ENDOCRINE FUNCTION AND GROWTH IN YOUNG PATIENTS WITH CHILDHOOD- OR ADOLESCENCE-ONSET EPILEPSY

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OULU 2004
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Academic Dissertation to be presented with the assent of the Faculty of Medicine, University of Oulu, for public discussion in the Auditorium L12 of the Department of Paediatrics and Adolescence, Oulu University Hospital, on September 3rd, 2004, at 12 noon.

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2004
Oulu, Finland

Abstract

Endocrine disorders are common in adults with epilepsy on antiepileptic drugs (AEDs). This study aimed to evaluate endocrine function, lipid metabolism and growth in patients with childhood- or adolescence-onset epilepsy.

Altogether 148 patients with epilepsy on carbamazepine (CBZ), oxcarbazepine (OXC), valproate (VPA) or lamotrigine monotherapy during pubertal maturation and 124 healthy controls participated in this population-based cohort study. Boys and young men (n = 140) underwent cross-sectional evaluation once, and girls and young women (n = 132) twice at an approximate interval of 6 years. Gonadal structure and serum reproductive and thyroid hormone and lipid concentrations were evaluated and growth data were gathered.

Elevated serum testosterone and androstenedione levels and polycystic ovary syndrome (PCOS) were common in female subjects whose medication, especially VPA, continued into young adulthood. Serum reproductive hormone concentrations and ovarian structure were similar in patients off medication and controls in young adulthood. CBZ was associated with elevated serum sex hormone binding globulin and decreased dehydroepiandrosterone sulfate levels and VPA with elevated serum androstenedione concentrations in male patients. Testicular structure was similar in patients and controls. CBZ, OXC and VPA were associated with changes in serum thyroid hormone, thyrotropin and lipid levels during pubertal maturation in female patients, but these levels returned to normal after withdrawal of medication. Linear growth and final height were normal in female patients, but overweight was common if they had been obese and on VPA medication during pubertal maturation.

Elevated serum androgen levels, PCOS and overweight are common if epilepsy and AED use, especially VPA, continue into young adulthood. These untoward changes or alterations in serum thyroid hormone or lipid concentrations are not present in young women with medication withdrawn. CBZ and VPA are associated with changes in serum sex hormone levels in boys and young men with epilepsy. Epilepsy and AED use during pubertal maturation does not seem to affect growth.

Keywords: adolescent, child, epilepsy, gonadal steroid hormones, gonads, growth, lipids, thyroid hormones
To my family
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Oulu, August 2004

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<th>Description</th>
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<tr>
<td>A</td>
<td>androstenedione</td>
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<tr>
<td>AED</td>
<td>antiepileptic drug</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<tr>
<td>B</td>
<td>breast</td>
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<tr>
<td>BMI</td>
<td>body mass index</td>
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<td>CBZ</td>
<td>carbamazepine</td>
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<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>CV</td>
<td>coefficient of variation</td>
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<tr>
<td>DHEA</td>
<td>dehydroepiandrosterone</td>
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<td>dehydroepiandrosterone sulfate</td>
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<tr>
<td>E₂</td>
<td>estradiol</td>
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<td>EEG</td>
<td>electroencephalogram</td>
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<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<td>F</td>
<td>female</td>
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<td>FSH</td>
<td>follicle-stimulating hormone</td>
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<tr>
<td>FT</td>
<td>free testosterone</td>
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<tr>
<td>FT₃</td>
<td>free triiodothyronine</td>
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<tr>
<td>FT₄</td>
<td>free thyroxine</td>
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<tr>
<td>G</td>
<td>genital maturation</td>
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<td>G</td>
<td>generalized epilepsy</td>
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<tr>
<td>GnRH</td>
<td>gonadotropin-releasing hormone</td>
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<tr>
<td>HA</td>
<td>hyperandrogenism</td>
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<tr>
<td>HDL-C</td>
<td>high-density lipoprotein cholesterol</td>
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<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
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<tr>
<td>IGF-1</td>
<td>insulin-like growth factor 1</td>
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<tr>
<td>IGFBP-1</td>
<td>insulin-like growth factor binding protein 1</td>
</tr>
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<td>IGFBP-3</td>
<td>insulin-like growth factor binding protein 3</td>
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<td>L</td>
<td>localization-related epilepsy</td>
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<td>LDL-C</td>
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<td>lamotrigine</td>
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<td>Full Form</td>
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<tr>
<td>M</td>
<td>male</td>
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<tr>
<td>17-OH-Prog</td>
<td>17-hydroxyprogesterone</td>
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<td>OR</td>
<td>odds ratio</td>
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<td>P</td>
<td>progesterone</td>
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<td>PCO</td>
<td>polycystic ovaries</td>
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<td>PCOS</td>
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<td>prolactin</td>
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<td>RIA</td>
<td>radioimmunoassay</td>
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<tr>
<td>SAS</td>
<td>Statistical Analysis System</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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<td>standard deviation score</td>
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<td>SHBG</td>
<td>sex hormone binding globulin</td>
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<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
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<td>testosterone</td>
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<td>Tanner pubertal stages I-V</td>
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<td>thyroxine</td>
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<td>TBG</td>
<td>thyroxine-binding globulin</td>
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<td>total cholesterol</td>
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<td>thyroglobulin antibody</td>
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<td>TGs</td>
<td>triglycerides</td>
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<td>TRH</td>
<td>thyrotropin-releasing hormone</td>
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<tr>
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<td>thyrotropin</td>
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<tr>
<td>VPA</td>
<td>valproate</td>
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<td>WHO</td>
<td>World Health Organization</td>
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List of original articles

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1 Introduction

Epilepsy is one of the most common neurological disorders during childhood and adolescence. In Finland, up to 6000 children and adolescents suffer from epilepsy, and most of them need daily antiepileptic medication (Keränen et al. 1997). In addition to seizure freedom, which is the main goal of therapy, the long-term tolerability of antiepileptic medication is important as well. Many types of endocrine and metabolic dysfunction, e.g. hyperandrogenism (HA), menstrual disorders, polycystic ovaries (PCO), hyposexualy, impaired potency and changes in serum thyroid hormone concentrations (Herzog et al. 1986a, Isojärvi et al. 1989c, Isojärvi et al. 1993b) and lipid profile (Isojärvi et al. 1993c), have been seen in adults with epilepsy taking antiepileptic medication. HA was also detected in girls with epilepsy taking valproate (VPA) during pubertal maturation (Vainionpää et al. 1999), and VPA has been associated with weight gain in children and adolescents with epilepsy (Biton et al. 2003). Changes in thyroid function (Yüksel et al. 1993, Verrotti et al. 2001) and serum lipid concentrations (Verrotti et al. 1998, Eiris et al. 2000) have also been observed in children with epilepsy on different medication regimens.

Even though childhood and adolescence are sensitive periods for hormonal and metabolic dysfunctions, the long-term consequences of antiepileptic drug (AED) use during pubertal maturation are poorly known. This study aimed to evaluate reproductive endocrine function, serum thyroid and lipid status and growth in patients with childhood- or adolescence-onset epilepsy.
2 Review of literature

2.1 Childhood- or adolescence-onset epilepsy

2.1.1 Epidemiology

Epilepsy is a common neurological disorder, which affects 0.5-2% of total population at all ages. There is a peak in incidence in childhood, especially within the first year of life (Hauser et al. 1993). The incidence of childhood-onset epilepsy is 40-100/100 000 per year and the prevalence 0.8-1% (Keränen et al. 1997). In the early 1990’s, the point prevalence of active epilepsy among Finnish children up to 15 years old was 0.4-0.7% (Sillanpää 1992, Eriksson & Koivikko 1997). Over 50% of all epilepsies begin during childhood and adolescence (Keränen et al. 1997).

2.1.2 Classification and etiology

Epileptic disorder is a clinical diagnosis characterised by unexpected and recurrent paroxysmal clinical symptoms classifiable as epileptic seizures. Epileptic seizures are caused by electric outbursts in the neuronal activity of brain, which are often self-limited (Blume et al. 2001). Seizures can be classified as either focal-onset (partial), which means that there is initial activation in only part of one cerebral hemisphere, or generalized, if both cerebral hemispheres are involved (Commission on Classification and Terminology of the International League Against Epilepsy 1981, Engel 2001). In addition to only classifying seizure types, accurate assessment of the type of epilepsy and the specific epilepsy syndrome has prognostic value and enables the selection of appropriate treatment (Commission on Classification and Terminology of the International League Against Epilepsy 1989, Engel 2001).
In children under five years of age, generalized seizures and epilepsies or epileptic syndromes are most prevalent, while in older age groups localization-related epilepsies are the most common (Hauser et al. 1993). It has been possible to classify 96% of seizures and nearly 90% of epilepsies and epileptic syndromes in certain populations of children (Eriksson & Koivikko 1997, Sillanpää et al. 1999), even at the time of the initial diagnosis, by using the criteria introduced by International League Against Epilepsy (Berg et al. 2000). Of epilepsy syndromes, usually approximately 60% are localization-related and 25-29% generalized, while 4-12% cannot be classified into either of these categories (Berg et al. 1999, Sillanpää et al. 1999). In a Finnish population-based cohort, the etiology of childhood-onset seizures was idiopathic in 28%, possibly symptomatic (cryptogenic) in 22% and symptomatic in 50% of cases (Sillanpää et al. 1999). In symptomatic epilepsy there is a known cause for seizures, e.g. genetic disorder, congenital brain malformation, perinatal injury, infection, metabolic disease, neoplasm, injury or vascular disease (Waltimo 1983, Keränen et al. 1997).

Idiopathic benign childhood epilepsy with centrotemporal spikes, i.e. Rolandic epilepsy, is the most common idiopathic localization-related epilepsy syndrome, and it accounts for approximately 5-17% of all epilepsies in children under 16 years of age in the Western countries (Sidenvall et al. 1996, Eriksson & Koivikko 1997, Callenbach et al. 1998, Berg et al. 1999, Sillanpää et al. 1999). Temporal, occipital and frontal lobe epilepsies are common cryptogenic or symptomatic localization-related epilepsies during childhood and adolescence, and they may represent over 30% of all epilepsies in these age groups (Sillanpää et al. 1999). Approximately 20% of epilepsies during childhood and adolescence are idiopathic generalized epilepsy syndromes (Eriksson & Koivikko 1997, Berg et al. 1999, Sillanpää et al. 1999). Of these, childhood absence epilepsy represents 3 to 12% and juvenile absence epilepsy approximately 1 to 2% of all epilepsies in children under 16 years of age (Sidenvall et al. 1996, Eriksson & Koivikko 1997, Callenbach et al. 1998, Berg et al. 1999, Sillanpää et al. 1999). As for other idiopathic generalized epilepsy syndromes, juvenile myoclonic epilepsy accounts for approximately 1-2% (Sidenvall et al. 1996, Eriksson & Koivikko 1997, Callenbach et al. 1998, Berg et al. 1999, Shinnar et al. 1999, Sillanpää et al. 1999) and tonic-clonic epilepsies occurring at random and on awakening for 6-11% of all cases of epilepsy during childhood and adolescence (Shinnar et al. 1999, Sillanpää et al. 1999). Idiopathic generalized epilepsies mostly begin during childhood, adolescence and young adulthood. The West and Lennox-Gastaut syndromes are examples of the relatively rare cryptogenic or symptomatic generalized epilepsies during childhood. The proportion of West syndrome has been shown to vary within 3-8% and that of Lennox-Gastaut syndrome within 0.7-6% of all epilepsies in children and adolescents (Sidenvall et al. 1996, Eriksson & Koivikko 1997, Callenbach et al. 1998, Berg et al. 1999, Sillanpää et al. 1999).

2.1.3 Prognosis

Prognosis varies depending on the etiology and type of the epileptic syndrome (Sillanpää et al. 1999). The majority of patients with epilepsy diagnosed in childhood are seizure-
free by the time they grow up, especially if they had idiopathic epilepsy, responded rapidly to medication and had no additional risk factors (Thurston et al. 1982, Sillanpää et al. 1998). Of all seizure types, benign childhood epilepsy with centrotemporal spikes has the best prognosis for remission (Sillanpää et al. 1999, Berg et al. 2001b). Juvenile absence and juvenile myoclonic epilepsies with a need for long-term therapy are exceptions among idiopathic generalized syndromes, which usually have good prognosis (Sillanpää et al. 1999). Cryptogenic and symptomatic epilepsy syndromes, i.e. West and Lennox-Gastaut syndromes, appear to have the worst prognosis in terms of both remission (22%) and mortality (38%) when followed up for over 30 years (Sillanpää et al. 1999).

Up to 20-30% of all patients with epilepsy do not reach remission on currently available medication (Keränen et al. 1997). In one Finnish study, the prevalence of childhood refractory epilepsy was 17% (Eriksson & Koivikko 1997). In another study, 10% of children with newly diagnosed epilepsy developed refractory disease during the first two years after diagnosis (Berg et al. 2001a). Cryptogenic or symptomatic etiology, high initial seizure frequency and focal slowing in electroencephalogram (EEG) correlate with drug resistance (Berg et al. 2001a). In addition, concurrent neuroimpairment and early onset of epilepsy correlate with refractory epilepsy (Eriksson & Koivikko 1997). Atonic or complex partial seizures are also considered poor prognostic factors (Camfield et al. 1985, Sillanpää et al. 1999).

Adult patients with childhood-onset epilepsy have a higher occurrence of psychiatric and psychosomatic disorders and social and educational problems than general population (Jalava & Sillanpää 1996, Sillanpää et al. 1998). Increased mortality was found in patients with symptomatic seizures, but mortality was similar to that in general population among patients with idiopathic seizures in a historical cohort (Olafsson et al. 1998). In another study, no difference in overall mortality was found between patients with generalized and partial-onset seizures (Sillanpää et al. 1999).

2.1.4 Treatment

Proper classification of seizures and accurate diagnosis of the type of epilepsy and epileptic syndrome provide a rational and individual basis for treatment. Antiepileptic medication should be individually tailored for every subject. Some epilepsy syndromes are known to be benign with infrequent seizures. On the other hand, some syndromes are severe, progressive and often drug-resistant with frequent seizures. Some syndromes appear to be responsive to certain AEDs, while some other AEDs may aggravate the same syndrome (Genton et al. 2000).

A risk of recurrence of seizures at all ages varies within 33-61% after one unprovoked seizure, regardless of whether or not antiepileptic medication is started (Hirtz et al. 1984, Shinnar et al. 1996, Hauser et al. 1998). In addition, following relapse 43-90% of children have more than two seizures (Hirtz et al. 1984, Camfield et al. 1985). Thus, in most epilepsies there is a case for starting medication after two unprovoked seizures (Camfield et al. 1985, Hauser et al. 1998).
The primary goal of treatment is remission of epilepsy. The goal of complete freedom from seizures may be achieved in some epilepsies, whereas in some other epilepsy syndromes it may be sufficient to aim at better seizure control and as normal life as possible. In clinical practice, it is often necessary to seek a balance between freedom from seizures and AED tolerability, which are important in determining the overall usefulness of therapy.

Epilepsy treatment aims at monotherapy. Nearly 50% of all patients with newly diagnosed epilepsy reach remission on an adequate first-line AED (Kwan & Brodie 2001). Symptomatic epilepsy, EEG slowing, and high initial seizure frequency are associated with a poor remission rate (Berg et al. 2001a). Most of the patients whose seizures continue during treatment with AEDs already have them during the first year of therapy (Camfield et al. 1985, Hauser et al. 1998). Polytherapy may be justified if remission is not reached with one AED. In rational polytherapy, two or more AEDs with different mechanisms of action are combined, which may enhance efficacy (Deckers et al. 2000).

More treatment options have become available during the recent decades. In addition to older traditional AEDs, such as phenobarbital, phenytoin, primidone, carbamazepine (CBZ) and VPA, many new AEDs, including oxcarbazepine (OXC), lamotrigine (LTG), gabapentin, levetiracetam, remacemide, tiagabine, topiramate, vigabatrin and zonisamide, have been introduced since the early 1990’s. Alternatives to antiepileptic medication, i.e. epilepsy surgery, vagus nerve stimulator and ketogenic diet, may be treatment choices in special cases of epilepsy (Coppola 2004).

There is no evidence-based consensus of the optimal time for AED discontinuation. Seizure freedom for at least two years before AED withdrawal is associated with fewer relapses than AED withdrawal after a shorter period of seizure freedom in children with localization-related epilepsy according to a recent meta-analysis of five controlled trials (Sirven et al. 2004). Children with generalized tonic-clonic seizures have had longer remission times after withdrawal of AEDs than children with localization-related epilepsy (Thurston et al. 1982). No significant differences in the seizure relapse rate (50% and 37%) were seen in children with generalized epilepsy between AED withdrawal after 1 or 3 years of treatment (Braathen et al. 1996). However, there is not enough evidence to ensure the optimal time for safe discontinuation of AEDs in children with generalized seizures (Sirven et al. 2004). In idiopathic generalized epilepsies, treatment is often needed for a long period, with the exception of childhood absence epilepsy, in which 1 to 3 years’ remission before AED withdrawal is recommended (Panayiotopoulos 2001).

2.1.4.1 Localization-related epilepsy

Almost all available AEDs have some efficacy in localization-related epilepsies. Old AEDs were adopted into clinical use before the era of large clinical trials. Thus, there are no placebo-controlled studies of their efficacy. However, the old AEDs primidone, phenobarbital and phenytoin should not be used as first-line drugs in children, because there are equally effective but better tolerated AEDs such as CBZ, VPA, OXC and LTG.
CBZ and VPA are regarded as first-line therapies in localization-related epilepsies in childhood (Coppola 2004). Even though they have been shown to be almost equally effective in the treatment of focal-onset seizures in children (Verity et al. 1995), there was some evidence in a meta-analysis for better efficacy of CBZ (Marson et al. 2002).

OXC and LTG have been effective both as add-on drugs and as monotherapy for localization-related epilepsy in children and adolescents (Guerreiro et al. 1997, Glauser et al. 2000, Nieto-Barrera et al. 2001, Duchowny et al. 2002, Bang & Goa 2003). However, no differences in efficacy as add-on therapy for localization-related epilepsy emerged between second-generation AEDs, such as LTG, OXC, gabapentin, levetiracetam, remacemide, tiagabine, topiramate, vigabatrin and zonisamide, in a meta-analysis and a comparative review, and they may have quite similar efficacy compared to older AEDs but better tolerability (Marson et al. 1996, Marson & Chadwick 2001). Newer AEDs have been compared either with placebo or with older AEDs in these studies, which have mostly been conducted in adults. Therefore, the new AEDs, with the exception of OXC, LTG, topiramate and gabapentin, are not yet recommended as first- or second-line therapy in children (Coppola 2004).

VPA and LTG are regarded as first-line drugs for idiopathic generalized epilepsies (Panayiotopoulos 2001). VPA has activity against all types of seizures, but LTG may have better tolerability, especially with regard to weight and reproductive and metabolic functions (Stephen et al. 2001, Biton et al. 2003). No difference in efficacy for primary generalized epilepsies in children was detected between CBZ and VPA in a multicentre clinical trial (Verity et al. 1995). Nor was there any evidence to prefer VPA over CBZ for generalized tonic-clonic seizures according to a meta-analysis (Marson et al. 2002). However, the confidence intervals were too wide to assess the equivalence of these AEDs, and the age distribution of the subjects with generalized seizures indicated that a large number of patients may have had their seizures misclassified (Marson et al. 2002).

VPA and ethosuximide are commonly used for childhood and juvenile absence epilepsy, although there are no placebo-controlled studies of their efficacy. However, no differences in their efficacy have been seen in small randomised comparative trials with follow-up times of 3 months to 4 years (Callaghan et al. 1982, Sato et al. 1982). The efficacy of LTG for absence epilepsies has been reported compared to placebo in a responder-enriched study with 4 weeks’ follow-up (Frank et al. 1999) and in a few non-randomised open-labelled studies (Besag et al. 1995, Buoni et al. 1999). It is not possible to identify the drug of choice for absence epilepsies (Posner et al. 2003). Meanwhile, VPA and ethosuximide are regarded as principal treatments and LTG as second-line therapy for absence epilepsies. Ethosuximide is effective only against absence seizures.

