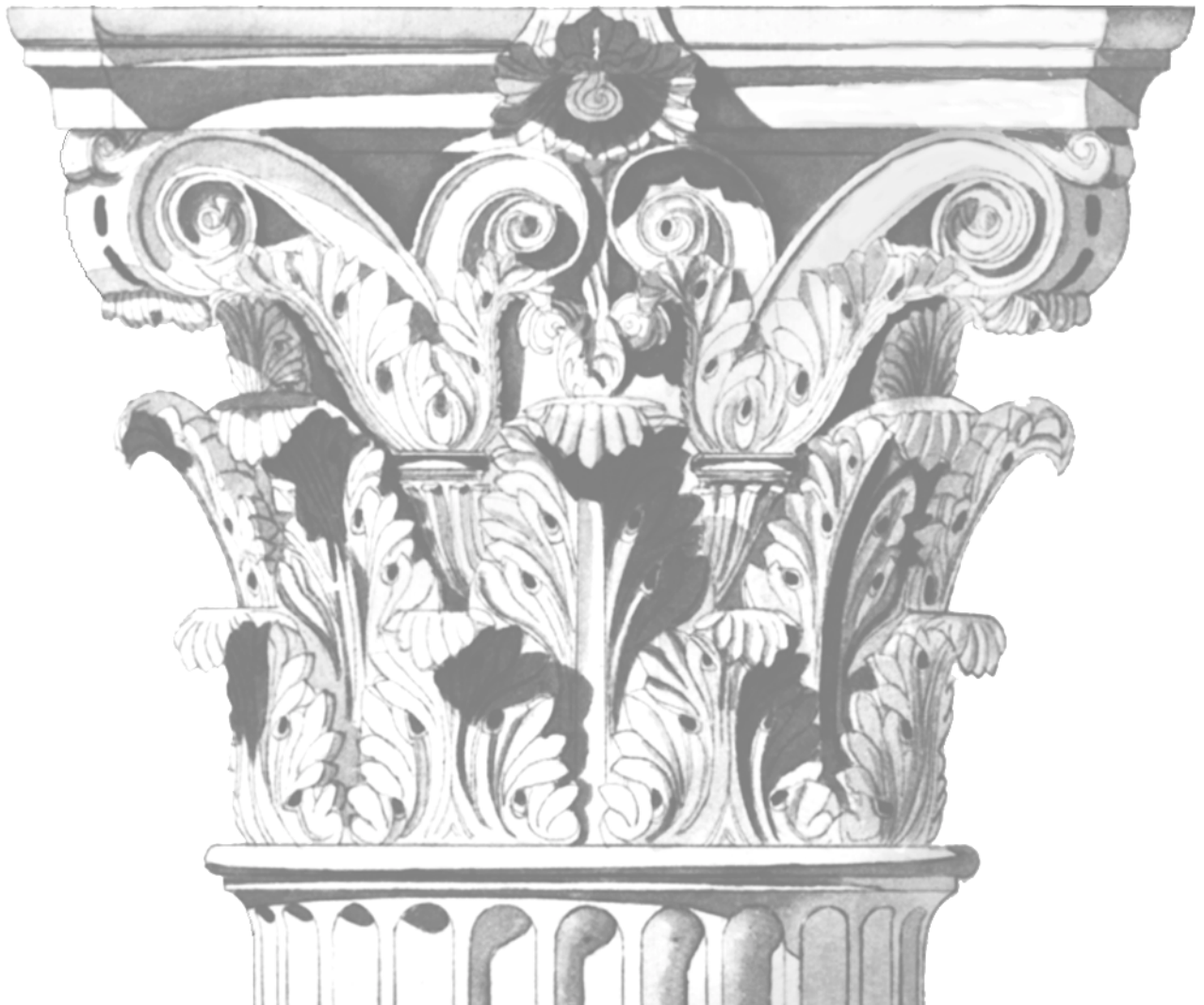


**REPRODUCTIVE ENDOCRINE  
EFFECTS OF ANTIEPILEPTIC  
DRUGS - WITH SPECIAL  
REFERENCE TO VALPROATE**

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OULU 2000



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- WITH SPECIAL REFERENCE TO  
VALPROATE**

Academic Dissertation to be presented with the assent of the Faculty of Medicine, University of Oulu, for public discussion in Auditorium 8 of the University Hospital of Oulu, on February 4th, 2000, at 12 noon.

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*To my dear family*

**Johanna Rättyä, Reproductive endocrine effects of antiepileptic drugs –with special reference to valproate**

Departments of Neurology and Pediatrics, FIN-90014, University of Oulu, Finland  
2000

Oulu, Finland

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***Abstract***

Previous observations have indicated that reproductive endocrine disorders are common among patients with epilepsy. Valproate (VPA) treatment is associated with hyperandrogenism, polycystic ovaries, and obesity in women. Carbamazepine (CBZ) may also induce endocrine disorders, while the hormonal effects of oxcarbazepine (OXC) are poorly known. The aim of this study was to elucidate the effects of antiepileptic drugs on reproductive hormones, linear growth and pubertal maturation in patients with epilepsy.

Altogether 223 patients taking VPA, CBZ, or OXC monotherapy for epilepsy and 103 healthy age- and sex-matched volunteers participated in the study. Seventy-eight girls and 90 men with epilepsy participated in the cross-sectional parts of the study. Thirty-nine adult patients with newly diagnosed epilepsy participated in a 3-month longitudinal study and VPA was replaced with lamotrigine (LTG) in 16 women with VPA-related endocrine disorders in a 1-year longitudinal study. The girls were between 8-18 years, the women 17-41 years and the men 17-51 years of age.

None of the antiepileptic drugs studied significantly influenced linear growth or pubertal development in girls with epilepsy, but hyperandrogenemia, increased number of ovarian follicles, and weight gain were observed in prepubertal, pubertal and postpubertal girls taking VPA for epilepsy. Increased serum testosterone levels were observed in half of the women after the first 3 months of VPA medication, and high serum concentrations of androgens were common (prevalence 57 %,  $p < 0.001$ ) in men taking long-term VPA treatment. The women with VPA-related hyperandrogenism and polycystic ovaries were also found to present other features of insulin resistance (i.e. hyperinsulinemia, centripetal obesity, and an unfavorable serum lipid profile). Reproductive endocrine disorders associated with VPA treatment in women began to normalize after VPA was replaced by LTG. CBZ reduced the bioactivity of androgens, whereas OXC did not have similar effects. Serum concentrations of sex hormone-binding globulin (SHBG) were increased and dehydroepiandrosterone sulfate decreased already during the first months of CBZ treatment. Serum hormone levels were normal in patients with low OXC doses ( $< 900$  mg/d), but serum concentrations of testosterone, gonadotropins and SHBG were high in men with a daily OXC dose  $\geq 900$  mg.

The adverse reproductive endocrine effects of antiepileptic drugs should be considered at the beginning of and during antiepileptic medication.

*Keywords:* growth, hyperinsulinemia, sex hormones, weight gain



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Oulu, January 1, 2000

Johanna Rättyä

## Abbreviations used in the text

ACTH	adrenocorticotropin
AED	antiepileptic drug
B	breast
BMI	body mass index
CBZ	carbamazepine
CT	computed tomography
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
E <sub>2</sub>	estradiol
EEG	electroencephalography
FAI	free androgen index
FSH	follicle-stimulating hormone
fT	free testosterone
GABA	γ-aminobutyric acid
GH	growth hormone
IGF-I	insulin-like growth factor I
IGFBP-1	insulin-like growth factor binding protein 1
IGFBP-3	insulin-like growth factor binding protein 3
LH	luteinizing hormone
LTG	lamotrigine
MRI	magnetic resonance imaging
OXC	oxcarbazepine
PCOS	polycystic ovary syndrome
PH	pubic hair
SD	standard deviation
SHBG	sex hormone-binding globulin
T	testosterone
VPA	valproate



## List of original articles

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

- I Rättyä J, Vainionpää L, Knip M, Lanning P & Isojärvi JIT (1999) The effects of valproate, carbamazepine, and oxcarbazepine on growth and sexual maturation in girls with epilepsy. *Pediatrics* 103:588-593.
- II Vainionpää LK, Rättyä J, Knip M, Tapanainen JS, Pakarinen AJ, Tekay A, Myllylä VV & Isojärvi JIT (1999) Valproate-induced hyperandrogenism during pubertal maturation in girls with epilepsy. *Ann Neurol* 45:444-450.
- III Rättyä J, Pakarinen A, Knip M, Repo M, Myllylä VV & Isojärvi JIT. Early reproductive endocrine effects of valproate and carbamazepine -A 3 month prospective study. Submitted.
- IV Rättyä J, Turkka J, Pakarinen A, Knip M, Kotila MA, Lukkarinen O, Myllylä VV & Isojärvi JIT. Reproductive endocrine effects of valproate, carbamazepine, and oxcarbazepine in men with epilepsy. Submitted.
- V Isojärvi JIT, Rättyä J, Myllylä VV, Knip M, Koivunen R, Pakarinen A, Tekay A & Tapanainen JS (1998) Valproate, lamotrigine, and insulin-mediated risks in women with epilepsy. *Ann Neurol* 43: 446-451.



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## 1. Introduction

The term *epilepsy* does not refer to a specific disease, or even a single syndrome, but rather to a broad category of symptom complexes arising from a number of disordered brain functions that themselves may be secondary to a variety of pathologic processes. (Engel & Pedley 1997). The prevalence of epilepsy is 0.7-0.8 % around the world (Hauser 1997). According to the Finnish Social Insurance Institution more than 45 000 patients received antiepileptic medication in Finland in 1996, but only 31 000 of them were outpatients with epilepsy and 5000-6000 of them were children. (Keränen *et al.* 1997). Epilepsy begin most often in childhood or adolescence (Sillanpää 1994, Hauser 1997). A few years of medication may be sufficient to treat some benign forms of epilepsy, but some epilepsies require life-long medication (Morton & Pellock 1996). Today, the prognosis of epilepsy is good: more than 70 % of patients with newly diagnosed epilepsy will become seizure free or enter substantial remission with antiepileptic drugs (AEDs) (Cockerell *et al.* 1995).

Epilepsy and hormones are connected in a complicated manner. It is known that epilepsy itself may affect the hormonal balance and, furthermore, hormones may provoke or inhibit seizures (Jabbari & Huott 1980, Newmark & Penry 1980, Cummings *et al.* 1995, Herzog 1996). It has been also shown that AEDs have effects on endocrine functions (MacPhee 1988, Isojärvi 1989, Isojärvi *et al.* 1990, 1992, 1993, Duncan *et al.* 1999). Valproate (VPA) has a wide-spectrum antiepileptic efficacy and it is used both in localization related and generalized epilepsies in adults, as well as in children and adolescents (Scheuer & Pedley 1990, Davis *et al.* 1994, Morton & Pellock 1996). A very high occurrence of reproductive endocrine disorders has recently been reported among women taking VPA for epilepsy. These endocrine disorders are characterized by polycystic ovaries, hyperandrogenism, and menstrual disorders (Isojärvi *et al.* 1993, 1996). Carbamazepine (CBZ) is one of the most widely used first-line AEDs. The use of CBZ is also associated with changes in endocrine and metabolic function, which are probably due to hepatic P450 enzyme system induction (Isojärvi *et al.* 1995a,b). The endocrine effects of the recently introduced AEDs have not been studied.

Further knowledge about hormonal adverse effects of AEDs is essential when tailoring antiepileptic medication individually to each patient in order to minimize the risk for AED-related endocrine disorders.

## **2. Review of literature**

### **2.1. Epilepsy**

#### ***2.1.1. General aspects***

Epilepsy is a complicated disorder involving disturbances of brain function at multiple levels. It is characterized by occasional seizures with various clinical manifestations: decrease of consciousness, involuntary movements, abnormal sensory phenomena, increased autonomic activity, and/or transient disturbances of behavior. Epileptic seizures result from excessive, synchronous, abnormal firing patterns of neurons that are located predominantly in the cerebral cortex. Epilepsy may be determined as a predisposition to periodic and excessive discharges of electrical activity of cerebral neurons. (Browne & Feldman 1983, Waltimo 1983, Engel & Pedley 1997)

#### ***2.1.2. Epidemiology***

The incidence of epilepsy is 24-53 /100 000 persons/year in industrialized countries, and it may be higher in developing countries. The age-specific incidence is highest during the first year of life, and falls during childhood, adolescence, and early adulthood, but increases again in the elderly beginning from 50-60 years of age. According to the Rochester study, the age-specific cumulative incidence of epilepsy is 0.7 % in 10 year-old children, 1.1 % in 20 year-old adults, 1.7 % in 40 year-old and 3.2 % in 80 year-old persons, and only 50 % of epilepsies begin in childhood or adolescence (Hauser *et al.* 1993). However, according to most of the other epidemiological studies 75-90 % of epilepsies begin before 20 years of age (Sillanpää 1994). The prevalence of epilepsy is 0.7-0.8 % worldwide and the lifetime cumulative incidence of epilepsy is 1-3 %. In most population-based studies the incidence is higher in males than in females. (Hauser 1997) According to the Finnish Social Insurance Institution more than 45 000 patients received antiepileptic medication in Finland in 1996, but only 31 000 of them were patients of outpatient clinics and 5000-6000 of them were children (Keränen *et al.* 1997).

### **2.1.3. Etiology**

All factors causing pathologic structural (neoplastic, malformative, inflammatory and destructive lesions) or functional changes in the brain are risk factors for epilepsy (Vinters *et al.* 1993). The most important etiologies of epilepsy in infancy and childhood are metabolic defects, congenital malformations, infections, genetic diseases, and perinatal injuries, while later in life the most important causes are trauma, brain tumors and vascular diseases of the brain. In addition, there are always patients with unresolved etiology of epilepsy (Lühdorf *et al.* 1986, Nelson & Ellenberg 1987). Also a positive family history may be considered an important risk factor for epilepsy. However, inheritance of epilepsy is complicated and still poorly known, and only a small proportion of cases may be attributable to single-gene disorders. (Lehesjoki & Pitkänen 1999)

### **2.1.4. Classification**

Epilepsy is called *symptomatic* if the etiology is known. In *cryptogenic epilepsy* there is presumed to be a pathologic process in the brain causing epileptic activity, although it is not detected by existing examination methods. *Idiopathic epilepsies*, which are much more common in children than in adults, comprise independent, intrinsic epileptic syndromes. The summary of International Classification of Epilepsies and Epileptic Syndromes is presented in Table 1 (Commission on Classification and Terminology of the International League Against Epilepsy 1989).

### **2.1.5. Treatment**

The goals of epilepsy therapy are to improve the patient's quality of life, and to control seizures without the patient having to tolerate adverse effects of the drug. The diagnosis of epilepsy should be confirmed and the type of seizures determined before starting antiepileptic medication. Therapy should be tailored to the individual patient according to etiology and type of syndrome or seizures; some drugs may even aggravate certain epileptic syndromes. The drug recommendations for various types of epilepsy are presented in Table 2 according to Keränen *et al.* 1997. In general, early intervention seems to prevent the epileptic process from becoming chronic. (Scheuer & Pedley 1990, Pellock & Willmore 1991, Dam 1997)

*Table 1. International classification of epilepsies and epileptic syndromes. (Commission on Classification and Terminology of the ILAE, 1989)*

Epilepsy / Epileptic syndromes	
1.	Localization-related (focal, local, partial)
1.1.	Idiopathic (with age-related onset) <ul style="list-style-type: none"> <li>- Benign childhood epilepsy with centrotemporal spikes</li> <li>- Childhood epilepsy with occipital paroxysm</li> <li>- Primary reading epilepsy</li> </ul>
1.2.	Symptomatic <ul style="list-style-type: none"> <li>- Chronic progressive epilepsia partialis continua of childhood</li> </ul>
1.3.	Cryptogenic The symptomatic and cryptogenic categories comprise syndromes of great individual variability that are based on: <ul style="list-style-type: none"> <li>- Seizure types (according to the International Classification of Epileptic Seizures)</li> <li>- Anatomic localization: <ul style="list-style-type: none"> <li>Temporal, frontal, parietal, and occipital lobe epilepsies</li> </ul> </li> <li>- Bi- and multilobar epilepsies</li> <li>- Etiology (in symptomatic epilepsies)</li> <li>- Specific modes of precipitation</li> </ul>
2.	Generalized
2.1.	Idiopathic (with age-related onset, in order of age) <ul style="list-style-type: none"> <li>- Benign neonatal familial convulsions</li> <li>- Benign neonatal convulsions</li> <li>- Benign myoclonic epilepsy in infancy</li> <li>- Childhood absence epilepsy (pyknolepsy)</li> <li>- Juvenile absence epilepsy</li> <li>- Juvenile myoclonic epilepsy (impulsive petit mal)</li> <li>- Epilepsy with grand mal (GTC) seizures on awaking</li> <li>- Other idiopathic generalized epilepsies not defined above</li> <li>- Epilepsies with seizures precipitated by specific modes of activation</li> </ul>
2.2.	Cryptogenic or symptomatic (in order of age) <ul style="list-style-type: none"> <li>- West syndrome (infantile spasms, Blitz-Nick-Salaam-Krämpfe)</li> <li>- Lennox-Gaustaut syndrome</li> <li>- Epilepsy with myoclonic-astatic seizures</li> <li>- Epilepsy with myoclonic absences</li> </ul>
2.3.	Symptomatic
2.3.1.	Nonspecific etiology <ul style="list-style-type: none"> <li>- Early myoclonic encephalopathy</li> <li>- Early infantile epileptic encephalopathy with suppression-burst</li> <li>- Other symptomatic generalized epilepsies not defined above</li> </ul>
2.3.2.	Specific syndromes (see the original reference)
3.	Epilepsies and syndromes undetermined whether focal or generalized
3.1.	With both generalized and focal seizures <ul style="list-style-type: none"> <li>- Neonatal seizures</li> <li>- Severe myoclonic epilepsy in infancy</li> <li>- Epilepsy with continuous spike-waves during sleep</li> <li>- Acquired epileptic aphasia (Landau-Kleffner syndrome)</li> <li>- Other undetermined epilepsies not defined above</li> </ul>
3.2.	Without unequivocal generalized or focal features (e.g., many cases of sleep-grand mal)
4.	Special syndromes
4.1.	Situation-related seizures (Gelegenheitsanfälle) <ul style="list-style-type: none"> <li>- Febrile convulsions</li> <li>- Isolated seizures or isolated status epilepticus</li> <li>- Seizures due to acute metabolic or toxic factors such as alcohol, drugs, eclampsia</li> </ul>

Monotherapy (treatment with only one AED at the time) should be the therapeutic aim. However, substitution of another drug may be mandated by failure of the first drug. Occasionally a concomitant use of more than one drug may be necessary, but polytherapy is associated with several possible negative aspects: adverse effects and chronic toxicity, impaired cognitive function, drug interactions, increased seizure frequency, poor patient compliance, and higher economic costs. (Keränen *et al.* 1997) About 25 % of patients fail to respond to prescribed 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. Additional diagnostic testing and investigations are indicated, and other forms of therapy should be considered, if seizures occur frequently after 1-2 years of adequate medical treatment. (Keränen *et al.* 1997) Other therapies are e.g. epilepsy surgery, vagus nerve stimulation, or conditioning and behavior modification techniques. (Dam 1997)

Drug therapy can generally be tapered off after the patient has been seizure free for 2-5 years. A short duration of treatment (two seizure-free years) may be sufficient in benign epilepsies, such as localization related idiopathic childhood epilepsies or childhood absence epilepsy. However, there are epileptic syndromes, e.g. juvenile myoclonic epilepsy, which require a life-long medication. Seizure control may also be difficult to achieve in localization related symptomatic or cryptogenic epilepsies. Withdrawal of medication should be done slowly over several months in order to prevent a relapse. (Keränen *et al.* 1994, Morton & Pellock 1996)

*Table 2. The drug recommendations for various types of epilepsy (Keränen et al. 1997).*

Type of epilepsy	Medication
Localization-related epilepsies	oxcarbazepine carbamazepine valproate phenytoin lamotrigine
(adjunctive therapy)	vigabatrin tiagabine gabapentin topiramate
Generalized seizures	
Absence seizures and myoclonus epilepsies	valproate ethosuximide (absence seizures) lamotrigine
Tonic-clonic seizures	valproate carbamazepine oxcarbazepine phenytoin lamotrigine

### 2.1.6. Antiepileptic drugs

In general, there are no significant differences in the effectiveness of the standard AEDs (including phenytoin, carbamazepine (CBZ), and valproate (VPA)), and new AEDs (e.g. oxcarbazepine (OXC), lamotrigine (LTG), topiramate, vigabatrin and gabapentin) when they are adequately used in relation to the type of seizure. The newer drugs appear to be better tolerated, but are much more expensive than the standard AEDs. (Brodie & Dichter 1996, Dichter & Brodie 1996, Marson *et al.* 1996, Perucca 1996) The general properties of the commonly used AEDs are discussed in the next chapters, and their adverse endocrine effects are discussed in greater detail in Chapter 2.5.

#### 2.1.6.1. Valproate

Valproic acid was used as an organic solvent until Meunier and coworkers found that the compound had a potent antiepileptic activity, which has led to a widespread use of its sodium salt in the treatment of seizures (Meunier *et al.* 1963). VPA has wide-spectrum antiepileptic efficacy both in localization related and generalized epilepsies. It is a drug of choice in generalized myoclonic and absence seizures during childhood and adolescence and it is also widely used for treatment of epilepsy in adult patients (Davis *et al.* 1994, Morton & Pellock 1996). The exact mechanism of action of VPA is unknown, but it is likely that more than one mechanism is responsible for VPA's anticonvulsant properties. VPA affects the voltage-dependent sodium channels in neural membrane reducing the burst firing and it also increases the concentration of gamma-amino butyric acid (GABA) in the brain (Loscher 1981). Furthermore, there is also evidence that VPA reduces transmission mediated by excitatory neurotransmitters (Whittle & Turner 1982). Moreover, VPA appears to activate calcium and potassium conductances in studies on hippocampal slices (Franceschetti *et al.* 1986). The enzyme inducing properties of VPA are equivocal. The drug is a well known inhibitor of the oxidative metabolism of other drugs (e.g. phenytoin, ethosuximide), but according to some studies it appear to be a weak enzyme inducer in patients with epilepsy. (Levy & Koch 1982, Perucca *et al.* 1984)

The majority of adverse effects during VPA treatment are mild to moderate in severity, appear early in therapy, and do not require dosage adjustment. The most common adverse effects of VPA are gastrointestinal disturbances, weight gain, and neurological (e.g. somnolence, fatigue, tremor, dizziness). Rare severe adverse effects like hepatotoxicity and haematologic changes during VPA treatment have also been reported. (Dreifuss & Langer 1988, Davis *et al.* 1994). The use of VPA is also associated with reproductive endocrine disorders in women taking VPA for epilepsy (Isojärvi *et al.* 1993, 1996).

#### 2.1.6.2. Carbamazepine and oxcarbazepine

CBZ is a derivative of iminostilbene and is chemically related to tricyclic antidepressants (Galbraith 1972). It is the drug of choice for the treatment of partial seizures with or

without generalization (Loiseau & Duche 1995, Bird *et al.* 1996). CBZ affects the voltage-dependent sodium channels in neural membranes reducing the burst firing (McLean & MacDonald 1986). Diplopia, headache, dizziness and nausea are the most common adverse effects of CBZ and limit the dose in many patients with refractory epilepsy. CBZ can also cause rash, mild leukopenia, and rare severe adverse effects are e.g. blood dyscrasias and toxic hepatitis. (Mattson *et al.* 1992, Brodie & Dichter 1996) CBZ is strongly bound to serum proteins and metabolized almost entirely by the liver (Morselli *et al.* 1975). It induces the cytochrome P450 enzyme system in the liver, which leads to stimulated hepatic functions (Perucca *et al.* 1984).

OXC is the 10-keto analogue of CBZ and has comparable anticonvulsant efficacy in partial seizures with or without generalization to CBZ, but it has a distinct pharmacokinetic profile. OXC and CBZ have different metabolic pathways in the liver: instead of oxidation, OXC is metabolized mainly by reduction to its active metabolite, 10,11-dihydro-10-hydroxy-carbamazepine. (Faigle & Menge 1990) Neither OXC nor its monohydroxy derivative induces the oxidative P450 enzyme system to an extent similar to that of CBZ (Patsalos *et al.* 1990, Larkin *et al.* 1991). Furthermore, OXC may be better tolerated than CBZ, cause fewer idiosyncratic reactions and have fewer interactions with other drugs. However, mild and transient neurologic and gastrointestinal adverse effects e.g. drowsiness, dizziness, ataxia, headache, diarrhea, nausea, and vomiting are reported during OXC medication (Dam *et al.* 1989, Grant & Faulds 1992). In addition, hyponatremia may be a problem during OXC treatment especially in female patients. (Pendelbury *et al.* 1989, Grant & Faulds 1992, Huuskonen *et al.* 1998)

### 2.1.6.3. Other antiepileptic drugs

Phenytoin is a standard AED, which is still one of the most frequently used AEDs in the world. It is indicated in focal seizures with or without generalization and its anticonvulsant action is mediated due to the voltage dependent sodium channels (MacDonald 1989, Brodie & Dichter 1996). Phenytoin can cause a range of dose-related (e.g. nystagmus, ataxia, nausea, vomiting, gingival hypertrophy, depression, drowsiness, cognitive difficulties) and idiosyncratic adverse effects (e.g. rash, blood dyscrasias, hepatotoxic effects) (Brodie & Dichter 1996). Phenytoin also induces the microsomal enzyme system in the liver (Perucca *et al.* 1984) and may cause endocrine disorders (Isojärvi 1989). In addition to phenytoin, CBZ and VPA other "old AEDs" are e.g. phenobarbital, primidone and benzodiazepines.

