* 1995). Long-term illness or medication was defined as lasting more than six months. High TG, low HDL cholesterol levels, HI and high insulin/BMI values were defined as values higher than the mean +2 SD of the control subjects. The criteria used to define the MBS were according to The National Cholesterol Education Program Adult Treatment Panel III described in Table 8.

4.2.5 Statistical analyses

The Kruskal-Wallis test for non-parametric analysis of variance and the Mann-Whitney U-test were used for the statistical analyses, as the data were unevenly distributed. P-value less than 0.05 was considered statistically significant. Chi-square or cross-tabulation tests were used for comparison of prevalence of different phenomena between the patients and the control subjects.

5 Results

5.1 Body weight

The mean BMI was similar in the patients and the control subjects. Forty of the 81 (49%) patients who received VPA and 25/51 (49%) of the control subjects were obese. The obese VPA-treated patients had higher WHR (0.92 ± 0.08) than the lean patients (0.86 ± 0.07 ; $p<0.001$) consistent with visceral obesity (study I). The men taking VPA were not more obese than the men taking CBZ, OXC or the control men (study II).

Twenty-seven of 51 (53%) of the patients and 24 (53%) of the control subjects were obese (Study III&IV). Seventeen (55%) of the male patients and 10 (50%) of the female patients were obese in contrast to 18 (78%) and six (29%) in the control subjects, respectively. Weight gain was the most common side effect reported by 45% of the patients. Furthermore, among the 51 patients there were 42 patients who had weight and height documented in their hospital records prior to the VPA medication. Twenty (48%) of these patients had had an increase in their BMI of 10% or more during the VPA treatment and were considered to have experienced indisputable weight gain. Body weight was not related to seizure type.

5.2 Self-reported side effects

Tremor was reported by 37% of the VPA-treated patients. Those who reported to have tremor had significantly higher fasting serum insulin levels (13.4 ± 8.3 mU/L) than those who had not experienced tremor (10.0 ± 4.3 mU/L; $p<0.05$). The patients with tremor had a higher daily dose of VPA (1222 ± 330 mg/day) than those who did not have tremor (965 ± 338 mg; $p<0.05$) as well as higher serum concentrations of VPA (469.1 ± 161.5 umol/L and 360 ± 130 umol/L, respectively; $p<0.01$).

The other commonly reported side effects shown in Table 4, were not associated with any identifiable laboratory abnormalities.

Table 4. Other self-reported side effects reported by patients on VPA monotherapy.

Experienced side effect	Number (%) of patients with different side effects.
Weight gain	23 (45.1)
Tremor	19 (37.3)
Hair loss	9 (17.6)
Irregular menses	4/20 (20.0)
Infertility	2 (3.9)
Others	9 (17.6)

5.3 Fasting serum insulin and insulin-related parameters in valproate treated patients

The differences in serum insulin, PI and CP concentrations as well as in plasma glucose levels and HOMA index when compared to control subjects in different substudies are summarized in Table 5.

Table 5. Summarized findings of differences in insulin and insulin-related parameters in VPA treated patients when compared to control subjects.

Insulin	PI	CP	Glucose	Insulin/BMI	HOMA	PI/I	CP/I
↑	↔	↔	↓	↑	↑	↓	↓

↑ = higher; ↔ = no difference; ↓ = lower; PI = proinsulin; CP = C-peptide; BMI = body mass index; HOMA = homeostasis model assessment; PI/I = proinsulin to insulin ratio; CP/I = C-peptide to insulin ratio

Serum insulin levels were higher in the obese (11.3 ± 6.0 mU/l) than in the lean patients (7.4 ± 5.4 mU/l; $p < 0.001$) on VPA. The mean serum insulin concentrations were higher in the VPA-treated patients (9.2 ± 6.0 mU/l) than in the control subjects (5.0 ± 4.5 mU/l; $p < 0.001$) despite similar BMI values, when all subjects were included in the comparison. The obese male patients on VPA had higher serum insulin levels (11.7 ± 5.9 mU/l) than the obese male control subjects (5.3 ± 2 mU/l; $p < 0.001$), although these two groups had BMI values of the same magnitude. Similarly a significant difference was also seen in serum insulin concentrations between the obese female patients treated with VPA (9.8 ± 6.0 mU/l) and the obese control women (5.4 ± 2.4 mU/l; $p < 0.01$). Furthermore, serum insulin levels were higher both in lean male (8.05 ± 7.1 mU/l) ($p < 0.05$) and lean female (6.7 ± 2.9 mU/l; $p < 0.01$) patients compared to the lean control subjects of same sex and with similar BMI (5.7 ± 9.8 mU/l and 4.1 ± 3.0 mU/l, respectively). (Study I)