In juvenile myoclonus epilepsy, VPA is still the drug of choice with efficacy in almost 90% of patients (Panayiotopoulos et al. 1994, Panayiotopoulos 2001). LTG and topiramate also have some efficacy in juvenile myoclonus epilepsy, but LTG may
aggravate myoclonic jerks. CBZ is also known to worsen this type of epilepsy (Genton et al. 2000).

Of the other newer AEDs, levetiracetam and zonisamide may also have some efficacy in idiopathic generalized epilepsies, but gabapentin has no effect, and vigabatrin and tiagabine may even exacerbate absence status (Panayiotopoulos 2001).

2.1.4.3 Mechanisms of action and pharmacokinetics of AEDs

Modern drug development aims to find new AEDs, which do not only suppress seizures but also have neuroprotective effects to prevent epileptogenesis. Meanwhile, the currently available AEDs are known to have mainly anticonvulsant effects with various mechanisms of action, which partly account for the differences in their clinical efficacy.

The blockade of the neuronal voltage-dependent sodium channels is the principal anticonvulsant effect of many old AEDs, such as phenytoin, CBZ and VPA, and also of the newer AEDs OXC and LTG. VPA, topiramate and gabapentin have various mechanisms of action. The mechanism of action of levetiracetam is not fully known, but it affects N-type calcium channels. Ethosuximide has an effect on calcium T-channel conductance. Tiagabine and vigabatrin are GABAergic drugs: tiagabine is a GABA uptake inhibitor, whereas vigabatrin inhibits GABA-transaminase. (Keränen et al. 1997, Shorvon 2000) Below, the pharmacokinetic characteristics of CBZ, OXC, VPA and LTG will be discussed in more detail.

CBZ undergoes hepatic epoxidation and conjugation. It has an active first metabolite CBZ-10,11-epoxide, which is hydrolysed further to CBZ 10,11-trans-dihydrodiol. Over 75% of CBZ is bound to plasma proteins. (Shorvon 2000.) CBZ is metabolised in the liver and has dose-dependent autoinduction (Liu & Delgado 1994). Being a liver enzyme inducing drug, CBZ has many interactions with other drugs, and it may enhance the metabolism of many hormones (Perucca et al. 1984, Crawford et al. 1990).

OXC, a keto-derivative of CBZ, was initially developed to provide a compound chemically similar enough to CBZ to mimic its efficacy while improving its side-effect profile (Larkin et al. 1991). Anticonvulsive action is mainly based on blockade of the neuronal sodium channels (Bang & Goa 2003). OXC is rapidly further hydroxylated to its major metabolite, 10-monohydroxy derivative, which is responsible for the antiepileptic action of the drug (Bang & Goa 2003). 67% of OXC and 38% of 10-monohydroxy derivative are bound to plasma proteins. The 10-monohydroxy derivative is further conjugated to a glucuronide and finally excreted into urine (Shorvon 2000). OXC may also induce certain hepatic P450 isoenzymes and increase the metabolism of oral contraceptives (Patsalos et al. 1990, Fattore et al. 1999), but as a whole, it has been reported to be a weaker inducer of the liver (CYP) P450 enzyme system than CBZ in children and adolescents with epilepsy (Sallas et al. 2003) and in healthy subjects (Larkin et al. 1991).

VPA was originally used as an organic solvent. Even though VPA was licensed in the 1960s as an AED in Europe, its mechanism of antiepileptic action is partly uncertain, but it may have effects on GABA glutaminergic activity, on the N-methyl-D-aspartate
subtype of glutamate receptors and on calcium T and potassium conductance in addition to inducing sodium channel blockade (Lösch 2002). Its clinical structure, a molecule of branched-chain fatty acid, is similar to that of endogenous fatty acids. There are no other AEDs structurally related to VPA. Approximately 90% of VPA is bound to plasma proteins. VPA is oxidated and conjugated in the liver. VPA has over 30 metabolites, which may not have antiepileptic activity, but may enhance some adverse effects. VPA is also an inhibitor of liver enzymes. (Davis et al. 1994, Shorvon 2000.)

LTG is a triazine compound, and it is chemically unrelated to any other AED. It has broad-spectrum antiepileptic efficacy, as it also affects the function of calcium channels in addition to the main mechanism of sodium channel blockade. 55% of LTG is bound to plasma proteins. LTG has no active metabolites, undergoes hepatic glucuronidation and is mainly eliminated renally as glucuronide (Peck 1991). Other AEDs, which have liver enzyme inducing or inhibiting properties, may strongly affect LTG metabolism. (Pellock 1994, Shorvon 2000.)

2.1.4.4 Untoward long-term consequences of AEDs

Adverse effects of AEDs are quite common. They may occur as acute idiosyncratic reactions regardless of dose or be dose-dependent. Most adverse effects are transient and appear early in therapy, but some are consequences of the chronic toxicity of long-term AED treatment.

The chronic toxicity of AEDs may affect many organs: blood (e.g. leucopenia), skin (e.g. acne, hirsutism), connective tissue (e.g. gingival hypertrophy), immune system (e.g. systemic lupus erythematosus), liver (e.g. changes in metabolism, hepatitis) and nervous system (e.g. memory, cognition, progressive ataxia, peripheral neuropathy). Especially older AEDs are suggested to cause untoward cognitive and behavioural effects, although medication may also have positive influences on cognition by eliminating seizures (Calandre et al. 1990). No difference in cognition between phenytoin and CBZ was seen in healthy subjects in a randomized cross-over study (Meador et al. 1991), and normal intelligence after prenatal exposure to CBZ was also seen in children at the mean age of seven years (Gaily et al. 2004). The cognitive effects of newer AEDs are not entirely known, but the results reported so far seem to promise more favourable cognitive profiles for them (Aldenkamp et al. 2002, Bang & Goa 2003).

In general, unfavourable effect of AEDs on bone health have been detected in children and adolescents (Sheth et al. 1995, Kafali et al. 1999, Guo et al. 2001, Rieger-Wettengel et al. 2001, Farhat et al. 2002). Low bone density was seen irrespective of vitamin D levels in patients with epilepsy regardless of the AED used (Farhat et al. 2002). In controlled studies, VPA has been associated with reduced bone mineral density and mineralization in children with epilepsy (Sheth et al. 1995, Kafali et al. 1999) and CBZ with abnormal bone turnover (Verrotti et al. 2002). Nevertheless, other controlled studies failed to confirm these findings associated with the use of CBZ (Sheth et al. 1995, Akin et al. 1998, Kafali et al. 1999) or VPA (Akin et al. 1998). As a whole, the new AEDs seem to be better tolerated than the older drugs, but their long-term consequences are not yet fully
established. The long-term effects of AEDs on growth and endocrine and metabolic functions will be discussed in more detail later in this thesis.

### 2.2 Reproductive endocrine system

#### 2.2.1 Female subjects

**2.2.1.1 Hypothalamus-pituitary-ovarian axis**

The classic type of interaction between the endocrine and nervous systems is feedback control, which also connects the hypothalamic and pituitary functions with ovarian function (Figure 1). The hypothalamus secretes gonadotropin-releasing hormone (GnRH) in a pulsatile fashion into the hypothalamic-pituitary portal vascular system and enhances the release of glycoprotein gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland, also in a pulsatile manner. Gonadotropins regulate follicle growth, ovulation, corpus luteum and the synthesis of estrogen, progesterone (P) and inhibin in the ovaries, which, on the other hand, have feedback effects on the release of gonadotropins from the pituitary. (Carr 1998.)

![Fig. 1. Schematic representation of the hypothalamic-pituitary-gonadal axis. + stimulative effect; - inhibitory effect; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; E<sub>2</sub>, estradiol; P, progesterone; T, testosterone.](image-url)
2.2.1.2 Serum circulating reproductive hormones

The secretion of gonadotropins varies during the female life span. After two peaks (*in utero* and a few months after birth), gonadotropin levels are low during childhood. The hypothalamic-pituitary axis is very sensitive to estrogen, and the circadian pattern in gonadotropin secretion may already be observed in prepuberty (Jakacki *et al.* 1982). The pituitary becomes very sensitive to GnRH during puberty. Gonadotropin serum concentrations rise at puberty, and their pulsatile secretion continues throughout the fertile life until menopause. FSH stimulates the development of ovarian follicles, and LH is a steroidogenic gonadotropin. (Carr 1998.)

Ovaries, being the target organs for gonadotropins, secrete three major steroids: estrogens, progestagens and androgens, which all are derived initially from cholesterol (Figure 2). The steroidogenic pathway of the ovaries is essentially similar to that of the adrenal cortex. Estrogens, with estradiol (E2) as the most potent form and the major ovarian estrogen, promote the growth and development of the uterus, fallopian tubes, vagina, ductal system of the breast and other secondary sexual characteristics. Progesterones are also needed for the implantation of ovum and the maintenance of pregnancy. Androstenedione (A) is the most abundant androgen secreted by the ovaries. It can be converted further to testosterone (T) and estrogen either in the ovary or in extraglandular tissue, e.g. adipose tissue (Carr 1998). E2 and T are bound in serum with high affinity to sex hormone binding globulin (SHBG), which is synthesized in the liver (Petra 1979). SHBG regulates the bioavailability of sex steroids. The SHBG level varies during the menstrual cycle due to hormonal control, i.e. estrogen administration increases, whereas T decreases plasma SHBG.

![Steroidogenesis in gonads and in adrenal glands](image)

**Fig. 2. Steroidogenesis in gonads and in adrenal glands.** Estradiol and progesterone are the major hormones secreted from the ovaries, and testosterone is the major hormone secreted from the testes. Above the marked line is adrenal steroidogenesis, and the pathways only occurring in adrenal glands are marked as gray. All white pathways are found in gonadal steroidogenesis.
2.2.1.3 Menstrual cycles

Menstrual cycles are cyclic responses to the pulsatile variations of gonadotropin levels. The hypothalamus, pituitary, ovaries and genital tract are all involved in the regulation of normal menstrual cycles, which initially begin during gonadarche at puberty. The age of menarche is influenced by many factors (e.g. genetic and nutritional factors). The mean or median age at the onset of menstruation varies within 12.0-13.5 years among European girls and within 12.2-12.9 among girls in the USA (Parent et al. 2003). The mean age of menarche among Finnish girls is 13.3 years (Rimpelä & Rimpelä 1993). The secular decrease in menarcheal age witnessed during the last century seems to have discontinued or at least slowed down in white girls, but is still suggested to be evident in black girls, who undergo menarche few months earlier than white girls (Herman-Giddens et al. 1997, Freedman et al. 2002).

The median length of the ovulatory period in healthy fertile women is 28 days with a range of 25 to 30 days. The length of the period depends mostly on the length of the follicular phase, because the luteal phase lasts quite constantly for 14 days. Regular menstrual cycles in adults are defined as cycles with a maximum minus minimum length of under 4 days and with intermenstrual variation intervals between 21 to 35 days (Polson et al. 1988). The hormonal interactions during the menstrual cycle are complex, but quite well established. Menstrual periods are sensitive to hormonal dysfunction due to complex hormonal interactions. Therefore, the presence of normal menstrual cycles implies that ovulation and the level of gonadotropins, estrogen, P and adrogens are adequate. (Carr 1998.)

Anovulatory cycles are common during adolescence for at least 1.4 years after menarche (Apter et al. 1987), and they may lead to infrequent menstruation, especially prolonged intervals between the menstruations (i.e. oligomenorrhea). However, the menstrual disorders in adolescence are not always caused by an immature hypothalamic-pituitary axis, but they may be an early sign of an endocrine disorder, such as polycystic ovary syndrome (PCOS), which is typically associated with menstrual disorders also in adulthood (van Hooff et al. 1999, Franks 2002).

2.2.1.4 Ovaries

In the ovary, the reproductive unit is a follicle, which consists of a germ cell surrounded by endocrine cells. Active follicular growth and atresia in the ovaries occur already during infancy and childhood (Fauser & van Heusden 1997). In response to rising levels of gonadotropins, especially FSH, ovarian follicles undergo final maturation at puberty (Fauser & van Heusden 1997, Britt & Findlay 2002), and normal ovarian function is dependent on the function of the pituitary-gonadal axis. In a healthy mature ovary, up to 95% of follicles are in a nongrowing phase throughout the female’s fertile life, and only a few undergo the final growing cascade (Fauser & van Heusden 1997). Enlargement of an oocyte is the first step in the growth of a follicle, as the surrounding layers of granulosa cells proliferate to form the primary follicle. The stroma of the follicle undergoes
differentiation into layers called theca interna and externa. This secondary follicle contains LH receptors, and its granulosa cells express FSH receptors. E2, FSH and insulin-like growth factor one (IGF-I) stimulate the proliferation and differentiation of granulosa cells. IGF-binding proteins (IGFBP) are produced by ovarian follicular cells and may antagonize the effects of IGFs and gonadotropins on these cells (Cataldo 1997). LH is the main regulator of androgen synthesis in theca cells in a normal ovary, but insulin and IGF-I may also stimulate steroid production in addition to proliferation of cells in human thecal cells (Bergh et al. 1993, Duleba et al. 1998). Synchronized follicular growth, ovulation and corpus luteum function make up the cyclic cascade. (Carr 1998.)

Ovarian volume increases during puberty from 2.8 to 15 mL. A mature healthy ovary is up to 5 cm in length, up to 3 cm in width and up to 1.5 cm in thickness. The ovary has three different zones: the germinal epithelium and the follicles are in the outer cortex, the stroma forms the central medulla, and the blood vessels enter in the hilum. (Carr 1998.)

Ovarian disorders may cause sexual precocity, menstrual irregularities, androgen excess and infertility. Previous epidemiological series have shown 10-23% of women to have PCO in ultrasonography, with no endocrine or metabolic changes suggestive of PCOS (Polson et al. 1988, Farquhar et al. 1994, Borgfeldt & Andolf 1999). The prevalence of PCO has turned out to be higher (26-33%) in adolescents and young women (Bridges et al. 1993, Michelmore et al. 1999) and multicysticity to be even a normal finding in healthy pubertal ovary. Even in the absence of full universal consensus concerning the diagnostic criteria, the prevalence of PCOS has usually been 4-10% in the female population of reproductive age (Hopkinson et al. 1998, Knochenhauer et al. 1998, Guzick 2004). PCOS is accepted as one of the most common reproductive endocrinopathies in women, and it was first described by Stein and Leventhal (Stein & Leventhal 1935). The ultrasonographic criteria of PCO widely used in Europe were defined by Adams and coworkers (Adams et al. 1985) and criteria of PCOS by Homburg (Homburg 1996). According to some criteria, used especially in the USA, the diagnosis of PCOS may be assessed based on serum hormone levels and without ultrasonography finding of PCO (Fox et al. 1991). PCOS is characterised by anovulation, menstrual disorders and infertility problems. HA, acne, acanthosis nigricans, hirsutism and obesity are often visible signs of this common syndrome (Hopkinson et al. 1998, Guzick 2004). Moreover, factors predisposing to type 2 diabetes and cardiovascular diseases, e.g. insulin resistance, hyperinsulinemia and dyslipidemia, are frequent in subjects with PCOS (Hopkinson et al. 1998, Guzick 2004). These frequently associated health implications make this gynaecological syndrome also a clinically relevant multisystem metabolic endocrinopathy (Homburg 1996, Hopkinson et al. 1998).
2.2.2 Male subjects

2.2.2.1 Hypothalamus-pituitary-testicular axis

Testicular function is regulated by the hypothalamic-pituitary system similarly to ovarian regulation in female subjects (Figure 1). GnRH is secreted by the hypothalamus, and it enhances the production of LH and FSH in the pituitary. These gonadotropins further regulate testicular function. The primary target sites for LH are Leydig cells, and those for FSH are Sertoli cells (Means & Vaitukaitis 1972). (Griffin & Wilson 1998.)

2.2.2.2 Serum circulating reproductive hormones

LH stimulates Leydig cells to produce steroids, and FSH induces Sertoli cells to support spermatogenesis. FSH indirectly also stimulates steroidogenesis by inducing maturation of Leydig cells and increasing the number of LH receptors in their cell surface. Gonadotropins up- and down-regulate their own receptors in testicular cells. With the exception of peak in serum LH, FSH, T and inhibin B concentrations after few postnatal months (Tapanainen et al. 1982, Andersson et al. 1998), serum gonadotropin and testicular steroid levels are low during childhood until puberty. Serum adrenal steroid levels usually increase before the increase in testicular steroid synthesis during puberty. (Griffin & Wilson 1998.)

Serum T is a major testicular hormone, and similarly to all other steroids, it is derived from cholesterol (Figure 2). The process from cholesterol to T includes five enzymatic steps, of which the conversion of cholesterol to pregnenolone is most often the rate-limiting phase. T is a potent androgen, but it is largely bound to albumin and SHBG in plasma (Dunn et al. 1981). Approximately 2% of total T is present in an unbound form as free T. In addition, testes secrete other steroids: dihydrotestosterone, androsterone, A, 17-hydroxyprogesterone, P and pregnenolone (Hammond et al. 1977). A is converted to estrogen extraglandularly for example in adipose tissue. In addition to the regulation of hypothalamic-pituitary feedback and spermatogenesis, androgens control sexual maturation in puberty and potency and libido in adults. A glycoprotein inhibin B is considered a marker of Sertoli cell function in mature testis (Andersson et al. 1998). Because both spermatogenesis and steroidogenesis are sensitive and complex events, many internal and external factors (e.g. drugs, stress, diet) may affect these functions (Jørgensen et al. 2001, Parent et al. 2003). (Griffin & Wilson 1998.)

2.2.2.3 Testes

The main testicular functions, spermatogenesis and steroidogenesis, take place in different parts of the testes. 80% of mature testis is composed of seminiferous tubules,
which contain Sertoli and germ cells and 20% of interstitial tissue with Leydig cells. Androgen hormones are produced by Leydig cells in the interstitium, and sperm is produced in seminiferous tubules in close contact with Sertoli cells. Sertoli cells are the main cell type in the testes during prepuberty and puberty, but germ cells are the most common cell type in adult testis. Adult type Leydig cells appear during puberty after stimulation by LH. Boys usually reach spermarche between the ages 11 and 15 (Nielsen et al. 1986), and at the bone age of 17 sperm reaches its adult motility and morphology and semen its adult volume. A mature testis is approximately 20 mL in volume and 4.5 cm in length. (Janczewski & Bablok 1985, Grumbach & Styne 1998, Toppari & Huhtaniemi 1999.)

2.3 Thyroid function

Normal thyroid function is essential for normal growth, development and metabolic homeostasis. The hypothalamus secretes thyrotropin-releasing hormone (TRH), which stimulates the anterior pituitary lobe to produce thyrotropin (TSH), which further stimulates the growth of the thyroid gland and the synthesis and release of thyroid hormones, mainly l-thyroxine (T4) (Figure 3). The biosynthesis of thyroid hormones includes iodide uptake, iodination and coupling and retrieval from thyroglobulin. Plasma contains many different iodothyronines, but T4 is the most common of them. T4 must undergo deiodination to a more active form of l-triiodothyronine (T3), which mainly takes place in the liver, kidney and muscle, but also in the thyroid gland, pituitary, central nervous system and possibly cardiac muscle. T3 and T4 regulate the synthesis and secretion of TSH by negative feedback. Several agents, e.g. iodide, inhibit the release of thyroid hormones from the thyroid gland. Therefore, a healthy, active hypothalamic-pituitary-thyroid axis maintains a sensitive balance between serum thyroid hormones and TSH. (Surks & Ocampo 1996, Larsen et al. 1998.)

In blood, most thyroid hormones are in a reservoir bound to specific serum-binding proteins, i.e. thyroxine-binding globulin (TBG) and transthyretin, and a minority to albumin and lipoproteins. Only a minority of circulating T4 and T3 are in the free, biologically active forms (FT4 and FT3), and TBG is the major regulator of the quantities of these free fractions. Since the conversion of T4 to T3 is influenced by many extrathyroid factors, T4 is a more appropriate hormone than T3 to be measured for thyroidal activity. Serum FT4 measurement together with the assessment of serum TSH is a clinically relevant means to examine the function of the thyroid gland. (Surks & Ocampo 1996, Larsen et al. 1998.)
2.4 Serum lipids

Lipids are needed as an energy source, as precursors for steroidogenesis and bile acids and as extra- and intracellular messengers. Lipids are classified into fatty acids, cholesterol (TC) and complex lipids, i.e. triglycerides (TGs) and phospholipids. Lipids are water-insoluble and thus need lipoproteins as vehicles for transport in blood. High-density lipoprotein cholesterol (HDL-C) contains an approximately equal amount of lipids as proteins, but low-density lipoprotein cholesterol (LDL-C) and very-low-density lipoprotein cholesterol are cholesterol-rich forms. All cell surfaces have LDL receptors, which further regulate the serum levels of cholesterol keeping the intracellular cholesterol level quite constant (Brown & Goldstein 1986). Because the liver is the principal organ for the synthesis and elimination of lipoproteins, alterations in hepatic function have some influence on plasma lipoprotein levels. (Mahley et al. 1998.)

