Concentration-effect relations of anti-asthma medications. Studies on inflammation markers
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Citation for published version (APA):
Chapter 8

The effect of hydrocortisone on plasma tyrosine concentration and lymphocyte counts in healthy subjects

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Clin Drug Invest, in press.
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ABSTRACT

Objective: Under corticosteroid therapy, plasma tyrosine concentrations are lowered due to induction of tyrosine amino transferase. Lymphocytopenia is considered to be a non-genomic effect of corticosteroids. The objective of this study was to test the hypothesis that by using PK/PD modelling, both genomic and non-genomic effects of hydrocortisone (HC) can be described by in essence the same physiologic model.

Methods: Seven healthy subjects were studied for 24 hours on two occasions on which they received either no drug or 300 mg HC orally.

Results: Tyrosine decreased on average maximally 15% on the control day and 50% on the test day. Lymphocytes decreased on average maximally 30% on the control day, and 75% on the test day. The nadirs of the tyrosine lowering occurred on average two hours later than that of the lymphopenia. The mean estimated plasma concentration of HC that gives 50% of the maximum attainable drug effect was 378 ± 186 mg/L (EC\textsubscript{50}) for the increase of tyrosine plasma concentration and 142 ± 42 mg/L (IC\textsubscript{50}) for the lymphocytopenic effect. The effects on the control day could be fitted to in essence the same physiologic model, using identical parameter values as were found on the HC day.

Conclusions: This study shows that spontaneous diurnal variations of tyrosine and lymphocytes plasma concentrations under controlled conditions can largely be described by fluctuations of endogenous HC. These effects may be used as an indicator for the actions of exogenous corticosteroids, which gives the possibility to study concentration dependencies for different corticosteroids for both genomic and non-genomic effects.

INTRODUCTION

Corticosteroids have a wide array of physiological and pharmacological effects. Their mechanisms of action can be divided into two modes: genomic (gene-mediated effects or so called indirect effects) and non-genomic effects (non-gene-mediated or so-called direct effects). Decreases of plasma tyrosine and blood lymphocytes after administration of corticosteroids are examples of these
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respective effects. The principal objective of this study was to test the hypothesis that HC concentration-effect relationships for both genomic and non-genomic effects can be estimated by PK/PD modelling, using in essence the same physiologic model.

Tyrosine blood concentration is an easily accessible and measurable marker of corticosteroid action. The aromatic amino acid tyrosine in the body is either synthesised from phenylalanine or absorbed after food intake. Tyrosine is a precursor of thyroxine, and hydroxylation of tyrosine is the first step in the formation of either dopa, which is converted to adrenaline -via dopamine- and noradrenaline, or melanin. However, tyrosine is mainly degraded to fumarate and acetoacetate. The first step of this degradation pathway is the transamination of tyrosine in p-hydroxyphenylpyruvate by the tyrosine amino transferase enzyme tyrosine-α-ketoglutarate-transaminase (TAT). Apart from auto-induction by its substrate, this enzyme is also induced by cortisol. Tyrosine amino transferase and the influence of corticosteroids on its activity has been extensively studied, mainly in rats. Pharmacokinetic-Pharmacodynamic (PK/PD) modelling of this effect of corticosteroids has been done in rat, using results of ex vivo measurements. In one study the relationship between cortisone and tyrosine was investigated in man. Cortisone 300 mg administered in divided doses during 24 hours reduced the tyrosine plasma concentration in humans within 24 hours of its administration. The results were compatible with corticosteroid mediated induction of an enzyme, most probably tyrosine aminotransferase. Corticosteroid induced lymphopenia is regarded to be a direct effect. The precise mechanism of action is not known though. Tyrosine plasma concentration and lymphocyte counts show both a diurnal rhythm.

Corticosteroids are widely used in the treatment of inflammatory diseases. Their therapeutic effects can often be discerned relatively fast -hours (e.g. anaphylactic reaction) to days (e.g. SLE)- in comparison to the adverse reactions, which mostly become apparent much later (e.g. osteopenia). Quantifiable markers of activity early in chronic treatment regimens could be useful to find the lowest effective dose or to optimize dosing schedules when corticosteroids are combined with other drugs (e.g. in asthma with possible interaction with beta-2-agonists in order to minimise the risk of late adverse reactions.
In vivo studies on kinetics and action of corticosteroids with the help of PK/PD modelling techniques have proven to be successful giving insight in many aspects of the effects of this class of drugs. As effect parameter the disappearance of lymphocytes or other leukocytes from the blood has mostly been used. The direct concentration dependency of these effects indicated that they are most likely not gene-mediated. We are not aware of studies on gene-mediated effects of corticosteroids in humans, which were done in vivo with linkage of pharmacokinetics and pharmacodynamics. The physiologic model of Jusko describes the action of corticosteroids by the kinetics of the corticosteroid receptor and its ligand in the nucleus and the resulting change in TAT concentrations. Various other indirect response models allow quantitation of the effects of corticosteroids.