Men receiving VPA had higher levels of fasting serum insulin (11.0 ± 16.7 mU/l) than the control men (4.8 ± 2.0 mU/l; $p < 0.01$). (Study II)

The VPA-treated patients with epilepsy had significantly lower fasting plasma glucose concentrations ($p < 0.001$) and significantly higher fasting serum insulin ($p < 0.001$) levels than the control subjects. Serum PI and CP levels were similar in the patients and the control subjects, but the patients had lower PI/I ($p < 0.01$) and CP/I ($p < 0.001$) ratios than the control subjects. (Study III).

Additional analyses were performed based on the BMI of the study subjects. These analyses showed that the obese VPA-treated patients had significantly lower fasting plasma glucose levels ($p<0.01$) and higher serum insulin concentrations ($p<0.001$) than the obese control subjects. In addition they had lower PI/I ($p<0.01$) and CP/I ratios ($p<0.001$) than the obese control subjects. Proinsulin levels tended to be higher in the obese patients ($p=0.06$) than in the obese control subjects. Similarly, the lean VPA-treated patients had higher serum insulin concentrations ($p<0.001$) and lower PI/I ($p<0.01$) and CP/I ratios ($p<0.001$) than the lean control subjects. They also had lower fasting plasma glucose concentrations ($p<0.05$) than the lean control subjects. (Table 6)

Among the VPA-treated patients eight (15.7%) were considered to have HI in absolute terms in contrast to two (4.4%) in the control group. This difference was not statistically significant, $p=0.072$. The findings were similar regardless of sex or type of epilepsy.. (Study IV)

Table 6. Insulin and other insulin-related parameters in valproate treated patients and in control subjects.

Subjects	Glucose, mmol/L	Insulin pmol/L	Proinsulin pmol/L	C-peptide pmol/L	Proinsulin/ insulin	C-peptide/ insulin
Patients						
All (51)	4.72±0.35 ^{SS}	11.30±6.23 ^{SSS}	3.01±1.78	390±290	0.30±0.14 ^{SSS}	35.48±24.09 ^{SSS}
Obese (27)	4.84±0.27 ^{**}	12.19±6.04 ^{***}	3.34±1.93	460±320	0.30±0.11 ^{**}	37.88±24.90 ^{***}
Lean (24)	4.58±0.40 [#]	10.30±6.42 ^{###}	2.64±1.55	300±220	0.31±0.18 ^{##}	32.79±23.33 ^{###}
Control						
All (45)	5.12±0.58	6.28±4.86	2.35±0.87	480±270	0.53±0.36	94.27±61.85
Obese (24)	5.28±0.63	6.43±3.66	2.67±0.86	580±300	0.55±0.39	107.06±70.63
Lean (21)	4.92±0.45	6.20±6.17	2.02±0.75	370±200	0.50±0.33	78.98±48.66

BMI = Body Mass Index; WHR = Waist Hip Ratio; HOMA = Homeostasis Model Assessment;

N = number; * $p<0.05$, ** $p<0.01$, *** $p<0.001$ when compared to the obese control subjects; # $p<0.05$, ## $p<0.01$,

$p<0.001$ when compared to the lean control subjects; § $p<0.05$, SS $p<0.01$, SSS $p<0.001$ when compared to all control subjects

5.3.1 Insulin to BMI ratio

The insulin/BMI ratio was significantly higher in VPA-treated patients than in control subjects. The ratio was similarly high in obese and lean, and in male (0.47±0.27) and female (0.42±0.21) patients compared to their control subjects (0.27±0.16 and 0.22±0.13, respectively) ($p<0.001$ in all comparisons). (Table 7)

A high insulin/BMI ratio was seen in nine (17.6%) patients in contrast to two (4.4%) control subjects ($p<0.05$). The patients with high insulin/BMI ratio were younger (25.1±8.5 years) than the ones with a lower ratio (32.8±12.3 years) ($p<0.05$), and they had started VPA medication at a younger age (19.9±8.4 years) than those with a lower ratio (26.9±12.4 years; $p<0.05$), although the mean duration of medication was the same, 6.2 vs. 6.3. (Study IV)

5.4 Fasting serum lipid levels in patients treated with valproate, oxcarbazepine, or carbamazepine

Serum lipid levels in VPA-treated men (II) did not differ from those of the male patients on OXC or CBZ or male control subjects, but the VPA-treated obese patients had high serum TG (n=7, 25%) and insulin levels (n=7, 35%) more often than the obese patients treated with CBZ (n=1, 5%, and n=0, respectively) or OXC (n=1, 9% and n=0, respectively) or the obese control subjects (n=1, 5% and n=1, 5%, respectively).