Cholesterol is an essential component of cell membranes, and a precursor for all steroids. A diet of animal origin and cholesterol biosynthesis especially in the liver are the main sources of serum cholesterol in humans (Dietschy et al. 1993). TGs store up fatty acids and are composed of three fatty acid molecules esterified to a glycerol molecule (Mahley et al. 1998). In adipose tissue, lipoprotein lipase activity releases the free fatty acids from TGs, and glucose and insulin promote the conversion of free fatty acids to TGs. Insulin also inhibits free fatty acid release through the hormone-sensitive lipase. Insulin and glucose also stimulate the formation of free fatty acids in the liver. (Mahley et al. 1998.)
2.5 Growth

2.5.1 Height

The rate of growth is highest during fetal life. During the first two years of life, the mean growth velocity is 15 cm per year, which decreases to approximately 6 cm per year in middle childhood. However, the first peak in postnatal growth takes place during the mid-growth spurt, usually at the age of 6 to 8 years, and the second peak during the adolescent growth spurt (Tanner 1962). The adolescent growth spurt takes place at the average age of 9.5 to 14.5 years and at pubertal stage 2 to 3 in girls, and at 10.5 to 16 years of age and pubertal stage 3 to 4 in boys (Tanner 1962, Grumbach & Styne 1998). After menarche, girls grow 3 to 11 cm (average 7 cm), which is approximately 2.5% of their final height (Dunkel 2000). The growth takes place in a definite sequence under complex hormonal control. The growth hormone stimulates IGF-I production, and they are the most important regulators of postnatal growth (Juul et al. 1995). Secretion of growth hormone can be indirectly measured by the levels of IGF-I and IGFBP-3, concentrations of which rise during pubertal maturation (Juul et al. 1995). Gonadal steroids increase serum growth hormone and further IGF-I secretion, and steroids also directly affect cartilage and bone. Thyroid hormones and adrenal androgens also participate in linear growth during puberty. (Carr 1998, Grumbach & Styne 1998.)

Estrogen, formed through aromatization from T, regulates the epiphyseal fusion of the long bones in both genders. Final height has been attained when the epiphyses of the long bones are completely closed. Since the epiphyses do not all fuse at the same time, bone age can be measured as a way of timing skeletal maturation. Assessment of bone age from wrist x-rays is a widely used standard method (Greulich & Pyle 1959). Onset of puberty can also be seen from bone age: in early puberty, bone age is 11 years in girls and 13 years in boys (Grumbach & Styne 1998). Male adolescents reach approximately 99% of their final height by the bone age of 17 years and female adolescents by the bone age of 15 years (Greulich & Pyle 1959, Dunkel 2000).

In 1978, the World Health Organization (WHO) created a model growth chart, which provides a graphic representation of the development and changes of growth and weight over time. Body height differs in different populations. Thus, it is necessary to have validated national standards for growth. The modified chart widely used in clinical practice in Finland was updated in 1993. Height is assessed in relation to age and sex, and it is possible to examine height in relation to mid-parental height. Up to approximately 46% of the variation in final height is expected by the variation in parental heights. Weight is assessed in relation to the subject’s height in the Finnish growth charts. (Sorva et al. 1989, Sorva et al. 1990.)
2.5.2 Weight

Prepubertal boys and girls have similar amounts of body mass and fat. An evident increase in lean body mass is seen in girls at the age of 6 years and in boys at the age of 9.5 years. However, body fat distribution differs between boys and girls already during adolescence. Girls have more often pear-shaped and boys apple-shaped fat distribution. Women have more body fat than men, but men have greater body mass (Rico et al. 1993). Triceps skinfold and waist hip ratio are used as measurements of body fat distribution. Body mass index, BMI = weight (kg) divided by the square of height (m), is widely used instead of absolute weight to assess the shape of the body in adults. Normal values for BMI vary with age, sex and pubertal stage. Thus, weight for height is used to assess weight in children. (Grumbach & Styne 1998.)

2.5.3 Pubertal maturation

Age at the onset of puberty is determined by genetic, internal and environmental factors (Parent et al. 2003). The Tanner and Whitehouse pubertal stage classification standards for both genders are in wide use (Tanner 1962, Tanner & Whitehouse 1976). The onset of adrenal androgen secretion is known as adrenarche, which is independent from pituitary-gonadal maturation. Pubertal maturation is mainly regulated by sex hormones and gonadotropins. The first signs of pubertal development are often seen about a year before the adolescent height spurt.

The first clinical sign of puberty in girls is most often the maturation of breasts, thelarche, which is controlled primarily by ovarian estrogens (Parent et al. 2003). The mean age at the onset of puberty in white girls is 10.6 years with wide normal limits between 8 and 13 years in Europe (Grumbach & Styne 1998, Parent et al. 2003). After that, adrenal and ovarian androgens induce the growth of pubic and axillary hair, which, together with peak in linear growth, take place at a median age of 11.4 years (Carr 1998). Menses begin at the mean or median age of 12.0-13.5 years in European girls and at the mean age of 13.3 years in Finnish girls (Rimpelä & Rimpelä 1993, Parent et al. 2003). Moderate obesity advances whereas morbid obesity or malnutrition delays the onset of menarche (Grumbach & Styne 1998). E₂, the principal ovarian hormone, is mostly responsible for the development of secondary female characteristics (Carr 1998).

The first sign of pubertal development in boys is the growth of the testes and scrotum. The mean age of boys at the onset of puberty is 11 years, with wide normal limits from 9 to 13.5 years (Grumbach & Styne 1998, Parent et al. 2003). Usually, this is followed by the growth of pubic hair. The penis begins to grow about one year after that, concurrently with the adolescent height spurt. The maturation of the penis usually correlates with the pubic hair stage, because both are under androgen control. Pubic hair growth is followed two years later by axillary and facial hair in boys. Many other pubertal signs are also seen, e.g. enlargement of the larynx and consequent deepening of the voice, sweat gland enlargement and increasing muscular strength. (Tanner 1962, Grumbach & Styne 1998.)
2.6 Epilepsy and endocrine function

Endocrine function is under cerebral control, and brain activity is affected by many hormones. Thus, some hormones may affect seizure activity in epilepsy, e.g. high serum T4 may predispose to status epilepticus (Jabbari & Huott 1980), and estrogen may lower the seizure threshold level, whereas P may raise it. In catamenial epilepsy, seizures are known to occur when P levels decline rapidly at the end of the luteal phase of the menstrual cycle (Mattson & Cramer 1985, Herzog et al. 1997).

On the other hand, overpresentation of reproductive endocrine disorders (Herzog et al. 1986a, Herzog et al. 1986b, Bilo et al. 1988) and changes in serum hormone concentrations, especially sex hormones (Isojärvi et al. 1988, Macphee et al. 1988, Isojärvi et al. 1989a, Isojärvi et al. 1989b) and thyroid hormones (Bentsen et al. 1983, Isojärvi et al. 1989c, Larkin et al. 1989), have been described in patients with epilepsy in both genders since the 1980’s. The direct influence of epileptic discharges on the hypothalamic-pituitary function, which causes increased secretion of pituitary hormones may be partly responsible for reproductive endocrine changes in patients with epilepsy (Dana-Haeri et al. 1983, Bilo et al. 2001, Herzog et al. 2003a). Especially localization-related epilepsy, temporal lobe origin in particular, has been suggested to underlie reproductive endocrine disorders (Herzog et al. 1986a, Herzog et al. 1986b, Cummings et al. 1995, Herzog et al. 2003b). Animal studies also support the view that temporolimbic seizures may cause reproductive endocrine dysfunction in both genders (Feeney et al. 1998, Edwards et al. 1999). Specific AEDs may also influence hormonal functions (Isojärvi et al. 1988, Macphee et al. 1988, Isojärvi et al. 1989a, Isojärvi et al. 1989b, Isojärvi et al. 1993b, Vainionpää et al. 1999, Morrell et al. 2003) and further the development of detected endocrine disorders in patients with epilepsy (Isojärvi et al. 1993b, Morrell et al. 2002, Isojärvi et al. 2004). These effects of AEDs are proposed to be mediated mainly through induced changes in the pharmacokinetics and metabolism of the hormones.

Although the underlying reasons are not fully known, sexual dysfunction and reproductive endocrine disorders involving infertility in addition to the lower marriage rate may explain the lower childbirth rates of patients with epilepsy compared to the average population (Dansky et al. 1980, Webber et al. 1986, Schupf & Ottman 1996, Jalava & Sillanpää 1997, Wallace et al. 1998, Artama et al. 2004).

2.7 Effects of AEDs on reproductive endocrine system

2.7.1 Female patients with epilepsy

Reproductive endocrine disorders have been reported in female subjects with epilepsy of all ages (Herzog et al. 1986b, Isojärvi et al. 1993b, Vainionpää et al. 1999, Morrell et al. 2002). Serum reproductive hormone changes may result in menstrual disorders and
PCOS, which may have an impact on fertility in women with epilepsy. Ovulatory dysfunction, premature ovarian failure and hyperinsulinism are also more frequent in female patients with epilepsy than in the average female population (Morrell et al. 2002). Reproductive endocrine changes are often associated with specific AEDs.

2.7.1.1 Carbamazepine

Reproductive endocrine changes have been observed in female patients with epilepsy taking CBZ. The findings regarding serum gonadotropin and prolactin levels have been inconsistent in different studies (Dana-Haeri et al. 1984, Isojärvi et al. 1989a, Isojärvi 1990), but serum SHBG levels have been found consistently to increase and dehydroepiandrosterone sulfate (DHEAS) levels often to decrease in women with epilepsy during CBZ medication (Levesque et al. 1986, Isojärvi 1990, Isojärvi et al. 1995b, Rättyä et al. 2001a). A progressive rise in serum SHBG levels was reported in a prospective study (Isojärvi et al. 1995b).

Liver enzyme induction by CBZ may accelerate the hepatic plasma clearance of certain hormones and enhance the production of binding proteins of some hormones, e.g. SHBG (Connell et al. 1984a, Perucca et al. 1984, Brunet et al. 1995). CBZ has been shown to increase the metabolism of oral contraceptives containing ethinylestradiol and progestagens (Crawford et al. 1990). Moreover, the increase of serum SHBG concentrations leads to reduced serum free bioactive sex steroid (E2 and T) levels (Isojärvi 1990, Isojärvi et al. 1995b, Rättyä et al. 2001a), which may contribute to reproductive disorders. However, menstrual cycles and ovarian structure appear to be normal in women taking CBZ for epilepsy (Isojärvi et al. 1993b, Murialdo et al. 1997, Murialdo et al. 1998, Bauer et al. 2000, Isojärvi et al. 2001a, Morrell et al. 2002).

2.7.1.2 Oxcarbazepine

Reproductive endocrine function during OXC treatment in women with epilepsy is poorly investigated, and there are no reports of abnormalities up to date. OXC is a weaker inducer of liver enzymes than CBZ (Patsalos et al. 1990, Isojärvi et al. 1995c), and its possible endocrine effects may be dose-dependent (Rättyä et al. 2001b). However, OXC has been shown to increase the metabolism of ethinylestradiol and progestogens of oral contraceptives, which effect has also been seen with many other liver enzyme inducing AEDs (Fattore et al. 1999). The changes in serum SHBG and DHEAS seen in men with epilepsy during CBZ normalized after replacement of CBZ by OXC (Isojärvi et al. 1995c), but high serum T, gonadotropin and SHBG concentrations have been detected in men with epilepsy taking high doses of OXC (Rättyä et al. 2001b). Furthermore, infertility was observed in an animal study in female monkeys during OXC exposure (Lockard et al. 2000). Thus, there is evidence to suggest that endocrine or metabolic effects may also be possible in women taking OXC.
2.7.1.3 Valproate

There is a strong link between VPA treatment and reproductive endocrine disorders in female patients with epilepsy. Elevated serum androgen levels and hyperandrogenism (HA) (Isojärvi et al. 1993b, Murialdo et al. 1998, Rättyä et al. 2001a, Stephen et al. 2001, Morrell et al. 2003) as well as menstrual disorders (Margraf & Dreifuss 1981, Svalheim et al. 2003) with ovulatory failure (Morrell et al. 2002) and with PCO or PCOS (Isojärvi et al. 1993b, Isojärvi et al. 2001a, Betts et al. 2003) have been described in association with VPA medication in women with epilepsy. Hyperinsulinism has been reported in association with these reproductive endocrine disorders (Isojärvi et al. 1996, Isojärvi et al. 2001a, Stephen et al. 2001).

PCO and menstrual irregularities have been found in approximately 40-60%, and PCOS in 39-49% of women on VPA monotherapy in some studies (Isojärvi et al. 1993b, Isojärvi et al. 2001a). Isojärvi et al. found elevated serum T levels and PCO to be most common in women with VPA started before the age of 20 (Isojärvi et al. 1993b). HA was also seen in up to 58% of girls with epilepsy taking VPA during pubertal maturation at the age of 8 to 18 years (Vainionpää et al. 1999), but HA was not associated with hyperinsulinism or increased frequency of PCO in VPA-treated girls. Ovarian structure (Murialdo et al. 1997, Murialdo et al. 1998, Luef et al. 2002b), menstrual cycles (Murialdo et al. 1997, Bauer et al. 2000, Luef et al. 2002b) and the frequency of PCOS (Bauer et al. 2000, Stephen et al. 2001) have been normal or at least similar to those seen in patients on other AEDs in some studies of women on VPA monotherapy. Although reproductive endocrine disorders have been associated with the use of VPA (Isojärvi et al. 1993b, Isojärvi et al. 1996, Murialdo et al. 1997, Isojärvi et al. 1998, Morrell et al. 2002, Betts et al. 2003), data on the prevalence of PCOS in patients with epilepsy are variable, at least partly due to the different diagnostic criteria of PCO and PCOS (Murialdo et al. 1997, Morrell et al. 2002, Betts et al. 2003). Nevertheless, an increased PCOS frequency has been observed in relation to the use of VPA in non-epileptic patients with bipolar disorder (O’Donovan et al. 2002), and cystic changes in ovaries have also been seen in animal studies on VPA exposure (Tauboll et al. 1999).

In general, the precise pathogenesis of PCO is unknown. It is known that the rate-limiting steps in androgen biosynthesis are affected in PCOS (Barnes et al. 1989), which leads to accumulation of 17-hydroxyprogesterone and A. But the causes of the increased activity of this pathway have not been established. LH, as a major regulator of androgen synthesis in ovary, is only elevated in some patients with PCO. Thus, some other mechanisms are also involved. IGF-I is known to potentiate the expression of LH receptors in healthy ovary (Adashi et al. 1985), and it stimulates LH-induced androgen production in the ovary (Barbieri et al. 1986). A decrease in IGFBP-1 levels may cause increased bioavailability of IGF-I in patients with PCOS. High serum T concentrations may arrest follicular maturation and influence the development of PCO. Also inadequate FSH level is hypothesized to be a cause of anovulation in patients with PCOS (Guzick 2004).

In addition, the exact mechanisms behind the increased frequency of PCOS and the enhancement of androgen production associated with VPA are still under research. Today, it seems likely that obesity, hyperinsulinism and low serum IGFBP-1 levels resulting in
increased bioactivity of IGF-I, associated with the use of VPA in some women, may ultimately result in reproductive endocrine disorders (Isojärvi et al. 1996, Isojärvi et al. 2001a), but this is not considered the main reason for HA and PCOS in women taking VPA. PCOS and HA have also been common in VPA-treated lean women with epilepsy with normal serum insulin and IGFBP-1 levels (Isojärvi et al. 2001a) and HA also with normal serum insulin levels in girls taking VPA for epilepsy (Vainionpää et al. 1999). Recently, biomedical evidence has supported the main view that VPA potentiates androgen biosynthesis directly in human ovarian theca cells (Nelson-DeGrave et al. 2004), and T conversion to estrogen has also been seen to be inhibited upon VPA exposure in porcine ovarian follicular cells in vivo (Taubøll et al. 2003).

2.7.1.4 Lamotrigine

There are only a few studies on the effects of LTG on reproductive health in women with epilepsy, and these studies have mostly compared the reproductive endocrine effects of LTG to those of VPA. LTG has been shown to have favourable effects on serum T concentrations and ovarian structure when substituted for VPA (Isojärvi et al. 1998). Normal serum androgen levels were also seen in some other studies (Stephen et al. 2001, Morrell et al. 2003), and LTG was not considered a predictor of ovulatory failure, as was VPA (Morrell et al. 2002). Reproductive endocrine changes have been associated with weight gain in VPA-treated patients with epilepsy (Isojärvi et al. 1996, Morrell et al. 2003), but LTG has been found to be a weight-neutral AED (Biton et al. 2001, Biton et al. 2003). LTG does not affect the metabolism of oral contraceptives (Crawford 2002).

Furthermore, in experimental studies on non-epileptic animals, ovarian morphology and serum T, FSH, LH, insulin and P levels remained unchanged, but serum estrogen concentrations dropped during LTG exposure (Sveberg Røste et al. 2001b, Sveberg Røste et al. 2003b).

2.7.2 Male patients with epilepsy

Reproductive dysfunction is often reported in male patients with epilepsy. Reproductive endocrine disorders, reduced potency, diminished sexual interest (Toone et al. 1983, Dana-Haeri et al. 1984, Mattson et al. 1985, Herzog et al. 1986a), changes in testicular volume (Isojärvi et al. 2004) and sperm abnormalities (Taneja et al. 1994, Sveberg Røste et al. 2003a, Isojärvi et al. 2004) are suggested to be manifestations of this dysfunction. These disorders and changes in serum sex hormone levels are frequently seen in association with specific AEDs.

There are many studies that have assessed the reproductive health of men with epilepsy, but the reproductive endocrine function in boys with epilepsy during pubertal maturation is poorly defined. High serum T levels and diminished testicular size in young male subjects with epilepsy on different AEDs have been reported (El-Khayat et al.
The study of El-Khayat et al. included control subjects matched for age, but not for pubertal stage, and it was not possible to differentiate between the effects of various AEDs on the evaluated parameters (El-Khayat et al. 2003). In contrast, two other studies showed boys with epilepsy on CBZ (Verrotti et al. 2000) or on VPA (Verrotti et al. 2000, Balaguer Martinez et al. 2003) to have normal serum T levels.

2.7.2.1 Carbamazepine

Sexual dysfunction, e.g. reduced libido and potency, related to long-term CBZ exposure in some men with epilepsy may be linked to the elevation of serum SHBG and further to the reduction of free serum androgen levels (Isojärvi et al. 1995d). In addition to high serum SHBG levels, low serum DHEAS concentrations are also common in men with epilepsy on CBZ (Isojärvi et al. 1988, Macphee et al. 1988, Isojärvi et al. 1989b, Isojärvi et al. 1990, Isojärvi et al. 1995d, Rättyä et al. 2001a, Rättyä et al. 2001b), but the clinical consequences of the reduction of this weak androgen are unclear.

Poor sperm motility in human sperm have been detected following in vitro and in vivo exposure to CBZ (Chen et al. 1992) as well as morphological changes in sperm in clinical studies (Isojärvi et al. 2004). Low levels of free bioactive T have been suggested to lead to problems in spermatogenesis in men with CBZ. In vitro studies in rats have shown CBZ to inhibit T formation in Leydig cells (Kühn-Velten et al. 1990). In addition to CBZ, temporal lobe epilepsy has been seen to be associated with changes in serum gonadotropin and T levels, all of which hormones are needed for normal spermatogenesis. This may affect unfavourably semen quality and be responsible for the effects seen in men with localization-related epilepsy taking CBZ (Herzog et al. 1986a, Stoffel-Wagner et al. 1998, Isojärvi et al. 2004).