There are several new antiepileptic compounds with comparable antiepileptic efficacy in partial seizures with or without generalization, e.g. lamotrigine (LTG), topiramate, vigabatrin, tiagabine, and gabapentin. The new AEDs are mostly used as adjunctive therapy thus far. The newer AEDs may be better tolerated than the old ones, but severe adverse effects may also occur during treatment with the new AEDs (Perucca 1996). For example, psychiatric problems and visual field defects during vigabatrin treatment and several neurological symptoms and nephrolithiasis during topiramate medication have been reported. (Dichter & Brodie 1996, Kälviäinen *et al.* 1999). Lamotrigine (LTG) is an antiepileptic compound that appears to possess a broad spectrum of activity: in addition to

localization related epilepsies, lamotrigine also seems to have efficacy against generalized seizures. LTG affects voltage dependent sodium and calcium channels in the neural membranes of the brain reducing epileptic activity. Adverse effects of LTG are e.g. rash, nausea and dizziness. (Kälviäinen *et al.* 1993, Brodie *et al.* 1995, Leach & Brodie 1995)

## **2.2. The reproductive endocrine system**

### ***2.2.1. The hypothalamic-pituitary-unit***

The hypothalamus extends from the preoptic area and fornix anteriorly to the mamillary bodies posteriorly. The human pituitary is situated inferiorly to the hypothalamus within the pituitary fossa, above the sphenoid sinus. A stalk connects the pituitary and hypothalamus. Hypothalamic regulatory hormones are secreted into the portal vessel for transport to the pituitary. (Dawson 1958, Daniel & Prichard 1975, Bonneville *et al.* 1989) The release of each of the pituitary hormones is under the control by at least one hypothalamic hormone, and hypothalamic hormones are controlled in turn by neurotransmitters. Furthermore, the release of both hypothalamic and pituitary hormones are regulated by concentrations of hormones secreted by the peripheral target glands, i.e. by a feedback mechanism. The hypothalamic hormones are released in a pulsatile manner. Luteinizing hormone (LH), follicle stimulating hormone (FSH), adrenocorticotropin (ACTH), thyrotropin, growth hormone (GH) and prolactin are secreted by the anterior pituitary, while antidiuretic hormone and oxytocin are synthesized and secreted from supraoptic and paraventricular nuclei and transported axonally to the posterior pituitary. (Lechan 1987, Riskind & Martin 1995)

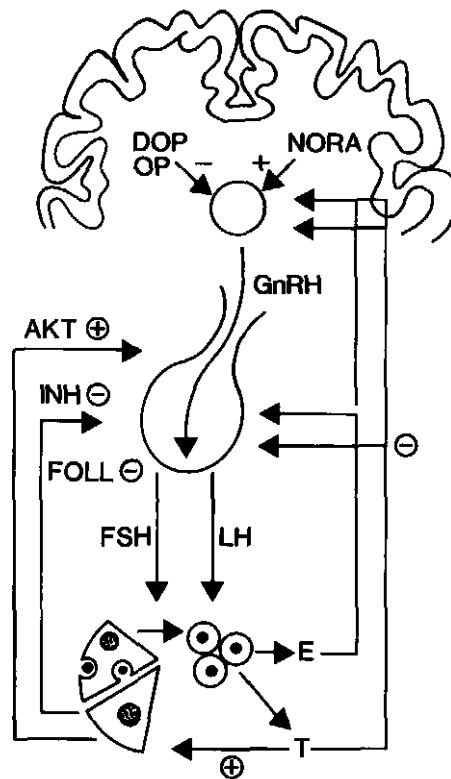
### ***2.2.2. The regulation of secretion of reproductive hormones in women***

The maturation of oocytes in the normal ovaries of women of reproductive age and production of female sex hormones as well as small amounts of androgens occur in periodic (menstrual) cycles. The basic functional unit of the ovary is a follicle, which consists of an outer layer of theca interna cells that encircle inner layers of granulosa cells. The maturation of this follicle includes the release of the oocyte, and the eventual transformation of the follicle into corpora lutea. These cyclic events are controlled by a number of hormones. (Ericson 1978, Irianni & Hodgen 1992)

The menstrual cycle can be divided into follicular, ovulatory, and luteal phases. FSH stimulates follicular maturation during the follicular phase, which starts with the first day of menstrual bleeding (Goodman & Hodgen 1983). According to a two-cell, two-gonadotropin hypothesis, LH stimulates the biosynthesis of androgens in the theca interna, and FSH regulates the conversion of androgens to estrogens in granulosa cells (Liu & Hsueh 1986). The hormonal interactions between the hypothalamus, pituitary gland, and the ovaries lead to a pre-ovulatory elevation of serum estrogen that in turn triggers the ovulatory gonadotropin surge. The luteal secretion of progesterone and

estrogens inhibits the release of gonadotropins, and ensures adequate uterine development for implantation of the fertilized egg. In the absence of blastocyst implantation, the corpus luteum regresses and progesterone secretion declines before menses. (Sherman & Korenman 1975, Pauerstein *et al.* 1978)

The negative feedback mechanism of gonadotropins is different in males and females. In both genders, FSH and LH are under the control of hypothalamic gonadotropin-releasing hormone. Serum levels of T regulate the release of gonadotropins in men, but hyperandrogenemia does not inhibit the secretion of gonadotropins in women, probably because women have approximately tenfold lower serum concentrations of T compared to that of men. Indeed, serum T concentrations above the normal *male* range do reduce LH pulse frequency in women. (Conway & Jacobs 1993). However, estrogens and progesterone regulate the gonadotropin feedback cycle in women, and the effect of estrogens on release of gonadotropin-releasing hormone and gonadotropins may be either inhibitory or stimulatory (Marshall 1995, Marshall & Kelch 1986). In addition to gonadotropins, estrogens, and progesterone, there are several ovarian peptide hormones (e.g., inhibin, activin), prostaglandins, regulatory proteins, growth factors and muco-



**Fig. 1.** The regulation of androgen synthesis in testis. DOP=dopamine, NORA=norepinephrine, OP=opioids, E=estrogen, T=testosterone, AKT=activin, INH=inhibin, FOLL=follistatin, + = stimulating effect, - = inhibiting effect (Huhtaniemi *et al.* 1992).

polysaccharides, which interfere with ovarian functions or have endocrine roles by acting outside the ovary. Also pituitary prolactin modulates luteal steroidogenesis together with LH, and other hormones, e.g. insulin and insulin-like growth factors have effects on ovarian androgen synthesis. Thus, the regulation of the ovarian hormonal function is very complicated. (McNeilly *et al.* 1982, Nobels & Dewailly 1992, Hsueh & Billig 1995)

### ***2.2.3. The regulation of secretion of reproductive hormones in men***

The regulation of androgen synthesis in testis is presented in Fig. 1 (Huhtaniemi *et al.* 1992). Testicular androgen production in Leydig cells is controlled by pituitary LH secretion, while spermatogenesis is stimulated by FSH (Bremner *et al.* 1981, Dufau 1988). The release of LH and FSH in turn is determined by the pulsatile release of hypothalamic gonadotropin-releasing hormone. Furthermore, the production of gonadotropins as well as the release of gonadotropin-releasing hormone is regulated by the negative feedback cycle by the testis. Numerous studies have shown that testosterone (T), estradiol (E<sub>2</sub>), and dihydrotestosterone exert a negative modulation on LH secretion, but the feedback system regulating the production of FSH is so far poorly known. However, several studies indicate that in addition to androgens e.g. inhibin and activin, which are peptide hormones secreted by Sertoli cells in testis, have a role in controlling the release of FSH. (Crowley *et al.* 1991)

In addition to gonadotropins, testicular androgen secretion is influenced by many other factors including prolactin, GH, insulin, insulin-like growth factors, other growth factors, and paracrine factors originating within the testis, e.g. inhibin, activin, prostaglandins E<sub>3</sub> and F<sub>2α</sub>, and partially uncharacterized factors secreted by Sertoli cells. (deKretser 1987, Pasquali *et al.* 1995, Erfurth 1996)

### ***2.2.4. Sex steroid synthesis in gonads***

The general steps in the sex steroid biosynthetic pathway and the subcellular localization of the major enzymes of steroid biosynthesis are similar in all sex steroid-producing glands, including the ovary, testis, adrenals, and placenta. The biosynthesis of sex steroids is presented in Fig. 2 (Handelsman 1995). However, in the adrenal cortex ACTH promotes the reaction, while it is stimulated by LH in the ovary and testis. (Huhtaniemi 1977, Waterman & Simpson 1985, Rommerts & van der Molen 1989, Simpson & Waterman 1995)

In the ovary the theca interna cells possess all the enzymes necessary for androgen biosynthesis, and the granulosa cells are capable of producing progestins and aromatizing androgens to estrogens. The luteal cells, which are derived from both granulosa and theca cells, contain necessary enzymes for both progestin and estrogen biosynthesis. The general pathway and the subcellular localization of the enzymes involved in the early steps of E<sub>2</sub> synthesis are the same as those involved in androgen biosynthesis in testis (Fig. 2). (Hsueh & Billig 1995) E<sub>2</sub> is the primary estrogen and androstenedione (ADION)

the most abundant androgen of ovarian origin. Estrone and E<sub>2</sub> are aromatized from ADION and T, respectively (Bjersing 1967, Brodie 1991).

The adult human testis produces daily approximately 7 mg of T, which is the major product of the Leydig cells, but the production of T diminishes with aging (Bremner *et al.* 1983). In addition, the testis produces smaller amounts of several weaker androgens and precursors of T, e.g. ADION and dehydroepiandrosterone (DHEA). The testis has also the capacity to secrete small amounts of 17 $\beta$ -estradiol (E<sub>2</sub>) and dihydrotestosterone, which is the most potent androgen. Furthermore, a small proportion of circulating T is metabolized to biologically active metabolites in certain target tissues; about 4 % of circulating T is converted to dihydrotestosterone and 0.2 % is converted to E<sub>2</sub> in various tissues. The majority of T is metabolized to inactive metabolites mainly in the liver, kidney, muscle, and adipose tissue predominantly by hepatic mixed function oxidases. (DeKretser *et al.* 1995)

### ***2.2.5. Function, synthesis, and regulation of adrenal sex steroids***

A characteristic property of the adrenal androgens is their conversion to stronger sex steroids, but they also participate directly in a number of physiological and pathological processes. The functions of adrenal androgens are partly unknown, but their serum concentrations are known to change e.g. in exercise, in stress and in various diseases. (Parker 1995) The adrenal cortex produces significant amounts of the T precursors and weak androgens DHEA, DHEA sulfate (DHEAS), and ADION (Haning *et al.* 1991a). The general pathways of adrenal androgen biosynthesis are similar to those of gonads, presented in Fig. 2. DHEAS is the steroid hormone found in the highest concentration in human circulation (Parker 1995). In women, adrenal androgens are important precursors for the synthesis of T and estrone (Haning *et al.* 1991a,b). Estrogens are not made in the normal adrenal in significant amounts (Parker 1995).

### ***2.2.6. Sex hormone-binding globulin***

The binding of steroid hormones to plasma proteins plays an important role in their distribution, biology, transport in the circulation, and probably also in the transport to their target cells. The most bioactive sex steroids, dihydrotestosterone, T and E<sub>2</sub>, are bound to sex hormone-binding globulin (SHBG), albumin, or possibly other proteins in the circulation, and only 1-2 % of those hormones circulate in free, non-protein bound form. SHBG is the most important binding protein of sex steroids, since it has high affinity and specificity for 17 $\beta$ -hydroxy steroids; it binds dihydrotestosterone and T with high affinity and E<sub>2</sub> with lower affinity. Approximately 40% of T is in association with albumin and 60 % with SHBG in men, while 30 % of T is bound to albumin and 70 % to SHBG in women. The clearance, bioavailability, and peripheral conversion of T, dihydrotestosterone and E<sub>2</sub> are strongly affected by the serum concentration of SHBG. (Hammond *et al.* 1980, Hammond *et al.* 1982, Siiteri *et al.* 1982, Moore *et al.* 1987, Petra 1991, Rosner *et al.* 1991)

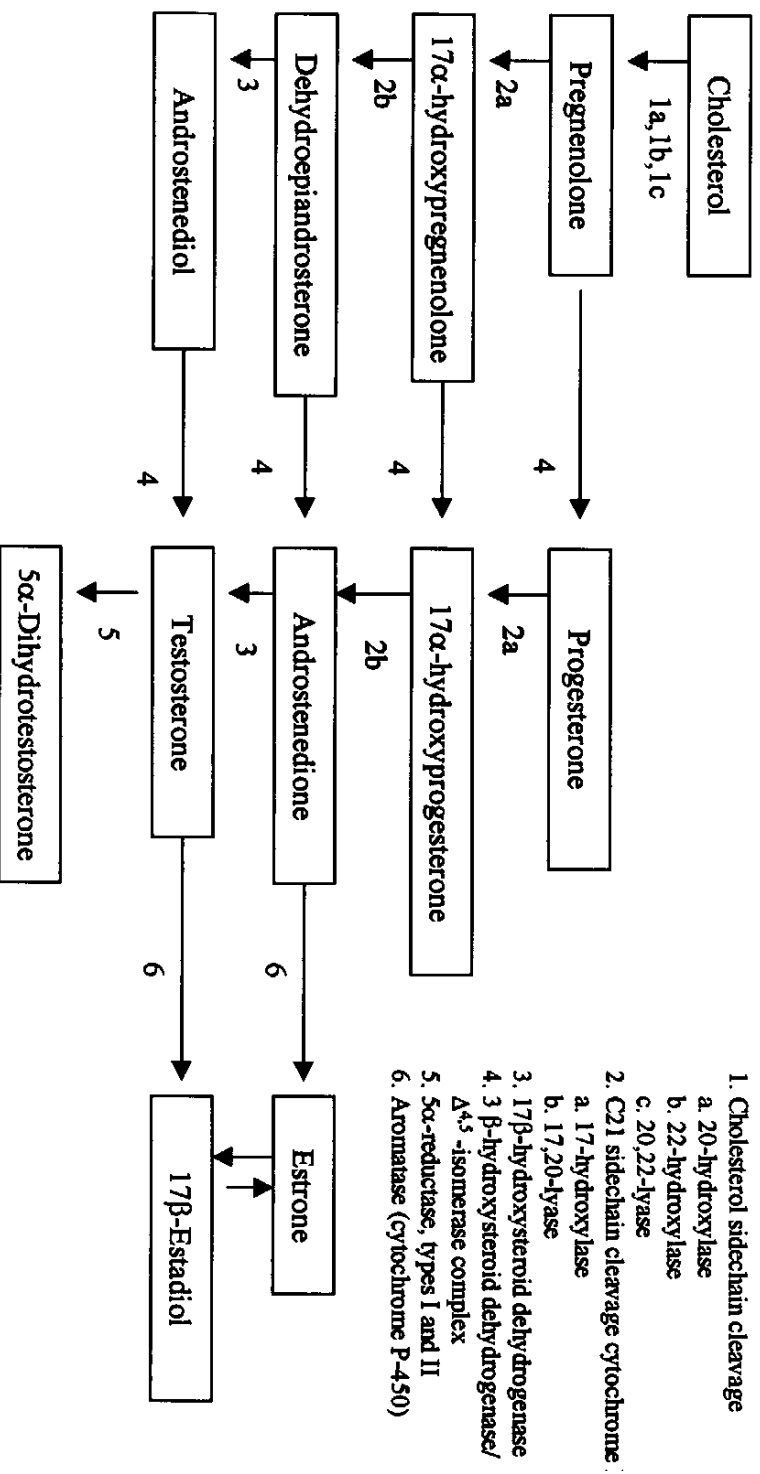


Fig. 2. The general steps of androgen biosynthesis (Handelsman 1995).

SHBG is a plasma glycoprotein, which is synthesized in the liver. Traditionally, sex steroids were considered to be the major regulators of SHBG. Androgens were considered to decrease, while estrogens were perceived to increase serum SHBG concentrations. However, *in vitro* studies have shown that androgens actually *increase* the secretion of SHBG from a hepatoma cell line (Lee *et al.* 1987). Recent studies indicate that also nutritional factors, probably mediated by insulin, are more important than sex steroids in determining the synthesis of SHBG: insulin and insulin-like growth factor-I (IGF-I) are shown to suppress SHBG production in the liver. Medical administration of hormones affects the production of SHBG more than the endogenous changes in hormone concentrations. (Siegberg *et al.* 1987, Plymate *et al.* 1988, Plymate *et al.* 1990, Petra 1991)

Altered serum concentrations of SHBG are clinically important, e.g. in women low serum levels of SHBG may lead to symptoms of hyperandrogenism, such as in some women with polycystic ovary syndrome (PCOS) (Nobels & Dewailly 1992). Respectively, high serum concentrations of SHBG in men may be associated with hypoandrogenic symptoms, e.g. disturbed sexual functions (Isojärvi 1995b).

Regulation of adrenal androgen secretion is poorly known at present (Parker 1995). ACTH is an important controlling factor under certain circumstances (Nieschlag *et al.* 1973). IGF-I has been found to stimulate DHEA secretion in human adrenal cells, and insulin causes a decrease in circulating DHEA and DHEAS concentrations (Pham-Huu-Trung *et al.* 1991, Nestler *et al.* 1989). Angiotensin may also stimulate adrenal androgen secretion in supraphysiological concentrations, as in salt-losing patients (Parker *et al.* 1983). Gonadotropins, prolactin, GH, estrogens, and prostaglandins of the A, E, and F series do not stimulate adrenal androgen secretion in physiological concentrations (Parker & Odell 1980)

### **2.2.7. Insulin, IGF-I, IGFBP-1 and IGFBP-3**

Insulin plays a central role in the regulation of a number of key metabolic processes. In addition to glucose, lipid and protein metabolism, and electrolytic balance, insulin also interferes -directly or indirectly- with several endocrine functions of human body. (DeFronzo *et al.* 1992) Insulin has effects e.g. on the production and function of sex hormones, growth hormone (GH), growth factors, and their binding proteins (Conway & Jacobs 1993). On the other hand several hormones, e.g. thyroid hormones, GH, glucocorticoids, prolactin and sex steroids, play a role in the regulation of insulin secretion in addition to gastrointestinal peptide hormones, glucagon and somatostatin (Samols *et al.* 1965, Felig *et al.* 1971, Ensink & Williams 1972, Alberti *et al.* 1973, Kalhan & Adam 1975, Landgraf *et al.* 1977). Therefore, the interactions between insulin and other hormones are relatively complicated. Insulin also affects serum lipid concentrations. Hyperinsulinemia may be associated with unfavorable changes in serum lipids, which increases the risk for cardiovascular diseases. (Conway & Jacobs 1993, Wild 1994) Insulin is secreted by pancreatic beta cells and released to circulation in equimolar amounts with C-peptide, which is a part of proinsulin. Therefore,

measurements of serum C-peptide concentrations may be used when examining insulin secretion. (Horwitz *et al.* 1975)

Insulin-like growth factors I and II (IGF-I and –II) are peptides related to insulin in structure and function (Rinderknecht & Hummel 1978). Circulating IGF-I is synthesized mainly in the liver, but also in several peripheral tissues, e.g. epiphyseal cartilage synthesizes IGF-I locally. IGF-I mediates the growth effect of GH, and interacts together with insulin in cell growth and replication. (Bang 1994) In addition IGF-I stimulates ovarian and adrenal sex steroid production, and it probably has various other functions as well (Pham-Huu-Trung *et al.* 1991, Nobels & Dewailly 1992).

Insulin-like growth factor binding proteins-1 and –3 (IGFBP-1 and –3) are proteins binding insulin-like growth factors, and they are mainly synthesized in the liver (Bang 1994). Insulin is the most important regulator of the hepatic synthesis of IGFBP-1, but IGFBP-3 synthesis is influenced by GH secretory status and also gonadal steroids interfere with regulation of IGFBP-3 levels during adolescence (Blum & Ranke 1990, Singh *et al.* 1990, Olivie *et al.* 1995). IGFBP-1 has an important role as a local regulator of the IGF-I function, since it acts as a competitive binding site for IGF-I, and thus reduces the interaction of IGF-I with its receptors. For example, serum levels of IGFBP-1 in polycystic ovary syndrome (PCOS) are low resulting in increased bioactivity of IGF-I in ovaries, which further stimulates the ovarian androgen synthesis. (Nobels & Dewailly 1992) The vast majority of circulating IGF-I, approximately 90 %, is bound to IGFBP-3. It has been shown that subnormal serum concentrations of IGF-I and IGFBP-3 are highly predictive of a subnormal GH secretion. Therefore, serum levels of IGF-I and IGFBP-3 may be used clinically when screening for GH deficiency. (Juul & Skakkebaek 1997)

### ***2.2.8. Polycystic ovary syndrome***

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women. It is characterized by hyperandrogenism, chronic anovulation, and metabolic disturbances, but the clinical and biochemical features are heterogeneous. Common clinical features due to hyperandrogenism are hirsutism, acne, and androgen-dependent alopecia. Many of these patients are obese. Anovulation manifests itself as menstrual disturbances and infertility. The diagnosis of PCOS is usually based on a combination of clinical, biochemical, and ultrasonographic criteria. (Franks 1995)

Biochemical features of PCOS are elevated serum levels of T, ADION, LH and insulin, and low serum concentrations of SHBG and IGFBP-1. Insulin appears to augment the ovarian androgen secretion and suppress the production of SHBG and IGFBP-1. Low circulating levels of SHBG leads to increased bioavailability of T and reduced intraovarian IGFBP-1 levels may increase the bioactivity of IGF-I, which further enhances the androgen production in the ovaries. Serum concentrations of FSH, estrogen, and progesterone are also changed due to anovulatory cycles; the normal cyclic secretion of these hormones is lacking. Hyperprolactinemia and impaired secretion of GH are less common biochemical features of PCOS. Women with PCOS have a greater degree of hyperinsulinemia and insulin resistance than weight-matched controls. Impaired glucose tolerance and type II diabetes mellitus are more prevalent in obese women with PCOS

than in weight-matched controls. These women have hyperlipidemia and a substantially increased risk of cardiovascular disease. However, all women with structural polycystic changes in their ovaries do not have PCOS with hyperandrogenemia and hyperinsulinemia. The pathogenic mechanisms of PCOS are still poorly defined. (Nobels & Dewailly 1992, Buyalos *et al.* 1995, Franks 1995) Typical features of PCOS have also been found in adolescent girls (Dramusic *et al.* 1991) and PCOS has been suspected to originate during pubertal development (Apter *et al.* 1994b).

### ***2.2.9. Endocrine regulation of growth and puberty in girls***

The onset of puberty is determined by maturational changes in the hypothalamus and activation of the hypothalamic pulse generator. There is still no consensus as to what constitutes the pulse generator. The inhibitory effect from the central nervous system is reduced and the pulsatile secretion of gonadotropin-releasing hormone stimulates the production and secretion of LH and FSH. (Crowley *et al.* 1985, Rasmussen *et al.* 1989, Bourguignon 1995) The gonadotropins are responsible for increased secretion of sex steroids, which in turn induces the development of the secondary sexual characteristics. The development of breasts is primarily controlled by estrogens secreted by the ovaries, while the growth of pubic and axillary hair is mainly under the influence of androgens secreted by the adrenal gland and ovaries. (Styne 1995)

The most important regulator of postnatal growth is the GH-IGF-I -system, which in addition to GH and IGF-I includes e.g. their binding proteins and receptors (Isaksson *et al.* 1982, Juul *et al.* 1995). GH secretion is regulated by a complex neuroendocrine control system that includes neurotransmitters, GH-releasing and -inhibiting hormones and feedback by hormonal substrates. IGF-I is the main mediator of the growth promoting actions of GH. The circulating concentration of IGF-I is regulated by GH, and it reflects the endogenous secretion of the latter (Bang 1994). The mean serum IGF-I level increases slowly in prepuberty, is highest in midpuberty, and is followed by a continuous slow fall after that. Girls reach maximal plasma IGF-I levels at an average age of 13.5-14.5 years, which is almost 2 years later than the average peak in growth rate. (Juul *et al.* 1994, Olivie *et al.* 1995)

In addition to the IGF-GH -system, the pubertal growth spurt is mediated by several other endocrine factors. Increasing sex steroid production during puberty stimulates the secretion of GH, and sex steroids also exert a direct effect upon epiphyseal cartilage and stimulate the local production of IGF-I (Attie *et al.* 1990, Rogol 1992). The type of steroids (androgen or estrogen) appears to determine the timing of the pubertal growth spurt, although both steroids appear to increase the total amount of GH (Brook and Hindmarsh 1992). Thyroid hormones are necessary in sufficient amounts to allow the pubertal growth spurt to proceed. Furthermore, insulin is necessary for growth, and puberty is characterized by hyperinsulinemia. There remain still several other biologically active, but insufficiently known substances, which may interfere with growth. However, the rate of linear growth slackens and finally ends in the late phase of puberty when epiphyseal cartilages are ossified by the effects of estrogens. (Rogol 1992, Hindmarsh & Brook 1995, Styne 1995, Smith & Korach 1996)

## **2.3. Normal linear growth and pubertal maturation in girls**

### ***2.3.1. Linear growth***

Linear growth and pubertal sexual maturation are genetically determined characteristics and influenced by a series of environmental and intrinsic factors, e.g. most endocrine disorders interfere with growth (Hindmarsh & Brook 1995). According to the infancy-childhood-puberty model, human growth comprises three distinct periods, the rapid growth phase in infancy, a phase of steady growth in childhood and a growth spurt in adolescence. Furthermore, growth in puberty can also be divided into three parts: 1) the slow growth rate in early puberty, 2) the growth spurt lasting on an average for 2 years and 3) the final slowing and the end of the growth. When the slowest phase is over in early puberty, girls grow an average of 28 (20-36) cm. (Karlberg *et al.* 1987) The growth spurt begins in girls prior to the onset of the secondary sexual characteristics and growth velocity is the fastest at approximately 12.0 years of age. (Tanner *et al.* 1976) After menarche, there is usually no more than 5 cm of growth remaining. Menarche correlates closely with a bone age of 13 years (Greulich & Pyle 1959, Styne 1995).

### ***2.3.2. Puberty***

The signs of puberty appear between 8 and 13 years of age (mean 10.5) in 95% of normal girls. Pubertal development lasts on an average for 4.2 years (range 1.5-6 yr). The stage of breast development is usually equal to that of pubic hair, but as different endocrine organs control these two processes, the stages of each phenomenon should be classified separately (Styne 1995). The peak velocity of linear growth occurs usually between breast stages 2 and 3 (Hindmarsh & Brook 1995). There has been a secular trend towards an earlier pubertal maturation, since the mean age at menarche among Finnish girls was 13.16 ( $\pm 0.02$ ) years in 1969, while that of their mothers had been 13.88 ( $\pm 0.02$ ) years (Kantero & Widholm 1971). However, according recent Danish study there has been a halt in this secular trend (Helm & Grolund 1998). The interaction between the endocrine system and the nutritional status is important, e.g. moderate obesity (up to 30 % above normal weight for age) correlates with an earlier menarche (Styne 1995).

### ***2.3.3. Evaluation of growth and puberty***

Human growth charts are essential instruments in evaluating linear growth, weight gain, and growth of the head (Tanner & Whitehouse 1976). There are several formulas used for assessing the target height and predicting the adult height of the child. The parental heights must be measured or bone age determined for these purposes (Hindmarsh & Brook 1995). Bone age determination is a method, in which an X-ray of the left hand and wrist is used for the evaluation of skeletal maturation in relation to patient's chronological age. The amount of remaining growth of a pubertal patient can be calculated from the

bone age. (Greulich and Pyle 1959). The physical changes associated with puberty may be described by an objective method developed by Tanner and Whitehouse. The breast and pubic hair development is classified into maturation stages 1-5 based on clinical examination. (Tanner and Whitehouse 1976) Also the age of menarche, as well as the maternal age of menarche, are important data in evaluating pubertal development (Styne 1995).

Biochemical assessment of growth and puberty comprises e.g. the analysis of serum concentrations of GH, gonadotropins and sex steroids. Release of GH and gonadotropins is pulsatile, and therefore special methods are needed for evaluating their secretion (Tapanainen & Knip 1992, Apter *et al.* 1994a). Those methods include e.g. stimulation tests, the monitoring of spontaneous nocturnal GH secretion with frequent sampling, urinary GH measurements, and quantification of IGF-I and IGFBP-3. (Hindmarsh & Swift 1995) The gonadotropin-releasing hormone (GnRH) stimulation test and measurements of spontaneous serum and urinary FSH and LH concentrations are useful tests for diagnosing precocious puberty. (Lee 1994, Neely *et al.* 1995)

## **2.4. Epilepsy and reproductive function**

### ***2.4.1. Effects of epilepsy on hormones***

Gastaut and Collomb recognized an association between epilepsy and hyposexuality in 1954. Patients with epilepsy suffer from sexual dysfunction and have reduced fertility more often than the average population of similar age (Dansky *et al.* 1980, Toone 1984, Webber *et al.* 1986). Those disorders may clinically appear e.g. as irregular menstrual cycles in women and as erectile dysfunction in men (Isojärvi *et al.* 1995a,b). The impaired reproductive functions are most often due to a disturbed hormonal balance. Indeed, it is well known that serum concentrations of several hormones in patients with epilepsy may differ from those in non-epileptic subjects of similar age. (Rodin *et al.* 1984, Toone 1984).

It is known that excess electrical activity during an epileptic seizure may affect the hypothalamic-pituitary axis, and may release hypothalamic and pituitary hormones. Transient increases in serum prolactin, LH and FSH levels have been observed both after generalized and partial seizures (Herzog *et al.* 1986a,b, Bilo *et al.* 1988). Reproductive endocrine disorders (hyperprolactinemia, hypogonadotropic hypogonadism, hypergonadotropic hypogonadism) and altered serum hormone levels are common especially in patients with refractory partial seizures of temporal lobe origin. About 50 % of these patients have been reported to suffer from reproductive endocrine disorders. (Herzog *et al.* 1986 a,b)

### ***2.4.2. Effects of hormones on epilepsy***

In addition to the effects of epilepsy on hormonal function, hormones may in turn affect seizure activity (Klein & Livingston 1950, Woodbury 1958, Logothetis *et al.* 1959, Bäckström *et al.* 1984). In catamenial epilepsy, the seizure frequency of female patients is altered during the menstrual cycle due to variable effects of female sex steroids on seizure susceptibility. E<sub>2</sub> seems to lower the seizure threshold, while progesterone has anticonvulsive properties. (Dickerson 1941, Laidlaw 1956, Bäckström 1976, Mattson & Cramer 1985, Herkes *et al.* 1993) Indeed, intermittent progesterone therapy and antiestrogenic clomiphene therapy have been reported to reduce the seizure frequency in women with catamenial epilepsy (Herzog 1986, Schachter 1988). Thyroid hormones are also known to have effects on epileptic activity, epileptic seizures may become manifest in association with thyrotoxicosis, and thyroxine may induce status epilepticus (Jabbari & Huott 1980, Sundaram *et al.* 1985). Accordingly epilepsy and hormones are connected in a complicated manner.