In the present study hydrocortisone is chosen as test drug, because this particular glucocorticoid is identical to the endogenous hormone cortisol - with therefore the same kinetic and dynamic behaviour - , which makes it easier to link observed effects to the corticosteroid concentrations. The endogenous and exogenous amount of the compound can simply be added so that a single concentration can be used for the concentration-effect relationship.

**METHODS**

**Subjects**

Seven healthy Caucasian male students (age range: 19-21 years) with a blank medical history participated in the study. The subjects were asked to avoid strenuous exercise the day before the test and to keep a normal sleep pattern the week before the study. None of the subjects were night shift workers. Inclusion criteria were a normal routine physical examination and history and a normal routine blood tests (full blood count, glucose, Na, K, AST, ALT and creatinine). Exclusion criteria were the use of medication or an acute medical problem in the preceding three months, and a history of illness or symptoms that were judged to be relevant. Smoking and alcohol consumption, other than for social use, were also exclusion criteria. Written informed consent was obtained.
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**Study design**
The study had an open, randomized, cross-over design. On the test day 300 mg hydrocortisone was administered as a single oral dose. On the control day no drug was given. The two days were at least one week apart. The subjects drank only tap water from the night before till the end of the experimental day, and also refrained from eating during that same period.

**Study procedures**
The study was approved by the Institutional Review Board. The participants arrived at the department of Clinical Pharmacology at 5.45 am. A cannula was inserted in the forearm from which a blank plasma sample and consecutive samples were obtained. The cannula was kept patent with a 10 U/ml heparin solution. The experiment lasted till the next morning 6:30 am. On one of the two days at 6:30 am 15 tablets of 20 mg hydrocortisone were swallowed with two glasses of water. At 0.25, 0.50, 1, 1.5, 2, 3, 4.5, 5.5, 7, 8, 9, 11, 12.5, 14, 16, 18, 20, 22, 24 hours after the drug intake blood was drawn. Each time 3 ml blood in EDTA for lymphocytes measurement and 10 ml blood in heparin for tyrosine and hydrocortisone measurements was collected. EDTA samples were taken to the Clinical Chemistry Laboratory of the hospital immediately after sampling. The heparin blood was centrifuged and the plasma was stored at -20 °C immediate after collection. Hydrocortisone and tyrosine measurements were done in the department of Clinical Pharmacology. During the day the subjects were seated in a comfortable chair and at night-time patients slept on the research unit of the department.

**Hydrocortisone assay**
Hydrocortisone was analysed with a high-performance liquid-chromatography assay with UV detection. The method as published by Rose and Jusko \(^20\), was applied with the modifications as described by Oosterhuis et al \(^21\). The intra- and inter-assay coefficients of variation were approximately 7%. The lower limit of detection was 20 ng/ml serum.
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Tyrosine assay
The tyrosine was analysed with high-performance liquid-chromatography assay with electro-chemical detection as described by Edwards et al. The intra- and inter-assay coefficients of variation were approximately 2%. The lower limit of detection was 1 mg/ml serum.

DATA ANALYSIS
A model based on the model described by Koopmans et al. was used to model hydrocortisone (HC) kinetics. This model has been shown to accurately describe the time course of plasma HC after exogenous HC administration. The HC concentration-time curves on the control day were fitted and described with a polynomial function. The results of these models were used as input for the concentration - effect model, for which the 'link' was made for both effects by in essence the same physiologic model as previously published by Jusko et al. Briefly, this model describes the influence of a drug on either an input or output rate of an effect parameter, or both. This influence can either be an inhibition or an increase of the rate. The relationship of the drug concentration and the rate constant was empirically described with an Emax formula. Finally, control day and experimental day data were fitted concurrently, using common parameter values. Goodness of fits was judged by correlation coefficients and visual inspection.

Pharmacokinetics
Hydrocortisone on test day
The exogenous HC data were modelled with a one compartment linear pharmacokinetic model with lagtime (Tlag) (h) for a single oral dose (equation 1). Ka (h⁻¹) is the absorption rate constant. Kel (h⁻¹) is the elimination rate constant. f is the fraction of the dose that is available systemically. Volume of distribution (L) incorporates f, giving V/f instead of V. The endogenous hydrocortisone production at the time that the dose was administered, T = 0, was assumed to be turned off immediately; hereafter endogenous HC disappears with the same elimination rate as estimated for the exogenous HC (equation 2).
Cortisol only returns to its baseline production rate when the exogenous administered hormone almost has disappeared. The reappearance of endogenous HC approximately 24 h after dosing, was modelled as an intravenous infusion that started at T = Tstart. In most subjects, only in the last blood sample could hydrocortisone be detected again. Therefore, Tstart was estimated by fitting the kinetic and dynamic data together to find the time that resulted in the best fit (equation 3). Total measured plasma concentration was the summation of three HC time curves (equation 4). The parameters were estimated by the nonlinear fitting program Scientist®.