2.7.2.2 Oxcarbazepine

OXC may also have reproductive endocrine effects in men with epilepsy. Serum reproductive hormone levels normalized (SHBG concentration decreased and DHEAS levels increased) after substitution of OXC for CBZ in twelve men with epilepsy in a six-month follow-up study (Isojärvi et al. 1995c). In contrast, however, high doses of OXC were observed to be associated with altered serum sex hormone levels in men with epilepsy (Rättyä et al. 2001b). Serum concentrations of T, gonadotropins and SHBG were high in patients taking high (≥ 900 mg) daily doses of OXC (Rättyä et al. 2001b). In another study, men with epilepsy taking OXC were found to have sperm abnormalities more often than healthy subjects (Isojärvi et al. 2004). Reproductive endocrine effects of OXC have, as yet, not been studied in young male subjects.
2.7.2.3 Valproate

In contrast to the earlier perceptions that VPA does not affect male reproductive health (Macphee et al. 1988, Isojärvi et al. 1990), high serum androgen (Rättyä et al. 2001a, Rättyä et al. 2001b, Isojärvi et al. 2004) and low gonadotropin levels (Isojärvi et al. 1990, Rättyä et al. 2001b) have been observed in men with epilepsy taking VPA. Moreover, cases of VPA-linked male infertility (Curtis et al. 1992, Yerby & McCoy 1999) have been reported. In animal studies, administration of VPA was associated with testicular atrophy and reduced spermatogenesis (Walker et al. 1990, Berner et al. 1999, Sveberg Røste et al. 2001a), and in vitro human studies showed sperm motility to be impaired (Chen et al. 1992). Recently, sperm abnormalities (Sveberg Røste et al. 2003a) and poor sperm motility in association with small testicular volume was also reported in VPA-treated men with generalized epilepsy (Isojärvi et al. 2004). However, the underlying mechanisms are unknown. VPA is known to have effects on mitochondria (Ponchaut & Veitch 1993), which are essential for the energy production of cells, especially spermatozoa (Ruiz-Pesini et al. 1998).

2.7.2.4 Lamotrigine

Only a few studies have been performed to evaluate the reproductive endocrine effects of LTG in male subjects. In one available study, 15 men with LTG for at least two years had no changes in serum reproductive hormone levels (Stephen et al. 2001). In animal studies, testicular morphology (Berner et al. 1999, Sveberg Røste et al. 2003b) and serum T, LH and FSH concentrations were normal in non-epileptic rats with LTG (Sveberg Røste et al. 2003b).

2.8 Thyroid function in patients with epilepsy

It is well documented that AEDs may alter serum thyroid hormone levels both in adults and in children. Normal serum baseline thyroid hormone levels have been observed in untreated newly diagnosed patients with epilepsy in controlled studies (Isojärvi et al. 1989c, Larkin et al. 1989, Yüksel et al. 1993). In many studies with variable methodologies CBZ has been seen to decrease serum thyroid hormone concentrations in patients with epilepsy of both genders and all ages. Consistent findings have included a decrease of T₄ and FT₄ levels and, more rarely, a reduction in T₃ and FT₃ levels in both controlled cross-sectional (Larkin et al. 1989, Isojärvi et al. 1993a, Tiihonen et al. 1995, Eiris-Puñal et al. 1999, Isojärvi et al. 2001b, Verrotti et al. 2001, Çaksen et al. 2003) and uncontrolled (Bentsen et al. 1983) and controlled prospective follow-up studies (Strandjord et al. 1981, Isojärvi et al. 1989c, Yüksel et al. 1993). Only a few studies have suggested an increase of serum TSH in patients with epilepsy taking CBZ (Eiris-Puñal et al. 1999) whereas the serum basal TSH level (Strandjord et al. 1981, Isojärvi et al. 1989c,
Yüksel et al. 1993, Isojärvi et al. 2001b, Verrotti et al. 2001) and the TSH response to TRH have remained unaltered in most controlled cross-sectional and follow-up studies in patients with epilepsy taking CBZ (Larkin et al. 1989, Tiihonen et al. 1995, Verrotti et al. 2001). Also, serum TBG levels have been seen to decrease in patients with epilepsy on CBZ (Strandjord et al. 1981, Bentsen et al. 1983, Isojärvi et al. 1989c, Eiris-Puñal et al. 1999). Connell et al. also showed a reduction in serum T₄, FT₄ and T₃ levels in healthy men exposed to CBZ, whereas their serum FT₃, TBG and basal and stimulated TSH concentrations remained unchanged (Connell et al. 1984b).

Isojärvi et al. found CBZ-related serum T₄ and FT₄ reductions to normalize during the first six months following replacement of CBZ with OXC in adults with epilepsy (Isojärvi et al. 1995a). However, low serum T₄ and FT₄ levels with unchanged serum TSH levels were later found in men with epilepsy taking OXC for a longer period (Isojärvi et al. 2001b).

Reports of VPA-related thyroid hormone changes at all ages are contradictory. No alterations in thyroid hormone or TSH levels in patients with epilepsy taking VPA have been found in many controlled studies (Larkin et al. 1989, Isojärvi et al. 2001b, Verrotti et al. 2001, Çaksen et al. 2002). However, both decreased (Bentsen et al. 1983, Eiris-Puñal et al. 1999) and increased (Isojärvi et al. 1992) concentrations of serum thyroid hormone have been reported in patients on VPA. Moreover, elevated concentrations of serum TSH have also been observed during VPA treatment (Isojärvi et al. 1992, Eiris-Puñal et al. 1999).

Even though many changes in serum thyroid hormones and TSH have been reported during different AEDs in patients with epilepsy, thyroid function after AED withdrawal is poorly studied.

2.9 Serum lipids in patients with epilepsy

Increased serum total cholesterol (TC), HDL-C, LDL-C and TG concentrations have been reported in both prospective studies on adults (Isojärvi et al. 1993c) and controlled studies on children and adolescents receiving CBZ (Verrotti et al. 1998). In a prospective study, Isojärvi et al. found consistently elevated serum TC and HDL-C levels during five-year follow-up, and transiently elevated serum LDL-C and TG levels were also observed during the first year of CBZ treatment (Isojärvi et al. 1993c). All these lipid levels increased in prospective studies on children taking CBZ (Verrotti et al. 1998, Yilmaz et al. 2001), and in cross-sectional controlled studies, mostly serum TC and LDL-C (Söüzier et al. 1997, Eiris et al. 2000), but occasionally also HDL-C levels (Eiris et al. 2000) have been high in children with CBZ.

Favourable effects of OXC on lipid metabolism have been seen in men with epilepsy, whose serum TC levels normalized after replacement of CBZ with OXC in a two-month follow-up study (Isojärvi et al. 1994). On the other hand, elevation in serum TC and LDL-C concentrations, but not HDL-C or TG levels, were found in an uncontrolled study on children with newly diagnosed epilepsy after starting OXC (Serdaroglu et al. 2003).
As a whole, the effects of OXC on serum lipid levels are poorly characterized, and there are no controlled studies on children.

The results on serum lipid levels during VPA treatment for epilepsy are contradictory. No effects of VPA on serum lipid metabolism (Sözüer et al. 1997), and low TC and LDL-C levels (Eiris et al. 2000) have been detected in controlled studies. In a prospective controlled study on children and adolescents, serum HDL-C was seen to increase, whereas LDL-C and TGs decreased during VPA (Verrotti et al. 1998). TG levels have been found to be higher in VPA-treated patients than ones taking LTG (Stephen et al. 2001). However, only obese VPA-treated women had low HDL-C/TC ratios and high TG levels in a controlled study (Isojärvi et al. 2001a). The effects of LTG on serum lipid balance are poorly known. The HDL-C/TC ratio has been observed to increase in women with epilepsy receiving LTG after withdrawal of VPA (Isojärvi et al. 1998). Higher concentrations of serum HDL-C, LDL-C and TC were found in women with LTG compared with VPA in one cross-sectional study (Morrell et al. 2003).

Verrotti et al. suggested no permanent effects of CBZ or VPA on lipid metabolism (Verrotti et al. 1998), but serum lipid metabolism after withdrawal of AEDs has been poorly evaluated.

2.10 Growth in patients with epilepsy

2.10.1 Height

Physical maturation can be adversely affected by a number of internal and external factors. However, the adult height of patients with epilepsy was similar to that of the general population (McGowan 1983, Kurlemann et al. 1997), but men with localization-related epilepsy had shorter height than men with primary generalized epilepsy (McGowan 1983). Patients with epilepsy on different AEDs have also had normal linear growth (Tada et al. 1986, Macardle et al. 1987), but shorter stature in boys with epilepsy compared to age-matched healthy control subjects has also been reported (El-Khayat et al. 2003).

Intrauterine exposure to AEDs has been shown to have some negative influences on postnatal growth (Gaily & Granström 1989, Wide et al. 2000). The findings on the effects of specific AEDs upon growth are inconsistent. Untoward effects of VPA and LTG on growth have been suggested, but the target height of the patients was not assessed in these studies (Novak et al. 1999, Guo et al. 2001). In a cross-sectional study, Guo et al. reported that combined therapy with VPA and LTG was associated with lower height than either of these AEDs administered as monotherapy. Low bone mineral density and reduced bone formation were also seen in patients on long-term VPA and LTG polytherapy in that study. Patients on polytherapy showed reduced physical activity compared with monotherapy patients. Moreover, they also had more severe disease than patients on monotherapy with these drugs (Guo et al. 2001). However, there are many studies reporting normal growth in patients taking CBZ (Kurowski et al. 1993,
Kurlemann et al. 1997, Rättyä et al. 1999), OXC (Rättyä et al. 1999), VPA (Kurowski et al. 1993, Kurlemann et al. 1997, Rättyä et al. 1999) or LTG therapy (Überall 2001). Neither epilepsy type nor AEDs have had any unfavourable influence on adult height in relation with target height in patients who started AEDs before their prepubertal growth spurt (Kurlemann et al. 1997).

2.10.2 Weight

Excessive weight gain has been reported in 8-14% of adult patients treated with CBZ (Mattson et al. 1992, Corman et al. 1997) and in 29% of children on CBZ (Easter et al. 1997). However, not all controlled studies confirm this association of weight gain in children taking CBZ (Rättyä et al. 1999). Increased appetite, hyponatremia and edema associated with impaired secretion of antidiuretic hormone have been suggested to underlie the weight gain sometimes seen in CBZ users (Lampl et al. 1991). The use of LTG has not been shown to influence weight (Biton et al. 2001, Überall 2001, Biton et al. 2003), and stable weight has also been reported during OXC treatment (Rättyä et al. 1999).

VPA, an AED traditionally widely used in children, is well documented to be associated with weight gain in adult patients with epilepsy (Dinesen et al. 1984, Corman et al. 1997) and also in children and adolescents of both genders (Egger & Brett 1981, Novak et al. 1999, Demir & Aysun 2000, Biton et al. 2003, Wirrell 2003). VPA-associated weight gain typically appears early during treatment, often within the first 3-6 months after the initiation of VPA. The frequency of weight gain seemed to increase during pubertal maturation in girls with epilepsy taking VPA (Rättyä et al. 1999). One randomized clinical trial involving more than 200 children revealed no difference in the percentage of excessive weight gain in patients receiving either CBZ (29%) or VPA (26%) (Easter et al. 1997).

Weight gain as an adverse effect of VPA has been known for decades (Völzke & Doose 1973), but the reports of reproductive endocrine disorders associated with weight gain in women (Isojärvi et al. 1996, Isojärvi et al. 2001a, Morrell et al. 2003) and also in girls with epilepsy taking VPA are more recent (Vainionpää et al. 1999). VPA-related obesity is also associated with metabolic changes (Verrotti et al. 1999, Demir & Aysun 2000, Isojärvi et al. 2001a, Stephen et al. 2001, Luef et al. 2002a). Obese adult patients taking VPA have had high serum insulin (Isojärvi et al. 1996, Isojärvi et al. 1998, Isojärvi et al. 2001a, Pylvänens et al. 2003) and IGF-I concentrations. Insulin inhibits the production of IGFBP-1 in the liver, which may lead to elevated bioactive IGF-I levels (Cataldo 1997).

Although VPA-related weight gain is well documented, the underlying mechanisms are still unclear. It is considered to be mediated through VPA-induced inhibition in beta-oxidation of fatty acids (Ponchaut & Veitch 1993, Biton 2003), which may result in lipid accumulation due to impairment of energy utilization. Decreased energy expenditure or increased appetite and elevated leptin levels have also been suggested as explanations of VPA-related weight gain (Verrotti et al. 1999).
There are only a few reports of pubertal maturation in epilepsy. Normal pubertal maturation has been observed in girls with epilepsy on CBZ or OXC monotherapy (Rättyä et al. 1999). Pubertal development has also been found to be unaffected during VPA monotherapy in both genders (Lundberg et al. 1986, Rättyä et al. 1999, Balaguer Martinez et al. 2003), even though low serum gonadotropin levels have been found in association with the use of VPA (Lundberg et al. 1986), and case reports (Cook et al. 1992) and animal data have suggested VPA to slow down pubertal maturation (Snyder & Badura 1995, Snyder & Badura 1998). Delayed pubertal development has been suggested in boys with epilepsy on different AEDs (El-Khayat et al. 2003).
3 Aims of the study

The aims of this study were:
1. To evaluate the effects of epilepsy and AEDs on reproductive endocrine function and gonadal structure in children, adolescents and young adults with childhood- or adolescence-onset epilepsy.
2. To study the effects of epilepsy and AEDs on serum thyroid hormone and lipid levels in girls and young women with epilepsy during pubertal maturation and thereafter.
3. To evaluate the effects of epilepsy and AEDs on linear growth, final height and weight in girls and young women with childhood- or adolescence-onset epilepsy.
4 Subjects and methods

This thesis was done during the years 2000-2004 at the Departments of Paediatrics and Adolescence and Neurology in the Oulu University Hospital in Finland. The approvals of the local ethics committee and written informed consent from all subjects or their parents were obtained before the study. The principles of the Declaration of Helsinki were observed.

4.1 Background of the study

The study cohort of girls and young women with epilepsy and their healthy control subjects was initially recruited during the years 1993-1995. At that time, the female subjects were 8 to 18 years old and the patients were seen at the Departments of Paediatrics or Neurology in Oulu University Hospital for epilepsy. During these years, the female subjects participated in studies of reproductive endocrine function and ovarian ultrasonography (Vainionpää et al. 1999) and growth (Rättyä et al. 1999) (Figure 4).

The same female study cohort participated in the second cross-sectional evaluation during the years 2000-2002. The follow-up results of the second evaluation are reported in this thesis, i.e. the present reproductive endocrine function and growth studies of girls and young women (Study I and IV) are sequel to these two previous studies. The studies of thyroid function and serum lipid levels (Study III and Study IV) are also based on this same female study population (Figure 4), and the results of the first and second evaluations are published in this thesis. The cohort of male patients with epilepsy and their control subjects was initially identified for this thesis during the years 2000-2002 (Study II, Figure 5).
Fig. 4. Flow chart of the studies in female subjects with childhood- or adolescence-onset epilepsy and healthy controls (Studies I, III and IV). Subjects excluded from analysis in Study I: 1 patient with premature ovarian failure; in Study III: 2 controls with thyroid disease; in Study IV: 1 patient and 1 control with thyroid disease. *Previously published (Rättyä et al. 1999, Vainionpää et al. 1999).

Fig. 5. Flow chart of the study in boys and young men with childhood- and adolescence-onset epilepsy and healthy controls (Study II). CBZ, carbamazepine; OXC, oxcarbazepine; VPA, valproate; LTG, lamotrigine.
4.2 Study design

Reproductive endocrine function and ovarian structure in girls and young women with childhood- or adolescence-onset epilepsy were investigated using a population-based cross-sectional study (Study I). One population-based cross-sectional evaluation was carried out to assess the reproductive endocrine function and testicular volume and structure in male patients with epilepsy during pubertal maturation (Study II). Serum thyroid hormone and lipid levels in girls and young women with epilepsy were examined using a prospective population-based cohort study, which included two cross-sectional evaluations at average time interval of 5.8 years (SD 1.0 year) (Studies III and IV). Growth in female subjects was examined prospectively using a longitudinal follow-up study (Study IV).

4.3 Subjects

4.3.1 Female subjects

A population-based cohort of girls and young women with epilepsy participated in this study (Figure 4). The cohort comprised a total of 78 patients at the beginning of the study. All patients were taking CBZ, OXC or VPA as monotherapy for epilepsy and were visiting the Departments of Paediatrics or Neurology in Oulu University Hospital at the first evaluation during the years 1993-1995. The cohort of patients consisted of all 8- to 18-year-old female subjects on monotherapy for epilepsy who were seen at Oulu University Hospital at the time of the study and who gave their informed consent to participate. Patients with an illness or condition other than epilepsy with possible effects on hormonal functions, contraceptive pills, mental retardation or brain damage or former corticosteroid treatment for therapy-resistant epilepsy were excluded from the study.

Fifty-four healthy female subjects agreed to participate in the study as control subjects. They were recruited from local primary or secondary schools and matched for age and pubertal stage of the patients on the first evaluation.

The number of subjects who consented to participate in the second evaluation and the number of subjects excluded from the second evaluation in different studies as well as the reasons for exclusion are shown in Figure 4. Fourteen participants in Study I and 13 participants in Study IV were not able to come for follow-up visit, but they agreed to take part by completing questionnaires. Twenty-four subjects were taking oral contraceptives and two subjects cyclic progestines at the second evaluation. The use of these hormonal regimens had no significant effect on the findings of the studies I, III or IV, except on serum SHBG and FT4 levels. Therefore, the subjects taking hormone therapy were excluded from the analysis of serum SHBG and FT4 concentrations. The clinical characteristics and antiepileptic medication of the subjects in the different studies are shown in Tables 1 and 2.
Table 1. Demographics of the subjects in the four studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Medication</th>
<th>N</th>
<th>Age (SD)</th>
<th>Pubertal stage y</th>
<th>Type of epilepsy L/G</th>
<th>Duration of drug therapy, y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I</td>
<td>(F)</td>
<td>Patients</td>
<td>69</td>
<td>18.9 (3.0)</td>
<td>0/6/63</td>
<td>45/24</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Off medication</td>
<td>42</td>
<td>17.7 (2.4)</td>
<td>0/4/38</td>
<td>34/8</td>
<td>4.4 (1.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>On medication (See Table 2)</td>
<td>27</td>
<td>20.6 (3.1)</td>
<td>0/2/25</td>
<td>11/16</td>
<td>5.2 (3.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls</td>
<td>-</td>
<td>17.4 (3.3)</td>
<td>0/16/35</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Study II</td>
<td>(M)</td>
<td>Patients</td>
<td>70</td>
<td>13.0 (3.8)</td>
<td>23/33/14</td>
<td>54/16</td>
<td>2.9 (2.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CBZ</td>
<td>28</td>
<td>11.7 (3.0)</td>
<td>11/15/2</td>
<td>28/0</td>
<td>2.1 (1.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OXC</td>
<td>12</td>
<td>14.2 (4.7)</td>
<td>3/4/5</td>
<td>12/0</td>
<td>3.0 (1.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VPA</td>
<td>25</td>
<td>13.4 (3.8)</td>
<td>8/12/5</td>
<td>9/16</td>
<td>3.5 (3.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LTG</td>
<td>5</td>
<td>15.5 (3.1)</td>
<td>1/2/2</td>
<td>5/0</td>
<td>4.3 (2.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls</td>
<td>-</td>
<td>13.6 (4.0)</td>
<td>23/33/14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Study III</td>
<td>(F) 1. evaluation</td>
<td>Patients</td>
<td>78</td>
<td>12.6 (3.1)</td>
<td>31/24/23</td>
<td>52/26</td>
<td>3.0 (1.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CBZ</td>
<td>19</td>
<td>12.7 (3.3)</td>
<td>7/8/4</td>
<td>19/0</td>
<td>4.1 (2.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OXC</td>
<td>18</td>
<td>12.7 (3.0)</td>
<td>8/5/5</td>
<td>16/2</td>
<td>1.8 (0.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VPA</td>
<td>41</td>
<td>12.5 (3.0)</td>
<td>16/11/14</td>
<td>17/24</td>
<td>3.0 (2.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls</td>
<td>-</td>
<td>12.5 (3.3)</td>
<td>20/13/21</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. evaluation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Patients</td>
<td>37</td>
<td>17.7 (2.3)</td>
<td>0/3/34</td>
<td>31/6</td>
<td>4.4 (2.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>On medication (See Table 2)</td>
<td>27</td>
<td>20.6 (3.1)</td>
<td>0/2/25</td>
<td>11/16</td>
<td>5.2 (3.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls</td>
<td>-</td>
<td>17.4 (3.4)</td>
<td>0/13/27</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Study IV</td>
<td>(F) 1. evaluation</td>
<td>Patients</td>
<td>77</td>
<td>12.6 (3.1)</td>
<td>31/23/23</td>
<td>52/25</td>
<td>2.8 (2.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CBZ</td>
<td>19</td>
<td>12.7 (3.3)</td>
<td>7/8/4</td>
<td>19/0</td>
<td>4.1 (2.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OXC</td>
<td>18</td>
<td>12.7 (3.0)</td>
<td>8/5/5</td>
<td>16/2</td>
<td>1.8 (0.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VPA</td>
<td>40</td>
<td>12.5 (3.1)</td>
<td>16/10/14</td>
<td>17/23</td>
<td>2.8 (1.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls</td>
<td>-</td>
<td>12.7 (3.4)</td>
<td>17/11/21</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. evaluation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Patients</td>
<td>42</td>
<td>17.7 (2.4)</td>
<td>0/4/38</td>
<td>34/8</td>
<td>4.4 (1.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>On medication (See Table 2)</td>
<td>27</td>
<td>20.6 (3.1)</td>
<td>0/2/25</td>
<td>11/16</td>
<td>5.2 (3.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls</td>
<td>-</td>
<td>17.7 (3.3)</td>
<td>0/14/33</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Studies I, III and IV were based on the same population-based cohort. Values are number of subjects or means (SD). CBZ, carbamazepine; OXC, oxcarbazepine; VPA, valproate; LTG, lamotrigine; F, female; M, male; Pre, Prepuberty; Pub, Puberty; Post, Postpuberty; L, localization-related epilepsy; G, generalized epilepsy.
Table 2a. Initial and current medication of the female patients on the second evaluation (Studies I, III and IV).