In Study IV serum TG concentrations were higher in all patients ($p<0.05$), and tended to be higher in obese ($p<0.06$) and lean ($p<0.09$) patients than in their respective control subjects. Fasting serum HDL cholesterol levels were lower in all VPA-treated patients ($p<0.01$) and in the obese ($p<0.01$) and lean VPA-treated patients ($p<0.01$) than in their respective control subjects (IV). (Table 7). There were no significant differences in the total cholesterol levels or in the LDL cholesterol levels between the VPA-treated patients and the control subjects.

Furthermore, the serum TG concentrations were significantly higher 2.12 ± 1.44 mmol/L in the patients with HI when compared to patients without HI (1.32 ± 0.73 mmol/L ($p<0.05$)).

5.5 Thyroid function in valproate-treated patients treatment

VPA-treated patients had significantly higher serum TSH levels than the control subjects ($p<0.05$) whereas there were no significant differences in the free T4 levels. (Table 7)

Table 7. Fasting serum lipid, thyroid hormone levels and insulin to body mass index - ratio in valproate-treated patients.

Subjects	Ins/BMI	cholesterol mmol/L	HDL mmol/L	LDL mmol/L	HDL/tot	TG mmol/L	TSH mU/L	FT4 pmol/L
Patients								
All (51)	$0.45\pm 0.25^{***}$	5.13 ± 1.09	$1.34\pm 0.37^{**}$	3.16 ± 1.02	0.27 ± 0.09	$1.4\pm 0.91^*$	$2.33\pm 1.40^*$	14.63 ± 1.79
Obese(27)	$0.440\pm .21^{###}$	5.45 ± 1.06	$1.31\pm 0.38^{##}$	3.40 ± 1.10	$0.25\pm 0.09^{\#}$	1.75 ± 1.08	2.55 ± 1.64	14.29 ± 1.71
Lean (24)	$0.46\pm 0.29^{+++}$	4.77 ± 1.02	$1.40\pm 0.36^{++}$	3.40 ± 1.10	0.30 ± 0.08	1.13 ± 0.55	2.09 ± 1.04	15.0 ± 1.83
Controls								
All (45)	0.25 ± 0.16	5.16 ± 0.98	1.55 ± 0.40	2.98 ± 0.89	0.31 ± 0.10	1.11 ± 0.78	1.87 ± 1.13	15.55 ± 2.46
Obese(24)	0.26 ± 0.17	5.42 ± 0.9	1.39 ± 0.34	3.40 ± 0.85	0.27 ± 0.1	1.35 ± 1.0	1.97 ± 1.34	15.66 ± 2.81
Lean(21)	0.24 ± 0.13	4.91 ± 1.0	1.72 ± 0.41	2.56 ± 0.71	0.36 ± 0.08	0.85 ± 0.37	1.73 ± 0.81	15.46 ± 2.10

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ when compared to lean control subjects; # $p<0.05$, ## $p<0.01$, ### $p<0.001$ when compared to all control subjects; $^{\#}$ $p<0.05$, $^{##}$ $p<0.01$, $^{###}$ $p<0.01$ when compared to obese control subjects;

Ins/BMI=insulin to body mass index ratio; HDL = high density lipoprotein; LDL=low density lipoprotein;

HDL/tot = high density lipoprotein to total cholesterol ratio, TSH = thyroid stimulating hormone, FT4 =free T4

5.6 Blood pressure, HOMA index and occurrence of metabolic syndrome in VPA-treated patients

Blood pressure levels were similar in the VPA-treated patients and the control subjects. The number of subjects with high blood pressure was similar among the VPA-treated patients to that of the control subjects. The HOMA index was significantly higher in all obese and lean VPA-treated patients compared to the control subjects ($p < 0.001$ in all comparisons). (Table 9).

The occurrence of the MBS and the components of it are given in Table 8 for the patients on VPA and control subjects. MBS was observed in nine (17.6%) patients (eight males, one female) in contrast to seven (15.6%) control subjects (four male, three females). This difference was not statistically significant.

Table 8. Occurrence of metabolic syndrome and its components in VPA-treated patients and control subjects.