## **2.5. Antiepileptic drugs and reproductive function**

AEDs have been shown to affect the reproductive endocrine balance in patients with epilepsy (Mattson & Cramer 1985, MacPhee *et al.* 1988, Isojärvi *et al.* 1988-1996, Geisler *et al.* 1997). Several studies indicate that the use of older AEDs (CBZ, phenytoin, phenobarbital and primidone) induce reproductive dysfunction in patients with epilepsy (Mattson *et al.* 1985, Isojärvi 1995a,b,c). The effects of new AEDs (e.g. OXC, LTG) on endocrine function are not known.

### ***2.5.1. Valproate and reproductive hormonal function***

#### ***2.5.1.1. Hormonal effects of valproate in children and adolescents***

Weight gain has been reported in children taking VPA for epilepsy (Egger & Brett 1981, Novak *et al.* 1999) and some previous studies have suggested that pubertal maturation is retarded during VPA treatment in animal models and may be also slowed in pediatric patients (Cook *et al.* 1992, Snyder & Badura 1995). It has been suggested that VPA may slow pubertal maturation by altering the neurochemical system that normally plays an important role in timing the maturation of the gonadotropin-releasing hormone pulse generator (Snyder & Badura 1998). However, in other reports, pubertal development has been unaffected by VPA treatment in adolescents with epilepsy (Lundberg *et al.* 1986), and serum gonadotropin levels and gonadotropin secretion in the gonadotropin-releasing hormone test is normal or decreased in pediatric patients taking VPA for epilepsy (Masala *et al.* 1981, Lundberg *et al.* 1986). Retarded skeletal growth has also been reported in some patients treated with VPA (Invitti *et al.* 1988, Cook *et al.* 1992). On the other hand, it has been suggested that chronic VPA administration has little or no effect

on skeletal maturation in prepubertal children (Legido *et al.* 1992, Snyder & Badura 1995). Consequently, the present knowledge of the effects of VPA on linear growth, pubertal maturation, and hormonal function in pediatric patients is insufficient and controversial.

### 2.5.1.2. Hormonal effects of valproate in women

A very high occurrence (over 50 %) of hyperandrogenism and polycystic ovaries has been found among women taking VPA for epilepsy. These abnormalities were more frequent in women who had started VPA treatment before the age of 20 years than in women who had started the treatment later. Serum levels of T, free testosterone (fT), and DHEAS were higher in women on VPA than in the controls, but their serum ADION, E<sub>2</sub>, and gonadotropin concentrations were normal. Serum SHBG levels were lower in women on VPA with polycystic ovaries or hyperandrogenism than in VPA-treated women with normal ovaries. (Isojärvi *et al.* 1993, 1996) The mechanism by which VPA could cause polycystic ovaries and elevated serum androgen concentrations is unclear.

GABA neurons seem to modulate hypothalamic gonadotropin-releasing hormone secretion, which is important in the pulsatile secretion of gonadotropins. Therefore, it has been previously suggested that VPA may affect gonadotropin secretion due to its GABAergic properties (Elias *et al.* 1982, Everitt & Keverne 1986). However, according to previous studies VPA do not alter pulsatile LH secretion in women (Lado Abeal *et al.* 1991, 1996, Popovic *et al.* 1996). Furthermore, PCOS is usually associated with hyperandrogenism, elevated serum LH and FSH concentrations, and enhanced secretory responses of LH to gonadotropin-releasing hormone (Yen 1980, Nobels & Dewailly 1992), whereas the VPA-treated women with polycystic ovaries or hyperandrogenism had normal serum gonadotropin levels (Isojärvi *et al.* 1993).

According to Isojärvi *et al.* 1996, hyperandrogenism and polycystic ovaries appeared to be related to obesity, hyperinsulinemia and low serum IGFBP-1 concentrations in women on long-term VPA therapy. Weight gain is a common side effect of VPA; it has been observed over in half of patients (children and adults) on VPA treatment (Egger & Brett 1981, Dinesen *et al.* 1984, Corman *et al.* 1997). The pathogenesis of VPA-related weight gain is not known. Differences have not been found between weight gainers and stable patients with regard to age, sex, pretreatment overweight, duration of treatment, dosage or serum levels of VPA. According to previous studies, patients receiving VPA appear to have low energy expenditure and it has been suggested that VPA-induced impairment in fatty acid beta oxidation may be of importance (Dinesen *et al.* 1984, Ponchaut & Veitch 1993, Gidal *et al.* 1996). Obesity was associated with hyperinsulinemia in VPA-treated women (Isojärvi *et al.* 1996). However, short-term exposure to VPA did not affect fasting serum insulin or C-peptide levels in a 1-month longitudinal study on 8 patients starting treatment with VPA (Breum *et al.* 1992). Similarly, no increase in serum C-peptide levels was observed after administration of VPA to 6 volunteers for 6 days (Kusunoki *et al.* 1988).

It has been proposed that increased insulin levels may result in hyperstimulation of the ovaries, which leads to hyperandrogenemia and finally to structural changes characteristic

of polycystic ovaries (Pasquali & Casimirri 1993). This could be due to a direct stimulatory effect or be mediated by suppressed IGFBP-1 concentrations that lead to an increase in levels of bioactive IGF-I. Therefore, polycystic ovaries and hyperandrogenism observed in women during long-term VPA treatment has thought to be mediated through altered insulin and IGF-I function (Isojärvi *et al.* 1996).

According to a previous report, short-term exposure to VPA stimulates GH release (Coiro *et al.* 1991), and on the other hand, increased GH secretion may cause insulin resistance and hyperinsulinemia (Nobels & Dewailly 1992). However, according to Isojärvi *et al.* 1996, it is unlikely that VPA-related hyperinsulinemia could be caused by increased GH secretion, since circulating levels of IGF-I and IGFBP-3 were normal in VPA-treated women with hyperinsulinemia and circulating IGF-I and IGFBP-3 are known to reflect GH secretion (Blum & Ranke 1991, Olivié *et al.* 1995). Furthermore, Invitti *et al.* reported in 1988 that VPA would actually decrease the secretion of several pituitary hormones.

### *2.5.1.3. Hormonal effects of valproate in men*

According to previous reports VPA has no significant effect on reproductive endocrine function in male patients, but the number of VPA-treated men in these studies has been small (n=10 and n=7) (MacPhee *et al.* 1988, Isojärvi *et al.* 1990). Serum T levels were normal, serum gonadotropin levels low, and serum levels of progesterone and the FAI ratio high in men on VPA (MacPhee *et al.* 1988, Isojärvi *et al.* 1990). Geisler *et al.* 1997 reported low serum FSH levels, but serum levels of LH, SHBG, DHEAS, ADION, T, and estrone sulfate were normal in men on VPA. The reports on serum basal prolactin levels and responses of prolactin secretion to thyrotropin-releasing hormone and metaclopramide in male epileptic patients treated with VPA are conflicting, but the levels and the responses have mostly been normal (Franceschi *et al.* 1984, MacPhee *et al.* 1988, Isojärvi *et al.* 1990). Therefore, VPA has been suggested to be a safe AED from the endocrine point of view in male patients.

### *2.5.2. Carbamazepine and reproductive endocrine function*

CBZ induces the cytochrome P450 enzyme system in the liver, which leads to stimulated hepatic functions (Perucca *et al.* 1984). Many hormones are metabolized via this enzyme system and, therefore, CBZ-related changes in serum concentrations of some hormones may be explained by enzyme-inducing properties of CBZ. Also the hepatic production of hormone binding proteins may be induced during CBZ medication. (Connell *et al.* 1984, Perucca *et al.* 1984, Isojärvi 1989)

The use of CBZ is associated with a progressive increase in serum levels of SHBG resulting in a low free androgen index (FAI) ratios reflecting decreased serum levels of free, bioactive T in male patients (Dana-Haeri & Richens 1981, Dana-Haeri *et al.* 1982, Toone *et al.* 1984, Isojärvi *et al.* 1988, MacPhee *et al.* 1988, Isojärvi *et al.* 1990, 1995b, Duncan *et al.* 1999). Serum T and free T levels have been reported to be normal during

CBZ monotherapy (Lühdorf *et al.* 1977, MacPhee *et al.* 1988, Isojärvi *et al.* 1988, 1989b, 1995b). The discrepancy between low FAI values and unchanged serum free T concentrations may be explained by displacement of T from serum albumin by AEDs, as suggested before (Isojärvi *et al.* 1988, 1989b, 1990). Low serum E<sub>2</sub> levels and low E<sub>2</sub>/SHBG ratios have been reported in female patients on CBZ (Isojärvi 1995a). The rise of serum SHBG levels is probably caused by increased synthesis due to the liver enzyme induction (Connell *et al.* 1984, Isojärvi *et al.* 1989, 1990). Serum gonadotropin levels have mainly been found to be normal during CBZ treatment (Isojärvi *et al.* 1988, 1989a, 1989b, 1990), but also high (Dana-Haeri *et al.* 1984, MacPhee *et al.* 1988) and low (Isojärvi *et al.* 1995a) LH levels have been reported. Serum concentrations of DHEAS have been observed to be low during CBZ medication (Levesque *et al.* 1986, MacPhee *et al.* 1988, Isojärvi *et al.* 1988, 1989b, 1990, Geisler *et al.* 1997, Duncan *et al.* 1999). The reports on serum basal prolactin levels and prolactin secretion responses to thyrotropin-releasing hormone or metoclopramide in male epileptic patients treated with CBZ are conflicting, but the levels have mostly been normal (Dana-Haeri *et al.* 1984, Franceschi *et al.* 1984, MacPhee *et al.* 1988, Isojärvi *et al.* 1988, 1989a) and the responses slightly elevated or unchanged (Dana-Haeri *et al.* 1984, Franceschi *et al.* 1984, MacPhee *et al.* 1988, Isojärvi *et al.* 1988) Furthermore, the use of CBZ has been reported to decrease the bioavailability of ethinyloestradiol and levonorgestrel when added to a contraceptive treatment (Crawford *et al.* 1990).

CBZ has also been found to influence thyroid function; serum thyroxine and free thyroxine levels are low (Yeo *et al.* 1978, Connell *et al.* 1984, Haidukewych & Rodin 1987, Isojärvi *et al.* 1992, 1990) serum thyroid hormone concentrations serum thyrotropin levels and thyrotropin responses to thyrotropin-releasing hormone were normal in patients on CBZ according to most studies (Yeo *et al.* 1978, Connell *et al.* 1984, Isojärvi 1989, Isojärvi *et al.* 1990). An accelerated metabolism of thyroid hormones in the liver has been postulated to be the reason also for decreased serum thyroid hormone levels in epileptic patients on CBZ treatment. (Connell *et al.* 1984, Isojärvi *et al.* 1990) Because thyrotoxicosis and thyroxine may induce epileptic seizures or status epilepticus, the reducing effect of CBZ on serum thyroxine and free thyroxine levels might contribute to the anticonvulsive properties of this drug (Jabbari & Huott 1980, Sundaram *et al.* 1985, Isojärvi *et al.* 1990).

Clinical manifestations of reproductive endocrine disorders during long-term CBZ treatment may comprise sexual dysfunction and menstrual disturbances (Isojärvi *et al.* 1995a,b).

### ***2.5.3. Oxcarbazepine and reproductive endocrine function***

OXC is thought to be a safe AED with regard to endocrine and metabolic effects, since previous studies have shown that CBZ induced changes in endocrine and metabolic function normalized after CBZ was replaced with OXC in men with epilepsy. According to these reports the function of the liver P450 enzyme system became normal and there was concomitant normalization of serum SHBG and DHEAS concentrations after CBZ medication was replaced by OXC. (Isojärvi *et al.* 1994, 1995c,d) Also FAI ratios tended

to increase, but there were no changes in serum T, fT, prolactin, and gonadotropin levels after CBZ had been replaced by OXC (Isojärvi *et al.* 1995c). Substituting CBZ for OXC affected also serum levels of thyroid hormones, since serum levels of thyroxine and free thyroxine increased and serum thyrotropin levels decreased to normal after the medication was changed (Isojärvi *et al.* 1995d). However, OXC may also induce liver enzymes when given at high doses (Patsalos *et al.* 1990) and it is reported to decrease the bioavailability of ethinylestradiol and levonorgestrol of oral contraceptive (Klosterskov Jensen *et al.* 1992, Fattore *et al.* 1999). Accordingly, additional studies on the endocrine effects of OXC are warranted.

#### ***2.5.4. Other antiepileptic drugs and reproductive endocrine function***

Phenytoin, phenobarbital, and primidone are known to have adverse endocrine effects. AED polytherapy resulted in a significant increase in prevalence of impotence and prominent changes in serum hormone concentrations in patients with epilepsy, e.g. low serum concentrations of thyroid hormones, high serum levels of T, SHBG and LH, low fT levels and low FAI ratios have been reported (Christiansen & Lund 1975, Barragry *et al.* 1978, Yeo *et al.* 1978, Dana-Haeri *et al.* 1982, Franceschi *et al.* 1984, Toone *et al.* 1984, Haidukewych & Rodin 1987, MacPhee *et al.* 1988, Isojärvi *et al.* 1990). However, patients on AED polytherapy suffer often from severe epilepsy, and also epilepsy per se is known to affect serum hormone levels in patients with frequently occurring seizures (see Chapter 2.4.1.).

11-22 % of the patients taking CBZ, phenytoin, phenobarbital or primidone for epilepsy have been reported to suffer from sexual dysfunction (Mattson *et al.* 1985). These AEDs are known to induce the hepatic P450 enzyme system (Perucca *et al.* 1984), which is thought to be the reason for altered serum levels of several hormones and their binding proteins. The use of phenobarbital or phenytoin is associated with high serum levels of SHBG and T, and FAI ratios of patients taking these AEDs have been reported to be low (Isojärvi *et al.* 1990, Mattson & Cramer 1985). According to previous studies serum DHEAS, thyroxine and free thyroxine levels were also low, but serum gonadotropin concentrations were normal in patients on phenytoin medication (Dana-Haeri *et al.* 1984, Levesque *et al.* 1986, MacPhee *et al.* 1988, Isojärvi *et al.* 1988, 1990). The reports on serum basal prolactin levels and prolactin responses to thyrotropin-releasing hormone and metaclopramide stimulation in male epileptic patients treated with phenytoin or AED polytherapy are conflicting, but they have mostly been normal or slightly increased (Dana-Haeri *et al.* 1984, Rodin *et al.* 1984, MacPhee *et al.* 1988, Isojärvi *et al.* 1989b).

The hormonal effects of new AEDs (e.g. LTG, topiramate, vigabatrin, tiagabine, gabapentin) have not been studied in patients with epilepsy.

### **3. Purpose of the present study**

The purpose of the present study was to evaluate reproductive endocrine effects of antiepileptic drugs, particularly the effects of VPA, in patients with epilepsy. More precisely defined this study aimed to:

1. assess the effects of VPA, CBZ, and OXC on linear growth and on pubertal development in girls with epilepsy (I),
2. elucidate reproductive endocrine influence of long-term VPA treatment in young girls and men (II, IV),
3. elucidate reproductive hormonal changes during the first 3 months of VPA treatment in patients with newly diagnosed epilepsy (III),
4. clarify the reversibility of VPA-related reproductive endocrine disorders in women by replacing VPA with LTG (V), and
5. compare the long-term reproductive endocrine effects of CBZ and OXC in men (IV).

## **4. Subjects and methods**

The study was carried out in the Departments of Neurology and Pediatrics, University of Oulu, with the approval of the Ethics Committee of the Medical Faculty of the University of Oulu and according to the principles of the Declaration of Helsinki. The Departments of Gynecology and Obstetrics, Clinical Chemistry, Radiology, and Urology in the University of Oulu, and the Department of Neurology in the University Hospital of Helsinki also participated in the present study.

### **4.1. Subjects**

The initial series was comprised of 78 girls (8-18 years) who were consecutive patients visiting the Outpatient Departments of Pediatrics or Neurology at the Oulu University Hospital, during 1993-1995, and 145 adult patients (women ranging in age from 17-41 and men ranging in age from 17-51) visiting the Outpatient Departments of Neurology at the Oulu (n=139) and Helsinki (n=6) University Hospitals. Inclusion criteria were epilepsy and AED monotherapy, and exclusion criteria were progressive brain disease, other chronic diseases or chronic medications besides epilepsy and AED, pregnancy, lactation, and use of hormonal contraceptives. The clinical characteristics of the patients and study design are presented in Table 3. The control groups comprised altogether 103 healthy sex- and age-matched volunteers. The control girls were recruited from primary and secondary schools (Kastellin ala-aste, yläaste ja lukio).

The epilepsy of all these patients was well controlled, i.e. they had less than one seizure per month (patients with severe epilepsy are usually on AED polytherapy, and were thus excluded from the studies). The daily AED dose of the patients had been unchanged for at least 1 year before the studies. None of the patients in series III had been on AED medication during the 2 years prior to participating in the study.

*Table 3. Study design and clinical characteristics of participants.*

Series	Study design	No. of subjects		AED/ controls	Age, yr mean (SD or range)	Type of seizure			Duration of present therapy, yr, mean (SD)
		F	M			G	L	GL	
I-II	Cross-sectional (retrospective growth analysis)	41		VPA	12.5 (3.0)	24	10	7	3.0 (1.9)
		19		CBZ	12.7 (3.3)	0	8	11	4.1 (2.6)
		18		OXC	12.7 (3.0)	2	9	7	1.8 (0.7)
		54		Controls	12.5 (3.0)	-	-	-	-
III	Longitudinal	10		VPA	28.7 (9.2)	4	0	6	0
			12	VPA	32.7 (11.2)	1	0	11	0
			7	CBZ	29.7 (7.4)	2	1	4	0
			10	CBZ	30.2 (9.8)	1	0	9	0
IV	Cross-sectional		21	VPA	30.4 (8.8)	10	1	10	5.2 (3.0)
			40	CBZ	34.5 (8.5)	4	3	33	8.8 (6.6)
			29	OXC	30.0 (9.2)	8	3	18	2.4 (1.6)
			25	Controls	35.9 (2.5)	-	-	-	-
V	Longitudinal	16		VPA	29.8 (19-39)	13	0	3	9.0 (5.7)
		24		Controls	29.8 (19-39)	-	-	-	-

F= female, M= male, AED= antiepileptic drug, VPA= valproate, CBZ= carbamazepine, OXC= oxcarbazepine, G = generalized epilepsy, L = localization related epilepsy without generalization, LG = localization related epilepsy with generalization

## 4.2. Methods

### 4.2.1. Study design

The study design was based on longitudinal, cross-sectional, and retrospective parts (Table 3). The girls (I, II) and the men (IV) were examined once. In addition, the growth data on 78 girls with epilepsy and 49 control girls were collected retrospectively (I). One girl on VPA was excluded from the growth analysis, because she had taken phenobarbital at the time when VPA treatment was started and the growth data of one girl on CBZ was missing. In series II we evaluated the reproductive endocrine disorders in the girls on VPA (the same girls as in series I), and the control group was completed with five new girls (n=54). Patients with newly diagnosed epilepsy were examined before AED medication was started, and 1 and 3 months thereafter (III). The patients with VPA-related endocrine disorders were first studied during VPA medication, and follow-up examinations were performed 2,6, and 12 months after valproate medication was replaced with LTG. The control women, however, were examined only once (V).

### 4.2.2. Clinical methods

All participants were clinically examined by the author or by one of the collaborators. All patients underwent a structured interview with special emphasis on reproductive function. Information on the age at menarche and the history of their menstrual cycle and the first

day of the last period, and information on changes in libido or potency were obtained using a questionnaire. In addition, the parents of participating girls were interviewed in order to have data on their pubertal development and parental height. The data on the medical history were collected from the hospital records. Growth data of the girls were collected from the records compiled at the Oulu University Hospital, child welfare clinics and by school nurses.

The epilepsy type was classified according to the recommendations of the International League Against Epilepsy Classification (Commission on Classification and Terminology of the International League Against Epilepsy 1989, Table 1). The AED was used according to generally accepted guidelines; CBZ or OXC were used particularly for treatment of patients with localization related epilepsy, whereas VPA was used in the treatment of patients with localization related or generalized epilepsy (Table 2). CT or MRI of the brain and EEG were performed on each patient.

The height of the girls was measured to the nearest 0.1 cm with a Harpenden wall mounted stadiometer, and their weight was determined to the nearest 0.1 kg on electronic scales. Target height, which is an estimate of their adult height, was determined based on parental heights according to Tanner and Whitehouse 1976. Relative heights and weights of the girls were assessed based on Finnish growth charts (Sorva *et al.* 1984). Relative height represents the deviation between absolute height and expected height for age and sex, and is expressed as a standard deviation score (SDS). A relative weight was calculated respectively from the subject's actual height divided by the their expected weight for height and multiplied by 100. The height and weight of the adults were measured to the nearest 0.5 cm and 0.5 kg. Body mass index (BMI) was calculated from the formula: weight in kilograms divided by the square of height in meters. Obesity was defined as a relative weight exceeding 120 % in girls or a BMI exceeding 25 kg/m<sup>2</sup> in adults. Body fat distribution was determined by measuring waist, thigh and hip circumferences, and by calculating the waist-hip- and waist-thigh ratios (I, II, IV, V). Furthermore, bone ages of prepubertal and pubertal girls were evaluated by the method of Greulich & Pyle 1959, and the stage of puberty was assessed according to Tanner and Whitehouse 1976. Individual growth charts were drawn for each girl starting from 1 year of age. Girls were classified as prepubertal if there were no clinical signs of puberty, that is, breast 1 (B1) and pubic hair 1 (PH1). Girls were classified as pubertal when they had clinical signs of puberty (B2-4 or PH2-4), whereas girls with full sexual maturation (B5PH5) or adult bone age were classified as postpubertal.

Table 4 summarizes the AEDs that the patients were taking and the serum hormone and binding protein concentrations assayed in each part of the study (I-V). Blood samples were drawn at 8.00 a.m. after an overnight fast for the analysis of serum LH, FSH, T, fT, ADION, DHEA, DHEAS, E<sub>2</sub>, SHBG, prolactin and progesterone. A second blood sample was obtained at 8.30 a.m. for another analysis of serum gonadotropins in series IV (Apter *et al.* 1994a). Furthermore, plasma concentrations of IGF-I, serum levels of insulin, IGFBP-1 and IGFBP-3 were studied in series I, II, IV and V, and serum concentrations of cholesterol, triglycerides and high-density lipoprotein (HDL) in series V.

Table 4. Description of the study (series I-V) by AEDs that patients were taking and by the serum hormone and binding protein concentrations assayed.

Series	I	II	III	IV	V
Medication	VPA,CBZ, OXC	VPA	VPA, CBZ	VPA, CBZ, OXC	VPA→ LTG
T	-	+	+	+	+
fT	-	-	-	+	-
DHEA	-	-	+	+	-
DHEAS	-	+	+	+	-
ADION	-	+	+	+	-
E <sub>2</sub>	-	+	+	+	-
Progesterone	-	-	+	+	-
Prolactin	-	-	+	+	-
LH	-	+	+	+	-
FSH	-	+	+	+	-
SHBG	-	+	+	+	-
Insulin	+	+	-	+	+
IGF-I	+	+	-	+	-
IGFBP-1	+	+	-	+	-
IGFBP-3	+	+	-	+	-

+ = included, - = not included, VPA = valproate, CBZ = carbamazepine, OXC = oxcarbazepine, LTG = lamotrigine, → = VPA replaced by LTG, T = testosterone, fT = free testosterone, DHEA = dehydroepiandrosterone, DHEAS = DHEA-sulfate, ADION = androstenedione, E<sub>2</sub> = estradiol, LH = luteinizing hormone, FSH = follicle stimulating hormone, SHBG = sex-hormone binding globulin, IGF-I = insulin-like growth factor no. 1, IGFBP-1 and -3 = IGF-binding proteins 1 and 3

An ultrasound examination of the endometrium and ovaries was performed transabdominally in young girls (II) and vaginally in adult women (V) using a Toshiba SSA-270A apparatus (Toshiba Co, Tokyo, Japan) equipped with a 3.75 Mhz curvilinear probe (PVF-375 MT), and all results were stored as hard copies. The volume of the ovary was calculated using the prolate ellipsoid formula: dimension 1 x dimension 2 x dimension 3 x 0.52 (cm<sup>3</sup>). The ovarian follicle size was determined by measuring the maximum follicle diameter. The ultrasonographic criteria used for the diagnosis of polycystic ovaries were those described by Adams *et al.* 1986.

The menstruating women were examined in the early follicular phase (on days 3-7) of the menstrual cycle, and the women with amenorrhea, the girls before menarche, and men were examined at random. The patients were advised to maintain their regular lifestyles during the longitudinal parts of the study (III, V).

The free androgen index (FAI) was calculated from the formula  $100 \times \text{serum T (nmol/l)} / \text{serum SHBG (nmol/l)}$ . The SHBG/E<sub>2</sub> ratio was calculated from the formula  $100 \times \text{serum E}_2 \text{ (pmol/l)} / \text{serum SHBG (pmol/l)}$ . The reference ranges of hormones were defined as the mean respective values of controls  $\pm 2$  standard deviation (SD). The girls whose serum T levels exceeded the mean +2 SD in the control girls of the same pubertal stage were considered to have hyperandrogenemia (serum T > 1.1 nmol/l for prepubertal girls, > 2.0 nmol/l for pubertal and postpubertal girls).

### 4.2.3. Laboratory methods

Serum samples were frozen at  $-20^{\circ}$  C until analyzed. The details of different assays according series IV and V are given in Table 5. Since the serum samples of different series were analyzed at different times, there were slight variation in details of some of the laboratory methods (See the original articles). All the assays were performed according to the instructions of the reagent manufacturers. The effect of analytical bias on the results was eliminated by the internal quality control of the laboratory.

Table 5. Characteristics of the assays used.