<table>
<thead>
<tr>
<th>Initial medication</th>
<th>Off medication</th>
<th>Current medication (Studies I, III, and IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I/III/IV CBZ</td>
<td>17/15/17</td>
<td>CBZ 3</td>
</tr>
<tr>
<td>Study I/III/IV OXC</td>
<td>17/15/17</td>
<td>12/10/12</td>
</tr>
<tr>
<td>Study I/III/IV VPA</td>
<td>35/34/35</td>
<td>18/17/18</td>
</tr>
</tbody>
</table>

Table 2b. Demographics of the female patients on medication on the second evaluation (Studies I, III and IV).

<table>
<thead>
<tr>
<th>Current Medication</th>
<th>N</th>
<th>Age (SD)</th>
<th>Pubertal stage</th>
<th>Type of epilepsy</th>
<th>Duration of drug therapy, y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>27</td>
<td>20.6 (3.1)</td>
<td>0/2/25</td>
<td>11/16</td>
<td>5.2 (3.5)</td>
</tr>
<tr>
<td>CBZ</td>
<td>5</td>
<td>19.7 (3.6)</td>
<td>0/0/5</td>
<td>3/2</td>
<td>3.7 (3.2)</td>
</tr>
<tr>
<td>OXC</td>
<td>9</td>
<td>20.8 (3.0)</td>
<td>0/0/9</td>
<td>5/4</td>
<td>5.1 (3.2)</td>
</tr>
<tr>
<td>VPA</td>
<td>7</td>
<td>20.9 (3.3)</td>
<td>0/1/6</td>
<td>1/6</td>
<td>6.3 (4.2)</td>
</tr>
<tr>
<td>LTG</td>
<td>3</td>
<td>23.5 (0.1)</td>
<td>0/0/3</td>
<td>2/1</td>
<td>2.4 (3.0)</td>
</tr>
<tr>
<td>Polytherapy</td>
<td>3</td>
<td>18.2 (1.8)</td>
<td>0/1/2</td>
<td>0/3</td>
<td>8.5 (2.0)</td>
</tr>
</tbody>
</table>

Values are number of subjects or means (SD). CBZ, carbamazepine; OXC, oxcarbazepine; VPA, valproate; LTG, lamotrigine; Pre, Prepuberty; Pub, Puberty; Post, Postpuberty; L, localization-related epilepsy; G, generalized epilepsy

4.3.2 Male subjects

This is a population-based cohort of 7- to 20-year-old boys and young men with epilepsy attending the Departments of Paediatrics and Adolescence, and Neurology in Oulu University Hospital during the years 2000-2002 (Study II, Figure 5). The cohort included all consenting patients with epilepsy in this age group, who fulfilled the inclusion criteria, which were the use of CBZ, OXC, VPA or LTG monotherapy for more than one year for epilepsy and no history of brain damage, mental retardation, steroid treatment for intractable epilepsy or any illness possibly affecting hormonal functions. Mild asthma or allergic diseases were allowed. Seventy of the 76 patients who met the inclusion criteria gave their consent to participate in this study. Seventy healthy male volunteers were recruited to serve as control subjects. They were randomly selected from primary or secondary schools in Oulu, and they were matched for age and pubertal stage with the patients. The clinical characteristics of the participants are shown in Table 1.
4.4 Methods

4.4.1 Clinical examination

All the subjects were clinically examined with special attention to growth, pubertal development, possible symptoms and signs of endocrine disease. The medical history (e.g. previous diseases and medications, EEG findings and brain magnetic resonance imaging) was obtained by interview and review of the hospital records. Seizures were classified according to the International Classification of Epileptic Seizures (Commission on Classification and Terminology of the International League Against Epilepsy 1981), and epilepsy type was classified according to the International Classification of Epilepsies and Epileptic Syndromes (Commission on Classification and Terminology of the International League Against Epilepsy 1989).

4.4.1.1 Growth

Height was measured to the nearest 1.0 mm using a Harpenden stadiometer (Holtain, Crymych, UK), and weight to the nearest 0.1 kg on a digital scale. Relative height, weight for height, and mid-parental height were assessed using the Finnish growth charts (Sorva et al. 1990). Relative height means the deviation between absolute height and mean height for age and sex, and it is presented as a standard deviation score (SDS). Weight for height is derived from absolute weight divided by mean weight for height and multiplied by 100 (%). Weight for height exceeding 120% was considered the limit of obesity in female subjects on the first evaluation (Study IV) and weight for height exceeding 130% in male subjects (Study II). The BMI was calculated as weight divided by the square of height (kg/m²). On the second evaluation, the female subjects with BMI exceeding 25 kg/m² were considered obese (Studies I and IV) (Kiddy et al. 1990). Head, hip and waist circumferences were also measured. Waist-hip ratio as a measurement of fat distribution was assessed (Study IV). Bone age was evaluated from the subjects with growing potential on the basis of an x-ray of the left hand and wrist (Greulich & Pyle 1959). Bone age related to chronological age was reported as SDS. Individual growth charts were drawn based on the growth data collected in the study examination, from the records of school nurses and from clinical visits to Oulu University Hospital. Attainment of final height was confirmed by the growth chart (growth less than 0.5 cm during the preceding six months) and bone age. The frequency and intensity of weekly physical exercise, daily dietary habits and history of weight reductions of each subject were interviewed based on a questionnaire.
4.4.1.2 Pubertal maturation

The stage of puberty was classified according to Tanner and Whitehouse (Tanner 1962, Tanner & Whitehouse 1976). Girls and young women were categorized into three pubertal stages, and boys and young men primarily into five Tanner pubertal stages (T I-V). The male pubertal groups T II-IV were combined for statistical analysis. Subjects were classified as prepubertal with no clinical signs of puberty: breast (B) 1 and pubic hair (PH) 1 in girls and genital maturation (G) 1 (T I) in boys. Subjects were considered as pubertal with clinical signs of pubertal development B2-4 or PH2-4 in girls and G2-4(-5) (T II-IV) in boys, i.e. based on the T II definition of the larger testis being more than 20 mm in length, to assure that every prepubertal subject was truly in prepuberty. Subjects were considered as postpubertal when their pubertal maturation was completed: B5 PH5-6 or B4 PH4 with adult bone age (Greulich & Pyle 1959) in female subjects and G5 (T V) with adult bone age (Greulich & Pyle 1959) in male subjects. (Tanner 1962, Tanner & Whitehouse 1976, Sorva et al. 1990.)

Menstrual cycles were defined as irregular if the intermenstrual variation was more than seven days or cycle duration more than 35 days or less than 21 days at least once during the preceding six months. Nine of the pubertal girls had their menarche less than six months before the second clinical examination, and they were not included in the analysis of menstrual disorders.

4.4.2 Ultrasonography of the gonads

4.4.2.1 Ovaries

Ultrasonography is the golden standard in the examination of ovarian structure. Toshiba SSA-370A ultrasound device (Toshiba Powervision 6000; Toshiba Co., Tokyo, Japan) equipped with a 6.0-MHz curvilinear transvaginal probe or a 3.5-MHz transabdominal probe was used to examine the ovaries and uterus. Two experienced gynaecologists performed the examinations. The transvaginal probe was used only when examining young adult subjects with their consent. The examination of ovarian structure included assessment of the number and size (maximum diameter) of ovarian follicles. The examinations were performed during the early follicular phase of the menstrual cycle.

The following ultrasonographic criteria were used for the diagnosis of PCO: 8 or more subcapsular follicles of 2 to 8 mm in diameter detected in one two-dimensional plane in either of the ovaries. PCOS was defined according to Homburg (Homburg 1996). In postpuberty, PCO verified by ultrasonography, and irregular menstrual cycles, or elevated serum T or clinical signs of HA (e.g. hirsutism or acne) or both were required for a diagnosis of PCOS. In addition, other endocrine disorders underlying anovulation or HA (e.g. thyroid diseases and hyperprolactinemia) were excluded.
4.4.2.2 Testes

The testes were examined by one experienced radiologist, who was blinded to clinical findings and medication regimens, using a Toshiba PowerVision 8000 (Toshiba Medical Systems, Tochigi-Ken, Japan) real-time scanner with a multifrequency 12-MHz linear transducer with a 58-mm contact surface. Testicular volume and structure and possible abnormal findings (e.g. cysts) were recorded. Both testes were examined in the longitudinal (Long) and transverse (Transv) planes, searching for a plane with the appearance of maximal length and a plane with the appearance of maximal transversal area. Epididymis was not included in the testicular measurements. The height (Long), width (Transv 1) and thickness (Transv 2) of testes were calculated as the means of three measurements on the scanner display using electronic calipers. Testicular volumes were presented as the corrected sum volumes of both testes. Testicular volume was calculated by using the prolate ellipsoid formula as Long x Transv 1 x Transv 2 x 0.52 (cm³, mL).

4.5 Laboratory assays

Venous blood samples were obtained between 8 and 10 a.m. after an overnight fast and before the first daily drug dose. Blood samples were taken during the early follicular phase of the menstrual cycle (1.-7. days) from girls with menstruation and at random from girls without menarche or with at least three months of secondary amenorrhea. If needed, blood samples were taken after the application of a local anesthetic (EMLA; Astra, Södertälje, Sweden) on the skin. Serum samples were frozen at -20ºC until analyzed by the specific methods (Table 3). Some laboratory variables were classified as either elevated or decreased based on the mean serum concentration of specific assay +/- 2 SD in the control subjects at various pubertal stages. These reference limits for the assays are presented in Table 4. All the patients on medication had serum drug concentrations within the therapeutic range in this study.
Table 3. Sensitivity and intra- and inter-assay coefficients of variation (CV) of the methods used for assays in the different studies.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Method</th>
<th>Sensitivity</th>
<th>Intra-assay CV %</th>
<th>Inter-assay CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Assays used in all studies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBZ</td>
<td>Fluorescence polarization immunoassay system, AxSym Plus analyzer</td>
<td>0.5 µmol/L</td>
<td>1.2</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>(Abbott Diagnostic Division, Irving, TX, U.S.A.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VPA</td>
<td>as above</td>
<td>5.0 µmol/L</td>
<td>1.8</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>Mono-hydroxycarbazepine (active metabolite of OXC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPLC (Keränen et al. 1990)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LTG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPLC (Fraser et al. 1995)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Assays used in studies I and II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>Immunologic chemiluminescence method, Bayer ADVIA Centaur analyser</td>
<td>0.6 nmol/L</td>
<td>5.7</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>(Tarrytown, NY, U.S.A.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRL</td>
<td>as above</td>
<td>10.0 mU/L</td>
<td>2.3</td>
<td>8.8</td>
</tr>
<tr>
<td>P</td>
<td>as above</td>
<td>4.6 nmol/L</td>
<td>4.6</td>
<td>7.7</td>
</tr>
<tr>
<td>SHBG</td>
<td>Two-site fluoroimmunometric method, Wallac Ltd. (Turku, Finland)</td>
<td>0.05 nmol/L</td>
<td>4.4</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>the 1235 AutoDELFIA (Turku, Finland) automatic system</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>as above</td>
<td>0.03 IU/L</td>
<td>1.4</td>
<td>2.6</td>
</tr>
<tr>
<td>LH</td>
<td>as above</td>
<td>0.03 IU/L</td>
<td>2.3</td>
<td>4.2</td>
</tr>
<tr>
<td>DHEAS</td>
<td>RIA, Diagnostic Products Co. (Los Angeles, CA, U.S.A.)</td>
<td>0.05 µmol/L</td>
<td>4.5</td>
<td>5.5</td>
</tr>
<tr>
<td>A</td>
<td>as above</td>
<td>0.07 nmol/L</td>
<td>5.0</td>
<td>8.6</td>
</tr>
<tr>
<td>E2</td>
<td>RIA, Orion Diagnostica (Turku, Finland)</td>
<td>5 pmol/L</td>
<td>4.5</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>Assays used in studies I, III, IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>ELISA, (Andersen et al. 1993)</td>
<td>0.5 mU/L</td>
<td>&lt;7.5</td>
<td>&lt;9.3</td>
</tr>
<tr>
<td>IGF-I</td>
<td>RIA, (Diagnostic System Laboratories, Webster, TX, U.S.A.). Plasma samples were extracted with acid ethanol before the assay.</td>
<td>1.0 nmol/L</td>
<td>&lt;4.6</td>
<td>&lt;9.0</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>Enzymometric modification of an immunoradiometric method using a monoclonal antibody (MoAb 6035)</td>
<td>0.4 µg/L</td>
<td>&lt;4.0</td>
<td>&lt;10.0</td>
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<tr>
<td>IGFBP-3</td>
<td>RIA, (Diagnostic System Laboratories, Webster, TX, U.S.A.)</td>
<td>30 µg/L</td>
<td>&lt;5.0</td>
<td>&lt;9.0</td>
</tr>
<tr>
<td>T4</td>
<td>RIA, Orion Diagnostica (Turku, Finland)</td>
<td>&lt;5.0 nmol/L</td>
<td>4.7</td>
<td>4.9</td>
</tr>
<tr>
<td>TSH</td>
<td>Two-site fluoroimmunometric method, Wallac Oy (Turku, Finland)</td>
<td>&lt;0.03 mU/L</td>
<td>2.0</td>
<td>3.5</td>
</tr>
</tbody>
</table>
Table 3. Continued.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Method</th>
<th>Sensitivity</th>
<th>Intra-assay CV %</th>
<th>Inter-assay CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assays used in studies III and IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I evaluation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FT₄</td>
<td>Chiron Diagnostics automated chemiluminescence System, ACS:180 analyzer (Medfield, MA, U.S.A.)</td>
<td>1.3 pmol/L</td>
<td>2.5</td>
<td>5.1</td>
</tr>
<tr>
<td>FT₃</td>
<td>as above</td>
<td>1.6 pmol/L</td>
<td>1.4</td>
<td>2.0</td>
</tr>
<tr>
<td>TPO-ab</td>
<td>RIA, Brahms Diagnostica (Berlin, Germany)</td>
<td>as above</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG-ab</td>
<td>Enzymatic colorimetric method, Boehringer Mannheim GmbH Analyzer (Boehringer Mannheim GmbH, Mannheim, Germany)</td>
<td>as above</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>Enzymatic colorimetric method, Roche Diagnostics (Rotkreuz, Switzerland), Cobas Integra 700 analyzer (F. Hoffmann-La Roche Ltd, Basel, Switzerland)</td>
<td>as above</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C</td>
<td>Calculated by the Friedewald formula (Friedewald et al. 1972)</td>
<td>as above</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGs</td>
<td></td>
<td>as above</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C</td>
<td></td>
<td>Calculated by the Friedewald formula (Friedewald et al. 1972)</td>
<td>as above</td>
<td></td>
</tr>
<tr>
<td>II evaluation</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>FT₄</td>
<td>Immunologic chemiluminescence method, Bayer ADVIA Centaur analyzer (Tarrytown, NY, U.S.A.)</td>
<td>2.0 pmol/L</td>
<td>3.9</td>
<td>4.9</td>
</tr>
<tr>
<td>FT₃</td>
<td>as above</td>
<td>1.0 pmol/L</td>
<td>1.6</td>
<td>1.8</td>
</tr>
<tr>
<td>TC</td>
<td>Enzymatic colorimetric method, Roche Diagnostics (Rotkreuz, Switzerland), Cobas Integra 700 analyzer (F. Hoffmann-La Roche Ltd, Basel, Switzerland)</td>
<td>as above</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C, LDL-C, TGs</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Assays used in study II</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DHEA</td>
<td>RIA, Diagnostic Products Co. (Los Angeles, CA, U.S.A.)</td>
<td>0.14 nmol/L</td>
<td>5.2</td>
<td>5.6</td>
</tr>
<tr>
<td>17-OH-Prog</td>
<td>as above</td>
<td>0.21 nmol/L</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>FT</td>
<td>as above</td>
<td>0.52 pmol/L</td>
<td>3.8</td>
<td>4.2</td>
</tr>
<tr>
<td>Inhibin B</td>
<td>Solid-phase sandwich ELISA method (Serotec, Oxford, England)</td>
<td>&lt;15.0 ng/L</td>
<td>&lt;7.0</td>
<td>&lt;7.0</td>
</tr>
<tr>
<td>Insulin</td>
<td>Immunological chemiluminescence method, Bayer ADVIA Centaur analyser (Tarrytown, NY, U.S.A.)</td>
<td>0.1 mU/L</td>
<td>2.9</td>
<td>4.7</td>
</tr>
</tbody>
</table>

CBZ, carbamazepine; VPA, valproate; OXC, oxcarbazepine; LTG, lamotrigine; T, testosterone; PRL, prolactin; P, progesterone; SHBG, sex hormone binding globulin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; DHEAS, dehydroepiandrosteroone sulfate; A, androstenedione; E₂, estradiol; IGF-I, insulin-like growth factor I; IGFBP-1 and -3, insulin-like growth factor binding protein 1 and 3; T₈, thyroxine; TSH, thyrotropin; FT₈, free thyroxine; FT₃, free triiodothyronine; TPO-ab, thyroid peroxidase antibody; TG-ab, thyroglobulin antibody; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TGs, triglycerides; LDL-C, low-density lipoprotein cholesterol; DHEA, dehydroepiandrosterone; 17-OH-Prog, 17-hydroxyprogesterone; FT, free testosterone; HPLC, high-performance liquid chromatography; RIA, radioimmunoassay; ELISA, enzyme-linked immunosorbent assay.
| Table 4. Reference limits for classified laboratory variables at different pubertal stages. |
|---------------------------------|-----------------|--------|--------|--------|
| Variable                        | Reference limit based on | Gender | Prepuberty | Puberty | Postpuberty |
| Studies I and II                | Serum T levels above/at the mean serum T concentration | F | - | ≥ 2.2 nmol/L | ≥ 2.4 nmol/L |
|                                | + 2 SD in the control subjects at different pubertal stages | M | > 1.3 nmol/L | > 26.9 nmol/L | > 28.8 nmol/L |
| Hyperandrogenism                | Serum A levels above/at the mean serum A concentration | F | - | ≥ 11.6 nmol/L | ≥ 16.5 nmol/L |
|                                | + 2 SD in the control subjects at different pubertal stages | M | > 3.9 nmol/L | > 13.5 nmol/L | > 18.5 nmol/L |
| Elevated A                      | Serum insulin levels above the mean serum insulin concentration | M | > 8.8 mU/L | > 15.5 mU/L | > 11.6 mU/L |
|                                | + 2 SD in the control subjects at different pubertal stages | | | | |
| Hyperinsulinism                 | Serum T4 levels under/at the mean serum T4 concentration | F | I | ≤ 85.9 nmol/L | ≤ 58.3 nmol/L | ≤ 67.4 nmol/L |
|                                | - 2 SD in the control subjects at different pubertal stages | | | | |
| Decreased T4                    | Serum FT4 levels under/at the mean serum FT4 concentration | F | I | ≤ 12.4 pmol/L | ≤ 10.1 pmol/L | ≤ 11.5 pmol/L |
|                                | - 2 SD in the control subjects at different pubertal stages | | | | |
| Decreased FT4                   | Considered to be positive, recommended by the manufacturer | F | I | > 60 U/mL | > 60 U/mL | > 60 U/mL |
|                                | Considered to be positive, recommended by the manufacturer | F | I | > 60 U/mL | > 60 U/mL | > 60 U/mL |
| Study IV                        | Serum TC levels above the mean serum TC concentration | F | I | > 5.7 mmol/L | > 5.7 mmol/L | > 5.7 mmol/L |
|                                | + 2 SD in the control subjects at different pubertal stages | | | | |

SD, standard deviation; F I, the first evaluation of the female subjects; M, male subjects; T, testosterone; A, androstenedione; T4, thyroxine; FT4, free thyroxine; TPO-ab, thyroid peroxidase antibody; TG-ab, thyroglobulin antibody; TC, total cholesterol.
4.6 Statistics

The null hypothesis of the data analysis was that there would be no difference in serum hormonal and lipid levels, growth, weight or ovarian findings in female subjects between patients on medication, off medication and control subjects, and that there would be no difference in the hormonal levels or testicular volume or structure between male patients and their control subjects or between the different medication groups. The alternative hypothesis was that there would be changes in serum concentrations, growth, weight and ovarian or testicular findings depending on medication.