Risk factor (Defining level)	Patients		Controls	
	Men (31) n (%)	Women (20) n (%)	Men (23) n (%)	Women (22) n (%)
Abdominal weight gain (Waist circumference)				
men (>102 cm)	7 (23)		5 (22)	
women (>88 cm)		6 (30)		5 (23)
High Density Lipoprotein Cholesterol				
men (<1.03 mmol/L)	8 (26)		3 (13)	
women (<1.28 mmol/L)		5 (25)		5 (23)
Triglycerides (<1.28 mmol/L)	11 (35)	2 (10)	4 (17)	1 (4)
Fasting glucose (>6.11 mmol/L)	0	0	2 (9)	0
Blood pressure Systolic (>130 mmHg)/	18 (58)	10 (50)	12 (52)	6 (26)
Diastolic (>85 mmHg)	8 (26)	6 (30)	6 (26)	4 (17)
No. of subjects with metabolic syndrome	8 (26)	1 (5)	4 (17)	3 (13)

5.7 Other metabolic parameters in valproate-treated patients

FFA levels tended to be higher in the VPA-treated obese patients ($p < 0.06$) than in the obese control subjects. In contrast, the lean VPA-treated patients tended to have lower FFA levels than the lean control subjects ($p < 0.07$). (Table 9)

Serum uric acid levels were higher in all patients ($p < 0.01$), in the obese patients ($p < 0.01$) and even in the lean patients ($p < 0.01$) on VPA when compared to their respective control subjects. (Table 9) (Study IV)

Table 9. Serum free fatty acid (FFA), uric acid concentrations, and HOMA index in VPA-treated patients (IV)

Subjects	FFA mmol/l	uric acid μ mol/l	HOMA
Patients			
all (51)	0.53 \pm 0.24	309.2 \pm 67.9**	2.37 \pm 1.4\$\$\$
obese (27)	0.60 \pm .20	329.4 \pm 64.2##	2.61 \pm 1.4 **
lean (24)	0.46 \pm 0.25	286.46 \pm 65.8++	2.11 \pm 1.4####
Controls			
all (45)	0.51 \pm 0.17	267.6 \pm 67.3	1.45 \pm 1.18
obese (24)	0.50 \pm 0.18	298 \pm 73.12	1.55 \pm 1.0
lean (21)	0.52 \pm 0.17	230.9 \pm 99.6	1.40 \pm 1.4

** p< 0.01, *** p<0.001 when compared to all control subjects; ## p< 0.01, ### p< 0.001 when compared to the obese control subjects; ++p<0.01, +++ p< 0.001 when compared to lean control subjects.

Serum leptin levels were higher in the obese patients (8.8 \pm 6.6 μ g/l) than in the lean patients (4.2 \pm 3.1 μ g/l) (p<0.01), and they were also higher in the obese control subjects than in the lean ones (6.9 \pm 3.6 μ g/l, 3.1 \pm 0.9 μ g/l, respectively, p<0.01). Serum leptin levels were higher in the female patients (20.0 \pm 13.7 μ g/l) than in the male patients (6.1 \pm 4.2 μ g/l) (p<0.001). There was no statistically significant difference in serum leptin levels between the patients treated with VPA and the control subjects. (Study I)

6 Discussion

6.1 General aspects

The purpose of this study was to evaluate the metabolic and endocrine effects related to the use of AEDs, especially VPA. Study I focused on serum insulin and leptin levels in 81 VPA-treated patients. In study II special interest was paid to male patients (n=102) on AEDs. At the time the study was started, there were no reports available on serum insulin levels in male VPA-treated patients. For studies III and IV, hospital records of 124 VPA-treated monotherapy patients from a cohort including 1386 patients with epilepsy (Ranua *et al.* 2004) were reviewed, and 74 eligible patients were identified. Fifty-one (69%) eventually agreed to participate in the study. This was a unique setting, but unfortunately the number of participating patients was small. A cross sectional study was done to evaluate the metabolic effects of VPA. Data on weight was collected retrospectively from the hospital records. As there is no data on the possible changes in body weight concerning the control subjects, and this must be considered when the results on this particular part are analysed.

The control groups consisted of very heterogeneous subjects and they were from different social-economical groups and of different age. However, this should not bias the conclusions, as the difference should lead to unfavourable changes in the control subjects' metabolic parameters. The possibility that VPA-related HI is associated with other metabolic changes was evaluated. Special attention was paid to possible association of these metabolic events with weight gain, other adverse effects related to VPA, and symptoms or signs of MBS. Furthermore, we tried to evaluate possible changes in insulin secretion and metabolism during VPA treatment and their role in VPA-related weight gain. The gold standard in evaluating insulin sensitivity is the insulin clamp technique, which was not possible in this group of patients due to their seizure susceptibility. The HOMA index may not be as good a marker of insulin sensitivity under conditions in which hepatic extraction of insulin is decreased. In addition, we wished to evaluate the role of leptin in VPA-related weight gain in men and women with epilepsy.