Assay	Method, Manufacturer	Typical sensitivity Values*	Typical coefficient of intra-assay variation (%) in the reference interval	Typical coefficient of interassay variation (%) in the reference interval
T	RIA, Orion Diagnostica, Turku, Finland	0.24 nmol/l	4.5	6.4
fT	RIA, Orion Diagnostica, Turku, Finland	0.52 pmol/l	3.8	4.2
E <sub>2</sub>	RIA, Orion Diagnostica, Turku, Finland	5.0 pmol/l	4.5	4.1
DHEA	RIA, Diagnostic Products Co, Los Angeles, CA	0.10 nmol/l	5.2	5.6
DHEAS	RIA, Diagnostic Products Co, Los Angeles, CA	0.05 $\mu$ mol/l	4.5	5.5
ADION	RIA, Diagnostic Products Co, Los Angeles, CA	0.07 nmol/l	5.0	8.6
Progesterone	ACS:180 automated chemiluminescence system, Chiron Diagnostics	0.05 nmol/l	4.6	7.5
Prolactin	ACS:180 automated chemiluminescence system, Chiron Diagnostics	6.4 mIU/l	2.1	5.4
FSH	Two-site fluoroimmunoassay method, Wallac Ltd, Turku, Finland	0.03 IU/l	1.4	2.6
LH	Two-site fluoroimmunoassay method, Wallac Ltd, Turku, Finland	0.03 IU/l	2.3	4.2
SHBG	Two-site fluoroimmunoassay method, Wallac Ltd, Turku, Finland	0.8 nmol/l	6.7	8.0
Insulin	Enzyme-linked immunosorbent assay**	0.5 mIU/l	<7.5	<9.3
IGF-I	RIA, Incstar, Stillwater, MN	1.0 nmol/l	5.1	<12
IGFBP-1	Immunoradiometric method with monoclonal antibody MoAb 6035	0.4 $\mu$ g/l	<4	<10
IGFBP-3	RIA, Diagnostic Systems Laboratories, Webster, TX	30.0 $\mu$ g/l	<5	<9
VPA	Fluorescence polarization immunoassay system, AxSym analyzer, Abbott Diagnostic Division, Irving TX	4.1 $\mu$ mol/l	1.8	2.7
CBZ	Fluorescence polarization immunoassay system, AxSym analyzer, Abbott Diagnostic Division, Irving TX	2.1 $\mu$ mol/l	1.2	3.2
OXC	High-pressure liquid chromatography***	0.1 $\mu$ mol/l	<5	<5
Cholesterol	Enzymatic determination with BM/Hitachi 911 Automatic Analyzer (Naka Works, Hitachi Ltd, Ibaraki-Ken, Japan) Kit: Cholesterol CHOD-PAP (Boehringer Mannheim, Mannheim, Germany)			
HDL-cholesterol	Enzymatic method using magnesium ion/dextran sulfate precipitation****			
Triglyceride	Enzymatic determination with BM/Hitachi 911 analyzer using a test kit, Triglycerides GPO-PAP (Boehringer Mannheim)			

\* Sensitivity = the result differs in 95% probability from zero value, \*\* See Andersen *et al.* 1993

\*\*\* See Keränen *et al.* 1990, \*\*\*\*See Finley *et al.* 1978

T = testosterone, fT = free testosterone, DHEA = dehydroepiandrosterone, DHEAS = DHEA-sulfate, ADION = androstenedione, E<sub>2</sub> = estradiol, LH = luteinizing hormone, FSH = follicle stimulating hormone, SHBG = sex-hormone binding globulin, IGF-I = insulin-like growth factor no. 1, IGFBP-1 and -3 = IGF-binding proteins 1 and 3, VPA = valproate, CBZ = carbamazepine, OXC = oxcarbazepine.

#### ***4.2.4. Statistical analysis***

Statistical evaluation was performed with Student's unpaired or paired t-test in the case of normal distribution when comparing quantitative data between the patients and controls. The Mann-Whitney two-sample test or the non-parametric Wilcoxon test was used in the case of skewed distribution or ordinal variables. The Kruskal-Wallis test was used when comparing three groups. Analysis of covariance was used for adjustment of age, when there was a difference in the mean age between the patients and the control men (IV). Repeated measures ANOVA and Fisher's LSD test, and ANOVA with Bonferroni correction was used in analyzing data of prospective parts of the study. Chi-square or cross-tabulation tests were used for comparison of prevalences of different phenomena between patient and control groups.

## **5. Results**

### **5.1. Growth and pubertal development in girls taking valproate, carbamazepine or oxcarbazepine for epilepsy (I)**

The results of the girls on VPA were analyzed in two different ways. In the first, the girls were divided into prepubertal and pubertal groups according to their pubertal stage at the onset of medication (I). For the second analysis, the girls and their controls were divided into prepubertal, pubertal, and postpubertal groups according to pubertal stage at the clinical examination (II).

The data of anthropometric measures are given in Table 6. Figures 3-5 present the growth curves of the patients and the control subjects. Time zero for the Figures 3a,b, 4a,b and 5a,b was the age when the AED was started, and thus, these figures show the effect of the AED on height and weight development in each group of patients. Fig. 3c show the average weight of the girls taking VPA for epilepsy and the control girls at 1-17 years of age.

#### ***5.1.1. Height, linear growth and bone age (I)***

The target height of the girls on VPA tended to be lower than that of the control girls ( $p=0.07$ ), but there were no differences in target heights between other patients and the control groups. The girls taking VPA or CBZ for epilepsy, and the girls who had started OXC in puberty were as tall as their age-matched controls at clinical examination, but the girls who had started OXC in prepuberty were on an average 5.5 cm shorter ( $p=0.03$ ) than the control subjects. The relative heights were similar in the patient and control groups.

The linear growth curves of the VPA- and OXC-treated girls were similar to those of the control subjects, except that the patients' curves ran at a level of approximately 0.3-0.5 SDS lower than the growth curve of the control group. The difference, however, was not statistically significant. The girls on OXC were significantly shorter than the control girls ( $p=0.006$ ) at the age of 15-17 years. The growth of girls on CBZ was similar to that of the controls. The growth spurt occurred between the age of 11 - 15 both in VPA-treated girls

and the control girls. The growth spurt in the CBZ-treated girls appeared to begin at the age of 13 years, while the growth spurt in the control girls started 2 years earlier. There was no notable increase in growth velocity in the mean linear growth curve of the girls on OXC medication. (data not shown)

The average age of the patients when the medication was started was 9.7 years for the girls taking VPA, 8.5 for the girls on CBZ and 10.9 for the girls taking OXC. The growth charts (Fig. 3a, 4a, 5a) did not show any change in growth velocity after the medication was initiated in any patient group, regardless of whether the patients started the AED before or during puberty.

Bone ages did not differ significantly between the patient groups and the controls.

### ***5.1.2. Weight (I, II)***

The girls on VPA were not obese according to their mean body mass index (BMI) (Table 6), although the BMI and relative weight of the VPA-treated girls were higher than those of the controls ( $p=0.03$  and  $0.04$ ). Only two out of seven postpubertal girls with hyperandrogenemia were definitely obese with BMIs of 27 and  $40 \text{ kg/m}^2$ . There were no differences in body fat distribution between the girls on VPA and the controls, i.e. their waist-hip- and waist-thigh ratios were similar. The older girls on VPA were more obese than the younger ones, since the prepubertal girls on VPA had a mean BMI similar to that of the controls ( $17.1$  vs.  $16.5 \text{ kg/m}^2$ ), the mean BMI tended to be higher in pubertal girls ( $19.9$  vs.  $18.0 \text{ kg/m}^2$ ), and was significantly higher in postpubertal girls on VPA compared to the controls ( $22.9$  vs.  $19.7 \text{ kg/m}^2$ ,  $p = 0.03$ ) at clinical examination. The BMI of the girls on VPA with hyperandrogenemia tended to be higher than the BMI of VPA-treated girls with normal serum androgen levels in every pubertal group.

The patients on VPA started to gain weight slowly but progressively after the medication was initiated (Fig. 3b). The prevalence of obesity increased during medication from a frequency 17.5% at the initiation of therapy to 50% after 6 years of therapy ( $p=0.04$ ). This increase was significant after the fourth year of treatment. Weight gain was similar in girls who started VPA before puberty and those who started VPA during puberty, and the relative weight in these two patient groups was similar at clinical examination as well. The relative weight of the control group decreased when the growth velocity increased and there was also a plateau or even slight decrease in the weight curve of the VPA group at the same time. The weight gain was not reflected in an increased growth velocity in girls taking VPA for epilepsy.

BMI, relative weight, waist-hip, or waist-thigh ratios were similar in CBZ- and OXC-treated girls and their control subjects (Table 6). The weight development of CBZ- and OXC-treated girls also did not differ from that of the control subjects. The initiation of CBZ or OXC medication did not significantly affect weight (Fig. 4b and 5b).

*Table 6. Anthropometric measures of the girls with epilepsy and their control girls at clinical examination. \**

Subjects	No. of pairs	Age (yr)	Height (cm)	Relative Height (SDS)	Target height (SDS)	Weight (kg)	Relative weight (%)	Body mass index (kg/m <sup>2</sup> )	Waist-hip ratio	Waist-thigh ratio
Patients on VPA	40	12.4±3.1	149.5±15.3	-0.05±1.1	0.11±0.7	45.8±18.5	109.8±22.5	19.8±4.8	0.80±0.06	1.87 ±0.15
Control girls		12.4±3.0	150.2±14.8	0.04±1.0	0.41±0.6	41.5±11.8	100.6±11.2	18.0±2.5	0.77±0.09	1.82 ±0.21
p		>0.1	>0.1	>0.1	0.07	0.06	0.04	0.03	>0.1	>0.1
Patients on CBZ	18	12.7±3.3	152.7±14.3	0.46±1.2	0.13±0.6	44.5±12.6	104.4±16.8	19.0±3.3	0.80±0.07	1.90 ±0.20
Control girls		12.6±3.2	154.4±14.5	0.59±1.0	0.52±0.4	43.1±12.4	99.3±10.9	17.9±2.5	0.73±0.14	1.74 ±0.31
p		>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
Patients on OXC	18	12.7±3.0	146.5±12.5	-0.20±1.0	-0.06±0.7	40.8±10.0	101.4±11.9	18.0±2.3	0.78±0.04	1.86 ±0.12
Control girls		12.7±3.0	152.5±10.8	0.21±0.6	0.34±0.6	42.4±9.6	99.6±11.5	18.0±2.4	0.79±0.07	1.86 ±0.15
p		>0.1	0.05	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1

\*Values are means ± standard deviations (SD).

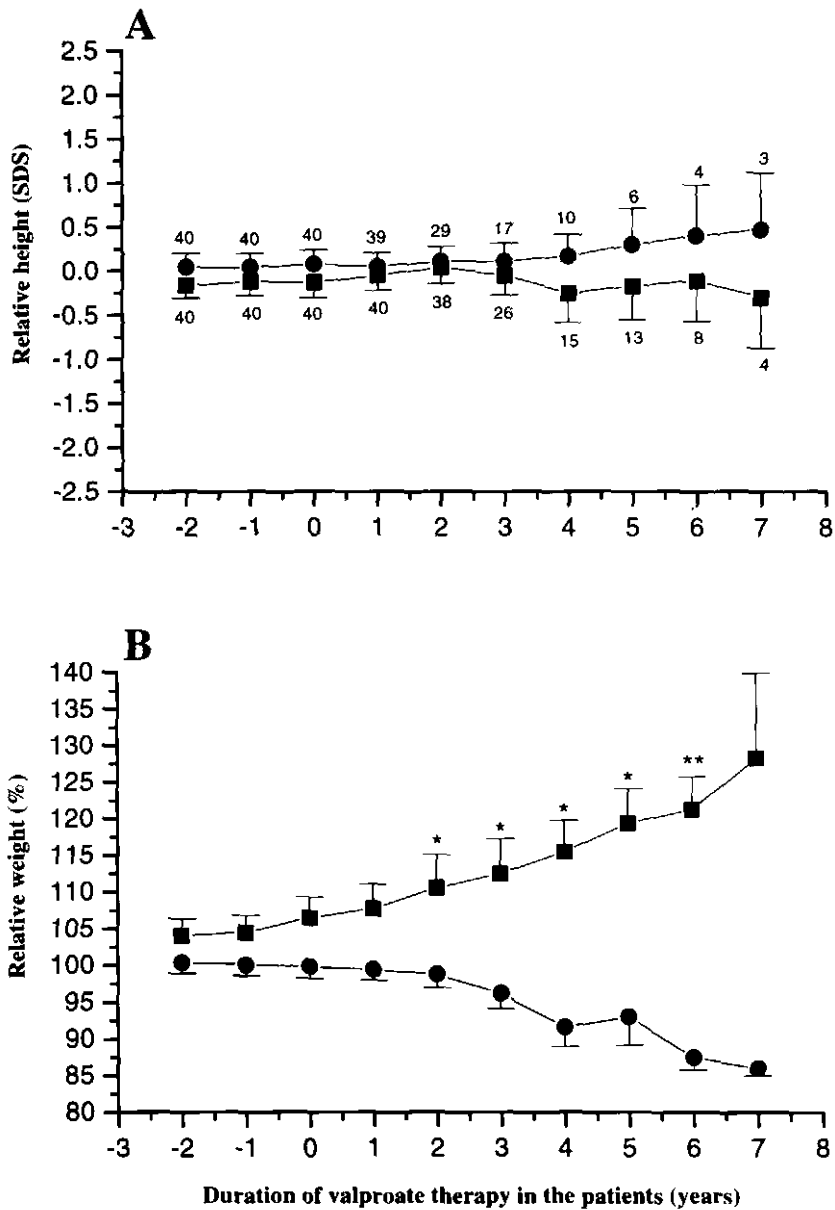


Fig. 3a,b. Mean relative height ( $\pm$ SEM) (A) and mean relative weight (B) in patients taking VPA for epilepsy (-■-), and in the control subjects (-●-) at the same ages, in relation to duration of therapy. Time-point zero is the onset of medication, and the negative numbers represent the time in years before medication was started. The patients and the control girls were approximately 9.7 years old at the time-point zero. The number of subjects at various time-points are expressed as numbers along the curves in figure A. \*  $p < 0.05$ , \*\*  $p < 0.001$

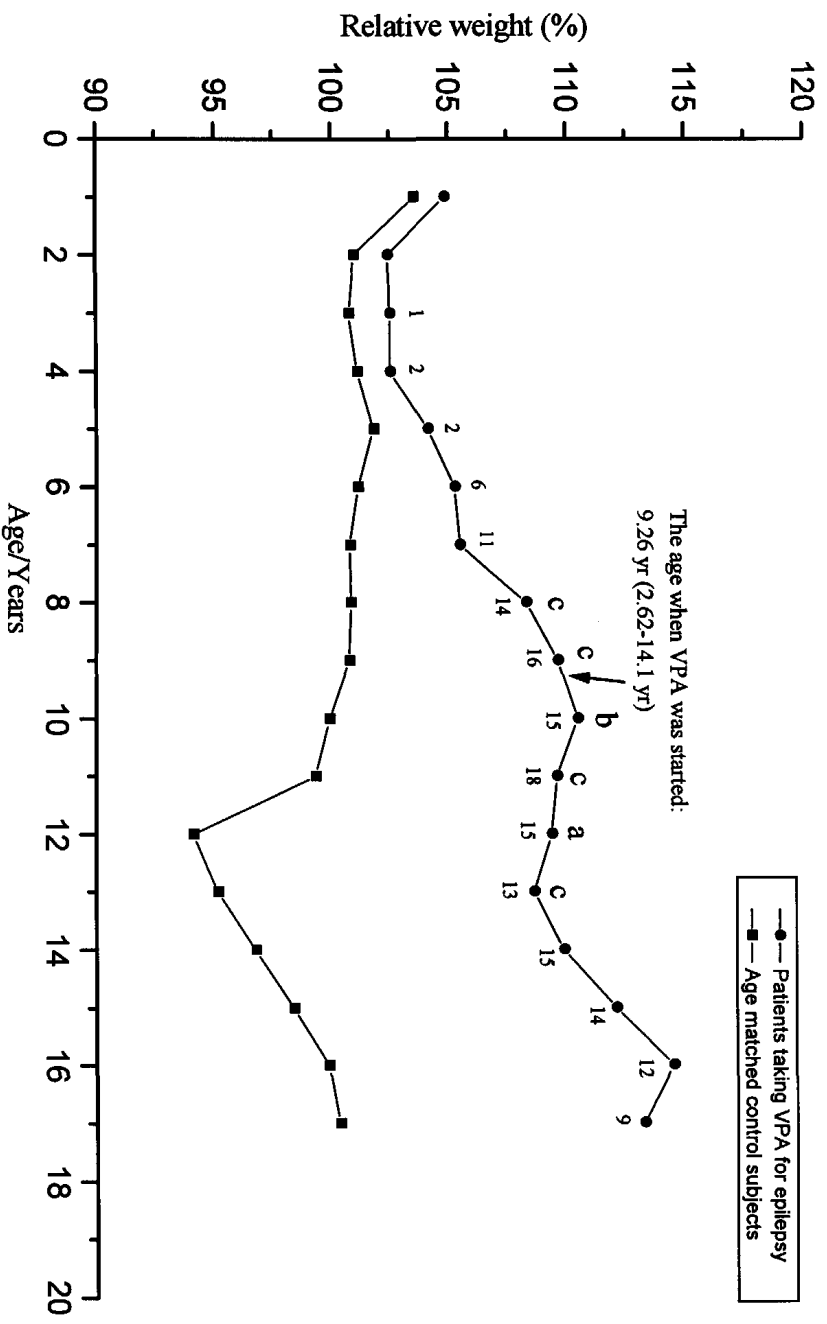


Fig. 3c. Weight curve of the patients taking VPA for epilepsy and their control subjects from 1 to 17 years of age. Numbers along the curve indicate the number of the patients taking VPA at the time. a:  $p < 0.001$ , b:  $p < 0.01$ , c:  $p < 0.02$

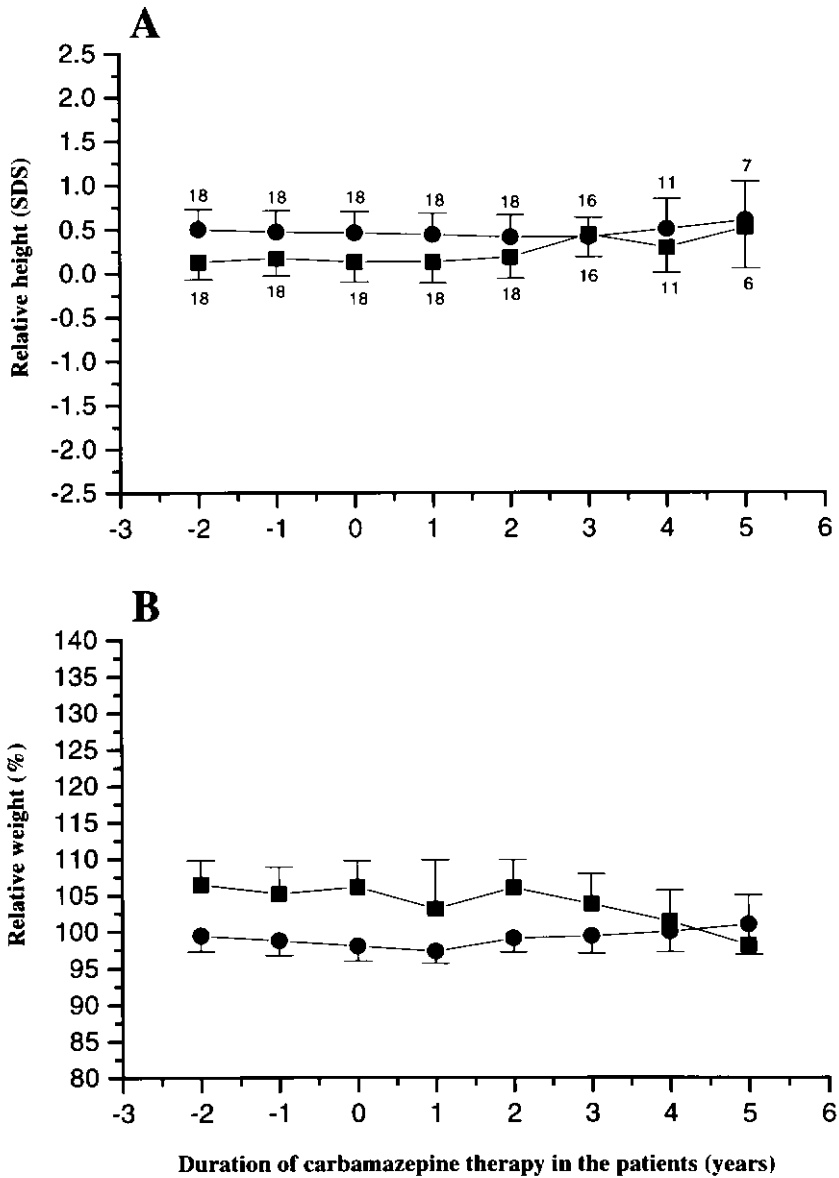


Fig. 4a,b. Mean relative height ( $\pm$ SEM) (A) and mean relative weight (B) in patients taking CBZ for epilepsy (-■-), and in the control subjects (-●-) at the same ages, in relation to duration of therapy. Time-point zero is the onset of medication, and the negative numbers represent the time in years before medication was started. The patients and the control girls were approximately 8.5 years old at the time-point zero. The number of subjects at various time-points are expressed as numbers along the curves in figure A.

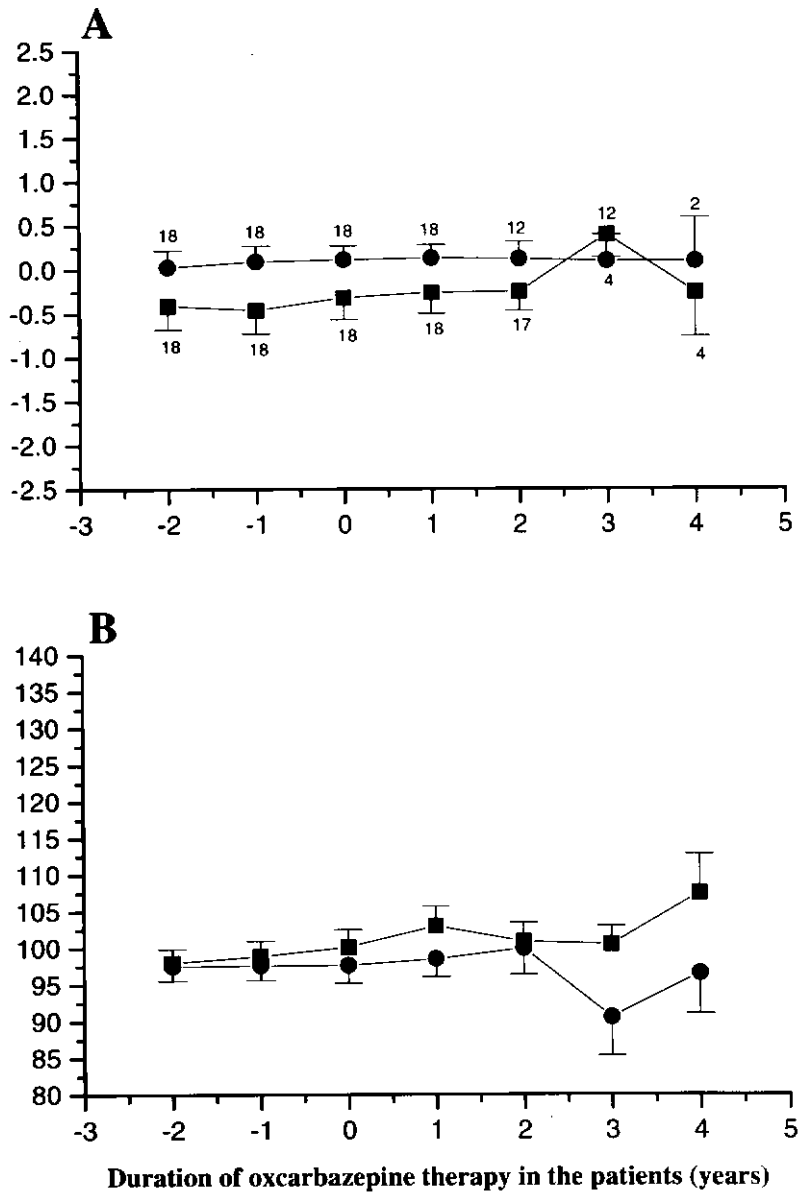


Fig. 5a,b. Mean relative height ( $\pm$ SEM) (A) and mean relative weight (B) in patients taking OXC for epilepsy (-■-), and in the control subjects (-●-) at the same ages, in relation to duration of therapy. Time-point zero is the onset of medication, and the negative numbers represent the time in years before medication was started. The patients and the control girls were approximately 10.9 years old at the time-point zero. The number of subjects at various time-points are expressed as numbers along th curves in figure A.

### ***5.1.3. Development of secondary sexual characteristics (I)***

According to the secondary sexual characteristics, pubertal development did not differ between the girls on VPA and those on CBZ, or between the girls who started OXC in puberty and their control subjects (Tanner & Whitehouse 1976). The pubertal development of girls who started OXC in prepuberty was delayed. The PH and B stages were more immature in the patients than in the control subjects ( $p=0.04$  and  $0.02$ , respectively). However, the pubertal maturation of the mothers of OXC-treated girls also tended to be delayed compared to the mothers of the control girls in terms of an older age at menarche (14.0 vs. 12.9 years).

### ***5.1.4. Menarche, menstruation, and ovaries (I, II)***

Seventeen (41 %) girls on VPA, eight (42 %) girls on CBZ, six (33%) girls on OXC, and 19 (39 %) control girls had experienced menarche. The mean age at menarche did not differ between the patients and the controls. Menstrual cycles and ovarian structure were analyzed in more details in girls taking VPA for epilepsy. The menstrual cycles were irregular in four of the 14 postpubertal patients on VPA (29%) and in eight of the 21 postpubertal control girls (38%). Three of the four postpubertal patients with menstrual disturbances had hyperandrogenemia.

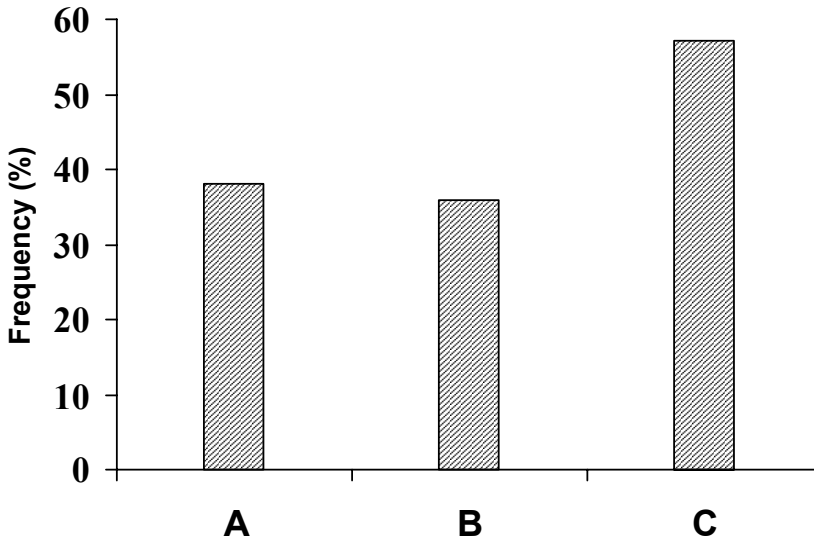
Fourteen of the 16 prepubertal girls on VPA and 16/20 control girls had one or both ovaries visible on ultrasonography. The respective numbers were 10/11 and 12/13 in the pubertal group, and 11/14 and 21/21 among postpubertal girls. VPA treatment had no significant effect on the ovarian volume in any of the groups. Polycystic ovaries, defined by the same criteria as in adult women (Adams *et al.* 1986), were seen in 14% ( $n=2$ ) of prepubertal patients (one with hyperandrogenemia) and none of the prepubertal control girls. Three of the pubertal patients (27%) and two (15%) of the controls had also polycystic ovaries, as well as two (18%) of the postpubertal girls and three (14%) of their control subjects. The pubertal participants and the postpubertal control girls with polycystic ovaries had normal serum androgen levels, but the serum T concentrations of the postpubertal patients on VPA with polycystic ovaries were high.

## **5.2. Reproductive endocrine effects of valproate**

### ***5.2.1. Endocrine effects of valproate in girls (I, II)***

Sixteen of the girls taking VPA for epilepsy were prepubertal, 11 pubertal and 14 postpubertal at the time of clinical examination. The mean serum T concentrations and FAI were significantly higher in the patients than in the control girls regardless of pubertal stage. In addition, the mean serum ADION concentration tended to be higher in pubertal VPA-treated girls than in the controls. There were no significant differences in mean serum concentrations of SHBG, DHEAS,  $E_2$ , LH, or FSH between the patients on

VPA and their control group. (Table 7a). Prevalence of hyperandrogenemia in VPA-treated girls in different pubertal stages is presented in Fig. 6. The youngest patient with hyperandrogenemia was only 8.4 years old. Only one pubertal control girl had high serum T levels.



**Fig. 6.** Frequency of hyperandrogenemia in prepubertal (A), pubertal (B), and postpubertal (C) girls taking VPA for epilepsy. Only one of the pubertal control girls had high serum testosterone levels.