The outcomes in female subjects were the outcome of epilepsy, HA, menstrual disorders, PCO, PCOS and the current frequency of reproductive endocrine disorders in subjects with HA in the initial cross-sectional study (Study I) as well as altered thyroid function (Study III), dyslipidemia, weight, growth and final height (Study IV). The outcomes of boys and young men were HA, other reproductive endocrine changes and reduced testicular size (Study II).

The Statistical Analysis System (SAS) for Windows (Release 8.02) was used to analyze the longitudinal growth and weight charts. All the other data were analyzed with the Statistical Package for the Social Sciences program (Version 11.5 SPSS Inc., Chicago, IL, U.S.A.) or with the StatsDirect (Version 2.2.3). Laboratory values are presented as means with standard deviation (SD) or with 95% confidence intervals (CI). Proportional differences with 95% CI are also shown.

Unpaired t-test was used to compare parametric variables (laboratory assays, final height, BMI, weight for height, testicular volume) between the two groups as appropriate, and a one-way analysis of variance (ANOVA) with Tukey’s or LSD post hoc tests were used to compare more than two groups. If homogeneity of variances was not assumed, the logarithmic transformation (ln) for variables was done before ANOVA, or the results were confirmed by Kruskal-Wallis test with Mann-Whitney U-test as post hoc. Bonferroni’s correction was used as appropriate. The standard normal deviate and chi-square tests were used to compare proportions, and Fisher’s exact test was used as needed. To find out the effect of age on the changes of specific hormone concentrations between two evaluations in female subjects, repeated-measures ANOVA was performed with age as a covariate. Finally, to control for confounding factors and to identify the independent predictors of specific results, stepwise binary logistic regression analysis was applied. The PROC MIXED model, in which it was possible to compare the whole-time linear growth and weight for height in the different groups and to fit fixed and random effects into the model, was used to analyze the growth and weight charts (Singer 1998).
5 Results

5.1 Reproductive endocrine function in patients with epilepsy

5.1.1 Girls and young women (Study I)

5.1.1.1 Menstrual cycles and ovarian structure

By the time of the second evaluation, mean 5.8 years (SD 1.0) after the first evaluation, medication had been withdrawn from 61% of the female subjects with epilepsy (Table 1). Thirty-two percent of the patients off medication, 56% of the patients still taking medication and 38% of the control subjects had menstrual disorders, P = 0.1. The frequency of PCO was higher in the patients taking medication, especially VPA, than in those off medication or the control subjects (Figure 6). PCO were found in 70% of the patients taking VPA (n=7/10) and in 50% of those taking some other AED (n=8/16), 95% CI of the difference -2% to 50%, P = 0.3. PCO were detected in 40% (n=2/5) of the patients on CBZ, 63% (n=5/8) of the patients on OXC and 33% (n=1/3) of the patients on LTG.

Thirty-eight percent of the patients on medication, 6% of the girls off medication and 11% of the control girls had PCOS, P = 0.005 (Figure 6). The difference in the frequency of PCOS between the patients taking VPA (63%) and another AED (25%) did not quite reach statistical difference, but the number of subjects was small (proportion difference 38%, 95% CI -4% to 70%, P = 0.07). The frequency of PCOS did not differ between the patients off medication and the control subjects (difference 5%, 95% CI -10% to 23%, P = 0.5).

Eighteen of the 41 patients initially taking VPA and one of the 54 control subjects had HA on the first evaluation during pubertal maturation (Vainionpää et al. 1999). After the follow-up, a high frequency of PCOS was seen in the patients still on medication, especially VPA, whereas none of the four patients off medication had PCOS (Table 5).
Fig. 6. Frequency of polycystic ovaries (PCO) and PCO syndrome (PCOS) in patients with epilepsy on VPA and other types of medication, patients without medication and controls. *P ≤ 0.008 compared with patients without medication or controls, standard normal deviate test with Bonferroni correction. VPA, valproate.

5.1.1.2 Serum reproductive hormones

The concentrations of serum reproductive hormones in postpubertal female subjects are shown in Table 6. There were no differences in any of the serum reproductive hormone levels in the patients off medication and the control girls. Serum T and A levels were increased in the patients on VPA medication.

HA, defined as exceeding the reference limit of T, was seen in 17% (n=4/24) of the postpubertal patients on medication, whereas none of the patients off medication or the control subjects had HA, P = 0.005. All ten girls and young women taking VPA treatment, either as mono- or polytherapy, had elevated serum A levels, whereas only one patient taking an AED other than VPA, one patient off medication and two control subjects had serum A concentrations above the reference level, P < 0.0005. There were only six patients in the pubertal group (Table 1). However, serum A levels were also high in the two pubertal girls on medication, one with VPA (22.7 nmol/L) and the other with VPA and LTG (34.5 nmol/L), compared with none of the patients off medication (mean 6.1 nmol/L, SD 2.7) or the control subjects (mean 6.8 nmol/L, SD 2.4; P < 0.02).

Serum A concentrations were high on the follow-up visit in the patients who had HA during VPA medication at the time of the first evaluation and were still on VPA medication on the second evaluation (Table 5). Serum androgen levels in the patients off medication after the follow-up were similar to the levels detected in the control subjects.
Table 5. Follow-up results of the frequency of polycystic ovary syndrome (PCOS) and serum androgen concentrations in subjects with hyperandrogenism (HA) in the initial population-based cross-sectional study (Vainionpää et al. 1999).

<table>
<thead>
<tr>
<th></th>
<th>Initial study</th>
<th>Current study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subjects N</td>
<td>Medication N</td>
</tr>
<tr>
<td>Patients on VPA</td>
<td>41</td>
<td>On medication</td>
</tr>
<tr>
<td>HA 18</td>
<td></td>
<td>On VPA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-VPA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Off medication</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Refused</td>
</tr>
<tr>
<td>Control subjects</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>HA 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are number of subjects or means (SD). VPA, valproate; A, androstenedione; T, testosterone

*P = 0.046 Chi-square test; †P = 0.018 and ‡P = 0.142 one-way analysis of variance;

§P < 0.03 compared with patients non-VPA and off medication, post hoc Tukey’s test. One control subject was excluded from post hoc analysis of serum A and T concentrations.
Table 6. Serum reproductive hormone and sex hormone binding globulin concentrations in postpubertal female subjects with childhood- and adolescence-onset epilepsy and controls.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>N</th>
<th>T</th>
<th>A</th>
<th>DHEAS</th>
<th>SHBG*</th>
<th>LH</th>
<th>FSH</th>
<th>PRL</th>
<th>P</th>
<th>E₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(nmol/L)</td>
<td>(µmol/L)</td>
<td>(nmol/L)</td>
<td>(JU/L)</td>
<td>(JU/L)</td>
<td>(mU/L)</td>
<td>(nmol/L)</td>
<td>(nmol/L)</td>
<td></td>
</tr>
<tr>
<td>Patients on medication</td>
<td>24</td>
<td>1.9 (0.7)†</td>
<td>18.8 (15.2)§</td>
<td>6.1 (3.5)</td>
<td>65.4 (23.6)</td>
<td>5.0 (3.4)</td>
<td>5.4 (1.8)</td>
<td>417.2 (257.7)</td>
<td>2.6 (1.2)</td>
<td>0.1 (0.1)</td>
</tr>
<tr>
<td>CBZ</td>
<td>5</td>
<td>2.0 (0.8)</td>
<td>11.1 (3.4)</td>
<td>4.0 (1.6)</td>
<td>75.0 (21.0)</td>
<td>7.4 (5.8)</td>
<td>6.6 (1.3)</td>
<td>291.8 (65.4)</td>
<td>2.4 (1.0)</td>
<td>0.1 (0.1)</td>
</tr>
<tr>
<td>OXC</td>
<td>9</td>
<td>1.7 (0.6)</td>
<td>11.0 (4.2)</td>
<td>6.6 (4.1)</td>
<td>60.0 (21.7)</td>
<td>3.8 (2.3)</td>
<td>4.8 (1.9)</td>
<td>475.7 (333.1)</td>
<td>2.5 (1.0)</td>
<td>0.1 (0.1)</td>
</tr>
<tr>
<td>VPA</td>
<td>6</td>
<td>2.2 (0.6)‡</td>
<td>30.4 (9.7)</td>
<td></td>
<td></td>
<td>8.0 (3.4)</td>
<td>63.0 (19.7)</td>
<td>4.3 (1.8)</td>
<td>5.1 (1.6)</td>
<td>513.2 (270.8)</td>
</tr>
<tr>
<td>LTG</td>
<td>2</td>
<td>1.2 (0.2)</td>
<td>9.9 (4.5)</td>
<td>5.2 (4.2)</td>
<td>23.0</td>
<td>6.7 (4.7)</td>
<td>6.7 (2.4)</td>
<td>233.5 (19.1)</td>
<td>2.0 (0.7)</td>
<td>0.1 (0.01)</td>
</tr>
<tr>
<td>VPA+CBZ</td>
<td>1</td>
<td>3.1</td>
<td>22.7</td>
<td>5.2</td>
<td>107.0</td>
<td>7.1</td>
<td>2.7</td>
<td>457.0</td>
<td>2.4</td>
<td>0.1</td>
</tr>
<tr>
<td>VPA+LTG</td>
<td>1</td>
<td>1.8</td>
<td>71.5</td>
<td>3.0</td>
<td>79.3</td>
<td>3.6</td>
<td>6.5</td>
<td>270.0</td>
<td>1.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Patients off medication</td>
<td>33</td>
<td>1.4 (0.5)</td>
<td>9.5 (2.6)</td>
<td>6.0 (2.5)</td>
<td>55.7 (26.2)</td>
<td>4.3 (2.1)</td>
<td>5.5 (1.9)</td>
<td>302.3 (171.5)</td>
<td>2.5 (1.0)</td>
<td>0.1 (0.1)</td>
</tr>
<tr>
<td>Controls</td>
<td>28</td>
<td>1.4 (0.5)</td>
<td>10.2 (3.2)</td>
<td>5.9 (3.4)</td>
<td>59.1 (23.3)</td>
<td>4.6 (2.5)</td>
<td>6.0 (2.1)</td>
<td>375.3 (227.5)</td>
<td>2.7 (1.1)</td>
<td>0.1 (0.1)</td>
</tr>
</tbody>
</table>

Values are means (SD). T, testosterone; A, androstenedione; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone binding globulin; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PRL, prolactin; P, progesterone; E₂, estradiol; *The use of oral contraceptives increases serum SHBG due to liver enzyme induction; results apply to patients with no oral contraceptives. CBZ, carbamazepine; OXC, oxcarbazepine; VPA, valproate; LTG, lamotrigine.

Compared with patients off medication and control subjects †P < 0.01; ‡P < 0.03 one-way ANOVA, post hoc Tukey; §P < 0.02, Kruskall-Wallis test, post hoc Mann-Whitney U test with Bonferroni’s correction. Compared with patients with no VPA, off medication and control subjects ‖P < 0.002 Kruskall-Wallis test, post hoc Mann-Whitney U test with Bonferroni’s correction.
5.1.2 Boys and young men (Study II)

5.1.2.1 Testicular structure

The mean testicular volumes or the frequency of varicose veins or epididymal cysts did not differ between the patients and the control subjects (Table 7). The mean testicular volume was larger in the prepubertal patients who had elevated serum A while taking VPA (2.5 mL, SD 0.8) than in their healthy control subjects (1.7 mL, SD 0.5), P = 0.04. Testicular volume was similar in pubertal and postpubertal patients regardless of the serum A levels.

Table 7. Results of testicular ultrasonography in patients with epilepsy on different antiepileptic drugs and age and pubertal stage matched control subjects at different pubertal stages.

<table>
<thead>
<tr>
<th>Group</th>
<th>Volume, mL</th>
<th>Varicose</th>
<th>Epididymal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Prepuberty</td>
<td>Puberty</td>
</tr>
<tr>
<td>Patients</td>
<td>59</td>
<td>2.1 (0.7)</td>
<td>18.9 (11.1)</td>
</tr>
<tr>
<td>CBZ</td>
<td>27</td>
<td>1.9 (0.6)</td>
<td>16.5 (12.7)</td>
</tr>
<tr>
<td>OXC</td>
<td>8</td>
<td>1.7 (0.6)</td>
<td>14.8 (7.1)</td>
</tr>
<tr>
<td>VPA</td>
<td>21</td>
<td>2.5 (0.8)</td>
<td>23.4 (9.2)</td>
</tr>
<tr>
<td>LTG</td>
<td>3</td>
<td>1.9</td>
<td>25.8</td>
</tr>
<tr>
<td>Controls</td>
<td>59</td>
<td>1.8 (0.5)</td>
<td>18.7 (11.8)</td>
</tr>
</tbody>
</table>

P value* 0.112 0.954 0.335 0.782 0.624

Values are mean (SD) sum volumes of both testes or number of subjects (%). Testicular volume was calculated by using the prolate ellipsoid formula as height x width x thickness x 0.52 (mL, cm³). CBZ, carbamazepine; OXC, oxcarbazepine; VPA, valproate; LTG, lamotrigine; *Comparison of all patients with controls, unpaired t-test (volumes) and standard normal deviate test (proportions). There were no differences in one-way analysis of variance between different medication groups.

5.1.2.2 Serum reproductive hormones

Serum reproductive hormone concentrations in male subjects are presented in Figure 7 and Table 8. Decreased serum DHEAS and increased serum SHBG levels were found in the pubertal CBZ-treated patients. The patients taking OXC or LTG had no changes in their serum DHEAS or other androgen levels or in their serum SHBG concentrations.
The VPA-treated male patients had higher serum A, but similar serum DHEAS and T levels compared to the patients taking other AEDs or the control subjects. Twenty-three percent of the patients (n=16/70) and 4% of the control subjects (n=3/70) were classified as having elevated serum A levels (proportion difference 19%, 95% CI of the difference 8% to 30%, P = 0.001). Elevated serum A levels were only seen in the patients taking VPA (64%, n=16/25) compared to none of the 45 patients on other AEDs, P = 0.0006. All prepubertal, 42% of the pubertal and 60% of the postpubertal patients taking VPA had elevated serum A concentrations. VPA medication also emerged as a significant independent predictive factor for elevated A levels in logistic regression analysis, and pubertal stage and weight for height were additional independent predictors (Table 9). Epilepsy type was not a significant predictor for serum A elevation. Elevated serum T levels were only seen in two patients with epilepsy (3%), both taking VPA, and in one control subject (1%), 95% CI of the proportion difference -5% to 9%, P = 0.6. Serum inhibin B concentrations were also measured and there were no statistically significant differences between the patients on different AEDs and the control subjects.

Fig. 7. Serum A (A) and DHEAS (B) levels in male patients with epilepsy on different antiepileptic drugs and controls at different pubertal stages. Horizontal lines indicate the serum A (A) and DHEAS (B) mean concentrations + 2 SD of the control subjects. A, androstenedione; DHEAS, dehydroepiandrosterone sulfate; CBZ, carbamazepine; OXC, oxcarbazepine; VPA, valproate; LTG, lamotrigine. *P < 0.006 (A); *P < 0.03 (B) compared to controls of the same pubertal stage, one-way analysis of variance with Tukey’s post hoc test.
Table 8. Serum reproductive hormone and sex hormone-binding globulin concentrations in male subjects.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>N</th>
<th>T</th>
<th>A*</th>
<th>DHEAS</th>
<th>SHBG</th>
<th>LH</th>
<th>FSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(nmol/L)</td>
<td>(nmol/L)</td>
<td>(µmol/L)</td>
<td>(nmol/L)</td>
<td>(IU/L)</td>
<td>(IU/L)</td>
<td></td>
</tr>
<tr>
<td>Prepuberty</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>23</td>
<td>0.7 (0.4)</td>
<td>3.8 (3.9)†</td>
<td>1.2 (0.9)</td>
<td>134.1 (37.7)</td>
<td>0.1 (0.1)</td>
<td>1.0 (0.7)</td>
</tr>
<tr>
<td>CBZ</td>
<td>11</td>
<td>0.7 (0.3)</td>
<td>1.8 (0.8)</td>
<td>0.8 (0.4)</td>
<td>133.3 (42.4)</td>
<td>0.1 (0.1)</td>
<td>1.0 (0.9)</td>
</tr>
<tr>
<td>OXC</td>
<td>3</td>
<td>0.3 (0.4)</td>
<td>1.3 (0.6)</td>
<td>1.3 (1.1)</td>
<td>126.3 (12.3)</td>
<td>0.1 (0.1)</td>
<td>1.0 (0.5)</td>
</tr>
<tr>
<td>VPA</td>
<td>8</td>
<td>0.8 (0.5)</td>
<td>8.7 (4.0)‡</td>
<td>1.5 (1.2)</td>
<td>136.2 (42.5)</td>
<td>0.1 (0.03)</td>
<td>0.9 (0.6)</td>
</tr>
<tr>
<td>LTG</td>
<td>1</td>
<td>0.9</td>
<td>2.2</td>
<td>2.1</td>
<td>149.2</td>
<td>0.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Controls</td>
<td>23</td>
<td>0.6 (0.4)</td>
<td>1.8 (1.0)</td>
<td>1.5 (1.0)</td>
<td>114.8 (27.6)</td>
<td>0.2 (0.2)</td>
<td>1.5 (1.0)</td>
</tr>
<tr>
<td>Puberty</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>33</td>
<td>11.3 (7.4)</td>
<td>7.0 (5.5)</td>
<td>2.7 (2.5)†</td>
<td>80.8 (45.1)†</td>
<td>2.6 (1.4)</td>
<td>3.4 (1.8)</td>
</tr>
<tr>
<td>CBZ</td>
<td>15</td>
<td>10.0 (8.2)</td>
<td>3.8 (2.1)</td>
<td>1.6 (1.1)§</td>
<td>92.7 (53.2)§</td>
<td>2.6 (1.8)</td>
<td>3.0 (1.4)</td>
</tr>
<tr>
<td>OXC</td>
<td>4</td>
<td>7.8 (4.6)</td>
<td>3.6 (1.2)</td>
<td>2.4 (1.1)</td>
<td>91.9 (22.3)</td>
<td>1.6 (0.5)</td>
<td>3.4 (2.0)</td>
</tr>
<tr>
<td>VPA</td>
<td>12</td>
<td>13.7 (7.2)</td>
<td>12.3 (5.6)†</td>
<td>3.8 (3.4)</td>
<td>68.4 (38.5)</td>
<td>2.9 (1.1)</td>
<td>3.9 (2.4)</td>
</tr>
<tr>
<td>LTG</td>
<td>2</td>
<td>14.7 (2.8)</td>
<td>7.0 (2.0)</td>
<td>5.5 (0.2)</td>
<td>42.9 (18.3)</td>
<td>3.3 (1.2)</td>
<td>3.9 (1.9)</td>
</tr>
<tr>
<td>Controls</td>
<td>33</td>
<td>9.8 (8.5)</td>
<td>6.3 (3.6)</td>
<td>4.2 (2.2)</td>
<td>56.9 (31.1)</td>
<td>2.8 (1.9)</td>
<td>3.5 (2.0)</td>
</tr>
<tr>
<td>Postpuberty</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>14</td>
<td>18.1 (6.5)</td>
<td>16.6 (10.8)</td>
<td>7.3 (3.8)</td>
<td>32.7 (20.3)</td>
<td>4.8 (1.8)</td>
<td>5.0 (3.3)</td>
</tr>
<tr>
<td>CBZ</td>
<td>2</td>
<td>20.1 (11.3)</td>
<td>9.4 (5.3)</td>
<td>3.8 (0.4)</td>
<td>29.1 (12.9)</td>
<td>6.0 (0.6)</td>
<td>4.3 (0.5)</td>
</tr>
<tr>
<td>OXC</td>
<td>5</td>
<td>20.0 (5.5)</td>
<td>12.2 (4.8)</td>
<td>6.7 (4.1)</td>
<td>35.0 (8.4)</td>
<td>4.9 (1.9)</td>
<td>4.9 (2.8)</td>
</tr>
<tr>
<td>VPA</td>
<td>5</td>
<td>15.6 (7.9)</td>
<td>27.1 (11.5)‖</td>
<td>7.6 (2.5)</td>
<td>36.9 (33.4)</td>
<td>4.9 (2.1)</td>
<td>5.6 (5.1)</td>
</tr>
<tr>
<td>LTG</td>
<td>2</td>
<td>17.7 (0.8)</td>
<td>8.5 (1.1)</td>
<td>11.6 (5.7)</td>
<td>20.3 (1.1)</td>
<td>3.1</td>
<td>4.4 (1.8)</td>
</tr>
<tr>
<td>Controls</td>
<td>14</td>
<td>17.7 (5.5)</td>
<td>11.9 (3.3)</td>
<td>7.5 (2.1)</td>
<td>26.8 (9.6)</td>
<td>4.0 (1.8)</td>
<td>3.8 (1.6)</td>
</tr>
</tbody>
</table>

Values are mean (SD) serum concentrations. T, testosterone; A, androstenedione; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone-binding globulin; LH, luteinizing hormone; FSH, follicle-stimulating hormone; CBZ, carbamazepine; OXC, oxcarbazepine; VPA, valproate; LTG, lamotrigine.