Previous reports on the metabolic effects of VPA are limited, and no data has been available on the occurrence of MBS in VPA-treated patients. Moreover, when the study

was started, no reports on serum insulin levels in lean patients or male VPA-treated patients had been published.

In this study VPA treated patients have higher serum insulin levels in relation to body mass index than the control subjects indicating that the high serum insulin levels are not a consequence of weight gain. Especially VPA –therapy started at a young age may often result in elevated serum insulin levels and associated other untoward metabolic changes. The VPA treated patients were not more obese than the control subjects. Furthermore, according to the present data high serum insulin levels are a consequence of compromised metabolism of insulin in the liver. However, it seems that the valproate-treated patients cluster risk factors for cardiovascular diseases, although the occurrence of MBS is not more common in VPA-treated patients than in the control subjects. Leptin does not play an independent role. The higher HOMA index in VPA –treated patients indicates that VPA treated patients could be more resistant to insulin. This result remains uncertain as the VPA treated patients have lower fasting plasma glucose levels than the control subjects.

Further studies are warranted to evaluate the relationship between body weight and high serum insulin levels in VPA treated patients.

6.2 Body weight

The BMI did not differ between the VPA-treated patients and the control subjects, although the occurrence of obesity among VPA-treated patients was similar to that in previous studies. Dinesen reported that 36 (57%) out of 63 patients gained more than 4 kg in weight during VPA treatment that lasted 9–74 months (mean 34). (Dinesen *et al.* 1984). In another study 44% of children gained weight during VPA therapy. (Egger & Brett 1981).

The present findings on 42 VPA treated patients based on retrospectively collected data on body weight before treatment are supported by Biton *et al.*, who showed in a prospective study an increase in BMI of 2.06 ± 1.5 over 8 months of VPA treatment in patients with initial BMI 26 ± 7 , i.e. a relative increase of about 8%. (Biton *et al.* 2001) That increase is of the same magnitude as that observed in our study.

Increases in body weight during VPA treatment have been associated with an increase in fatty tissue due to elevated appetite (Dinesen *et al.* 1984; Egger & Brett 1981), reduction of facultative thermogenesis and increased availability of long chain fatty acids due to competitive valproic acid binding to serum albumin (Vorum *et al.* 1993). The weight gain can sometimes be controlled by reducing caloric intake (Dinesen *et al.* 1984).

Obesity in VPA-treated female patients was associated with a high prevalence of PCO and hyperandrogenism, and other features of IR syndrome, e.g. visceral obesity and unfavourable changes in the serum lipid concentrations (Isojärvi *et al.* 1993c; Isojärvi *et al.* 1996) and obese women with VPA-related metabolic and endocrine disorders appear to cluster risk factors for cardiovascular disease. In a prospective study the increased serum insulin concentrations as well as unfavourable serum lipid profile returned to normal within 2 months after VPA was replaced with LTG in women with epilepsy, although weight reduction occurred more gradually and progressively during a 12 month

follow up (Isojärvi *et al.* 1998). VPA-treated patients have higher serum insulin levels both in lean and obese patients (Studies II&III). As presented in Chapter 6.4, the present data suggest that the high circulating insulin concentrations are due to decreased hepatic insulin degradation resulting in increased serum insulin levels eventually leading to weight gain (Matthews *et al.* 1985). One has, however, to keep in mind that the pathogenetic mechanisms behind VPA-related weight gain could be multiple as mentioned above.

Weight gain pose subjects to well documented health risks (Pi-Sunyer 1993), such as coronary artery disease (Willett *et al.* 1995), diabetes mellitus (Colditz *et al.* 1995), and hypertension (Huang *et al.* 1998). In addition, it has been postulated that weight gain is associated with endocrine disorders such as menstrual disturbances, PCO and hyperandrogenism in female subjects, seen also in VPA-treated female subjects (Isojärvi *et al.* 1996; Isojärvi *et al.* 2001a).