There were no differences in circulating levels of insulin, IGF-I, IGFBP-1 and IGFBP-3 between patients on VPA and the control girls (Table 8), except that the postpubertal girls on VPA had significantly decreased IGFBP-3 levels compared with their controls (3.4 vs. 3.8 mg/l,  $p=0.04$ ). Insulin, IGF-I, IGFBP-1 and IGFBP-3 levels were also compared between the girls on VPA with hyperandrogenemia and VPA-treated girls with normal serum androgen levels. The mean duration of VPA treatment in these two patient groups was similar. The girls on VPA with hyperandrogenemia at the prepubertal stage tended to have lower levels of IGFBP-1 compared with those without hyperandrogenemia (7.5 vs. 11.6  $\mu\text{g/l}$ ), and the pubertal patients with hyperandrogenemia had higher IGF-I levels ( $p=0.01$ ). When the pubertal and postpubertal girls were analyzed together, the mean concentration of IGFBP-1 in patients with hyperandrogenemia was significantly lower than that in girls without hyperandrogenemia (2.2  $\mu\text{g/l}$  vs. 5.6  $\mu\text{g/l}$ ;  $p=0.03$ ).

### ***5.2.2. Endocrine changes in women starting valproate medication (III)***

All ten women who started VPA medication completed the 3 months follow-up. None of them had seizures or serious adverse effects during the first 3 months of medication. Moreover, they did not have menstrual disorders or weight gain.

The mean serum levels of T, LH, and FSH increased ( $p=0.008$ ,  $p=0.005$ , and  $p=0.006$ , respectively) already during the first month of VPA treatment in women. After 3 months of VPA medication their mean serum concentration of SHBG was also increased ( $p=0.03$ ) and serum DHEAS level was decreased (0.04). The circulating levels of other hormones studied (DHEA, ADION,  $E_2$ , progesterone, prolactin) were not altered in women during the first 3 months of VPA treatment. (Table 7a)

All of the VPA-treated women had changes (exceeding 1 SD of the mean serum hormone level of all participants of same sex before the medication was started) in at least one serum reproductive hormone concentration (Fig. 8). Serum levels of T increased in half of the women on VPA (5/10), but they remained unchanged in the other five. There were no significant differences in serum levels of other hormones studied between women on VPA with increased serum T levels and those with stable serum T concentrations. Changes in serum levels of hormones other than T did not seem to be consistent in women after starting VPA treatment.

### ***5.2.3. Endocrine and ovarian changes in women replacing valproate with lamotrigine (V)***

VPA was replaced with LTG in 16 women with VPA-related endocrine disorders. Two patients with juvenile myoclonic epilepsy had to return to VPA medication because of increased seizure frequency, and one patient returned to VPA treatment because of a rash while on LTG. The follow-up was discontinued in one patient because of pregnancy. Thus, the 12-month follow-up was completed in 12 women. The women substituting LTG for VPA in the present study were previously identified as having hyperandrogenism or polycystic ovaries, and they also presented other features of insulin resistance (i.e. hyperinsulinemia, centripetal obesity, and unfavorable serum lipid concentrations). While still on VPA, the women also had higher BMIs, waist and hip circumferences, and waist/hip ratios than the control subjects.

The women lost weight during the first year after discontinuing VPA, and a significant decrease of BMI was observed after 6 months ( $p<0.01$ ) and 12 months ( $p<0.001$ ) (Table 9). Furthermore, a significant reduction of waist/hip ratio was also seen. Fasting serum insulin ( $p < 0.01$  after 2 and 12 months,  $p < 0.05$  after 6 months) and T levels decreased after the change of medication ( $p < 0.001$ ) (Fig. 7). Furthermore, there were favorable changes in serum lipid levels in these patients during the first year after VPA was replaced by LTG. When taking VPA, the women had low serum HDL-cholesterol and high serum triglyceride concentrations compared to the control subjects (HDL-cholesterol: 0.9 mmol/l vs. 1.3mmol/l in the control subjects,  $p<0.001$ , triglycerides: 1.5 vs. 1.1 mmol/l,  $p<0.05$ ) and low HDL/total cholesterol ratios (0.17 vs. 0.27 in the controls,  $p<0.001$ ). HDL-cholesterol levels and HDL cholesterol/total cholesterol ratios

increased ( $p < 0.001$  for both), and serum triglyceride levels decreased ( $p < 0.01$ ) during the first years after VPA was replaced by LTG. However, the concentrations of serum cholesterol were similar in women on VPA and in the control women and did not change during the first year after VPA was replaced by LTG.

The changes in BMI, menstrual cycle and ovarian structures in individual patients after replacing VPA with LTG are presented in Table 9. Seven women had menstrual disturbances during VPA medication, but the menstrual cycles became normal in five of them during the first year after VPA was replaced with LTG ( $p < 0.05$ ). Only one of the women did not have polycystic ovaries on either side while taking VPA. Two women had polycystic ovaries on one side, and 9 women had polycystic ovaries on both sides. After 1 year of LTG medication, 3 women had polycystic ovaries on one side and 4 women on both sides. Thus, the total number of polycystic ovaries decreased from 20 to 11 ( $p < 0.01$ ). At the beginning of the study the women with epilepsy had a higher mean number of follicles than the control subjects ( $p < 0.001$ ), but the number of ovarian follicles decreased after discontinuation of VPA, and no significant difference was found 1 year later. The mean volume of the ovaries tended to decrease during the first year after VPA was replaced with LTG, but the change did not reach statistical significance.

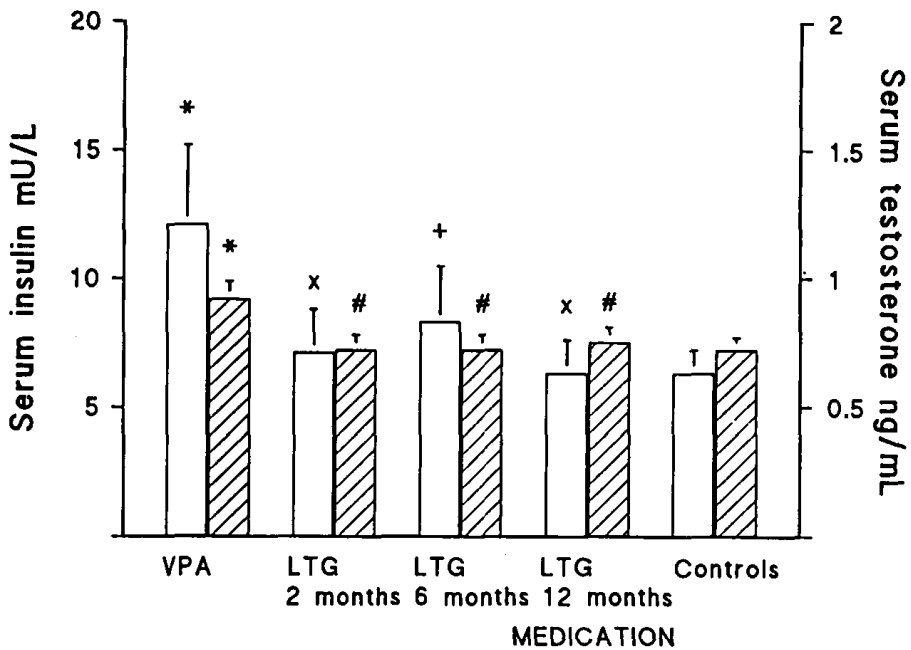


Fig. 7. The mean  $\pm$  SEM fasting serum insulin (left column) and testosterone (right column) levels in 12 women with epilepsy during VPA medication and 2, 6, and 12 months after replacing VPA with LTG and in 24 control women. \* $p < 0.05$ , + $p < 0.05$ , <sup>x</sup> $p < 0.01$ , <sup>#</sup> $p < 0.001$ .

**A. Women on VPA medication.**

no.	T	DHEAS	ADION	SHBG	LH	FSH
1	↑	-	-	↑	-	-
2	↑	-	-	-	-	-
3	↑	-	-	-	↑	-
4	↑	-	↑	-	↑	↑
5	↑	-	↑	-	-	-
6	-	↓	↓	-	-	-
7	-	↓	-	↑	-	↑
8	-	-	-	-	-	↑
9	-	↓	-	-	-	-
10	-	-	↑	-	-	↑

**C. Women on CBZ medication**

No.	T	DHEAS	ADION	SHBG	LH	FSH
1	↑	-	-	↑	-	-
2	↓	↓	-	↑	-	-
3	↓	↓	-	↑	↓	-
4	-	↓	↑	↑	-	-
5	-	↓	-	-	-	↓
6	-	↓	-	-	↓	-
7	-	↓	-	↑	↓	↓

**B. Men on VPA medication.**

no.	T	DHEAS	ADION	SHBG	LH	FSH
1	↑	-	↑	↑	↓	-
2	↑	↑	-	-	-	-
3	↓	↓	-	-	↓	-
4	-	↑	-	-	-	-
5	-	-	↑	-	-	-
6	-	-	↑	-	-	-
7	-	↓	-	-	-	-
8	-	↓	-	-	↓	-
9	-	-	↓	-	-	-
10	-	-	-	-	-	-
11	-	-	-	-	-	-

**D. Men on CBZ medication.**

no.	T	DHEAS	ADION	SHBG	LH	FSH
1	↑	-	↓	-	-	-
2	↑	↓	-	-	-	-
3	-	↓	-	-	-	-
4	-	↓	↓	-	-	-
5	-	↓	-	-	-	-
6	-	↓	-	-	-	-
7	-	-	↓	-	-	-
8	-	-	↓	-	-	-
9	-	-	-	-	-	-
10	-	-	-	-	-	-

**Fig. 8. Serum hormone changes in patients with newly diagnosed epilepsy during the first 3 months of valproate (VPA) or carbamazepine (CBZ) medication. T= testosterone, DHEAS= dehydroepiandrosterone, ADION= androstenedione, SHBG= sex-hormone binding globulin, LH= luteinizing hormone, FSH= follicle stimulating hormone. ↑ = serum hormone concentration increased, ↓ = serum hormone concentration decreased, - = serum hormone concentration did not change significantly.**

Table 7a. Serum hormone concentrations in female patients and the control subjects (Series II, III).

Series	No. of subjects	AED/ controls	Duration of AED treatment	T (nmol/l)	DHEAS ( $\mu$ mol/l)	ADION (nmol/l)	E <sub>2</sub> (nmol/l)	LH U/l	FSH U/l	SHBG (nmol/l)	FAI		
II	16	Prepubertal	VPA	2.3 years	1.0 $\pm$ 0.3 <sup>a</sup>	1.2 $\pm$ 0.9	1.9 $\pm$ 1.0	0.02 $\pm$ 0.01	0.11 $\pm$ 0.11	2.0 $\pm$ 0.9	107.6 $\pm$ 44.5	4.1 $\pm$ 2.2 <sup>a</sup>	
			Controls		0.5 $\pm$ 0.3	1.2 $\pm$ 1.0	1.3 $\pm$ 1.0	0.02 $\pm$ 0.01	0.06 $\pm$ 0.07	1.5 $\pm$ 0.7	98.4 $\pm$ 31.7	1.8 $\pm$ 1.7	
			Pubertal										
		11	Postpubertal	VPA	4.6 years	1.7 $\pm$ 0.8 <sup>d</sup>	2.1 $\pm$ 1.4	4.5 $\pm$ 2.5	0.07 $\pm$ 0.06	1.4 $\pm$ 1.0	3.9 $\pm$ 1.8	87.2 $\pm$ 55.6	10.7 $\pm$ 8.1 <sup>d</sup>
				Controls		1.0 $\pm$ 0.5	2.2 $\pm$ 1.3	3.3 $\pm$ 1.3	0.07 $\pm$ 0.05	2.4 $\pm$ 2.1	4.0 $\pm$ 2.2	82.0 $\pm$ 21.2	4.4 $\pm$ 2.6
				Controls									
	14	Postpubertal	VPA	3.1 years	2.4 $\pm$ 0.9 <sup>a</sup>	5.4 $\pm$ 3.1	8.1 $\pm$ 3.1	0.08 $\pm$ 0.03	3.9 $\pm$ 1.5	5.2 $\pm$ 1.7	69.2 $\pm$ 30.0	14.7 $\pm$ 11.4 <sup>a</sup>	
			Controls		1.1 $\pm$ 0.5	4.5 $\pm$ 2.1	8.1 $\pm$ 3.3	0.11 $\pm$ 0.04	4.4 $\pm$ 2.3	5.3 $\pm$ 1.1	60.1 $\pm$ 13.7	6.6 $\pm$ 2.5	
			Controls										
	III	10	VPA	0	1.4 $\pm$ 0.4	5.0 $\pm$ 1.8	7.5 $\pm$ 3.8	0.16 $\pm$ 0.14	2.6 $\pm$ 0.9	3.7 $\pm$ 1.3	53.2 $\pm$ 22.3	3.0 $\pm$ 1.3	
				1 months	1.8 $\pm$ 0.3 <sup>c</sup>	4.5 $\pm$ 1.3	9.1 $\pm$ 4.2	0.13 $\pm$ 0.06	3.7 $\pm$ 1.1 <sup>b</sup>	5.3 $\pm$ 1.5 <sup>c</sup>	63.7 $\pm$ 28.0	3.2 $\pm$ 1.2	
				3 months	1.7 $\pm$ 0.6 <sup>e</sup>	4.0 $\pm$ 1.8 <sup>d</sup>	9.1 $\pm$ 4.4	0.15 $\pm$ 0.11	3.5 $\pm$ 1.2 <sup>b</sup>	5.2 $\pm$ 2.1 <sup>d</sup>	71.5 $\pm$ 41.2 <sup>d</sup>	3.1 $\pm$ 1.7	
7		CBZ	0	2.1 $\pm$ 0.6	5.7 $\pm$ 1.9	9.6 $\pm$ 3.3	0.11 $\pm$ 0.05	4.9 $\pm$ 1.3	6.7 $\pm$ 3.0	53.9 $\pm$ 22.4	4.1 $\pm$ 1.5		
			1 months	2.1 $\pm$ 0.8	3.5 $\pm$ 1.8 <sup>c</sup>	10.5 $\pm$ 4.7	0.10 $\pm$ 0.06	3.8 $\pm$ 1.4	5.0 $\pm$ 0.9	74.2 $\pm$ 29.6 <sup>a</sup>	3.1 $\pm$ 1.2		
			3 months	2.0 $\pm$ 0.6	3.2 $\pm$ 1.6 <sup>b</sup>	9.9 $\pm$ 3.1	0.08 $\pm$ 0.07	3.9 $\pm$ 1.5 <sup>c</sup>	5.5 $\pm$ 1.6	83.5 $\pm$ 40.3 <sup>c</sup>	2.9 $\pm$ 1.4		

<sup>a</sup>: p = 0.001, <sup>b</sup>: p = 0.005, <sup>c</sup>: p = 0.01, <sup>d</sup>: p = 0.05, <sup>e</sup>: p < 0.1. Patients were compared with the control girls by paired t-test in series II and repeated measures of ANOVA was used in series III. AED=antiepileptic drug, VPA=valproate, CBZ=carbamazepine, OXC=oxcarbazepine, T= testosterone, DHEAS= dehydroepiandrosterone, ADION= androstenedione, E<sub>2</sub>= estradiol, LH= luteinizing hormone, FSH= follicle stimulating hormone, SHBG=sex-hormone binding globuline, FAI=free androgen index.

*Table 7b. Serum hormone concentrations in male patients and the control men (Series III, IV).*

Series	No. of subjects	AED/ controls	Duration of AED treatment	T (nmol/l)	DHEAS (µmol/l)	ADION (nmol/l)	LH (U/l)	FSH (U/l)	SHBG (nmol/l)	FAI
III	12	VPA	0	27.2±11.3	6.9±2.0	10.4±2.9	5.1±2.8	4.0±1.9	41.3 ±28.5	73.0±21.4
			1 months	25.6±9.1	7.4±2.3	10.4±3.4	4.2±1.9	3.4±2.1 <sup>e</sup>	41.1 ±18.3	64.7±17.0
			3 months	29.3±10.6	6.5±3.1	12.5±4.0	4.1±1.7 <sup>e</sup>	3.2±1.5 <sup>b</sup>	44.7 ±25.3	71.7±17.3
10	CBZ	0	23.6±7.5	7.2±3.6	9.2±3.7	4.7±1.6	4.6±3.8	30.9 ±10.1	81.6±32.5	
		1 months	22.9±6.7	3.7±1.7 <sup>b</sup>	7.9±3.5	5.8±2.0 <sup>d</sup>	4.7±3.7	38.2 ±9.8 <sup>d</sup>	62.3±19.5 <sup>e</sup>	
		3 months	25.9±8.8	3.7±2.4 <sup>b</sup>	7.1±3.0	5.0±1.7	4.8±3.3	39.5 ±11.7 <sup>a</sup>	67.7±20.6	
IV	21	VPA	5.2 years	19.4±9.2	7.8±4.3	13.0 ±5.9 <sup>a</sup>	3.4±1.6	2.7±1.0 <sup>d</sup>	36.7 ±16.2	57.1±22.0
			8.8 years	23.0±10.3	4.4±2.6 <sup>a</sup>	8.0±3.0	4.4±1.8	5.5±4.1	49.0 ±23.7 <sup>d</sup>	50.7±17.4
			2.4 years	24.4±7.3	7.3±2.2	9.0±3.0	5.2±3.9	6.5±5.9	45.1 ±18.6	60.2±23.3
25	Controls	0	19.7±6.2	7.5±2.8	7.7±2.4	4.0±1.3	4.4±2.2	37.3 ±18.0	56.5±13.6	

<sup>a</sup> : p = 0.001, <sup>b</sup> : p = 0.005, <sup>c</sup> : p = 0.01, <sup>d</sup> : p = 0.05, <sup>e</sup> : p < 0.1. Repeated measures of ANOVA was used in series III and patients were compared with the control men by independent t-test in series IV. AED=antiepileptic drug, VPA=valproate, CBZ=carbamazepine, OXC=oxcarbazepine, T= testosterone, DHEAS= dehydroepiandrosterone, ADION= androstenedione, E<sub>2</sub> = estradiol, LH= luteinizing hormone, FSH= follicle stimulating hormone, SHBG=sex-hormone binding globuline, FAI=free androgen index.

Table 8. BMIs, serum concentrations of insulin, insulin-like growth factor binding proteins -1 and -3 (IGFBP-1 and -3), and plasma concentration of IGF-I in girls and men with epilepsy and the control subjects in series I and IV, means  $\pm$  SD.

No. of subjects		AED/ controls	Duration of AED treatment (years)	Insulin (mU/l)	IGFBP-1 ( $\mu$ g/l)	IGF-I (nmol/l)	IGFBP-3 (mg/l)
Girls	Men						
38		VPA	2.8	5.8 $\pm$ 4.0	3.3 $\pm$ 0.6	38.8 $\pm$ 18.3	3.3 $\pm$ 0.6
38		controls		6.5 $\pm$ 3.0	3.6 $\pm$ 0.6	33.5 $\pm$ 12.8	3.6 $\pm$ 0.6
17		CBZ	4.1	7.0 $\pm$ 3.4	3.8 $\pm$ 0.6	51.7 $\pm$ 20.6 <sup>a</sup>	3.8 $\pm$ 0.6
17		controls		7.1 $\pm$ 3.5	3.6 $\pm$ 0.6	36.2 $\pm$ 14.5	3.6 $\pm$ 0.6
16		OXC	1.9	7.3 $\pm$ 4.9	3.7 $\pm$ 0.6	52.9 $\pm$ 25.3 <sup>b</sup>	3.7 $\pm$ 0.6
16		controls		6.7 $\pm$ 3.0	3.6 $\pm$ 0.7	35.9 $\pm$ 12.6	3.6 $\pm$ 0.7
	21	VPA	5.2	10.9 $\pm$ 11.7 <sup>c</sup>	2.4 $\pm$ 2.1	20.5 $\pm$ 3.9	3.1 $\pm$ 0.5
	40	CBZ	8.8	6.8 $\pm$ 4.2 <sup>a</sup>	3.5 $\pm$ 2.9	20.4 $\pm$ 4.9 <sup>c</sup>	3.0 $\pm$ 0.6
	29	OXC	2.4	5.5 $\pm$ 2.6 <sup>c</sup>	2.8 $\pm$ 2.1	21.3 $\pm$ 5.6 <sup>d</sup>	3.2 $\pm$ 0.7
	25	controls		3.2 $\pm$ 2.0	3.0 $\pm$ 2.2	17.1 $\pm$ 4.4	3.0 $\pm$ 0.5

<sup>a</sup> : p < 0.001, <sup>b</sup> : p < 0.005, <sup>c</sup> : p < 0.01, <sup>d</sup> : p < 0.05. Girls with epilepsy were compared with the control girls by paired t-test and men with epilepsy were compared with the control men by independent t-test. AED= antiepileptic drug, VPA= valproate, CBZ= carbamazepine, OXC=oxcarbazepine, IGF-I=insulin-like growth factor, IGFBP-1 and -3=IGF-binding proteins 1 and 3

Table 9. Changes in body mass index (BMI), menstrual cycle and ovarian structure in women replacing valproate (VPA) with lamotrigine (LTG) (Series V).

Patient number	Age (yr)	Duration of VPA medication (yr)		BMI (kg/m <sup>2</sup> )		Duration of menstrual cycle (days)		PCO	Ovdx/Ovsin After 12 months of LTG
		Before VPA*	During VPA	Before LTG	During LTG	During VPA	After 12 mo of LTG		
1	38	4	20.7	23.3	21.1	28	27	+/U	+/+
2	32	18	18.8	20.1	19.6	34	30	+/+	+/+
3	39	1.5	36.2	37.9	33.7	28	28	+/+	-/-
4	36	19	23.3	41.6	35.5	Amenor	30	+/+	-/-
5	33	14	24.4	31.7	30.1	28	28	+/+	U/+
6	22	9	24.6	37.5	32.1	Amenor	Amenor	-/-	-/-
7	20	5	29.6	31.6	33.8	Amenor	Olig	+/+	+/+
8	34	4	21.3	22.1	22.1	28	28	+/+	-/-
9	26	11	21.1	38.2	32.3	Olig	28	+/+	-/+
10	37	11	22.7	24.4	23.0	32-40	30	+/+	-/-
11	21	8	20.4	31.9	27.7	Olig	29	+/+	+/+
12	19	4	26.2	30.9	28.5	Olig	28	+/U	-/+
Mean (SD)		29.8 (7.6)	9.0 (5.7)	24.1 (4.8)	30.9 (7.1)	28.3 (5.6)			

\*Data obtained from hospital records. VPA=valproate, LTG= lamotrigine, BMI= body mass index, PCO=poly-cystic ovary, Ovdx=right ovary, Ovsin= left ovary, Amenor=amenorrhea, Olig=Oligomenorrhea, U=unidentifiable.

### ***5.2.4. Endocrine changes in men starting valproate medication (III)***

All twelve men completed the 3-month follow-up and none of them had any seizures or serious side effects during the first 3 months of medication. None gained weight significantly. The results are presented in Table 7b and Fig. 8.

The mean serum levels of FSH decreased ( $p=0.05$ ) and progesterone increased ( $0.001$ ) in men during the first month of VPA therapy. After 3 months of VPA treatment their serum DHEA levels were also increased ( $p=0.05$ ) and their LH levels tended to decreased ( $p=0.09$ ). The mean serum levels of the other hormones studied and the FAI were not changed in men on VPA. There was no consistent pattern of changes in the serum hormone levels of individual men at the beginning of VPA treatment.

### ***5.2.5. Hormonal and sexual function in men on long-term valproate therapy (IV)***

Elevated serum androgen levels (serum T, and/or ADION and/or DHEAS concentrations above the reference range) were observed in 12 (57%) patients on VPA but only in two (8%) control men ( $p<0.001$ ) (Fig. 9). Men taking VPA for epilepsy had higher mean serum ADION concentrations and lower serum progesterone levels than the control subjects ( $p<0.001$  and  $p=0.03$ , respectively), but the mean serum levels of other steroid hormones (T, E<sub>2</sub>, DHEA, DHEAS), and mean serum prolactin, LH and SHBG levels and the FAI ratio were similar in men taking VPA and in the control men (Table 7b). The E<sub>2</sub>/T ratio was higher ( $p=0.04$ ) and the mean serum concentration of FSH was lower in men on VPA than in the control men ( $p=0.02$ ). Serum insulin levels were higher in men on VPA than in the controls, although their mean BMIs were similar. There were no differences in the circulating concentrations of IGF-I, IGFBP-1 or -3 between men on VPA and the control subjects. (Table 8)

The VPA-treated patients with high serum androgen concentrations were more obese than the patients on VPA with normal serum androgen levels (BMI 26.2 kg/m<sup>2</sup> vs. 23.7 kg/m<sup>2</sup>,  $p=0.03$ ). Furthermore, serum SHBG concentrations were higher in men on VPA with high serum androgen levels than in VPA-treated men with normal serum androgens (43.8 vs 27.2 nmol/l,  $p=0.01$ ), but their serum levels of gonadotropins and insulin were similar.

One man (5%) on VPA reported decreased sexual functions, but according to hospital records his sexual dysfunction had started already when he was on CBZ medication. On the other hand, enhanced sexual function (increased libido, potency, or both) was reported by four patients (19%) on VPA. Three of the control men reported changed sexual function, but the differences in prevalence of changed sexual function between patients and controls did not reach statistical significance.

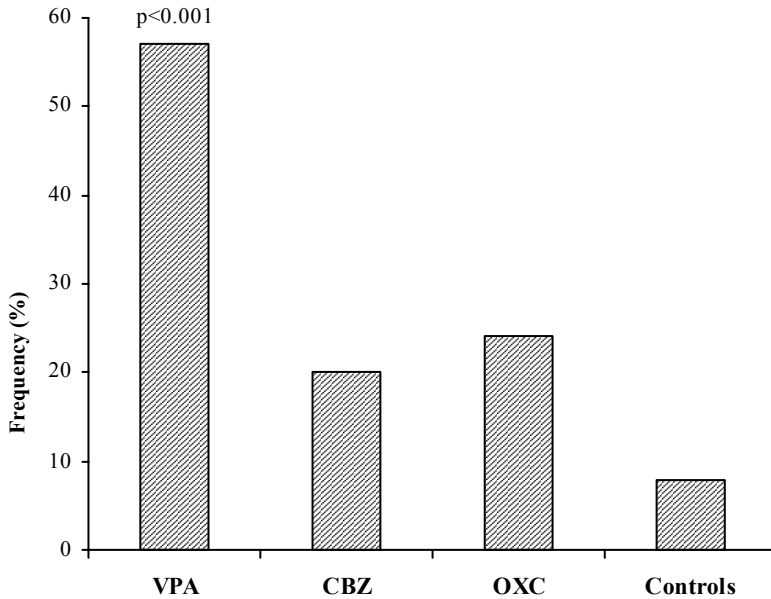


Fig. 9. Frequency of high serum androgen levels in men taking valproate (VPA), carbamazepine (CBZ) or oxcarbazepine (OXC) for epilepsy and in the control men.

### 5.3. Reproductive endocrine effects of carbamazepine and oxcarbazepine

#### 5.3.1. Circulating concentrations of insulin, IGF-I, IGFBP-1 and -3 in girls on carbamazepine or oxcarbazepine treatment (I)

The plasma concentrations of IGF-I were higher in the CBZ- and OXC-treated girls than in the control group ( $p<0.001$  and  $p=0.004$ ), but their serum concentrations of insulin, IGFBP-1 and IGFBP-3 were similar. (Table 8)

#### 5.3.2. Hormonal changes in patients starting carbamazepine medication (III)

All patients on CBZ (7 women, 10 men) completed the 3-month follow-up. None of the patients had any seizures or serious side effects during the first 3 months of medication. None of the women had menstrual disorders and none of the patients had gained weight significantly at the end of the 3-month period.

