Summary

This thesis presents studies with respect to the epidemiology of asthma in children with and without nocturnal symptoms as well as its underlying pathophysiology. Nocturnal respiratory symptoms are the clinical expression of nocturnal airflow limitation and indicate a more severe disease state, also named 'nocturnal asthma'. Much research has been performed in the past decades on the underlying mechanisms of nocturnal airflow limitation. This thesis set out to contribute to a better insight into its pathophysiology by investigating the role of serum cortisol levels.

In chapter 2 we provide an overview of the prevalence of nocturnal respiratory symptoms and the endogenous and exogenous factors that contribute to the pathophysiology of nocturnal airflow limitation in childhood asthma, as far as known at the start of this research. So far it was well established that endogenous factors may contribute to nocturnal airflow limitation i.e. the autonomic nervous system, inflammatory processes measured as changes in airway responsiveness, and differences in numbers of eosinophils and neutrophil granulocytes at night obtained from peripheral blood and/or bronchoalveolar lavage. One of the most important exogenous factors in the Netherlands is exposure to high house dust mite allergen levels. Another important contributing exogenous factor is environmental tobacco smoke.

Chapter 3 discusses the treatment strategies for children with nocturnal respiratory symptoms. Inhaled corticosteroids are still the treatment of choice when symptoms persist. They have been shown to reduce day- and nighttime symptoms, reduce the degree of airway responsiveness and decrease the number of asthma exacerbations. Long-acting β2-agonists have, by their long lasting effects clearly shown to reduce the number of nightly awakings.

In chapter 4 we describe the prevalence of awakening due to nocturnal respiratory symptoms, its influence on daytime activities, and exposure to parental cigarette smoking by a questionnaire in children with and without asthma. 555 children with doctor diagnosed asthma (0-16 years) and 763 control subjects (0-16 years) participated. FEV1 as % predicted was measured in all children with asthma ≥ 6 years, and in a random sample of 102 control subjects. Both groups suffered from awakening due to nocturnal
respiratory symptoms, the prevalence being significantly higher in children with asthma. Nocturnal cough was the most frequently reported symptom, yet in the age group 0-4 years it was not significantly different between control subjects (36%) and children with asthma (46%). The prevalence of nocturnal respiratory symptoms dropped with increasing age in both groups. Thirty five per cent of the asthma group and 11.5% of the control group reported that nocturnal respiratory symptoms affected daytime activities. As little as 34.3% of the asthma group reported their nocturnal symptoms spontaneously.

This study is the first one to compare the prevalence of nocturnal respiratory complaints in both healthy subjects and in children with asthma from 0-16 years. It shows that the prevalence of nocturnal cough is comparable in asthmatics and healthy children of age 0-4 years. Furthermore, it stresses once again the necessity that doctors ask specifically for nocturnal respiratory symptoms in children with asthma, since reduction of nocturnal symptoms has been shown to improve daytime activities.

New treatment strategies, such as early institution of inhaled corticosteroids and long-acting $\beta_2$-agonists have been introduced in the nineties to improve nocturnal asthma symptoms and lung function. In chapter 5 we investigated a cohort of 86 asthmatic children (0-16 years) of an outpatient clinic population who answered a questionnaire in 1991 and 1998. We assessed whether changes in therapeutic approach over this period of seven years resulted in a decreased prevalence of nocturnal respiratory symptoms due to airflow limitation. FEV$_1$ % predicted was measured in all asthmatic children 6 years and older (n=42). Furthermore we assessed predicting factors for nocturnal respiratory symptoms and changes in FEV$_1$ % predicted between 1991 and 1998.

The prevalence of awakening due to nocturnal respiratory symptoms significantly decreased over a period of seven years from 62% to 33%. Inhaled corticosteroid (ICS) use increased significantly from 59% in 1991 to 79% in 1998. In the group of children who used ICS on a daily basis during the whole seven years period, FEV$_1$ % predicted values were significantly higher in 1998 compared to 1991. Additional use of long-acting $\beta_2$-agonists in 1998 did not have a complementary effect on the FEV$_1$ % predicted values in 1998. These results support the idea that the use of long term ICS in children with nocturnal respiratory symptoms might have a beneficial influence on the development of their respiratory health.
We hypothesised that decreased serum cortisol levels may contribute to nocturnal airway obstruction in children with asthma. This might either be due to lower cortisol levels at night or to an overall 24-h lower serum cortisol level in children with nocturnal airflow limitation. In chapter 6 we investigated if endogenous cortisol levels are lower, and also whether the 24-h variation of cortisol is greater, in children with asthma than in control subjects and assessed the relationship between serum cortisol and nocturnal airflow limitation in children with asthma. Cortisol and FEV₁ were measured every 4 h over a period of 24 h; blood eosinophils, airway responsiveness to methacholine and adenosine-5'-monophosphate (AMP) at 0400 and 1600 h Children with asthma had lower cortisol levels than control subjects; only at midnight the difference was significant. Subjects with nocturnal asthma (24-h FEV₁ variation ≥ 15%) had significantly lower cortisol levels than control subjects at 0000, 0800 and 1200 h. A higher mean 24-h cortisol level in subjects with asthma was associated with a significantly higher FEV₁ as a percentage of the predicted value (FEV₁ % pred) at 0400, 0800 and 2000 h, yet not in control subjects. Higher 24-h cortisol variation was associated with lower FEV₁ % pred at all time points in both control subjects and at almost all time points in subjects with non-nocturnal asthma. There was no significant association between the level or variation of cortisol and the level of PD₂₀ methacholine (provocative dose of methacholine causing a 20% fall in FEV₁) and PD₂₀ AMP, or the number of blood eosinophils. Our data suggest that lower cortisol levels contribute to overall lower levels of FEV₁ in asthmatic children, and especially at night. This may be due to a lack of suppression of airway inflammation.