6.3 Other self-reported side effects

Another commonly reported side effect of VPA monotherapy was tremor. This is in accordance with previous studies (Karas *et al.* 1982). Interestingly, insulin levels were higher in patients with tremor, suggesting that tremor may be associated with increased sympathetic activity caused by high serum insulin levels (Bray 2004). The sympathetic dysfunction can predispose to obesity and type 2 diabetes (Nonogaki 2000). There are no previous reports on tremor and serum insulin concentrations in VPA-treated patients. The side effects reported here are in accordance with previous studies (Davis *et al.* 1994).

6.4 Fasting serum insulin and insulin-related parameters in valproate treated patients

Results of these studies show that VPA-treated patients have higher serum insulin levels in their peripheral circulation than the control subjects regardless of sex or BMI (Studies I–IV). Furthermore, male patients on VPA have higher circulating insulin concentrations than the patients treated with CBZ or OXC (Study II). Fasting serum PI and CP levels were similar in the patients on VPA treatment and in the control subjects. In addition, the PI to insulin (PI/I) and CP to insulin (CP/I) ratios were lower in the VPA-treated patients (Study III). Furthermore, the VPA-treated patients have lower fasting plasma glucose concentrations than the control subjects regardless of BMI or sex (III).

High serum insulin levels have been observed previously in female VPA-treated patients (Isojärvi *et al.* 1996; Isojärvi *et al.* 1998; Isojärvi *et al.* 2001), HI during VPA treatment has previously been suggested to be due to the development of IR (Isojärvi *et al.* 1998). Luef *et al.* found that VPA-treated patients had higher postprandial levels of serum insulin, CP and PI compared to patients treated with CBZ and/or LTG (Luef *et al.* 2002b; Luef *et al.* 2002c) possibly related to increased secretion of these hormones from pancreatic beta cells (Luef *et al.* 2003). Breum *et al.* did not find any changes in fasting

concentrations of pancreatic hormones, including insulin and CP before or during VPA treatment in a one-month prospective study with eight patients (Breum *et al.* 1992). On the contrary, Røste *et al.* found higher CP levels along with high serum insulin levels in men treated with VPA when compared to control subjects. (Røste *et al.* 2005). Thus, earlier data on serum PI and CP concentrations in VPA-treated patients is limited.

Insulin and CP are released in equimolar amounts from the pancreatic islets, whereas only a small proportion of PI and related intermediate cleavage forms are secreted into the portal vein (Horwitz *et al.* 1975). About half of the secreted insulin is removed from the circulation on its initial passage through the liver, whereas the hepatic extraction of CP is low. The liver is the primary organ for the degradation of insulin, while the kidney plays a major role in the removal of CP (Steiner *et al.* 1976).

The low PI/I and CP/I ratios in the VPA-treated patients appear to be due to the disproportionately increased serum insulin concentrations, because serum CP and PI concentrations were not increased in the VPA-treated patients. Circulating insulin concentrations are considered to reflect the posthepatic amount of insulin, whereas the CP and PI levels in the peripheral circulation reflect the amount of insulin initially secreted from the pancreatic beta cells (Horwitz *et al.* 1975). In general an increased PI/I ratio is associated with type 2 diabetes or predictive of progression to type 2 diabetes within 2–5 years in individuals at risk (Mykkänen *et al.* 1997). The pathogenetic mechanisms leading to high serum insulin levels during VPA treatment remain unknown or controversial. The present findings of low PI/I and CP/I ratios in VPA-treated patients indicate that insulin secretion from the beta cells is not increased during VPA treatment. Instead the hepatic extraction of insulin seems to be reduced, which is often the case in obese subjects (Kissebah 1991) and in subjects with glucose intolerance (Bonora *et al.* 1983). However decreased PI/I and CP/I ratios were observed both in non-obese and obese patients treated with VPA.

VPA, which is a known inhibitor of liver metabolism (Davis *et al.* 1994), could inhibit the hepatic degradation of insulin leading to high fasting serum insulin concentrations in the VPA-treated patients, while serum CP and PI levels would remain normal. The increased insulin concentrations in the peripheral circulation observed in both obese and lean VPA-treated patients can secondarily induce weight gain by increasing the uptake of glucose into adipose cells and by activating lipogenic and glycolytic enzymes thus stimulating lipogenesis (Nakae & Accilli 1999; Kersten 2001) leading to weight gain (Khan & Flier 2000).

6.4.1 Insulin to BMI ratio

The insulin to BMI ratio was higher in VPA-treated patients than in the control subjects, suggesting that the high serum insulin levels related to VPA treatment are independent of body weight. Insulin to BMI ratios have not been reported before in this context. The high insulin/BMI ratio was associated with a younger age at the start of VPA treatment. (Study IV).