The serum levels of SHBG increased ( $p=0.001$  in women and  $0.03$  in men) and levels of DHEAS decreased ( $p=0.009$  in women and  $0.002$  in men) in patients during the first month of CBZ medication. In women, serum LH concentrations also tended to be low ( $p=0.08$ ) and progesterone levels to be high ( $p=0.09$ ) after the 3 month follow-up. In addition, the FAI ratios decreased in men after starting CBZ medication ( $p=0.01$  after 1 month,  $p=0.1$  after 3 months), but the decrease in the  $E_2$ /SHBG ratios was not statistically significant in women. A transient increase was also observed in serum levels of LH in men on CBZ therapy. There were no significant changes in serum concentrations of the other hormones studied (T,  $E_2$ , ADION, DHEA, prolactin, FSH) in patients taking CBZ medication. (Table 7a, b)

### ***5.3.3. Endocrine effects of long-term carbamazepine treatment in men with epilepsy (IV)***

Seven men (18%) on CBZ complained of decreased libido or decrease potency, while three men (8%) on CBZ reported increased libido or increased potency. Elevated serum androgen levels (serum T, and/or ADION and/or DHEAS concentration above the reference range) were observed in eight (20%;  $p=NS$ ) of the CBZ-treated men.

Men taking CBZ for epilepsy had higher mean serum SHBG concentrations than the control subjects ( $p=0.02$ ). Their serum levels of DHEAS were lower, FAI values tended to be lower ( $p<0.001$  and  $p=0.08$ , respectively) and their circulating levels of insulin and IGF-I were higher than in the control men. (Table 8) However, the circulating concentrations of the other hormones studied were not significantly different between patients on CBZ medication and the control subjects, and their mean BMI's were also similar. Most of the endocrine effects of CBZ were not dose-dependent, but serum prolactin levels were lower in CBZ-treated patients with daily doses  $> 600$  mg/day than in patients with daily doses of 600 mg or less ( $p=0.01$ ).

### ***5.3.4. Reproductive endocrine effects of oxcarbazepine in men with epilepsy (IV)***

Five men (17%) on OXC reported decreased sexual functions. Four of these men were previously on CBZ medication and, according to hospital records, sexual dysfunction had already developed during CBZ treatment in one of these patients. One (3%) OXC-treated man reported enhanced sexual function (increased libido, potency, or both). Elevated serum androgen levels (serum T, and/or ADION and/or DHEAS concentration above the reference range) were observed in seven (24 %) of the OXC-treated men (Fig. 9). The differences in the prevalence of reported changes in sexual function or high serum androgen levels between men on OXC and the controls were not statistically significant.

The circulating levels of insulin and IGF-I were higher in men taking OXC for epilepsy than in the control men, but there were no significant differences in serum concentrations of the other hormones studied between the patients on OXC and the

control subjects. The serum concentrations of T, LH and SHBG tended to be higher in the OXC-treated men than in the control men ( $p = 0.08, 0.07$  and  $0.08$ , respectively). (Tables 7b, 8)

Patients with a daily OXC dose of less than 900 mg had serum hormone concentrations similar to those of the control men, but their circulating levels of insulin and IGF-I were higher ( $p=0.01$  for both). Patients with a high daily OXC dose ( $\geq 900$  mg/day) had increased serum levels of T, LH, FSH and SHBG when compared to the control subjects ( $p = 0.008, 0.003, 0.02$  and  $0.005$ , respectively). The mean daily OXC dose was  $1286 (\pm 449)$  mg in the men with high serum androgen levels. None of these men had an OXC dose of less than 900 mg/d. Men with normal serum androgen levels received a mean daily OXC dose of  $1002 (\pm 422)$  mg. However, the difference in daily OXC dose between patients with high and normal androgen levels was not statistically significant.

## 6. Discussion

### 6.1. General aspects

Treatment of epilepsy requires in most cases long-term, sometimes even a life-long antiepileptic medication. The majority of patients with recently diagnosed epilepsy will enter substantial remission with AED treatment. For these patients, epilepsy per se does not seem to be a problem because of good seizure control. However, the adverse effects of chronic antiepileptic medication may cause quality of life changes for these patients.

The purpose of this study was to clarify the reproductive endocrine effects of AED monotherapy in patients with epilepsy. Therefore, only patients of reproductive age (III-V) or young girls (I, II) on a single AED, with no diseases other than epilepsy, were included. Patients with intractable epilepsy, institutionalized or aged patients with epilepsy, and patients with symptomatic epilepsy caused by a progressive brain disease were not examined. However, the study patients in series I-IV with well-controlled epilepsy represent the majority of epileptic patients. Women with clinically observed VPA-related endocrine disorders were selected to series V.

Serum concentrations of most hormones fluctuate according to sex, age, diurnal rhythm, blood glucose levels, phase of the menstrual cycle, and environmental factors. Therefore, the participants were grouped into various substudies according to sex and age. Furthermore, for the statistical analyses they were divided into subgroups according to antiepileptic medication, and pubertal maturation (I, II), and blood sampling was standardized (see Chapter 4.2.2.). Frequent measurements over a long time period (e.g. blood specimens in every 10 minutes during 24 hours) would be needed for a fully reliable analysis of pulsatile secretions of some hormones (e.g. gonadotropins and prolactin) (Apter *et al.* 1994b), but studies on large groups of patients and controls preclude that type of analysis. Therefore, in clinical studies, analyses of hormones from one blood specimen have been considered sufficient (Connell *et al.* 1984a, b, Haidykewych & Rodin 1987, Kusunoki *et al.* 1988, Macphee *et al.* 1988, Isojärvi *et al.* 1989-1996, Herzog *et al.* 1991, Geisler *et al.* 1997). In the present study measurements of weight, height, body fat distribution, etc. as well as ultrasound examinations and bone age determinations were always done with the same equipment and by the same physicians in order to avoid random errors.

Both epilepsy per se and antiepileptic medications are known to affect serum concentrations of reproductive hormones and it may be difficult to distinguish the effect of epilepsy and the effect of treatment. However, most of the patients in this study were seizure-free during the present medication and there were no changes in the epileptic activity in patients completing the longitudinal parts of this study. Therefore, the results of this study indicate that the reproductive endocrine disorders in patients with epilepsy are primarily induced by antiepileptic medication and not by epilepsy per se.

A very high occurrence of reproductive endocrine disorders has been reported among women taking VPA for epilepsy (Isojärvi *et al.* 1993, 1996) and CBZ medication is also related to hormonal and menstrual disturbances in women (Isojärvi *et al.* 1995a). However, the effects of AEDs on hormonal function in young girls are not known. Since growth and sexual maturation in adolescence are regulated by a complex neuroendocrine system (Styne 1995), it is important to clarify whether AED-related hormonal disorders interfere with growth or pubertal maturation in girls. Previous knowledge about reproductive endocrine effects of VPA in men, as well as the development and reversibility of VPA-related endocrine disorders, has also been limited. Furthermore, the endocrine effects of OXC had not been previously studied.

## **6.2. Weight gain, linear growth and pubertal development in girls with epilepsy**

Linear growth and pubertal development did not differ between the girls with epilepsy and the control girls in the present study, but relative weight increased significantly in girls on VPA medication. Although VPA-related weight gain has been studied extensively, the present study is the first to compare the effects of VPA on weight gain at different pubertal stages and on the endocrine system during pubertal maturation.

Obesity was associated with VPA-related endocrine disorders in women in previous studies (Isojärvi *et al.* 1993, 1996), but the VPA-treated girls in this study were not obese, with the exception of two postpubertal girls. However, the mean BMI of VPA-treated girls was higher than that of the control girls, and their mean weight chart showed a progressive increase after VPA was started. The weight gain of VPA-treated girls was similar regardless of whether the medication was started before or during puberty. It has been suggested previously that VPA-related weight gain in children may be noticeable shortly after initiating the medication, but the doses of VPA used in those patients were high: 25-30 mg/kg (Egger & Brett 1981). In our study, the doses were smaller; averaging 16.5 mg/kg (8.7-27.5 mg/kg) and weight gain was not observed until after 2-3 years of medication. This may indicate that the higher the VPA dose the faster the weight gain. Previous studies suggest that VPA-related weight gain is progressive in nature (Isojärvi *et al.* 1996), which is confirmed by the weight curve of the VPA-treated girls in the present survey. Early in puberty, when the girls had their growth spurt, there was a 3-year plateau in weight gain, but at the same time the weight curve of the control subjects decreased sharply. In most previous studies pubertal and skeletal maturation have been normal in children taking VPA for epilepsy, but the results of some studies suggest that they may also be delayed (Lundberg *et al.* 1986, Cook *et al.* 1992, Sheth *et al.* 1995, Snyder &

Badura 1995, Snyder & Badura 1998). In the present study, neither VPA medication itself nor the associated weight gain seemed to affect linear growth or pubertal development, since mean relative height, bone age, and sexual maturation were similar in patients taking VPA and in the control subjects.

The patients who started to take OXC for epilepsy before puberty were shorter than their control subjects, although their target height and bone age were similar to those of the control girls. Moreover, their pubertal maturation was delayed according to the clinical signs of puberty. This may, however, be due to genetic factors, since the mothers of OXC-treated girls had experienced their menarche on an average more than 1 year later than the mothers of the controls. Linear growth and pubertal maturation of the girls who started OXC during puberty and girls on CBZ were similar to those of the control girls. Therefore, VPA, CBZ or OXC do not seem to affect the hormonal balance in prepubertal and pubertal girls to an extent that could lead to obvious disturbances in linear growth or in pubertal development.

### **6.3. Endocrine effects of valproate**

#### ***6.3.1. Reproductive endocrine effects of valproate in girls with epilepsy***

Recent studies have shown that 80 % of women who had started long-term VPA medication before the age of 20 years suffer from hyperandrogenism and/or polycystic ovaries, which are associated with hyperinsulinemia and obesity (Isojärvi *et al.* 1993, 1996). In the present study, hyperandrogenemia and ovaries with numerous follicles were also found in young girls on VPA. The frequency of hyperandrogenemia seemed to increase with pubertal development, as one third of the prepubertal and pubertal girls had elevated serum T concentrations, whereas more than half of the postpubertal girls were affected.

The finding of increased T already in prepubertal girls implies that VPA affects serum steroid concentrations already before and during the sensitive period when pubertal maturation is initiated. These data suggest that a mature reproductive endocrine system is not necessary for the development of VPA-related endocrine disorders. Excess production of adrenal steroids could be the explanation, especially since high serum levels of DHEAS, which is the main adrenal androgen, are reported to be associated with VPA-related endocrine disorders in adult women (Isojärvi *et al.* 1993, 1996). Indeed, adrenal androgens are known to function as precursors for stronger androgens like T, especially in women in pathologic stages (Haning *et al.* 1991a, b). However, serum levels of adrenal androgens including DHEAS, DHEA, and ADION did not differ between VPA-treated girls and their controls in the present study. Similarly, there were no differences in serum gonadotropin concentrations between girls on VPA and the control girls in the present study, although serum gonadotropin levels were observed to decrease during VPA treatment in pubertal patients in an earlier survey (Lundberg *et al.* 1986). The mean serum level of SHBG was also normal in prepubertal, pubertal and postpubertal girls on VPA in this study.

Hyperinsulinemia was common in women with VPA-related endocrine disorders in a previous survey (Isojärvi *et al.* 1996), but in the present study serum insulin levels were normal in girls taking VPA for epilepsy. There were also no differences in serum insulin levels between VPA-treated girls with hyperandrogenemia and the girls on VPA with normal serum androgen levels. The features of VPA-related endocrine disorders seemed to differ between adult women in previous studies and young girls in the present study. These girls had taken VPA for epilepsy approximately 2.8 years (range 0.8-10.3 y), while in the previous studies the women with VPA-related endocrine disorders had been taking VPA medication for a longer period of time (on an average  $7 \pm 5$  years) (Isojärvi *et al.* 1993, 1996). Furthermore, the mean duration of VPA therapy was  $9.0 \pm 5.7$  years in women with VPA-related endocrine disorders replacing VPA with LTG in the present study (V). The underlying pathogenic mechanisms of VPA-related endocrine disorders are still unknown, but they seem to be progressive in nature. The differences between girls and women taking VPA for epilepsy may arise both from a shorter duration of medication in the girls, and naturally from differences in the reproductive endocrine systems of adults, adolescents, and children. However, it is likely that the girls with hormonal changes, polycystic ovaries, and weight gain in the present study may suffer from obesity and reproductive endocrine problems in adulthood if their VPA medication is continued.

### ***6.3.2. Reproductive endocrine changes in women after starting valproate treatment***

In the present study, VPA induced hormonal changes in women already during the first 3 months of medication, but no clinical signs of reproductive endocrine disorders, e.g. menstrual disturbances, or weight gain could be observed during that time, although endocrine adverse effects are known to be common in women during long-term VPA treatment (Isojärvi *et al.* 1993, 1996). The mean serum levels of T, gonadotropins, and SHBG increased, but DHEAS decreased in women after initiating VPA medication.

There are no previous prospective data on endocrine function in women after starting VPA medication. In the present study, serum T concentrations increased in half of the women during the first 3 months of VPA therapy, a proportion that is similar to the frequency of VPA-induced hyperandrogenism in patients on long-term treatment (Isojärvi *et al.* 1993, 1996). It is possible that the women with increased serum T levels at the beginning of VPA therapy are those who develop VPA-related endocrine symptoms later during long-term medication. This may be clinically important in the treatment of women of reproductive age with epilepsy.

In a previous study, serum DHEAS levels have been reported to be higher and serum SHBG levels lower in women with hyperandrogenism than in women with normal serum T levels during long-term VPA treatment (Isojärvi *et al.* 1993, 1996). In this study the mean serum DHEAS level actually decreased and serum SHBG levels increased in women after starting VPA. The reasons for these changes are unclear. Since VPA does not induce liver enzymes, the increased SHBG levels are probably not mediated by direct hepatic effects. The production of SHBG is known to be regulated by several hormones,

e.g. by sex steroids, and thus an altered hormonal balance related to the initiation of VPA-treatment may be of importance. The patients with VPA-related endocrine disorders during long-term treatment were also obese and had high serum insulin levels, which are known to inhibit the synthesis of SHBG (Conway & Jacobs 1993, Isojärvi *et al.* 1996). Therefore, it is possible that although serum SHBG levels are increased during the first months of VPA therapy, hyperinsulinemia would decrease serum SHBG levels in women with endocrine disorders related to long-term VPA treatment. The reasons for the changes in serum DHEAS levels during VPA medication remain unclear.

Serum gonadotropin levels were increased in women on VPA after the first months of therapy in the present study, while serum  $E_2$  levels of women starting VPA were normal, although LH stimulates the production of  $E_2$  in women. High serum gonadotropin levels could be partly responsible for increased serum T levels in women starting VPA medication, but despite hyperandrogenism, serum gonadotropin levels were normal in women in a previous study (Isojärvi *et al.* 1996) and normal in girls in the present study during long-term VPA medication. However, due to the pulsatile secretion of gonadotropins further studies are needed to confirm the short-term effects of VPA on gonadotropin concentrations. Indeed, according to Lado Abeal *et al.* 1991 and 1996, short-term VPA administration did not affect LH secretion in women. Serum levels of other hormones studied (prolactin, progesterone, DHEA, ADION) remained normal in women during the first months of VPA medication in this study, and serum levels of these hormones were also reported to be normal in women during long-term VPA treatment (Isojärvi *et al.* 1993, 1996).

### ***6.3.3. Reproductive endocrine changes in women after replacing valproate with lamotrigine***

The women substituting LTG for VPA had previously identified hyperandrogenism and/or polycystic ovaries, and they were also found to present other features of insulin resistance (i.e. hyperinsulinemia, centripetal obesity, and unfavorable serum lipid concentrations). Thus, the VPA-induced changes in ovarian function are very similar to those found in PCOS (Isojärvi *et al.* 1993, Franks 1995, Isojärvi *et al.* 1996).

Serum T and insulin concentrations decreased already during the first 2 months after LTG was substituted for VPA. Normalization of the endocrine functions resulted in a gradual recovery of normal ovarian structures and regular menstrual cycles in most of the women during the first year after replacing VPA with LTG. The patients participating in this study were obese (BMI  $30.9 \pm 7.1$  kg/m<sup>2</sup> at the beginning), but they started to lose weight after changing the medication. In a previous study, patients with VPA-related weight gain also had increased fat-free mass (muscle) in addition to an increased amount of adipose tissue (Gidal *et al.* 1996), which may be due to anabolic effects of increased serum T levels. In the present study weight loss was more rapid during the first 2 months after the change of medication, 0.4 kg/m<sup>2</sup>/month, then decreased to approximately 0.2 kg/m<sup>2</sup>/month during the next 10 months of follow-up. It is possible that the decrease in serum T levels resulted in part of the decreases in BMIs shortly after the change of medication. A significant reduction in the waist/hip ratio was also seen in this study after

1 year of LTG therapy. A high waist/hip ratio indicates centripetal obesity, which is associated with insulin resistance and hyperinsulinemia more often than peripheral obesity of the female type (Evans *et al.* 1983, Pasquali & Casimirri 1993). Therefore, normalization of serum insulin levels may be of importance in weight loss after VPA was replaced with LTG.

In the present study unfavorable changes in serum lipid concentrations were also found in women taking VPA for epilepsy. Since hyperinsulinemia, insulin resistance, centripetal obesity, and unfavorable serum lipid profile are associated with increased risk for cardiovascular diseases (Reaven 1988, Wild *et al.* 1990, DeFronzo & Ferrannini 1991, Conway *et al.* 1992, Conway & Jacobs 1993, Wild 1994, Han *et al.* 1995, Reaven 1995), the results of the present study show that the women on VPA clustered risk factors for coronary heart disease. However, in addition to recovery of endocrine function and ovarian structure and weight loss, substituting LTG for VPA also resulted in a normalization of serum lipid levels. Therefore, replacing VPA with LTG may also reduce the risk for cardiovascular disease substantially in obese women with epilepsy and hyperandrogenism.

#### ***6.3.4. Reproductive endocrine effects of valproate in men***

VPA has been considered a safe AED from the endocrine point of view in male patients (MacPhee *et al.* 1988, Isojärvi *et al.* 1990, Geisler *et al.* 1997). The results of this study show that the use of VPA is also associated with changes in serum levels of reproductive hormones in men with epilepsy. Mean serum ADION levels were high and close to 60 % of the men on long-term VPA had serum T, ADION and/or DHEAS concentrations above the reference range. However, no consistent pattern of altered serum androgen levels was observed in men with newly diagnosed epilepsy during the first 3 months of VPA medication. Serum gonadotropin levels were low or tended to decrease both in men after starting VPA and in men on long-term VPA treatment, but VPA did not seem to disturb sexual function in men. Moreover, the men starting VPA medication did not gain weight during the first few months of medication and the men on long-term VPA treatment were not obese.

Serum androgen levels also seem to be modified in men taking VPA for epilepsy, which is similar to the situation in women. As is well known, pituitary gonadotropin secretion is controlled by a negative feedback mechanism, in which increased levels of T inhibit the production of gonadotropins in men (Crowley *et al.* 1991). Serum LH and FHS levels were decreased in men on VPA medication both after starting the treatment and during long-term medication in this study as well as in previous studies (MacPhee *et al.* 1988, Isojärvi *et al.* 1990, Geisler *et al.* 1997). This could theoretically be related to activation of this negative feedback mechanism as a consequence of excess T production stimulated by VPA. However, there were no differences in serum gonadotropin concentrations between patients on VPA with normal serum androgen levels and VPA-treated men with high serum androgen levels. It is possible that although VPA would affect androgen metabolism in men, an inhibitory regulation of androgen biosynthesis prevents excess T production.

Obesity and hyperandrogenism appear to be associated with hyperinsulinemia and altered circulating levels of IGF-I, IGFBP-1 and -3 in female patients taking VPA for epilepsy (Isojärvi *et al.* 1993, 1996). In the present study, the men on VPA with high serum androgen concentrations were also more obese than men with normal serum androgen levels, although obese men in general have lower serum androgen concentrations than men with normal weight (Mårin *et al.* 1992). The mean serum insulin level in men taking long-term VPA medication was high, but, in contrast to the situation in women on VPA medication, hyperinsulinemia was not associated with high serum androgen levels in VPA-treated men since we could not demonstrate any differences in circulating levels of insulin, IGF-I or IGFBP-3 between VPA-treated men with normal serum androgen levels and those with high serum androgen levels.

### ***6.3.5. Pathogenesis of valproate-induced reproductive endocrine disorders***

#### ***6.3.5.1. Hyperandrogenemia***

All VPA-treated patient groups studied (girls, women, men) had elevated serum levels of androgens; serum T levels were high in female patients, and serum levels of T precursors were high in men taking VPA for epilepsy. These changes were observed already after 1 month of VPA medication, while a normalization of biochemical hyperandrogenemia was observed in women after 2 months of discontinuing VPA treatment. Therefore, VPA directly or indirectly either increases the production of androgens, or decreases their metabolism.

The most important factor regulating sex steroid production is LH (Hall & Crowley 1995). It has been previously suggested that VPA may affect gonadotropin secretion due to its GABAergic properties, but, according to previous reports, short-term exposure to VPA did not increase pulsatile LH secretion in normal women (Lado Abeal *et al.* 1991, 1995, Popovic *et al.* 1996). It is well established that pituitary gonadotropin secretion is controlled by a negative feedback system, which is different in women and men (See Chapters 2.2.2. and 2.2.3.). Theoretically, the decreased serum levels of LH in VPA-treated men could be related to the activation of this negative feedback mechanism as a consequence of excess T production stimulated by VPA. It is also possible that the negative feedback mechanism prevents the further increase of serum androgen levels in men on VPA. VPA seems to have opposite effects on serum gonadotropin levels in men and women at least during the first months after starting the treatment. Furthermore, hyperandrogenemia was also observed in prepubertal girls taking VPA for epilepsy, although their hypothalamic-pituitary-ovarian axis should not have been induced yet. These suggest that the changes in serum gonadotropin levels in patients with epilepsy may be secondary consequences rather than primary phenomena.

Insulin and IGF-I also have effects on the production of gonadal steroids. High concentrations of insulin and free IGF-I are known to stimulate ovarian androgen synthesis (Nobels & Dewailly 1992). Therefore, hyperinsulinemia and low serum levels

of IGFBP-1 were suggested to be the reason for hyperandrogenism in women on VPA in the previous studies (Isojärvi *et al.* 1996). According to the results of this study, no significant differences were seen in serum insulin or IGFBP-1 levels between girls on VPA with or without hyperandrogenemia, or between men with or without elevated serum androgen levels. This suggests that altered androgen balance in patients taking VPA for epilepsy is probably not primarily a consequence of increased function of insulin or IGF-I. However, it is obvious that hyperinsulinemia and high concentrations of IGF-I in ovaries do increase the ovarian androgen production further during long-term VPA treatment.

Short-term exposure to VPA may stimulate GH release due to its GABAergic properties (deKretser 1987, Coiro *et al.* 1991). However, the results of the present study suggest that endogenous GH secretion is not increased on long-term VPA treatment, since circulating concentrations of IGF-I and IGFBP-3, which are known to reflect serum GH levels (Juil & Skakkebaek 1997) were normal in most of the VPA-treated patients. Furthermore, increased GH production would affect growth in children and adolescents, but linear growth of girls was not accelerated after starting VPA treatment or during long-term therapy. There were no changes in serum concentrations of other hormones studied, which may affect sex steroid production in patients taking VPA for epilepsy.

Since VPA-related hyperandrogenemia does not seem to be mediated by changes in serum concentrations of other hormones or binding proteins, it is possible that VPA may have a direct peripheral effect on androgen metabolism or biosynthesis, which are driven by a variety of enzymes (Inano *et al.* 1990, Handelsman 1995). Indeed, VPA may function as an enzyme inhibitor (Perucca *et al.* 1984). However, further studies are needed to resolve the pathomechanism of VPA-related hyperandrogenemia.

### 6.3.5.2. Hyperinsulinemia

According to the present study, serum insulin levels were similar in young girls on VPA and the control girls regardless of serum androgen levels at all stages of puberty, while it was high in VPA-treated adult women with hyperandrogenism (Isojärvi *et al.* 1996). Physiological hyperinsulinemia during puberty (Styne 1995) may be the reason for different insulin findings in young girl and adult women on VPA. The girls were hyperandrogenic, but not obese, and they had been on VPA for a shorter time than the women with VPA-related obesity and hormonal changes (3 vs. 9 years) had. Therefore, it is also possible that obesity and hyperinsulinemia develop after years of VPA treatment, whereas changes in serum androgen levels emerge earlier. However, men on long-term VPA in this study had high serum insulin levels, but their hyperinsulinemia was not associated with increased serum androgen levels.

In the present study, features of insulin resistance were seen in women with VPA-related endocrine disorders. It is possible that hyperinsulinemia would be connected to insulin resistance, which is known to be common in persons with abdominal obesity in general. According to the literature, androgens may also promote insulin resistance (Conway & Jacobs 1993). The relationship between insulin, possible insulin resistance, weight gain and hyperandrogenemia during VPA treatment is very complicated. Whether

VPA therapy induces hyperinsulinemia via insulin resistance, via hyperandrogenemia, or by causing weight gain and subsequent obesity remains open. Furthermore, development of hyperandrogenemia and hyperinsulinemia may also be independent phenomena.

### 6.3.5.3. *Weight gain*

Weight gain is a common side effect of VPA therapy, and it may arise from changes in energy expenditure, energy intake, or hormonal/biochemical parameters during VPA treatment (Egger & Brett 1981, Dinesen *et al.* 1984, Breum *et al.* 1992, Gidal *et al.* 1996, Isojärvi *et al.* 1996). The exact mechanism of VPA-related weight gain is unclear. VPA-induced impairment in fatty acid beta-oxidation may be of importance (Breum *et al.* 1992, Ponchaut & Veitch 1993). Previous studies on energy balance during VPA treatment do not consider hormonal disorders as important factors inducing weight gain. However, these studies process women and men together, which confuses the results of hormone measurements (Breum *et al.* 1992, Gidal *et al.* 1996).

Since VPA-related obesity is associated with hyperinsulinemia and hyperandrogenism in previous as well as in the present studies (Isojärvi *et al.* 1996), it is obvious that VPA-related reproductive endocrine changes and weight gain are interrelated. Obesity seen in women with VPA-related endocrine disorders is abdominal obesity (Isojärvi *et al.* 1996, V), which is associated with insulin resistance and hyperinsulinemia more often than peripheral obesity (Pasquali & Casimirri 1993). However, all women with VPA-related endocrine disorders are not obese (V). The men on VPA with high serum androgen levels had higher BMI than VPA-treated men with normal serum androgens. According to Gidal *et al.* 1996 persons gaining weight during VPA treatment not only have increased adipose tissue, but also increased fat-free mass (e.g. muscle). This implies that, in addition to hyperinsulinemia, increased serum androgen levels may be involved in VPA-related weight gain due to anabolic effects. The results of this study suggest that the hormone balance changes without weight gain in women starting VPA medication (III) and serum androgen and insulin concentrations became normal prior to weight loss in women discontinuing VPA treatment (V). Therefore, it is possible that VPA-related weight gain and obesity are consequences of persistent hyperinsulinemia and hyperandrogenism over several years of VPA treatment. However, further studies are needed to resolve the biochemical pathomechanisms of VPA-induced weight gain.

## 6.4. Reproductive endocrine effects of carbamazepine, oxcarbazepine, and lamotrigine

### 6.4.1. *Endocrine effects of carbamazepine*

The use of CBZ has been associated with diminished sexual function in men and menstrual disturbances in women with epilepsy in previous studies. These disorders are thought to be mediated by decreased bioactivity of sex steroids due to increased serum

concentrations of SHBG in CBZ treated patients. (Isojärvi *et al.* 1995 a, 1995b) The previous findings were confirmed by the results of this study. The endocrine effects of CBZ have been suggested to be due to an accelerated metabolism of hormones or stimulated production of binding proteins in the liver as a consequence of hepatic enzyme induction during CBZ treatment (Isojärvi *et al.* 1995a, 1995b). According to the present study, CBZ induces these hormonal disorders even at low daily doses ( $\leq 600$  mg/day).