*Analyzed after ln transformation due to non-homogeneity of variances in the different groups. Patient on LTG were excluded from the analysis of variance in prepuberty due to the small number of subjects. †P < 0.02 compared to control subjects, unpaired t-test; ‡P < 0.008 compared to control subjects, patients on CBZ and OXC; §P < 0.03 compared to control subjects; ‖P ≤ 0.02 compared to control subjects, patients on CBZ, OXC or LTG, one-way analysis of variance with Tukey’s post hoc test.
Table 9. Results of binary logistic regression analyses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Independent variables</th>
<th>OR (95% CI)</th>
<th>P value</th>
<th>Not in equation value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated A</td>
<td>Medication</td>
<td>0.0002</td>
<td>Age, year</td>
<td>0.223</td>
</tr>
<tr>
<td>(R-square 41%)</td>
<td>VPA vs. Controls</td>
<td>117.5 (12.5-1103.7)</td>
<td>Type of epilepsy</td>
<td>0.941</td>
</tr>
<tr>
<td>(No elevated A in patients on CBZ, OXC, or LTG)</td>
<td>Size of testes</td>
<td>0.284</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight for height, %</td>
<td>1.1 (1.04-1.2)</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage of puberty</td>
<td>0.007</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pub vs. Pre</td>
<td>0.1 (0.01-1.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post vs. Pre</td>
<td>0.01 (0.0003-0.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>Medication</td>
<td>0.003</td>
<td>Type of epilepsy</td>
<td>0.737</td>
</tr>
<tr>
<td>(I evaluation)</td>
<td>CBZ vs. Controls</td>
<td>25.5 (2.5-264.0)</td>
<td>Weight for height</td>
<td>0.784</td>
</tr>
<tr>
<td>(R-square 18%)</td>
<td>OXC vs. Controls</td>
<td>18.8 (1.9-186.9)</td>
<td>Age, year</td>
<td>0.260</td>
</tr>
<tr>
<td></td>
<td>VPA vs. Controls</td>
<td>4.3 (0.4-44.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage of puberty</td>
<td>0.044</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pub vs. Pre</td>
<td>0.2 (0.03-1.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post vs. Pre</td>
<td>0.2 (0.04-1.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td>Obesity (I evaluation)</td>
<td>41.3 (6.5-264.0)</td>
<td>Age, year</td>
<td>0.168</td>
</tr>
<tr>
<td>(II evaluation)</td>
<td>Medication (I evaluation)</td>
<td>0.015</td>
<td>Stage of puberty</td>
<td>0.123</td>
</tr>
<tr>
<td>(R-square 29%)</td>
<td>VPA vs. Controls</td>
<td>6.2 (1.04-37.3)</td>
<td>Type of epilepsy</td>
<td>0.665</td>
</tr>
<tr>
<td></td>
<td>CBZ vs. Controls</td>
<td>1.9 (0.2-19.8)</td>
<td>(No obese patients on OXC)</td>
<td></td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval; A, androstenedione; VPA, valproate; CBZ, carbamazepine; OXC, oxcarbazepine; LTG, lamotrigine; Pre, Prepubertal; Pub, Pubertal; Post, Postpubertal; Elevated A/Hypercholesterolemia, serum A/total cholesterol higher than the mean concentration + 2 SD in the controls of corresponding gender and pubertal stage; Obesity in Study IV (I evaluation) weight for height > 120%, (II evaluation) body mass index > 25 mg/kg^2.
The possible clinical consequences of high serum A levels in boys and young men were also analyzed. The mean bone age related to chronological age of the VPA-treated prepubertal and pubertal boys with high serum A was advanced (0.55 SDS, SD 0.9) compared to their control subjects matched for age and pubertal stage (-0.21 SDS, SD 0.5), P = 0.01. The mean pubic hair stages of the patients with high serum A and their control subjects were the same 2.9 (SD 2.1). Weight for height was 114.3% (SD 18.9) in the patients with elevated serum A and 105.3% (SD 11.2) in the control subjects, P = 0.1. On the other hand, 22% of the obese patients had elevated serum A levels compared to 23% of the normal-weight patients. The prevalence of hyperinsulinism was 13% both in the patients with high A and in their control subjects, and the respective prevalences of acne in these groups were 19% and 6%, P = 0.6.

5.2 Thyroid function (Study III)

Serum thyroid hormone levels during AED monotherapy and after withdrawal of medication in girls and young women with childhood- and adolescence-onset epilepsy are presented in Table 10. Serum T4 and FT4 concentrations were decreased in female patients with childhood- and adolescence-onset epilepsy taking CBZ and OXC monotherapy, whereas serum TSH was increased during VPA treatment on the first evaluation. Sixty-three percent (n=12/19) of the patients taking CBZ and 67% (n=12/18) of the patients taking OXC had serum T4 and/or FT4 concentrations below the lower limit of the assessed reference range compared to 10% (n=4/41) of the patients taking VPA, P < 0.0001. Serum thyroid peroxidase antibodies (TPO-ab) were positive in one patient on CBZ and one on VPA, in addition to which serum thyroglobulin antibodies (TG-ab) were also positive in three control subjects. All these subjects with positive antibody levels had normal serum thyroid hormone and TSH levels on the first evaluation. One control subject with positive antibodies developed hypothyroidism during the follow-up.

Serum thyroid hormone concentrations were normal in the patients who were off medication on the second evaluation regardless the original AED (Table 10). Decreased T4 and FT4 levels of the patients on CBZ or OXC and increased TSH levels of the patients on VPA at the first evaluation had become normalized by the second evaluation after the withdrawal of AED. The number of patients with the same AED on both evaluations was too small to allow any meaningful statistical analysis of their serum thyroid hormone concentrations (Table 2).
Table 10. Serum thyroid hormone and thyrotropin concentrations in girls with epilepsy and controls during pubertal maturation and after the follow-up period (mean 5.8 years, SD 1.0).

<table>
<thead>
<tr>
<th>Variable</th>
<th>I evaluation</th>
<th>II evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medication</td>
<td>Original medication</td>
</tr>
<tr>
<td></td>
<td>CBZ</td>
<td>OXC</td>
</tr>
<tr>
<td>N</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;, nmol/L</td>
<td>70.2 (10.9)*</td>
<td>74.9 (16.4)*</td>
</tr>
<tr>
<td>FT&lt;sub&gt;4&lt;/sub&gt;, pmol/L</td>
<td>11.5 (1.8)*</td>
<td>11.3 (1.8)*</td>
</tr>
<tr>
<td>FT&lt;sub&gt;3&lt;/sub&gt;, pmol/L</td>
<td>5.2 (0.6)</td>
<td>5.1 (0.5)</td>
</tr>
<tr>
<td>TSH, mU/L</td>
<td>2.3 (0.8)</td>
<td>2.1 (0.9)</td>
</tr>
</tbody>
</table>

Values are means (SD). On the first evaluation patients and controls were matched for gender, age and pubertal stage. On the second evaluation, only the results of subjects off medication are shown. T<sub>4</sub>, thyroxine; FT<sub>4</sub>, free thyroxine; FT<sub>3</sub>, free triiodothyronine; TSH, thyrotropin; CBZ, carbamazepine; OXC, oxcarbazepine; VPA, valproate. *P < 0.001 compared to controls and patients on VPA; †P < 0.01 compared to controls; one-way analysis of variance with post hoc LSD. FT<sub>4</sub> and FT<sub>3</sub> on the second evaluation Kruskal-Wallis test.

### 5.3 Serum lipids (Study IV)

Serum lipid levels during AED monotherapy and after withdrawal of medication in girls and young women with childhood- and adolescence-onset epilepsy are presented in Table 11. Patients taking VPA monotherapy had low serum HDL-C levels, and patients taking CBZ and OXC monotherapy had high serum TC and LDL-C concentrations. Hypercholesterolemia was also more common in the patients on CBZ (29%) or OXC (31%) than in the healthy control subjects (2%), both comparisons P < 0.002. CBZ and OXC also had the strongest association with high TC levels in the logistic regression analysis (Table 9). There were no statistically significant changes in serum lipid levels in the postpubertal patients after the withdrawal of medication (Table 11).
Table 11. Serum lipid concentrations in girls with epilepsy and controls during pubertal maturation and after the follow-up period (mean 5.8 years, SD 1.0).

<table>
<thead>
<tr>
<th>Variable</th>
<th>I evaluation</th>
<th>P Value</th>
<th>II evaluation</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medication</td>
<td></td>
<td>Original medication</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBZ</td>
<td>OXC</td>
<td>VPA</td>
<td>Controls</td>
</tr>
<tr>
<td>Number of subjects</td>
<td>17</td>
<td>16</td>
<td>38</td>
<td>46</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>5.1*</td>
<td>5.3*</td>
<td>4.7</td>
<td>4.3</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(4.6-5.7)</td>
<td>(4.8-5.8)</td>
<td>(4.5-5.0)</td>
<td>(4.1-4.5)</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.2</td>
<td>1.1</td>
<td>1.0*</td>
<td>1.3</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(1.1-1.4)</td>
<td>(1.0-1.2)</td>
<td>(0.9-1.1)</td>
<td>(1.2-1.3)</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.7†</td>
<td>2.9*</td>
<td>2.6</td>
<td>2.2</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(2.3-3.1)</td>
<td>(2.5-3.3)</td>
<td>(2.4-2.7)</td>
<td>(2.1-2.4)</td>
</tr>
<tr>
<td>TGs (mmol/L)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(0.9-1.2)</td>
<td>(0.9-1.2)</td>
<td>(0.9-1.2)</td>
<td>(0.7-0.9)</td>
</tr>
</tbody>
</table>

Values are means. On the first evaluation, patients and controls were matched for age and pubertal stage. On the second evaluation, only the results of postpubertal subjects off medication are shown. CI, confidence interval; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TGs, triglycerides; CBZ, carbamazepine; OXC, oxcarbazepine; VPA, valproate; ANOVA, one-way analysis of variance; CI, confidence interval. *P < 0.005; †P < 0.05 compared to control subjects, ANOVA with post hoc Tukey’s test.
5.4 Growth

5.4.1 Height (Study IV)

Linear growth did not differ between the female patients with epilepsy initially on different AEDs and the control subjects (Figures 8 and 9). The mean final height was 164.8 cm (SD 6.7) in 47 of the 68 patients and 165.5 cm (SD 5.6) in 30 of the 46 control subjects who had reached their final height. The difference between final height and mid-parental height was 0.04 SDS (95% CI -0.3 to 0.3) in the patients and -0.24 SDS (95% CI -0.5 to 0.1) in the control subjects, P = 0.21, and there were no significant differences between the patients on different AEDs, either.

Serum concentrations of growth markers, IGF-I and IGFBP-3, during AED treatment on the first evaluation have been reported earlier (Rättyä et al. 1999). On the second evaluation, there were no statistically significant differences in serum IGF-I levels between the postpubertal female patients, whether off medication or on medication, and the control subjects. The postpubertal patients still on VPA monotherapy had lower serum mean IGFBP-3 level (3.4 mg/L, SD 0.3) than the control subjects (4.5 mg/L, SD 0.7), P = 0.005. Serum mean IGFBP-3 level in the postpubertal patients off medication (4.5 mg/L, SD 0.8) was similar to that of in the control subjects.

5.4.2 Weight (Studies II and IV)

Thirteen percent of the male patients and 4% of the male control subjects were obese, the proportion difference being 9%, 95% CI of the difference -1% to 19%, P = 0.07. The frequency of obesity did not significantly differ between the male patients on different AEDs, either.

Girls and young women taking VPA at the beginning of the study had higher weight for height than those initially on CBZ or OXC (Figures 8 and 9). At the end of follow-up, the patients still on any AED had higher mean BMI (24.6 kg/m², SD 6.5; P < 0.002) and more often obesity (33%, P = 0.002) than the patients whose medication had been withdrawn (BMI 21.2 kg/m², SD 4.1; obesity 12%) or the control subjects (20.3 kg/m², SD 2.6; obesity 4%). The prevalence of obesity did not differ between the patients still on different AEDs (30% of the patients on VPA and 35% of patients on any other AED). Also, the mean BMI (P = 0.46) and the prevalence of obesity (95% CI of the proportion difference -4% to 22%, P = 0.18) did not differ in the patients off medication and the control subjects. There were no significant differences in weight reduction, physical exercise or dietary habits between the patients on different AEDs and the control subjects. VPA medication and obesity at the first evaluation were the only factors predictive of obesity on the second occasion (Table 9). Serum insulin and IGFBP-1 concentrations did not differ significantly in the female patients and the control subjects, but the obese female subjects had higher serum insulin concentrations (10.2 mU/L, SD 4.9) than the
non-obese subjects (6.5 mU/L, SD 3.3) on the second evaluation, P = 0.0002, and this difference was seen in all study groups irrespective of the type of medication.

Fig. 8. Mean relative height and weight for height charts until the second evaluation in patients initially (A) on carbamazepine (CBZ) and (B) on oxcarbazepine (OXC) and controls. The time point zero stands for the start of the respective medication. The number of subjects and the bars for SD are shown at each time point. Relative height is the deviation between absolute height and expected height for age and sex (SDS), and weight for height is (absolute weight/expected weight for height) x 100 (%) (Sorva et al. 1990). The PROC MIXED model (SAS) was used to analyze the charts (Singer 1998). SDS, standard deviation score.
Fig. 9. Mean relative height and weight for height charts until the second evaluation (A) in patients initially on valproate (VPA) and controls; and (B) in patients on medication other than VPA or off medication, with both groups initially on VPA. The time point zero stands for (A) the introduction of VPA and (B) the withdrawal of VPA. The number of subjects and the bars for SD are shown at each time point. Relative height is the deviation between absolute height and expected height for age and sex (SDS), and weight for height is (absolute weight/expected weight for height) x 100 (%) (Sorva et al. 1990). The PROC MIXED model (SAS) was used to analyze the charts (Singer 1998). SDS, standard deviation score.
6 Discussion

6.1 Reproductive endocrine function in patients with childhood- or adolescence-onset epilepsy

6.1.1 Girls and young women (Study I)

Childhood-onset epilepsy is known to have a good prognosis (Thurston et al. 1982, Sillanpää et al. 1998, Sillanpää et al. 1999). Most of the present female patients with childhood- and adolescence-onset epilepsy were free of seizures and medication after approximately six years of follow-up. Knowledge of the reproductive endocrine health in subjects with earlier epilepsy has so far been limited. In this research, the subjects with childhood- and adolescence-onset epilepsy had a favourable outcome of reproductive endocrine function if their epilepsy was remitted and their medication had been withdrawn before they reached adult age. Indeed, this was the case even if they had HA when taking VPA during puberty. The serum reproductive hormone levels, menstrual cycles and ovarian findings of patients off medication were similar to those of healthy control subjects and the observed frequencies of PCO and PCOS were similar to those seen in the general population (Polson et al. 1988, Knochenhauer et al. 1998, Koivunen et al. 1999).

Elevated serum androgen levels and frequent PCOS in the present girls and young women with childhood- or adolescence-onset epilepsy with long-term AED exposure, especially with VPA, agree with most of the previous reports on adult women (Isojärvi et al. 1993b, Isojärvi et al. 1996, Muraldo et al. 1997, Muraldo et al. 1998, Isojärvi et al. 2001a, Morrell et al. 2002, Betts et al. 2003, Morrell et al. 2003). Data on the frequency of PCOS in patients with epilepsy may be influenced by different diagnostic criteria and inconsistencies in the terminology of PCO and PCOS in the literature. However, increased frequency of PCOS has not been observed in all women with epilepsy taking VPA (Bauer et al. 2000, Stephen et al. 2001). The present findings indicate that patients with childhood- and adolescence-onset epilepsy on long-term medication, particularly
VPA, frequently already have reproductive endocrine abnormalities in adolescence and young adulthood.

VPA has also been reported to be associated with reproductive endocrine dysfunction in female patients with bipolar disorder (O'Donovan et al. 2002). In addition, non-epileptic animals have had PCO findings related to exposure to VPA (Taubøll et al. 1999), and there is also biomedical evidence indicating that VPA potentiates androgen biosynthesis in human ovarian theca cells (Nelson-DeGrave et al. 2004). The present results of a threefold increase in the circulating A concentration in female patients on VPA together with a minor increase in the peripheral concentrations of DHEAS also suggest that VPA is rather associated with stimulated ovarian androgen synthesis than with increased adrenal androgen production.

PCOS is one of the most common reproductive endocrinopathies in women with anovulation, menstrual disorders and infertility problems, which may have their roots in childhood (Franks 2002). Also, many other health implications, such as dyslipidemia and insulin resistance, are related to this clinically relevant endocrinopathy (Homburg 1996, Robinson et al. 1996, Hopkinson et al. 1998, Morin-Papunen et al. 2000, Guzick 2004).

It is important to consider reproductive endocrine functions when starting the antiepileptic therapy of a female patient with childhood- or adolescence-onset epilepsy, especially if the need for medication is likely to be long. Clinical signs of PCOS, e.g. HA and menstrual disorders, in young women with epilepsy may be indicators of adverse effects of AED. They should warrant further examinations of the reproductive endocrine function, to identify possibly treatable disorders. Easily available gynecological examinations, i.e. menstrual history, serum reproductive hormone measurements and ovarian ultrasonography, are needed for a diagnosis of reproductive endocrine disorders in female subjects.