6.5 Fasting serum lipid concentrations in patients treated with valproate, carbamazepine or oxcarbazepine

In male patients (II) on VPA there were more individuals with high serum insulin and TG concentrations than among the CBZ or OXC treated patients or control men. However the mean HDL-cholesterol/total cholesterol ratio and the serum TG levels in obese men treated with VPA did not differ from those of the control subjects (Study III).

In another group of VPA-treated patients (Study IV), lower serum HDL cholesterol and higher triglyceride concentrations were observed in all VPA-treated patients than in the total series of control subjects. The HDL/total cholesterol ratio was also reduced in the obese VPA-treated patients when compared to the obese control subjects. These findings are in accordance with previous findings in female patients on VPA, namely, the VPA-treated female patients cluster risk factors for cardiovascular disease, e.g. HI (Isojärvi *et al.* 1998), weight gain of androgen type and hyperandrogenism (Isojärvi *et al.* 1993a), with associated unfavourable alterations in serum lipid concentrations (Isojärvi *et al.* 2001).

The hyperinsulinaemic patients treated with VPA had higher serum TG concentrations that comparable to those of normal hyperinsulinaemic subjects. HI is known to be associated with increased IR even in subjects with normal glucose tolerance (Moro *et al.* 2003).

6.6 Thyroid function in valproate-treated patients

High TSH concentrations in VPA-treated subjects are in accordance with some previous studies, although the reports on the effects of VPA on serum concentrations of TSH and free T4 are controversial (Isojärvi *et al.* 1992; Fichsel & Knopfle 1978; Larkin *et al.* 1989). Higher TSH in VPA-treated patients compared to similarly obese control subjects suggest that VPA interferes with secretion, metabolism, or the feedback regulation of TSH secretion. It has been suggested that VPA may influence secretion of TSH via the GABAergic properties of the drug. On the other hand, weight gain is associated with elevated TSH concentrations in the general population (Pinkney & Kepelman 2004). (Study IV)

6.7 Blood pressure, HOMA index and occurrence of the metabolic syndrome in valproate-treated patients

HI is known to be associated with high BP mediated by altered sympathetic activity (Bray 2004; Kawasaki *et al.* 2000). However, the VPA-treated patients did not have elevated BP (Study IV) in the present study.

The VPA-treated patients (Study III) had a higher HOMA index than the control subjects. This is in accordance with one previous study by Luef who also reported a

higher HOMA index in VPA-treated patients than in patients treated with CBZ (Luef *et al.* 2004). An increased HOMA value does reflect a reduced sensitivity to insulin (Matthews *et al.* 1985). It is, however, questionable whether the HOMA index is a useful marker of IR in abnormal situations associated with e.g. reduced hepatic insulin extraction combined with unchanged insulin secretion from the pancreatic islets. The increased HOMA index in VPA treated patients most likely reflects decreased hepatic insulin degradation, especially since both obese and lean patients did have a higher HOMA index than their respective control group. The low fasting plasma glucose levels mentioned earlier also support the concept that HI in VPA-treated patients is not due to IR.

In this study (Study IV) the proportion of subjects meeting the criteria of the MBS was similar among the 51 VPA-treated patients and the 45 control subjects. There are no previous reports on this issue, although HI during VPA treatment has previously been suggested to be due to the development of IR (Isojärvi *et al.* 1998). The present observations do not support that concept. In general MBS is associated HI, but the high insulin concentrations in VPA-treated patients may be due to decreased hepatic metabolism of insulin, not weight gain. It is controversial whether HI for any cause (Reaven 1995), can lead to MBS. On the other hand, it has been suggested that the predictive value of increased serum insulin concentrations as an independent risk factor for CHD may decrease in the long run (Pyörälä *et al.* 1998).

6.8 Other metabolic parameters in valproate-treated patients

The present observations on fasting serum FFA concentrations in VPA treated patients were controversial. The fasting FFA levels were similar in the VPA-treated patients with epilepsy and in the control subjects. However, the obese patients tended to have higher serum FFA levels than the obese control subjects; but, in contrast, the lean patients tended to have lower serum FFA levels than the lean controls subjects. (Study IV)

VPA which is a fatty acid itself, has been suggested to compete with FFA for binding to albumin which may result in increased availability of FFA and induce excess secretion of insulin, which stimulates appetite and may result in weight gain (Vorum *et al.* 1993). On the other hand, FFAs inhibit glucose oxidation and have stimulatory effects on gluconeogenesis (Saloranta & Groop 1996; Groop *et al.* 1992). These changes should result in higher plasma glucose concentrations (Golay *et al.* 1985), for example in association with type 2 diabetes (Fraze *et al.* 1985). In general high serum insulin concentrations inhibit the release of FFA from adipose tissue followed by a fall in circulating plasma FFA levels (Mayes 1988) The findings of this study could be due to increased availability of FFAs in the obese patients, whereas in the lean patients the high insulin concentrations decrease the FFA concentration.