The mean serum levels of SHBG increased significantly both in women and in men during the first month of CBZ medication, and they are also high in patients on long-term CBZ treatment (Dana-Haeri & Richens 1981, Dana-Haeri *et al.* 1982, Toone *et al.* 1984, Isojärvi *et al.* 1988, MacPhee *et al.* 1988, Isojärvi *et al.* 1990, 1995b). This leads to decreased bioactivity of androgens (reflected by decreased FAI ratios) in patients on CBZ. A decreased  $E_2$ /SHBG ratio has also been previously reported in women on CBZ (Isojärvi 1995a), and similar trend was seen in women after starting CBZ in the present study. The liver enzyme inducing properties of CBZ may explain the increased serum SHBG levels during CBZ. However, changes in the T/  $E_2$  ratio have also been suggested to increase serum SHBG levels (Herzog 1995).

The mean serum concentrations of DHEAS decreased in both male and female patients during the first month of CBZ medication, and were also low in men on long-term CBZ treatment, which is consistent with previous reports (Isojärvi *et al.* 1995a, 1995b). In the present study, serum levels of DHEA, as well as levels of other sex steroids analyzed were normal in CBZ-treated patients both during short- and long-term medications. Metabolism of DHEAS is mainly driven by P450-enzymes (Haning 1991a, 1991b, Parker 1995), which are known to be induced by CBZ (Perucca *et al.* 1984). Therefore, metabolism of DHEAS may be changed during CBZ treatment.

In the present study, circulating insulin and IGF-I concentrations were higher in men treated with CBZ than in control men (IV), which has not been reported before. Circulating insulin, IGF-I and IGFBP-3 have also been reported to be high in adult women during long-term CBZ treatment (Isojärvi *et al.* 1996). Furthermore, plasma levels of IGF-I were high in girls taking CBZ for epilepsy in the present study (I). Insulin may also stimulate the production of IGF-I, but serum insulin levels were normal in girls on CBZ. Thus, it seems possible that CBZ directly stimulates the hepatic production of IGF-I, which is the main source of circulating IGF-I, but the pathomechanism of high peripheral insulin concentrations is unclear as well as the clinical significance of altered circulating levels of insulin and IGF-I.

Decreased bioactivity of sex steroids may lead to disturbed sexual function after years of CBZ treatment. In this study, 18 % of men taking CBZ for epilepsy reported decreased libido, impaired potency, or both, while 12 % of other patients with epilepsy reported impaired sexual function. Furthermore, all except one man (5/6) on VPA or OXC with reported impairment in sexual function had previously been treated with CBZ. Therefore, it is possible that decreased libido and potency in these men may arise from their previous CBZ treatment. A high serum SHBG concentration and low FAI ratio reflect reduced T bioactivity, and this may be the reason for impaired sexual function in five of seven men on CBZ who reported impaired sexual function in the present series. However, the serum androgen profiles were normal in the other two patients with these problems. Impaired sexual function can also be associated with epilepsy itself, psychological factors and situation of life. All of the men on CBZ who reported disturbed sexual function had

partial or secondary generalized epilepsy, and sexual dysfunction has been reported to be common especially in men with refractory partial epilepsy of temporal lobe origin (Herzog *et al.* 1986b). However, these patients did not have frequent seizures. 19% of patients on VPA, 8% on CBZ and 4% on OXC reported increased libido, potency, or both, but increased bioactivity of T (increased FAI) was found in only one of them.

#### ***6.4.2. Endocrine effects of oxcarbazepine differ from those of carbamazepine***

The endocrine effects of OXC have previously been studied only in male patients after changing their medication from CBZ to OXC. Replacement of CBZ by OXC resulted in a restoration of normal circulating levels of SHBG and DHEAS simultaneously with a decrease in the induction of the hepatic P450 enzyme system (Isojärvi *et al.* 1994, 1995c, 1995d). Therefore, OXC has been thought to be a safe AED from the endocrine point of view. However, in previous studies mean daily OXC doses were lower than in the present survey (913 ±251 mg/day vs. 1070 ±440 mg/day) (Isojärvi *et al.* 1995c). Observations in the present study suggest that reproductive endocrine changes are seen in men taking high daily doses of OXC (≥900 mg/d), while serum hormone concentrations were similar in patients with an OXC dose lower than 900 mg/d and in control men.

The mean serum levels of T, gonadotropins and SHBG were high in men on high daily OXC doses in the present study, but their FAI ratios were normal suggesting normal bioactivity of T. Therefore, CBZ and OXC medications seem to have different effects on reproductive endocrine function, since CBZ diminishes the bioactivity of androgens. However, the effects of OXC and CBZ were similar on insulin and IGF-I levels in patients with epilepsy, since circulating insulin and IGF-I concentrations were higher in patients treated with OXC or CBZ than in control men. The findings were not dose-dependent. In the present study, plasma IGF-I levels were also high in young girls taking OXC or CBZ for epilepsy.

Despite the close structural homology of OXC and CBZ, their reproductive endocrine effects seem to be different. Although OXC is not as potent an inducer of liver enzymes as CBZ, there is evidence that OXC may function as an enzyme inducer at high doses (Patsalos *et al.* 1990). Moreover, it is possible that OXC may induce different isoenzymes of the hepatic microsomal cytochrome P450-enzyme system than CBZ, which may explain the differences in endocrine effects between these two drugs. According to the results of this study, OXC enhances rather than inhibits androgen function in men in contrast to reproductive endocrine effects of CBZ. However, the prevalence of reported changes in sexual function was similar in men on OXC or CBZ.

### ***6.4.3. Endocrine effects of lamotrigine***

The results of the present study suggest that LTG therapy is not associated with changes in body weight, or in endocrine and metabolic functions, since the replacement of VPA with LTG resulted in both a weight reduction and normalization of fasting serum T, insulin and lipid levels. Furthermore, polycystic changes in ovaries started to recover and menstrual cycles became more regular after VPA was replaced by LTG. Since LTG has an efficacy comparable to VPA in generalized epilepsies, it seems to be an alternative medication for women of reproductive age.

## 7. Conclusions

1. VPA, CBZ or OXC do not affect linear growth or pubertal development in girls with epilepsy, despite endocrine changes associated with the use of these AEDs. Significant weight gain was observed in girls 2-3 years after starting VPA medication.
2. VPA has effects on reproductive endocrine function in young girls and men with epilepsy. Hyperandrogenemia, polycystic ovaries, and weight gain were observed in girls taking VPA for epilepsy, and the frequency of hyperandrogenemia increased with pubertal maturation. Increased serum androgen levels were seen in more than half of the men on long-term VPA treatment, which is similar to the frequency of hyperandrogenism observed in women on long-term VPA treatment in previous studies.
3. Changes in serum hormone concentrations occurred already after 1 month of VPA medication in patients with recently diagnosed epilepsy. Serum testosterone levels increased in half of the women during the first 3 months of VPA medication. The women with increased serum testosterone levels in the early phase of VPA therapy may be at increased risk for VPA-related endocrine symptoms later during long-term VPA treatment. Although changes in serum levels of reproductive hormones were also seen in men after starting VPA medication, no consistent pattern of hormonal changes was observed during the first 3 months of medication.
4. VPA-induced endocrine and metabolic disorders are at least partly reversible. The women substituting LTG for VPA in the present study were previously identified as having hyperandrogenism and polycystic ovaries, and they were also found to present other features of insulin resistance (i.e. hyperinsulinemia, abdominal obesity, and an unfavorable serum lipid profile). Replacing VPA with LTG resulted in a decrease in body weight and normalization of serum hormone and lipid levels, ovarian structure, and menstrual cycles during the first year after the change in medication.
5. The reproductive endocrine effects of CBZ and OXC are different. CBZ reduced the bioactivity of androgens, whereas OXC did not have similar effects. The CBZ-induced hormonal changes were already seen 1 month after starting the medication in both genders. OXC did not seem to affect reproductive endocrine function at low daily doses (< 900 mg) in men. The differences in endocrine effects of CBZ and OXC are likely due to their different properties as inducers of the microsomal P450 enzyme system in the liver.

## 8. References

- Adams J, Polson DW & Franks S (1986) Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. *BMJ* 293:355-358.
- Alberti KG, Christensen NJ, Christensen SE, Hansen AP, Iversen J, Lundbaek K, Seyer-Hansen K & Orskov H (1973) Inhibition of insulin secretion by somatostatin. *Lancet* 2:1299-1301.
- Andersen L, Dinesen B, Jorgensen PN, Puolsen F & Roder ME (1993) Enzyme immunoassay for intact human insulin in serum or plasma. *Clin Chem* 39:578-582.
- Apter D, Bützow T, Laughlin GA & Yen SSC (1994a) Increasing the clinical value of serum LH measurements by reducing short term variation. XX Spring Meeting of the Finnish Endocrine Society, abstr p. 20-21.
- Apter D, Bützow T, Laughlin GA & Yen SSC (1994b) Accelerated 24-hour luteinizing hormone pulsatile activity in adolescent girls ovarian hyperandrogenism: Relevance to the developmental phase of polycystic ovarian syndrome. *J Clin Endocrinol Metab* 79:119-125.
- Attie MK, Ramirez NR, Conte FA, Kaplan SL & Grumbach MM (1990) The pubertal growth spurt in eight patients with true precocious puberty and growth hormone deficiency: evidence for a direct role of sex steroids. *J Clin Endocrinol Metab* 71:975-983.
- Bäckström T (1976) Epileptic seizures in women in relation to variations of plasma estrogen and progesterone during the menstrual cycle. *Acta Neurol Scand* 54:321-347.
- Bäckström T, Zetterlund B, Blom S & Romano M (1984) Effects of continuous progesterone infusion on the epileptic discharge frequency in women with partial epilepsy. *Acta Neurol Scand* 69:240-248.
- Bang P (1994) Insulin-like growth factors (IGF)-I and IGF binding proteins: serum concentrations, bioavailability and effects of proteases (Thesis). Kongl Carolinska Medico Chirurgiska Institutet. Stockholm.
- Barragry JM, Makin HLJ, Trafford DJH & Scott DF (1978) Effect of anticonvulsants on plasma testosterone and sex hormone binding globulin levels. *J Neurol Neurosurg Psychiatr* 41: 913-914.
- Bilo L, Meo R, Nappi C, Annunziato L, Striano S, Coloa AM, Merola B & Buscaino GA (1988) Reproductive endocrine disorders in women with primary generalized epilepsy. *Epilepsia* 29:612-619.
- Bird CAK, Griffen BP, Milkazewska JM & Galbraith AW (1996) Tegretol (carbamazepine): a controlled trial of a new anticonvulsant. *Br J Psychiatry* 12:737-742.
- Bjersing L (1967) On the morphology and endocrine function of granulosa cells in ovarian follicles and corpora lutea. *Acta Endocrinol* 125:1-23.
- Blum WF & Ranke MB (1990) Insulin-like growth factor binding proteins (IGFBPs) with special reference to IGFBP-3. *Acta Paediatr Scand Suppl* 367:55-62.
- Blum WF & Ranke MB (1991) Plasma IGFBP-3 levels as clinical indicators. In: Spencer EM (ed) *Modern concepts of insulin-like growth factors*. Elsevier, New York, p. 381-393.

- Bonneville JF, Cattin F & Diemann JL (1989) Hypothalamic-pituitary region: computed tomography imaging. *Clin Endocrinol Metab* 3: 35-71.
- Bourguignon J-P (1995) The neuroendocrinology of puberty. *Growth, Genetics & Hormones* 11:1-6.
- Bremner WJ, Matsumoto AM, Sussman AM Paulsen CA (1981) Follicle stimulating hormone and human spermatogenesis. *J Clin Invest* 68: 1044-1052.
- Bremner WJ, Vitiello MV & Prinz PN (1983) Loss of circadian rhythmicity in blood testosterone levels with aging in normal men. *J Clin Endocrinol Metab* 56:1278-1281.
- Breum L, Astrup A, Gram L, Andersen T, Stokholm KH, Christensen NJ, Werdelin L & Madsen J (1992) Metabolic changes during treatment with valproate in humans: implication for untoward weight gain. *Metabolism* 41:666-670.
- Brodie A (1991) Aromatase and its inhibitors -an overview. *J Steroid Biochem Molec Biol* 40:255-261.
- Brodie MJ & Dichter MA (1996) Antiepileptic drugs. *N Engl J Med* 334:168-175.
- Brodie MJ, Richens A & Yuen AWC, for UK Lamotrigine/ Carbamazepine Monotherapy Trial Group (1995) Double-blind comparison of lamotrigine and carbamazepine in newly diagnosed epilepsy. *Lancet* 345:476-479.
- Brook CG & Hindmarsh PC (1992) The somatotrophic axis in puberty. *Endocrinol Metab Clin North Am* 21:767-782.
- Browne TR & Feldman RG (1983) Epilepsy: An overview. In: Browne TR & Feldman RG (eds) *Epilepsy. Diagnosis and management*. Little, Brown and Company, Boston & Toronto, p. 1-10.
- Buyalos RP, Pekonen F, Halme JK, Judd HL & Rutanen E-M (1995) The relationship between circulating androgens, obesity, and hyperinsulinemia on serum insulin-like growth factor binding protein-1 in the polycystic ovarian syndrome. *Am J Obstet Gynecol* 172:932-939.
- Christiansen P & Lund M (1975) Sexual potency, testicular function and excretion of sexual hormones in male epileptics. In: Janz D (ed) *Advances in epileptology: VIIth Epilepsy International Symposium*. Publishing Sciences Group, Berlin, p.190-191.
- Cockerell OC, Johnson AL, Goodridge DMG, Sander JWAS & Shorvon SD (1995) The remission of epilepsy: results from the National General Practice Study of Epilepsy. *Lancet* 346:140-144.
- Coiro V, Capretti L, Bianconi L, Castelli A, Cerri L, Roberti G, Marcato A, Volpi R & Chiodera P (1991) Reduction of baclofen-, but not sodium valproate-induced growth hormone release in type I diabetic men. *Horm Metab Res* 23:600-604.
- Commission on Classification and Terminology of the International League Against Epilepsy (1989) Proposal for revised classification of epilepsies and epileptic syndromes. *Epilepsia* 30: 389-399.
- Connell JMC, Rapeport WG, Beasall GM & Brodie MJ (1984a) Changes in circulating androgens during short term carbamazepine therapy. *Br J Clin Pharmacol* 17:347-351.
- Connell JMC, Rapeport WG, Gordon S & Brodie MJ (1984b) Changes in circulating thyroid hormones during short-term hepatic enzyme induction with carbamazepine. *Eur J Clin Pharmacol* 26:453-456.
- Conway GS, Agrawal R, Betteridge DJ & Jacobs HS (1992) Risk factors for coronary artery disease in lean and obese women with polycystic ovary syndrome. *Clin Endocrinol* 37:119-126.
- Conway GS & Jacobs HS (1993) Clinical implications of hyperinsulinemia in women. *Clin Endocrinol* 39:623-632.
- Cook JS, Bale JF Jr & Hoffman RP (1992) Pubertal arrest associated with valproic acid therapy. *Pediatric Neurol* 8:229-231.
- Corman CL, Leung NM & Guberman AH (1997) Weight gain in epileptic patients during treatment with valproic acid: a retrospective study. *Canadian J Neurol Sci* 24:240-244.
- Crawford P, Chadwick DJ, Martin C, Tjia J, Back DJ & Orme M (1990) The interaction of phenytoin and carbamazepine with combined oral contraceptive steroids. *Br J Clin Pharmacol* 30:892-896.
- Crowley WF, Filicori M, Spratt DI & Santoro NF (1985) The physiology of gonadotropin-releasing hormone (GnRH) secretion in men and women. *Rec Proc Horm Res* 41:473-531.
- Crowley WF Jr, Whitcomb RW, Jameson JL, Weiss J, Finkelstein JS & O'Dea LS (1991) Neuroendocrine control of human reproduction in the male. *Recent Prog Hormone Res* 47:27-67.
- Cummings LN, Giudice L & Morrell MJ (1995) Ovulatory function in epilepsy. *Epilepsia* 36:353-357.

- Dam M (1997) Goals of therapy. In: Engel J Jr. & Pedley TA (eds) *Epilepsy - a comprehensive textbook*. Lippincott-Raven Publishers, Philadelphia, vol. 2, p. 1103-1106.
- Dam M, Ekberg R, Loyning Y, Waltimo O & Jakobsen K (The Scandinavian Oxcarbazepine Study Group) (1989) A double-blind study comparing oxcarbazepine and carbamazepine in patients with newly diagnosed previously untreated epilepsy. *Epilepsy Res* 3:70-76.
- Dana-Haeri J, Oxley J & Richens A (1982) Reduction of free testosterone by antiepileptic drugs. *Br Med J* 284:85-86.
- Dana-Haeri J, Oxley J & Richens A (1984) Pituitary responsiveness to gonadotrophin-releasing and thyrotrophin-releasing hormones in epileptic patients receiving carbamazepine or phenytoin. *Clin Endocrinol* 20:163-168.
- Dana-Haeri J & Richtens A (1981) Effects of antiepileptic drugs on the hypothalamic-pituitary axis in epileptic patients. In: Dam M, Gram L, Penry JK (eds) *Advances in Epileptology: The XIIth Epilepsy International Symposium*. Raven Press, New York, NY, p. 469-475.
- Daniel PM & Prichard MML (1975) Studies of the hypothalamus and the pituitary gland. *Acta Endocrinol.* 201: 1-63.
- Dansky LV, Andermann E & Andermann F (1980) Marriage and fertility in epileptic patients. *Epilepsia* 21:261-271.
- Davis R, Peters DH & McTavish D (1994) Valproic acid. A reappraisal of its pharmacological properties and clinical efficacy in epilepsy. *Drugs* 47: 332-373.
- Dawson BH (1958) The blood vessels of the human optic chiasma and their relation to those of the hypophysis and hypothalamus. *Brain* 81:201-217.
- DeFronzo RA, Bonadonna RC & Ferrannini E (1992) Pathogenesis of NIDDM: A balanced overview. *Diabetes Care* 15:318-368.
- DeFronzo RA & Ferrannini E (1991) Insulin resistance: a multifaced syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* 14:173-194.
- Degen R & Rodin EA, eds (1991) *Epilepsy, sleep and sleep deprivation*. *Epilepsy Res: Suppl* 2.
- de Kretser DM (1987) Local regulation of testicular function. *Int Rev Cytol* 10:89-112.
- de Kretser DM, Risbridger GP, Kerr JB (1995) Basic endocrinology of the testis. In: DeGroot LJ (ed) *Endocrinology*, vol. 3. W.B. Saunders Company, Philadelphia, p 2307-2335.
- Dichter MA & Brodie MJ (1996) New antiepileptic drugs. *N Engl J Med* 334:1583-1590.
- Dickerson W (1941) The effect of menstruation on seizure incidence. *J Nerv Ment Dis* 94:160-169.
- Dinesen H, Gram L, Andersen T & Dam M (1984) Weight gain during treatment with valproate. *Acta Neurol Scand* 70:65-69.
- Dramusic V, Goh HH, Yang M & Ratnam SS (1991) Menstrual dysfunctions in adolescence. *Singapore J Obstet Gynecol* 22:69-75.
- Dreifuss FE & Langer DH (1988) Side effects of valproate. *Am J Med* 84 (Suppl 1A):34-41.
- Dufau ML (1988) Endocrine regulation and communicating functions of the Leydig cell. *Ann Rev Physiol* 50:483-508.
- Duncan S, Blacklaw J, Beastall GH & Brodie MJ (1999) Antiepileptic drug therapy and sexual function in men with epilepsy. *Epilepsia* 40:197-204.
- Egger J & Brett EM (1981) Effects of sodium valproate in 100 children with special reference to weight. *Br Med J* 29:377-581.
- Elias AN, Szekeres AV, Stone S & Valenta LJ (1982) A presumptive role for gamma-aminobutyric acid in the regulation of gonadotropin secretion in man. *Am J Obstet Gynecol* 144:72-76.
- Engel JJr. & Pedley TA (1997) Introduction: what is epilepsy? In: Engel JJr. & Pedley TA (eds) *Epilepsy - a comprehensive textbook*. Lippincott-Raven Publishers, Philadelphia, p. 1-10.
- Ensinck JW & Williams RF (1972) Hormonal and non-hormonal factors modifying man's response to insulin. In: Steiner DF, Freinkel N (eds) *Handbook of physiology*. Washington, American Physiological Society, p. 665.
- Erfurth EMT, Hargmart LE, Sääf M & Hall K (1996) Serum levels of insulin-like growth factor I and insulin-like growth factor-binding protein 1 correlate with serum free testosterone and sex hormone binding globulin levels in healthy young and middle-aged men. *Clin Endocrinol* 44:659-64.
- Ericson GF (1978) Normal ovarian function. *Clin Obstet Gynecol* 21:31.

- Evans DJ, Hoffman RG, Kalkhoff RK & Kissebach AH (1983) Relationship of androgenic activity to body fat topography, fat cell morphology and metabolic aberrations in premenopausal women. *J Clin Endocrinol Metab* 57:304-310.
- Everitt BJ & Keverne EB (1986) Reproduction. In: Lightman SL & Everitt BJ (eds) *Neuroendocrinology*. Blackwell Scientific, Boston, p. 472-537.
- Faigle JW & Menge GP (1990) Pharmacokinetic and metabolic features of oxcarbazepine and their clinical significance: comparison with carbamazepine. *Int Clin Psychopharmacol* 5:73-82.
- Fattore C, Cipolla G, Gatti G, Limido GL, Sturm Y, Bernasconi C & Perucca E. Induction of ethinylestradiol and levonorgestrel metabolism by oxcarbazepine in healthy women. *Epilepsia* 40:783-787.
- Felig P, Marliss EB & Cahill GF Jr (1971) Metabolic response to human growth hormone during prolonged starvation. *J Clin Invest* 50:411.
- Finley PR, Schifman RB, William RJ & Lichti DA (1978) Cholesterol in high-density lipoprotein: use of  $Mg^{2+}$ /dextran sulfate in its enzymatic measurement. *Clin Chem* 24:931-933.
- Franceschetti S, Hamon B & Heineman U (1986) The action of valproate on spontaneous epileptiform activity in the absence of synaptic transmission and on evoked changes in  $Ca^{++}$  and  $K^{+}$  in the hippocampal slice. *Brain Res* 386:1-11.
- Franceschi M, Perego L, Cavagnini F, Cattaneo AG, Invitti C, Caviezel F, Strambi LF & Smirne S (1984) Effects of long-term antiepileptic therapy on the hypothalamic-pituitary axis in man. *Epilepsia* 25:46-52.
- Franks S (1995) Polycystic ovary syndrome. *N Engl J Med* 333:853-861.
- Galbraith A (1972) Tegretol -background and introduction. In Wink CAS & Nicholls C (eds) *Tegretol in epilepsy*. Manchester, p. 132-133.
- Gastaut H & Colomb H (1954) Etude dy comportement sexuel ches les epileptiques psychomoteurs. *Ann Med Psychol* 112:657-696.
- Geisler J, Engelsen BA, Berntzen H, Geisler S & Lønning PE (1997) Differential effect of carbamazepine and valproate monotherapy on plasma levels of oestrone sulfate and dehydroepiandrosterone sulfate in male epileptic patients. *J Endocrinol* 153:307-312.
- Gidal BE, Anderson GD, Spencer NW, Maly MM, Murty J, Pitterle ME & Collins DM (1994) Valproic acid (VPA) associated weight gain in monotherapy patients with epilepsy. *Epilepsia* 35:S142.
- Gidal BE, Anderson GD, Spencer NW, Maly MM, Murty J, Pitterle ME, Collins DM & Davis LA (1996) Valproate-associate weight gain: potential relation to energy expenditure and metabolism in patients with epilepsy. *Epilepsy* 9:234-241.
- Goodman AL & Hodgen GD (1983) The ovarian triad of the primate menstrual cycle. *Rec Prog Horm Res* 39:1-73.
- Grant SM & Faulds D (1992) Oxcarbazepine. A review of its pharmacology and therapeutic potential in epilepsy, trigeminal neuralgia and affective disorders. *Drugs* 43: 873-888.
- Greulich WW & Pyle SI (1959) *Radiographic Atlas of Skeletal Development of the Hand and Wrist*. Stanford, CA: Stanford University Press.
- Haidukewych D & Rodin EA (1987) Chronic antiepileptic drug therapy: classification by medication regimen and incidence of decreases in serum thyroxine and free thyroxine index. *Ther Drug Monit* 9:392-398.
- Hall JE & Crowley WF Jr (1995) Gonadotropins and the gonad: Normal physiology and their disturbances in clinical endocrine diseases. In: Degroot LJ, Besser M & Burger HG (eds) *Endocrinology*, 3rd ed. Philadelphia, W.B. Saunders Company, p. 242-258.
- Hammond GL, Lähteenmäki P & Luukkainen T (1982) Distribution and percentages of non-protein bound contraceptive steroids in human serum. *J Steroid Biochem* 17: 375-380.
- Hammond GL, Nisker JA, Jones LA & Siiteri PK (1980) Estimation of percentage of free steroid in undiluted serum by centrifugal ultrafiltration-dialysis. *J Biol Chem* 255:5023-5026.
- Han TS, van Leer, Seidell JC & Lean MEJ (1995) Waist circumference action levels in the identification of cardiovascular risk factors: prevalence study in random sample. *BMJ* 311:1401-1405.
- Handelsman DJ (1995) Testosterone and other androgens: Physiology, pharmacology, and therapeutic use. In: Degroot LJ, Besser M & Burger HG (eds) *Endocrinology*, 3rd ed. Philadelphia, W.B. Saunders Company, p. 2351-2361.

- Haning RV Jr, Flood CA, Hackett RJ, Loughlin JS, McClure N & Longcope C (1991a) Metabolic clearance rate of dehydroepiandrosterone sulfate, its metabolism to testosterone, and its intrafollicular metabolism to dehydroepiandrosterone, androstenedione, testosterone and dihydrotestosterone in vivo. *J Clin Endocrinol Metab* 72:1088-1095.
- Haning RV Jr, Carlson IH, Flood CA, Hackett RJ & Longcope C (1991b) Metabolism of dehydroepiandrosterone sulfate (DS) in normal women and women with high DS concentrations. *J Clin Endocrinol Metab* 73:1210-1215.
- Hauser WA (1997) Incidence and prevalence. In: Engel J Jr. & Pedley TA (eds) *Epilepsy - a comprehensive textbook*. Lippincott-Raven Publishers, Philadelphia, p. 47-58.
- Hauser WA, Annegers JF & Kurland LT (1993) The incidence of epilepsy and unprovoked seizures in Rochester, Minnesota, 1935-1984. *Epilepsia* 34:453-468.
- Helm P & Grolund L (1998) A halt in the secular trend towards earlier menarche in Denmark. *Acta Obstet Gynecol Scand* 77:198-200.
- Herkes G, Eadie M, Sharbrough F & Moyer T (1993) Patterns of seizure occurrence in catamenial epilepsy. *Epilepsy Res* 15:47-52.
- Herzog A (1986) Intermittent progesterone therapy and frequency of complex partial seizures in women with menstrual disorders. *Neurology* 36:1607-1610.
- Herzog A (1995) Hormonal changes in epilepsy. *Epilepsia* 36:323-326.
- Herzog AG (1996) Temporolimbic brain dysfunction -role in reproductive endocrine disorders. *Fertil Steril*. 65:210-211.
- Herzog AG, Levesque LA, Drislane FW, Ronthal M & Schomer DL (1991) Phenytoin-induced elevation of serum estradiol and reproductive dysfunction in men with epilepsy. *Epilepsia* 32: 550-553.
- Herzog AG, Seibel MM, Schomer DL, Vaitukaisis JL & Geschwind N (1986a) Reproductive endocrine disorders in women with partial seizures of temporal lobe origin. *Arch Neurol* 43:341-336.
- Herzog AG, Seibel MM, Schomer DL, Vaitukaisis JL & Geschwind N (1986b) Reproductive endocrine disorders in men with partial seizures of temporal lobe origin. *Arch Neurol* 43:347-350.
- Hindmarsh PC & Brook CGD (1995) Normal Growth and its endocrine control. In: Brook CGD (ed) *Clinical Paediatric Endocrinology*. Blackwell Scientific Publications, Oxford, o.85-106.
- Hindmarsh PC & Swift PG (1995) An assesment of growth hormone provocation tests. *Arch Dis Child* 72:362-367.
- Horwitz DL, Starr JL, Mako ME, Blackard WG & Rubenstein AH (1975) Proinsulin, insulin, and C-peptide concentrations in human portal and peripheral blood. *J Clin Invest* 55:1278.
- Hsueh AJW & Billig H (1995) Ovarian hormone synthesis and mechanism of action. In: Degroot LJ, Besser M & Burger HG (eds) *Endocrinology*, 3rd ed. Philadelphia, W.B. Saunders Company, p. 2019-2030.
- Huhtaniemi I (1977) Studies on steroidogenesis and its regulation in human fetal adrenal and testis. *J Steroid Biochem* 8:491-497.
- Huhtaniemi I, Koskimies A & Pelkonen R (1992) Kivekset. In: Lamberg B-A, Koivisto V & Pelkonen R (eds) *Kliininen endokrinologia*. Kustannus OY Duodecim, 4th ed., Helsinki, p. 458-478.
- Huuskonen U, Pakarinen AJ, Moilanen E & Isojärvi JIT (1998) The role of gender and drug metabolites in carbamazepine- or oxcarbazepine-related hyponatremia. *Epilepsia* 39 (Suppl 6):126.
- Inano H, Ishii-Ohba H, Sugimoto Y, Ohta Y, Morikawa T, Yoshida M & Tamaoki B (1990) Purification and properties of enzymes related to steroid hormone synthesis. *Ann NY Acad Sci* 595:17-25.
- Invitti C, Danesi L, Dubini A & Cavagnini F (1988) Neuroendocrine effects of chronic administration of sodium valproate in epileptic patients. *Acta Endocrinol* 118:381-388.
- Irianni F & Hodgen GD (1992) Mechanism of ovulation. *Endocrinol Metab Clin North Am* 21:19-38.
- Isaksson OGP, Jansson J-O & Gause IAM (1982) Growth hormone stimulates longitudinal bone growth directly. *Science* 216:1237-1239.
- Isojärvi J (1989) Endocrine effects of anticonvulsant medication in patients with epilepsy. *Acta Univ Oul D* 194.
- Isojärvi JIT, Pakarinen AJ & Myllylä VV (1988) Effects of carbamazepine therapy on serum sex hormone levels in male patients with epilepsy. *Epilepsia* 29:781-786.