The newer AEDs have been shown to be effective against epilepsy, but their possible endocrine effects have not been widely studied and are not known. Meanwhile, however, the possible benefits of a change of antiepileptic therapy must always be balanced against efficacy, i.e. seizure control, and the known adverse effect profiles of alternative agents.

6.1.2 Boys and young men (Study II)

The high serum SHBG and low serum DHEAS levels in the present pubertal patients taking CBZ are consistent with the results of previous studies on adult men with epilepsy taking CBZ (Isojärvi et al. 1989b, Isojärvi et al. 1990, Isojärvi et al. 1995d, Verrotti et al. 2000, Rättyä et al. 2001a, Isojärvi et al. 2004). These consistent hormonal findings in patients with epilepsy taking CBZ have been assumed to be caused by liver enzyme induction of CBZ.

VPA was previously considered to have only minor effects on sex hormone levels in male subjects (Maclache et al. 1988, Isojärvi et al. 1990). However, as currently known, VPA may also have unfavourable effects on reproductive endocrine function in adult men (Rättyä et al. 2001a, Rättyä et al. 2001b, Isojärvi et al. 2004). The present findings of high serum A and unchanged serum T levels in VPA-treated boys and adolescents with
epilepsy are consistent with the previous findings in adult men with epilepsy (Rättyä et al. 2001b). Moreover, two other studies showed boys with epilepsy on VPA to have normal serum T and also serum A levels (Verrotti et al. 2000, Balaguer Martinez et al. 2003), but the patients and the controls were not matched for pubertal stage in these studies. On the other hand, one report suggested high serum T levels together with diminished testicular size in young male subjects with epilepsy on different AEDs, including VPA (El-Khayat et al. 2003). However, control subjects matched only for age, but not for pubertal stage, were included in that study, and it was not possible to differentiate between findings related to the use of different AEDs (El-Khayat et al. 2003).

Knowledge of the effects of OXC and LTG on male reproductive health is so far limited. The normal serum reproductive hormone levels associated with the use of OXC and LTG in the current young male patients with epilepsy suggest that these drugs may not affect reproductive endocrine function in young male patients and agree with the previously reported findings in men taking OXC for epilepsy (Isojärvi et al. 2004) and with the available animal data on the effects of LTG on male reproductive function in rats (Sveberg Røste et al. 2003b). However, the number of LTG- or OXC-treated subjects was small in the present study, and further studies are needed to confirm our preliminary findings.

Sperm abnormalities associated with small testicular volume have been reported in men with generalized epilepsy on VPA (Isojärvi et al. 2004). However, the serum FSH and inhibin levels, which are known to regulate spermatogenesis, were within the normal range (Isojärvi et al. 2004). Despite the normal testicular volume, sperm abnormalities were also found in men taking CBZ or OXC more often than in healthy control subjects according to the established WHO criteria (Isojärvi et al. 2004). In general, Finnish men have been reported to have good semen quality (Jørgensen et al. 2001). The impact of LTG on testes has not previously been studied, with the exception of one experimental animal study, in which testicular volume was unchanged in LTG-treated rats (Sveberg Røste et al. 2003b). Testicular volume was not decreased in male patients with childhood- or adolescence-onset epilepsy taking CBZ, OXC, VPA or LTG monotherapy in the current study. It was not possible to examine sperm quality, libido or potency in the present study because of the young age of the male subjects. Nevertheless, serum FSH and inhibin B levels as well as testicular volume and structure were normal in both pubertal and postpubertal male subjects. Thus, it seems that the endocrine regulation of spermatogenesis in young male patients with epilepsy is normal.

There are different views on whether the reproductive endocrine disorders detected commonly in patients with epilepsy are caused by AED or the epilepsy itself or both. In this study, VPA medication, but not the type of epilepsy, was considered as an independent predictor of elevated serum A levels. This supports the role of VPA medication in the development of elevated serum androgen levels in boys and young men. High serum A levels in boys and young men with epilepsy taking VPA may be caused by the effects of VPA on steroid synthesis, but the underlying mechanisms are not known. In theory, high serum A levels may be caused by VPA blocking the 17β-hydroxysteroid dehydrogenase enzyme, which converts A to T, leading to accumulation of serum A. Another explanation could be stimulation of the enzymatic cascade before this step. Since the adrenal contribution to A synthesis is significant, high serum A levels
together with the relatively normal serum T and LH concentrations and unaffected testicular size in the pubertal and postpubertal male subjects may reflect increased adrenal secretion of A during VPA medication. However, a concomitant effect on gonadal steroid synthesis cannot be excluded either, because larger testicular volume was found in prepubertal boys on VPA with elevated serum A, and VPA-treated patients with elevated serum A at all pubertal stages had normal serum levels of adrenal androgens, DHEAS and dehydroepiandrosterone.

Reproductive endocrine abnormalities may have a role in the development of sexual dysfunction, i.e. decreased libido and impaired potency, which have been reported in men with epilepsy, particularly associated with CBZ therapy (Isojärvi et al. 1995d, Rättyä et al. 2001b). The androgenic potency of serum A is relatively weak, and the consequences of elevated serum A levels in male subjects with epilepsy are not known. In this study, prepubertal patients taking VPA, who all also had high serum A levels, had enlarged testes and advanced bone age, which may reflect an acceleration in pubertal development due to A. In contrast, adult men taking VPA have been reported to have elevated serum A levels, but diminished testicular size (Isojärvi et al. 2004). Thus, in theory, the testicular atrophy seen in men with epilepsy on VPA could be explained by abnormal early testicular growth in patients taking valproate since childhood. Abnormal early testicular growth may lead to diminished final testicular size, because testicular size is mostly determined by the amount of Sertoli cells, which are known to undergo terminal differentiation. In general, children with severe androgen excess may suffer from precocious virilization and acne and have advanced bone age and puberty, which may predispose them to early epiphyseal fusion and short adult stature (Merke et al. 2002). Elevated serum A levels were associated with advanced bone age, but not with acne or abnormal virilization in the current male patients. Short stature and delayed sexual development together with high serum T levels in male patients with epilepsy on different AED combinations have been reported earlier in a study on subjects matched for age but not for pubertal stage (El-Khayat et al. 2003).

Reproductive endocrine changes during pubertal maturation may have potential effects on long-term health and sexual function. However, the long-term clinical consequences of reproductive hormonal changes in boys and young men with epilepsy taking AEDs remain to be established.

6.2 Thyroid function and serum lipid profile (Studies III and IV)

The present results of low serum T4 and FT4 levels in female patients with childhood- or adolescence-onset epilepsy taking CBZ agree with the previous reports on patients with epilepsy taking CBZ in both childhood and adulthood (Strandjord et al. 1981, Isojärvi et al. 1989e, Yüksel et al. 1993, Verrotti et al. 2001). In addition, low serum T4 and FT4 concentrations were similarly found in the current female patients with OXC. Consistent with this, low serum thyroid hormone concentrations have been reported in men taking OXC for epilepsy (Isojärvi et al. 2001b). As far as we know, this is the first study of serum thyroid function in children and adolescents with epilepsy taking OXC. The
frequency of positive serum TPO and TG antibodies was not increased in the present patients. Increased serum TSH levels together with normal serum thyroid hormone levels were found in current patients taking VPA. On the whole, however, the results of the controlled studies on the effects of VPA on serum thyroid hormone levels in children and adults have been inconsistent (Larkin et al. 1989, Isojärvi et al. 1992, Eiris-Puñal et al. 1999, Isojärvi et al. 2001b, Verrotti et al. 2001, Çaksen et al. 2002).

CBZ and OXC were the identified independent predictors of elevated serum TC levels in the current female subjects. This is in agreement with the previous reports of increased serum TC levels in children with epilepsy taking CBZ (Sözüer et al. 1997, Verrotti et al. 1998, Demircioğlu et al. 2000, Eiris et al. 2000). In these earlier studies, serum LDL-C levels were also high and HDL-C levels often low in patients treated with CBZ. Our finding of increased serum LDL-C concentrations associated with CBZ is in accordance with these earlier reports, but we did not find a decrease in serum HDL-C levels. Even though OXC has been marketed for more than 10 years for epilepsy in Europe, this is the first controlled study on lipid metabolism in children and adolescents on OXC. In adults with epilepsy, OXC has not been found to affect serum lipid concentrations (Isojärvi et al. 1994). In the current study, patients with OXC also had high serum TC and LDL-C levels of the same magnitude as the patients on CBZ. This agrees with what has been previously reported about lipid changes in children taking short-term OXC in an uncontrolled study (Serdaroglu et al. 2003). Decreased serum HDL-C concentrations associated with VPA have also been reported previously in adults (Isojärvi et al. 1998). The reference limits for hypercholesterolemia were calculated using the values of the control subjects matched for age and pubertal stage in the present study. The respective limits, 5.7 mmol/L at all pubertal stages, were somewhat higher than 5.5 mmol/L, which is given as the limit of hypercholesterolemia in the recommendations of the Finnish Pediatric Society for children in clinical practice (Salo et al. 1994).

CBZ may alter the peripheral metabolism of thyroid hormones and serum lipids by induction of the hepatic P450 enzyme system (Perucca et al. 1984, Patsalos et al. 1990, Isojärvi et al. 1992, Isojärvi et al. 1993c, Isojärvi et al. 1994, Sözüer et al. 1997, Vertortti et al. 1998). The mechanisms by which OXC may affect serum thyroid hormone and lipid concentrations are not known, although it has also been suggested that OXC may induce liver P450 enzymes to a certain degree (Patsalos et al. 1990, Rättä et al. 1999). CBZ may competitively displace T4 and T3 from serum-binding proteins (Surks & DeFesi 1996), which should lead to an increased free fraction of these hormones as well as to a previously noted reduction in serum TBG levels (Strandjord et al. 1981, Bentsen et al. 1983, Isojärvi et al. 1989c, Eiris-Puñal et al. 1999). In addition, CBZ has been suggested to have an inhibitory effect at the thyroidal level (Villa & Alexander 1987, Isojärvi et al. 1989c), which could explain the subnormal free T4 levels in association with normal serum TSH in patients on CBZ. Nevertheless, the reason for the failure of pituitary TSH secretion to rise in response to the fall in circulating T4, FT4 and T3 is unclear. Methodologic explanations of this discrepancy have also been given. Low FT4 and FT3 have been detected upon exposure to CBZ when analyzed by a commercial procedure from diluted serum, but not when analysed with an ultrafiltration method from undiluted serum (Surks & DeFesi 1996). At least partly, this may explain the decrease in serum FT4 concentrations and, further, the normal serum TSH levels in girls on CBZ and also on OXC, but not the decrease in their T4 levels.
Patients with epilepsy with abnormal serum thyroid hormone and/or serum TSH levels have been suggested to suffer from subclinical hypothyroidism (Isojärvi et al. 1995a, Eiris-Puñal et al. 1999, Verrotti et al. 2001), which is typically defined as an increased serum TSH concentration with normal serum thyroid hormones, especially FT₄ (Wiersinga 1995). The prevalence of subclinical hypothyroidism in female populations varies from 0.9 to 9.5% (Wiersinga 1995). Even though patients with subclinical hypothyroidism are, by definition, subjectively asymptomatic, this condition may cause dry skin, cold intolerance, depression, increased achilles tendon reflex time, prolonged systolic time intervals and decreased left ventricular ejection fraction (Wiersinga 1995). Concomitant improvement in diastolic heart function and increase in lowered thyroid hormones was seen in men with epilepsy when CBZ was replaced by OXC (Isojärvi et al. 1995a).

Thyroid status influences lipid metabolism in general, for instance by causing an increase in LDL-C and a decrease in HDL-C levels in patients with subclinical hypothyroidism (Wiersinga 1995). Thus, the elevated serum lipid levels observed in girls with epilepsy taking CBZ and OXC may also be associated with their observed low serum thyroid hormone concentrations. The present female patients taking CBZ and OXC did not have weight gain. Also, the linear growth and final height of the patients taking CBZ, OXC or VPA were unchanged, but the patients on VPA had weight gain and lowered serum HDL-C levels. However, in the present study, the reference limits for low serum thyroid hormones were calculated from the values of the control subjects, and the limits were not as low as the levels regarded as hypothyroid in clinical practice, and all the observed unfavourable serum thyroid hormone and lipid concentration changes disappeared after the withdrawal of medication. Moreover, according to a placebo-controlled study, patients receiving anticonvulsant drugs are chronically eumetabolic and do not need T₄ supplementation (Tiikonen et al. 1995). Otherwise healthy people with subclinical hypothyroidism appear to benefit from T₄ medication (Wiersinga 1995). The changes in serum thyroid hormone and TSH levels in the present study were not signs of subclinical hypothyroidism or preclinical compensated hypothyroidism, which is a sign of impending real thyroid disease. Thus, the clinical relevance of these hormonal changes remains unknown and is probably small. According to a large retrospective evaluation, the screening of thyroid function is not indicated in patients with epilepsy (Verma & Haidukewych 1994). However, when otherwise needed, serum TSH level seems to be the clinically the most relevant hormone assay to evaluate the true thyroidal state of patients with epilepsy taking CBZ, OXC or VPA.

Lipids are essential for normal metabolism, but elevated plasma TC and low HDL levels are known risk factors for atherosclerosis, which may start already in childhood or early adulthood (Berenson et al. 1992). In general, serum TC levels have been found to slowly decrease during the last 15 years in young adults in Finland (Juonala et al. 2004). According to the present study, both CBZ and OXC may alter serum lipid concentrations in a way that facilitates the development of atherosclerosis (de Chadarévian et al. 2003). This may result in untoward health consequences, especially after long-term AED exposure. However, the unfavourable lipid profile observed during treatment with any of the three AEDs in girls and adolescents normalised after withdrawal of medication. The consistency of the present results with the previous reports supports the view of transient

6.3 Height and weight (Studies II and IV)

Linear growth and final height were not affected in the current female subjects with childhood- and adolescence-onset epilepsy on long-term therapy with CBZ, OXC or VPA. Accordingly, normal linear growth (Tada et al. 1986, Macardle et al. 1987) and adult stature (McGowan 1983, Kurlemann et al. 1997) in patients with epilepsy on different AEDs have been reported. Also, normal growth has been detected in patients with epilepsy on CBZ (Kurowski et al. 1993, Kurlemann et al. 1997), OXC (Rättyä et al. 1999), LTG (Ueberall 2001) and VPA (Kurowski et al. 1993, Kurlemann et al. 1997), but conflicting results have also been presented in children (Novak et al. 1999, Guo et al. 2001) and also in animal studies (Wu et al. 2004).

VPA medication during pubertal maturation as an independent predictor of obesity in young adulthood is in accordance with the common notification of weight gain during VPA medication (Egger & Brett 1981, Rättyä et al. 1999, Biton et al. 2003). The etiology of obesity is certainly multifactorial, and the exact mechanisms of VPA-related weight gain are still unknown. However, weight gain during VPA medication is mainly considered to be caused by abnormal fat metabolism and to be mediated through VPA-induced inhibition of beta-oxidation of fatty acids, which may impair energy utilization and promote lipid accumulation (Breum et al. 1992, Ponchaut & Veitch 1993, Biton 2003). Increased appetite (Egger & Brett 1981, Corman et al. 1997) and decreased energy expenditure have also been suggested as possible explanations of weight gain during VPA medication. In general, obesity is related to high serum leptin levels, which suggest decreased sensitivity to leptin. As in other types of obesity, serum leptin levels were reported to increase in patients taking VPA for epilepsy and showing weight gain in a prospective study (Verrotti et al. 1999), but patients’ leptin levels were not different from the levels of obese control subjects in a controlled study (Pylvänen et al. 2002). In addition, high serum insulin levels are associated with obesity in general. Hyperinsulism is also associated with obesity in adult patients with epilepsy taking VPA (Isojärvä et al. 1996), but not in girls undergoing pubertal maturation (Rättyä et al. 1999). Furthermore, high serum insulin levels have also been reported in lean adults with VPA (Pylvänen et al. 2002).

In general, the hypothalamus is known to regulate appetite at the central nervous system level (Pesonen & Koulu 1994). It has not been studied whether neurotransmitters that regulate satiety and body fat storage (noradrenaline and many neuropeptides, e.g. neuropeptide Y and peptide YY) are involved in VPA-related weight gain. In one study, energy expenditure and food intake remained unchanged in patients started on VPA medication (Breum et al. 1992), and it has not been possible to reduce VPA-related weight gain by dietary reductions or enhanced exercise (Dinesen et al. 1984, Corman et al. 1997). Generally, children who become overweight due to increased energy intake
also experience accelerated linear growth. In the present study, the weight gain in female subjects taking VPA was not associated with changes in growth.

The baseline weight before starting AED has not unequivocally predicted the subjects who will gain weight during AED treatment (Corman et al. 1997, Novak et al. 1999). In general, it has been observed that obese adolescents continue to be obese as adults (DiPietro et al. 1994). It is not known whether the patients who gain weight and are obese for several years during VPA treatment are able to reach and maintain normal weight after withdrawal of VPA. Many patients had changed the AED between the two evaluations, which made drawing of conclusions somewhat difficult. However, VPA treatment and obesity during pubertal maturation predicted obesity in young adulthood in the present female subjects. Previously, the mean BMI decreased in adult women with epilepsy; but all patients who were obese on VPA remained obese after the replacement of VPA with weight-neutral LTG (Isojärvi et al. 1998). The follow-up time in that study was one year. However, the present results suggest that girls and adolescents who gain weight on long-term treatment with VPA during puberty remain obese if epilepsy continues into adulthood, even when VPA is replaced by some other AED. Interestingly, the subjects whose medication could be withdrawn had remained lean even during VPA medication, and they had normal weight equally often as their healthy female controls. The reason for this may be related to differences in the severity of epilepsy or genetic factors. In addition, after the use of antiepileptic medication for approximately three years, the present male patients showed no more obesity than the healthy control subjects, and there was no difference between the AEDs.

Overweight is an increasing problem world-wide both in adults and in adolescents (Flegal et al. 2002, Ogden et al. 2002, Juonala et al. 2004). It is not only a cosmetic problem affecting self-esteem, but it is well-known to predispose to many health problems. High blood pressure, hyperinsulinemia, diabetes mellitus and coronary artery disease as well as sleep apnea and osteoarthritis later in life may ensue from obesity in general (DiPietro et al. 1994, Must et al. 1999). Reproductive endocrine disorders (Isojärvi et al. 1996, Isojärvi et al. 2001a, Morrell et al. 2003) and metabolic changes (Verrotti et al. 1999, Isojärvi et al. 2001a, Stephen et al. 2001, Luef et al. 2002a) have been documented in associated with weight gain in women with epilepsy taking VPA. These many health implications and possibly also poor treatment compliance and discontinuation of medication make overweight an important and clinically relevant adverse effect of AED (Isojärvi et al. 1996). Therefore, it is also important to give advice concerning the diet and physical exercise to prevent weight gain for other reasons and to consider weight-neutral AEDs when otherwise possible, especially in the case of children and adolescents.
7 Conclusions

1. Childhood- or adolescence-onset epilepsy and AED use during pubertal maturation do not affect reproductive endocrine function or gonadal structure in female subjects who discontinue their medication before young adulthood. Elevated serum androgen levels and PCOS are common in female patients who remain on AEDs, especially VPA, until young adulthood. CBZ and VPA, but not OXC and LTG, are associated with some serum reproductive hormone changes in boys and young men with childhood- or adolescence-onset epilepsy.

2. CBZ, OXC and VPA are associated with changes in serum thyroid hormone or TSH levels in female patients with epilepsy during pubertal maturation. These changes do not reflect real thyroid diseases, and they do not warrant medical interventions. Serum thyroid hormone and TSH concentrations were normal after the withdrawal of medication in patients.

3. CBZ, OXC and VPA are associated with unfavourable changes in serum lipid levels during pubertal maturation in female patients with epilepsy. These changes in lipid concentrations are reversible after withdrawal of medication.

4. Neither epilepsy nor CBZ, OXC or VPA monotherapy affects linear growth or final height in female subjects. Epilepsy or these AEDs during pubertal maturation are not associated with obesity in young adulthood if the epilepsy remitted and the medication was withdrawn before young adulthood. Obesity is common in young women who were on VPA medication and gained weight during puberty, if their epilepsy continues and they remain on medication until adult age.
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