VPA-treated patients had significantly higher serum uric acid levels than the control subjects, irrespective of body weight.(Study IV). Higher uric acid levels have been reported earlier in patients taking VPA for epilepsy than in patients on enzyme-inducing antiepileptic drugs (Krause *et al.* 1987; Ring *et al.* 1991). In general hyperuricemia occurs frequently in subjects with weight gain, IR or dyslipidemia, because insulin

stimulates sodium and uric acid reabsorption in the renal proximal tubule (1995). The role of uric acid as a mediator of hypertension and renal disease is not well defined (Johnson *et al.* 2003). Even though uric acid can function as an antioxidant (Krause *et al.* 1987), it has been shown to impair endothelial function (Waring *et al.* 2000) and predict future development of hypertension (Selby *et al.* 1990).

High uric acid concentrations could be merely explained by the high serum insulin levels, as insulin is known to increase uric acid reabsorption in the proximal tubules of the kidney (Galvan 1995), but an effect of VPA on its metabolism is also possible. In any case the increased uric acid concentrations can be a risk factor for e.g. hypertension (Waring *et al.* 2000; Johnson *et al.* 2003).

Obese patients taking VPA, as well as obese control subjects, had higher serum concentrations of leptin than lean subjects. In addition, the female patients as well as the female control subjects had higher serum leptin levels than the male subjects. These findings are in accordance with previous observations based on the general population (Considine *et al.* 1996; Haffner *et al.* 1997). Serum leptin levels did not differ between the obese patients taking VPA and the obese control subjects, despite the higher serum insulin levels in VPA-treated patients. This is in accordance with the reports indicating that insulin may have a trophic effect on the adipocytes, eventually increasing the serum leptin levels, rather than having a direct effect on OB gene expression (Segal *et al.* 1996; Kolazinsky *et al.* 1996). Leptin activation seems to be similar in obese VPA-treated subjects to that seen in otherwise obese subjects. This has been observed earlier in female patients taking VPA in a longitudinal study (Verrotti *et al.* 1999). However, Lagace *et al.* reported that VPA decreases leptin secretion and mRNA levels in adipocytes *in vitro*, suggesting that VPA therapy may be associated with altered leptin homeostasis contributing to weight gain *in vivo* (Lagace *et al.* 2004). These findings suggest that leptin does not have an independent role in VPA-related weight gain.

7 Conclusions

1. The MBS was not more frequent among VPA-treated patients than among control subjects. Despite this observation and the similarity between the body weight of the patients and control subjects, the VPA-treated patients appear to cluster some of the risk factors for cardiovascular diseases. In particular, VPA therapy started at a young age may often result in increased circulating insulin levels and other untoward metabolic changes associated with HI. Furthermore, the serum uric acid concentrations were higher in VPA-treated patients than in control subjects, possibly reflecting HI. The observations on serum FFA concentrations were discordant, as obese VPA-treated patients tended to have increased FFA concentrations, whereas lean VPA-treated patients tended to have decreased FFA levels in the peripheral circulation. The results of the present study do not support an independent role for leptin in the pathogenesis of VPA-related weight gain.
2. VPA-treated patients have higher serum insulin levels in relation to their BMI than the control subjects implying that the HI seen in patients on VPA is not a consequence of weight gain. The present findings suggest that VPA does not stimulate insulin secretion, but may interfere with the hepatic insulin metabolism resulting in higher insulin concentrations in the peripheral circulation and, in parallel, reduced plasma glucose concentrations. The metabolic changes seen in VPA-treated patients appear irrespective of concomitant weight gain, suggesting that host-related factors might determine whether peripheral HI will contribute to the development of weight gain or not in these patients. More studies are needed to clarify the mechanism of possible differences in the metabolism of insulin and also in the peripheral action of insulin in VPA treated patients.
3. HI in VPA-treated patients with epilepsy is, however, associated with unfavourable changes in serum lipid concentrations. Patients treated with VPA had higher serum TG and lower HDL. CBZ and OXC do not seem to have any significant effects on serum lipid levels in men with epilepsy.

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