- Isojärvi JIT, Myllylä VV & Pakarinen AJ (1989a) Effects of carbamazepine on pituitary responsiveness to luteinizing hormone-releasing hormone, thyrotropin-releasing hormone, and metoclopramide in epileptic patients. *Epilepsia* 30:50-56.
- Isojärvi JIT, Pakarinen AJ & Myllylä VV (1989b) Effects of carbamazepine on the hypothalamic-pituitary-gonadal axis in male patients with epilepsy: a prospective study. *Epilepsia* 30:446-452.
- Isojärvi JIT, Pakarinen AJ, Ylipalosaari PJ & Myllylä VV (1990) Serum hormones in male epileptic patients receiving anticonvulsant medication. *Arch Neurol* 47:670-676.
- Isojärvi JIT, Pakarinen AJ & Myllylä VV (1992) Thyroid function with antiepileptic drugs. *Epilepsia* 33:142-148.
- Isojärvi JIT, Laatikainen TJ, Pakarinen AJ, Juntunen KT & Myllylä VV (1993) Polycystic ovaries and hyperandrogenism in women taking valproate for epilepsy. *N Engl J Med* 4: 1383-1388.
- Isojärvi JIT, Pakarinen AJ, Rautio A, Pelkonen O & Myllylä VV (1994) Liver enzyme induction and serum lipid levels after replacement of carbamazepine with oxcarbazepine. *Epilepsia* 35: 1217-1220.
- Isojärvi JIT, Laatikainen TJ, Pakarinen AJ, Juntunen KTS & Myllylä VV (1995a) Menstrual disorders in women with epilepsy receiving carbamazepine. *Epilepsia* 36: 676-681.
- Isojärvi JIT, Repo M, Pakarinen AJ, Lukkarinen O & Myllylä VV (1995b) Carbamazepine, phenytoin, sex hormones, and sexual function in men with epilepsy. *Epilepsia* 36: 366-70.
- Isojärvi JIT, Pakarinen AJ, Rautio A, Pelkonen O & Myllylä VV (1995c) Serum sex hormone levels after replacing carbamazepine with oxcarbazepine. *Eur J Clin Pharmacol* 47: 461-464.
- Isojärvi JIT, Airaksinen KEJ, Mustonen JN, Pakarinen AJ, Rautio A, Pelkonen O & Myllylä VV (1995d) Thyroid and myocardial function after replacement of carbamazepine by oxcarbazepine. *Epilepsia* 36:810-816.
- Isojärvi JIT, Laatikainen TJ, Knip M, Pakarinen AJ, Juntunen KT & Myllylä VV (1996) Obesity and endocrine disorders in women taking valproate for epilepsy. *Ann Neurol* 39: 579-584.
- Jabbari B & Huott AD (1980) Seizures in thyreotoxicosis. *Epilepsia* 21:91-96.
- Juul A, Bang P, Hertel NT, Main K, Dalgaard P, Jørgensen K, Müller J, Hall K & Skakkebaek NE (1994) Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. *J Clin Endocrinol Metab* 78:744-752.
- Juul A, Dalgaard P, Blum WF, Bang P, Hall K, Michaelsen KF, Müller J & Skakkebaek NE (1995) Serum levels of insulin-like growth factor (IGF)- binding protein-3 (IGFBP-3) in healthy infants, children, and adolescents: The relation to IGF-I, IGF-II, IGFBP-1, IGFBP-2, age, sex, body mass index, and pubertal maturation. *J Clin Endocrinol Metab* 80:2534-2542.
- Juul A & Skakkebaek NE (1997) Prediction of the outcome of growth hormone provocative testing in short children by measurement of serum levels of insulin-like growth factor I and insulin-like growth factor binding protein 3. *J Pediatr* 130:197-204.
- Kalhan SC & Adam PAJ (1975) Inhibitory effect of prednisone on insulin secretion in man: Model for duplication of blood glucose concentration. *J Clin Endocrinol Metab* 41:600.
- Kantero R-L & Widholm O (1971) The age of menarche in Finnish girls in 1969. *Acta Obstet Gynaecol Scand* 14(suppl):7-19.
- Karlberg J, Fryer JG, Engström I & Karlberg P (1987) Analysis of linear growth using a mathematical model. II. From 3 to 21 years of age. *Acta Paediatr Scand Suppl* 337:12-29.
- Keränen T (1994) Epilepsialääkityksen lopettaminen. *Duodecim* 110:1725-1729.
- Keränen T, Jolkkonen J & Klosterskov Jensen P (1990) Single-dose kinetics of oxcarbazepine after treatment cimetidine or erythromycin. *Epilepsia* 31: 641.
- Keränen T, Kälviäinen R & Sillanpää M (1997) Epilepsia potilaan lääkehoito. *Kapseli* 27:9-70.
- Klein R & Livingston S (1950) The effects of adrenocorticotrophic hormone in epilepsy. *J Pediatr* 37:733-739.
- Klosterskov Jensen P, Saano V, Haring P, Sventrup B & Menge GP (1992) Possible interaction between oxcarbazepine and an oral contraceptive. *Epilepsia* 33:1149-1152.
- Kusunoki M, Yamamura T, Ichii S, Fujita S, Nakai T & Utsunomiya J (1988) The effects of sodium valproate on plasma somatostatin and insulin in humans. *J Clin Endocrinol Metab* 67:1060-1063.
- Kälviäinen R, Keränen T & Riekkinen PJ Sr (1993) Place of newer antiepileptic drugs in the treatment of epilepsy. *Drugs* 46:1009-1024.

- Kälviäinen R, Nousiainen I, Mäntyjärvi M, Nikoskelainen E, Partanen J, Partanen K & Riekkinen P Sr (1999) Vigabatrin, a gabaergic antiepileptic drug, causes concentric visual field defects. *Neurology* 53:922-925.
- Lado Abeal J, Gabezas Agricola J, Paz Carreira JM & Cabezas-Cerrato J (1991) Influence of sodium valproate on late follicular phase pulsatile LH secretion in normal women. *Clin Endocrinol (Oxf)* 35:477-483.
- Lado Abeal J, Liz JL, Rey C, Febrero M & Cabezas-Cerrato J (1996) Effects of valproate-induced alteration of the GABAergic system on pulsatile luteinizing hormone secretion in ovariectomized women. *Eur J Endocrinol* 1996:135:293-298.
- Laidlaw J (1956) Catamenial epilepsy. *Lancet* 271:1235-1237.
- Landgraf R, Landraf-Leurs MM, Weissmann A, Horl R, von Werder K & Scriba PC (1977) Prolactin: a diabetogenic hormone. *Diabetologia* 13:99-104.
- Larkin JG, McKee PJW, Forrest G, Beastall GH, Park BK, Lowrie JI, Lloyd P & Brodie MJ (1991) Lack of enzyme induction with oxcarbamazepine (600 mg daily) in healthy subjects. *Br J Clin Pharmacol* 31:65-71.
- Leach JP & Brodie MJ (1995) Lamotrigine: clinical use. In: Levy R, Dreifuss FE, Mattson RE, et al. (eds) *Antiepileptic drugs*. Raven Press, New York, p. 889-895.
- Lechan RM (1987) Neuroendocrinology of pituitary hormone regulation. *Endocrinol Metab Clin North Am* 16: 475-501.
- Lee PA (1994) Laboratory monitoring of children with precocious puberty. *Arch Pediatr Adolesc Med* 148:369-376.
- Lee IR, Dawson SA, Wetherall JD & Hahnel R (1987) Sex hormone binding globulin secretion by human hepatocarcinoma cells is increased by both estrogens and androgens. *J Clin Endocrinol Metab* 64:825-831.
- Lehesjoki A-E & Pitkänen A (1999) Epilepsian geenit ja neurobiologia. *Duodecim* 115:583-594.
- Levesque LA, Herzog Ag & Seibel MM (1986) The effect of phenytoin and carbamazepine on serum dehydroepiandrosterone sulfate in men and women who have partial seizures with temporal lobe involvement. *J Clin Endocrinol Metab* 63:243-245.
- Levy RH & Koch KM (1982) Drug interactions with valproate acid. *Drugs* 24:543-556.
- Liu YX & Hsueh AJW (1986) Synergism between granulosa and theca-interstitial cells on estrogen biosynthesis by gonadotropin-treated rat ovaries: Studies on the two-cell, two-gonadotropin hypothesis using steroid antisera. *Biol Reprod* 35:27-36.
- Logothetis J, Harner R, Morrell F & Torres F (1959) The role of estrogen in catamenial exacerbation of epilepsy. *Neurology* 9:352-360.
- Loiseau P & Duche B (1995) Carbamazepine, clinical use. In: Levy RH, Mattson RH, Meldrum BS (eds) *Antiepileptic drugs*. 4th ed. New York, Raven Press, p. 555-565.
- Loscher W (1981) Valproate induced changes in GABA metabolism at the subcellular level. *Biochem Pharmacol* 30:1364-1366.
- Lundberg B, Nergårdh A, Ritzén EM & Samuelsson K (1986) Influence of valproic acid on the gonadotropin-releasing hormone test in puberty. *Acta Paediatr Scand* 75:787-792.
- Lühdorf K, Christiansen P, Hansen JM & Lund M (1977) The influence of phenytoin and carbamazepine on endocrine function: preliminary results. In: Penry JK (ed) *Epilepsy. The Eighth International Symposium*. Raven Press, New York, NY, p. 209-213.
- Lühdorf K, Jensen LK & Plesner AM (1986) Etiology of the seizures in the elderly. *Epilepsia* 27:458-463.
- MacDonald RL (1989) Antiepileptic drug actions. *Epilepsia* 30:S19-S28.
- MacPhee GJ, Larkin JG, Butler E, Beastall GH & Brodie MJ (1988) Circulating hormones and pituitary responsiveness in young epileptic men receiving long-term antiepileptic medication. *Epilepsia* 29: 468-475.
- Märin P, Holmäng S, Jönsson L, Sjöström L, Kvist H, Holm G, Lindstedt G & Björntorp P (1992) The effects of testosterone treatment on body composition and metabolism in middle-aged obese men. *Internat J Obes* 16:991-997.
- Marshall JC (1995) Regulation of gonadotropin secretion. In: Degroot LJ, Besser M & Burger HG (eds) *Endocrinology*, 3rd ed. Philadelphia, W.B. Saunders Company, p. 1993-2007.

- Marshall JC & Kelch RP (1986) Gonadotropin-releasing hormone: role of pulsatile secretion in the regulation of reproduction. *N Engl J Med* 315: 1459-1468.
- Marson AG, Kadir ZA & Chadwick DW (1996) New antiepileptic drugs: a systematic review of their efficacy and tolerability. *Br Med J* 313: 1169-1174.
- Masala A, Meloni T, Alagna S, Rovasio PP, Rasu S, Mele G & Franca V (1981) Effect of long-term treatment with sodium valproate on gonadotrophin and prolactin secretion in paediatric patients. *Br J Clin Pharmacol* 12:845-848.
- Mattson RH & Cramer JA (1983) Valproic acid. In: Browne TR & Feldman RG (eds) *Epilepsy: diagnosis and management*. Little, Brown, Boston, p. 225-233.
- Mattson RH & Cramer JA (1985) Epilepsy, sex hormones and antiepileptic drugs. *Epilepsia* 26(Suppl 1):S40-S51.
- Mattson RH, Cramer JA, Collins JF, Smith DB, Delgado-Escueta AV, Browne TR, Williamson PD, Treiman DM, McNamara JO, McCutchen CB, Homan RW, Crill WE, Lubozynski MF, Resenthal NP & Mayersdorf A (1985) Comparison of carbamazepine, phenobarbital, phenytoin, and primidone in partial and secondary generalized tonic-clonic seizures. *N Engl J Med* 313:145-151.
- Mattson RH, Cramer JA, Collins JF & VA Epilepsy Cooperative Study No. 264 Group (1992) A comparison of valproate with carbamazepine for the treatment of partial seizures and secondarily generalized tonic clonic seizures in adults. *New Engl J Med* 327:765-771.
- McLean MJ & MacDonald RL (1986) Carbamazepine and 10-11 epoxide produce use- and voltage-dependent limitation of rapidly firing action potentials of mouse central neurons in cell. *J Pharmacol Exp Ther* 238:727-738.
- McNeilly AS, Glasier A, Jonassen J & Howie PW (1982) Evidence for a direct inhibition of ovarian function by prolactin. *J Reprod Fertil* 65: 559-569.
- Meunier H, Garoz G, Meunier Y, Eymard P & Aimard P (1963) Propriétés antiépileptiques. *Thérapie* 18:435-438.
- Moore JW, Hoare SA, Quinlan MK, Clark GMG & Wang DY (1987) Centrifugal ultrafiltration dialysis for non-protein bound oestradiol in blood: Importance of the support. *J Steroid Biochem* 28:677-681.
- Morselli PL, Gerna M, de Maio D, Zanda G, Viani F & Garattini S (1975) Pharmacokinetic studies on carbamazepine in volunteers and in epileptic patients. In: Schneider H, Janz D, Gardner-Thorpe C, Meinardi H & Sherwin AL (eds) *Clinical Pharmacology of Antiepileptic Drugs*. Springer-Verlag NY inc., New York, NY, p. 166-180.
- Morton LD & Pellock JM (1996) Diagnosis and treatment of epilepsy in children and adolescents. *Drug* 51: 399-414.
- Neely EK, Wilson DM, Lee PA, Stene M & Hintz RL (1995) Spontaneous serum gonadotropin concentrations in the evaluation of precocious puberty. *J Pediatr* 127:47-52.
- Nelson KB & Ellenberg JH (1987) Predisposing and causative factors in childhood epilepsy. *Epilepsia* 28[Suppl 1]:S16-24.
- Nestler JE, Usiskin KS, Barlascini CO, Welty DF, Clore JN & Blackard WG (1989) Suppression of serum DHAS levels by insulin: An evaluation of possible mechanism. *J Clin Endocrinol Metab* 69:1040-1046.
- Newmark M & Penry K (1980) Katamenial epilepsy: a review. *Epilepsia* 21:281-300.
- Nieschlag E, Loriaux DL, Ruder HJ, Zucker IR, Kirschner MA & Lipsett MB (1973) The secretion of dehydroepiandrosterone and dehydroepiandrosterone sulfate in man. *J Endocrinol* 57:123-134.
- Nobels F & Dewailly D (1992) Puberty and polycystic ovarian syndrome: the insulin/insulin-like growth factor I hypothesis. *Fertil Steril* 58:655-666.
- Novak GB, Mayatal J, Alshansky A, Eviatar L, Sy-Kho R & Siddique Q (1999) Risk of excessive weight gain in epileptic children treated with valproate. *J Child Neurol* 14:490-495.
- Olivíe MAA, García-Mayor RV, Lestón DG, Sousa TR, Dominguez AS, Alvarez-Novoa R & Cortizas JA (1995) Serum insulin-like growth factor (IGF) binding protein-3 and IGF-I levels during childhood and adolescence. A cross-sectional study. *Pediatr Res* 38:149-155.
- Parker L (1995) Adrenal androgens. In: DeGroot LJ, Besser M & Burger HG (eds) *Endocrinology*, 3rd ed. Philadelphia, W.B. Saunders Company, p. 1836-1852.
- Parker L & Odell W (1980) Control of adrenal androgen secretion. *Endocr Rev* 1:392-410.

- Parker LN, Lifrak ET, Kawahara CK, Geduld SI & Kozbur XM (1983) Angiotensin II potentiates ACTH-stimulated adrenal androgen secretion. *J Steroid Biochem* 18:205-208.
- Pasquali R & Casimirri F (1993) The impact of obesity on hyperandrogenism and polycystic ovary syndrome in premenopausal women. *Clin Endocrinol (Oxf)* 39:1-16.
- Pasquali R, Casimirri F, de Iasio R, Mesini P, Boschi S, Chierici R, Flaminia R, Biscotti M & Vicennati V (1995) Insulin regulates testosterone and sex hormone-binding globulin concentrations in adult normal weight and obese men. *J Clin Endocrinol Metab* 80: 654-658.
- Patsalos PN, Zakrzewska JM & Elyas AA (1990) Dose dependent enzyme induction by oxcarbazepine. *Eur Clin Pharmacol* 39:187-188.
- Pauerstein CJ, Eddy CA, Croxatto HD, Hess R, Siler-Khodr TM & Croxatto HB (1978) Temporal relationship of estrogen, progesterone, and luteinizing hormone levels to ovulation in women and infrahuman primates. *Am J Obstet Gynecol* 130:876-886.
- Pellock JM & Willmore LJ (1991) A rational guide to routine blood monitoring in patients receiving antiepileptic drugs. *Neurology* 41: 961-964.
- Pendlebury SC, Moses DK & Eadie MJ (1989) Hyponatremia during oxcarbazepine therapy. *Hum Toxicol* 8:337-344.
- Perucca E (1996) The new generation of antiepileptic drugs: advantages and disadvantages. *Br J Clin Pharmacol* 42:531-543.
- Perucca E, Hedges A, Makki KA, Ruprah M, Wilson JF & Richens A (1984) A comparative study of the relative enzyme inducing properties of anticonvulsant drugs in epileptic patients. *Br J Clin Pharmacol* 18:401-410.
- Petra PH (1991) The plasma sex steroid binding protein (SBP or SHBG). A critical review of recent developments on the structure, molecular biology and function. *J Steroid Biochem Molec Biol* 40:735-753.
- Pham-Huu-Trung M, Villette J, Bogoyo A, Duclos JM, Fiet J & Binoux M (1991) Effects of IGF-I on enzymatic activity in human adrenocortical cells: Interactions with ACTH. *J Steroid Biochem Molec Biol* 39:903-909.
- Plymate SR, Matej LA, Jones RE & Friedl KE (1988) Inhibition of sex hormone-binding globulin production in the human hepatoma (hep G2) cell line by insulin and prolactin. *J Clin Endocr Metab* 67:460-464.
- Plymate SR, Hoop RC, Jones RE & Matej LA (1990) Regulation of sex hormone-binding globulin production by growth factors. *Metabolism* 39:967-970.
- Popovic V, Spremovic-Radjenovic S, Eric-Marinkovic J & Grossman A (1996) Effect of sodium valproate on luteinizing hormone secretion in pre- and postmenopausal women and its modulation by naloxone infusion. *J Clin Endocrinol Metab* 81:2520-2524.
- Ponchaut S & Veitch K (1993) Valproate and mitochondria. *Biochem Pharmacol* 46:199-204.
- Rasmussen DD, Gambacciani M, Schwartz W, Tueros VS & Yen SS (1989) Pulsatile gonadotropin-releasing hormone release from the human mediobasal hypothalamus in vitro: opiate receptor-mediated suppression. *Neuroendocrinology* 49:150-156.
- Reaven G (1988) Role of insulin resistance in human disease. *Diabetes* 37:1595-1607.
- Reaven G (1995) Pathophysiology of insulin resistance in human disease. *Phys Rev* 75:473-485.
- Rinderknecht E & Humbel RE (1978) Primary structure of human insulin-like growth factor II. *FEBS Lett* 89:283-286.
- Riskind PN & Martin JB (1995) Functional anatomy of the hypothalamic -Anterior pituitary complex. In: Degroot LJ, Besser M & Burger HG (eds) *Endocrinology*, 3rd ed. Philadelphia, W.B. Saunders Company, p. 151-159.
- Rodin E, Subramanian MC & Gilroy J (1984) Investigation of sex hormones in male epileptic patients. *Epilepsia* 25:690-694.
- Rogol A (1992) Growth and growth hormone secretion at puberty: the role of gonadal steroid hormones. *Acta Paediatr Scand* 383(suppl):15-20.
- Rommerts FFG & van der Molen HJ (1989) Testicular steroidogenesis. In: Burger HG, de Kretser DM (eds) *The testis*. New York, Raven Press, p.303-328.
- Rosner W, Hryb DJ, Khan S, Nakhla AM & Romas NA (1991) Sex hormone-binding globulin: anatomy and physiology of a new regulatory system. *J Steroid Biochem Molec Biol* 40:813-820.
- Schachter C (1988) Hormonal considerations in women with seizures. *Arch Neurol* 45:1267-1270.

- Scheuer ML & Pedley TA (1990) The evaluation and treatment of seizures. Current concepts. *N Engl J Med* 323:1468-1474.
- Sherman BM & Korenman SG (1975) Hormonal characteristics of the human menstrual cycle throughout reproductive life. *J Clin Invest* 55: 699-706.
- Sheth RD, Wesolowski CA, Jacob JC, Penney S, Hobbs GR, Riggs JE & Bodensteiner JB (1995) Effect of carbamazepine and valproate on bone mineral density. *J Pediatr* 127:256-262.
- Sieberg R, Nilsson CC, Stenman U-H & Widholm O (1987) The effect of oral contraceptives on hormone profiles of oligomenorrheic adolescent cycles. *Contraception* 35:29-40.
- Siiteri PK, Murai JT, Hammond GL, Nisker JA, Raymore WJ & Kuhn RW (1982) The serum transport of steroid hormones. *Recent Prog Horm Res* 38: 457-510.
- Sillanpää M (1994) Epilepsian epidemiologia. In: Larsen TA & Iivanainen M (eds) *Epilepsia*. Otava, Keuruu, Finland, p. 42-47.
- Simpson ER & Waterman MR (1995) Steroid hormone biosynthesis in the adrenal cortex and its regulation by adrenocorticotropin. In: Degroot LJ, Besser M & Burger HG (eds) *Endocrinology*, 3rd ed. Philadelphia, W.B. Saunders Company, p. 1630-1641.
- Singh A, Hamilton-Fairley D, Koistinen R, Seppälä M, James VH, Franks S & Reed MJ (1990) Effects of insulin-like growth factor type I (IGF-I) and insulin on the secretion of sex hormone binding globulin and IGF-I binding protein (IGFBP-1) by human hepatoma cells. *J Endocrinol* 124:R1-3.
- Smith EP & Korach KS (1996) Oestrogen receptor deficiency: consequences for growth. *Acta Paediatr* 417(suppl):39-43.
- Snyder PJ & Badura LL (1995) Chronic administration of sodium valproic acid slows pubertal maturation in inbred DBA/2J mice: skeletal, histological and RIA evidence. *Epilepsy Res* 20:203-211.
- Snyder PJ & Badura LL (1998) A potential mechanism of slowed pubertal maturation after chronic administration of sodium valproate acid. *Neurology* 50:922-925.
- Sorva R, Perheentupa J & Tolppanen E-M (1984) A novel format for growth chart. *Acta Paediatr Scand* 73:527-529.
- Styne DM (1995) Physiology of puberty. In: Brook CGD (ed) *Clinical Pediatric Endocrinology*. Blackwell Scientific Publications, Oxford, p. 234-252.
- Sundaram MBM, Hill A & Lowry N (1985) Thyroxine-induced petit mal status epilepticus. *Neurology* 35:1792-1793.
- Tanner JM & Whitehouse RH (1976) Clinical longitudinal standards for height, weight, height velocity and stages of puberty. *Arch Dis Child* 51:170-182.
- Tanner JM, Whitehouse RH, Marubini E & Resele LF (1976) The adolescents growth spurt of boys and girls of the Harpenden Growth Study. *Ann Hum Biol* 3:109-126.
- Tapanainen P & Knip M (1992) Evaluation of growth hormone secretion and treatment. *Ann Med* 24:237-247.
- Toone BK, Wheeler M, Nanjee M, Fenwick P & Grant R (1983) Sex hormones, sexual activity and plasma anticonvulsant levels in male epileptics. *J Neurol, Neurosurg, Psychiatr* 46:824-826.
- Toone BK, Edeh J, Fenwick P, Grant R, Nanjee ON, Purches AG & Wheeler M (1984) Hormonal and behavioral changes in male epileptics. In: Porter RJ, Ward AA Jr, Mattson RH, Dam M (eds) *Advances in Epileptology: XVth Epilepsy International Symposium*. Raven Press, New York, NY, p. 283-289.
- Vinters HV, Armstrong DL, Babb TL, Daumas-Duport C, Robitaille Y, Bruton CJ & Farrell MA (1993) The neuropathology of human symptomatic epilepsy. In Engel J Jr, ed. *Surgical treatment of the epilepsies*, 2nd ed. New York:Raven Press:593-608.
- Waltimo O (1983) Diagnosis of epilepsy. *Acta Neurol Scand*, suppl. 97:11-16.
- Waterman MR & Simpson ER (1985) Regulation of the biosynthesis of cytochrome P-450 involved in steroid hormone synthesis. *Mol Cell Endocrinol* 39:81-89.
- Webber MP, Hauser WA, Ottman R & Annegers JF (1986) Fertility in persons with epilepsy: 1935-1974. *Epilepsia* 27:746-752.
- Whittle SR & Turner SJ (1982) Effects of anticonvulsants on the formation of gamma-hydroxybutyrate from gamma-aminobutyrate in rat brain. *J Neurochem* 38:848-851.
- Wild RA (1994) Cardiovascular disease risks, insulin resistance, and androgen excess. *Semin Reprod Endocrinol* 12:38-44.

- Wild RA, Grubb B, Hartz A, Van Nort JJ, Bachman W & Bartholomew M (1990) Clinical signs of androgen excess as risk factors for coronary artery disease. *Fertil Steril* 54: 255-259.
- Woodbury D (1958) Relationship between the adrenal cortex and the central nervous system. *Pharmacol Rev* 10:276-336.
- Yen SS (1980) The polycystic ovary syndrome. *Clin Endocrinol* 12:177-207.
- Yeo PP, Bates D, Howe DG, Ratcliffe WA, Schardt CW, Heath A & Evered DC (1978) Anticonvulsants and thyroid function. *Br Med J* 1:1581-1583.