In view of the above demonstrated relationship between FEV₁ % pred values and serum cortisol levels we investigated whether substitution of low serum cortisol with intravenous hydrocortisone would improve FEV₁ variation and whether it would improve indirect measures of airway inflammation. This is outlined in chapter 7. Intravenous hydrocortisone was given during 24 h in a double blind randomised crossover design to 26 children with asthma. FEV₁ was measured every 4 h during 24 h, blood eosinophil numbers and airway responsiveness to methacholine and adenosine 5'-monophosphate (AMP) at 0400 and 1600 h. Infusion of hydrocortisone significantly increased cortisol levels in all patients during the night and morning hours. FEV₁ values were higher at all time points in children with nocturnal asthma which was significant at 0800 h during infusion of hydrocortisone. This was not observed in the non-nocturnal asthma group. The median (range) numbers of blood
eosinophils at 0400 h decreased significantly in the whole asthma group, from 0.61 (0.05-1.42) to 0.52 (0.05-1.79) $10^9/l$. PD_{20} methacholine did not significantly change, whereas PD_{20} AMP improved only significantly at 1600 h in the NA-group, from 72.0 (0.13-144.0) mg/ml to 144.0 (2.25-144.0) mg/ml. The current study shows that substitution of lower endogenous 24-h values of cortisol improves lung function in asthmatic children. Together with the previous observation that asthmatic children have lower FEV_1 values and cortisol levels than healthy children, this signifies the importance of cortisol in the pathophysiology of asthma. Furthermore a short period of hydrocortisone infusion reduces the number of circulating eosinophils, to be considered as a marker of inflammation, whereas it does not change the severity of airway hyperresponsiveness to methacholine. The improvement of AMP responsiveness was significant at 1600 h in only the non-nocturnal asthma group, whereas the number of eosinophils decreased only significantly at 0400 h. Thus, improvement in AMP responsiveness could not simply be explained by reduced airway inflammation. The underlying mechanism of the improvement in FEV_1 values is not fully clear. It is possible that the supranormal blood levels of cortisol reached by hydrocortisone treatment mainly causes decreased edema of the airway wall, thereby improving FEV_1 especially in the children with more severe airway wall edema, i.e. the nocturnal asthma group. Thus edema reduction may play a large role, next to a theoretically plausible anti-inflammatory effect.

Eosinophils and lymphocytes are believed to be key effector cells of airway wall inflammation in asthma, releasing pro-inflammatory mediators and cytokines. Next to eosinophils and lymphocytes, neutrophils may play an additional role, especially during exacerbations of asthma and viral respiratory infections. It may be proposed that activation of monocytes and/or epithelial cells can function as a source of the chemokine IL-8. Corticosteroids are known for their potent anti-inflammatory activity in allergic asthma. Decrease of serum cortisol levels at night may be of great importance in the loss of control of the inflammatory response. In chapter 8 we present the results of an additional study that might contribute to explain the observed improvement in FEV_1 by cortisol as described in chapter 7. This study investigated 16 children with moderately severe asthma. It was designed to investigate whether a day and night difference exists in IL-4, IL-5, IL-8 and IFN-γ production of concanavaline A stimulated peripheral blood mononuclear cells (PBMC's), important cytokines in the inflammatory process of allergic asthma. Furthermore, we performed this study to identify whether
substitution of low serum cortisol levels with intravenous hydrocortisone would decrease those cytokines, in order to explain the observed increase in FEV₁ during substitution of intravenous hydrocortisone. Median (range) values of IFN-γ, IL-4, IL-5 and IL-8 produced by PBMC’s did not significantly differ, neither between day- and nighttime values, nor between the measurements with saline at 0400 and 1600 h and with hydrocortisone. The earlier findings of improvement in nocturnal and morning FEV₁ values after substitution of low endogenous cortisol levels with a low dose intravenous hydrocortisone could not be explained by a decreased release of the above mentioned cytokines in peripheral blood PBMC’s. We hypothesise that glucocorticosteroids play an indirect role in eosinophilic and neutrophilic airway inflammation. This may well run via a strong inhibition of epithelial tissue cell. Thus the positive effect of glucocorticosteroids on FEV₁ values may be explained by the effect on the activation state of local tissue cells thereby suppressing airway inflammation and vascular leakage.