**Equation 1.**
\[ HC_{exo} = \frac{(Dose \times Ka)}{(Volume \times f \times (Ka - Kel))} \times \left( e^{-Kel \times (T - Tlag)} - e^{-Ka \times (T - Tlag)} \right) \]

**Equation 2.**
If \( T \leq T_{lag} \): \( HC_{endo1} = HC_{T=0} \)
If \( T > T_{lag} \): \( HC_{endo1} = HC_{T=0} \times e^{-Kel \times (Tlag - T)} \)

**Equation 3.**
If \( T > T_{start} \): \( HC_{endo2} = \frac{\text{Infus}}{(Volume \times Kel)} \times (1 - e^{-kel \times (T - Tstart)}) \)

**Equation 4.**
\( HC = HC_{endo1} + HC_{endo2} + HC_{exo} \)

**Cartoon 1.**
Hydrocortisone on control day

The endogenous hydrocortisone versus time curve on the control day, when the variation of the concentrations is caused by a variable but unknown production rate, was fitted and described with a polynomial equation (equation 5). The order necessary to describe the data was found by visual inspection.

Equation 5.
\[ \text{HC}(T) = C + C1 \times T + C2 \times T^2 + C3 \times T^3 \] and so on.

Pharmacodynamics

The lymphocytes and tyrosine depletion were described with differential equations describing models in which respectively the influx and output rate constants are influenced by HC concentration according to a sigmoid Emax formula. These models are based on physiologic models as described by Jusko. A modification introduced by us is that we allowed rate constants to be influenced directly by HC concentrations according to an Emax formula. The data were fitted to the models with the nonlinear fitting program Scientist. Common dynamic parameter values were used for the two days except for the tyrosine-input rates and the baseline values for the lymphocyte concentrations. For each person the time courses of the two effects were fitted simultaneously. Differences between EC_{50} values for the HC efficacy on the tyrosine disappearance or IC_{50} values for the lymphocyte appearance, and the elimination rate constants values for tyrosine, Kdis and KdisHC, and Keff for lymphocytes were tested with the two-sided T-test for paired data. Correlation coefficients were compared with the Wilcoxon non-parametric test.

Lymphocytes

It was assumed that lymphocytes are being lost from the circulation with a linear rate constant and that the constant influx is controlled by HC concentration. Thus the lymphocyte count (L^-1) was described by a linear efflux rate constant, Keff (h^-1), and a constant influx, Inmax, that was controlled by HC concentration according to equation 6. The influx, Inmax, is influenced by HC according to a sigmoidal Emax formula, with IC_{50} (mg/L) being the (inhibitory) plasma concentration that gives 50% drug effect and with a maximal influx with zero HC
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concentration and no influx with high HC concentration. N is the sigmoidicity factor that determines the steepness of the concentration drug effect curve. The baseline values E0 for the lymphocytes at T = 0 were estimated by Scientist® as a parameter. E0 was separately estimated for the control day (E0control) and the hydrocortisone day (E0). (cartoon 2)

Equation 6.
\[
\text{Lymphocyte}' = \text{Inmax} * \left(1 - \frac{(\text{HC}^n)}{(\text{IC}_{50}^n + \text{HC}^n)}\right) - \text{Keff} * \text{Lymphocyte}
\]

At T = 0: E = E0 on the HC day; E = E0control on the control day.

Cartoon 2.

[Diagram of Equation 6]

Tyrosine

It was assumed that before exogenous HC administration tyrosine was being formed at a constant rate, as no dietary intake took place, and that in this case the disappearance was controlled by HC concentration as has been described previously 1.28. We did not observe a time delay between an increase of HC in the circulation and the start of the tyrosine decline. An immediate but slow acting modulation of the tyrosine disappearance rate satisfyingly described the data. The tyrosine plasma concentration was described by a constant influx, Input (h⁻¹), a constant linear efflux rate, Kdis (h⁻¹), and an efflux rate, KdisHC (h⁻¹), that was influenced by HC according to a sigmoidal Emax formula; EC_{50} (mg/L) being the plasma concentration that gives 50% of maximum drug effect. N is the sigmoidicity factor that determines the steepness of the concentration drug effect.
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curve. Zero drug concentration gives no drug induced efflux and high HC concentration maximal induced efflux (equation 7). In contrast to the lymphocyte counts, tyrosine (Tyr0) at the start of each day was not based on the actually measured baseline value. This was because the tyrosine measurements showed larger variations than the lymphocytes. In view of the low endogenous HC concentrations in the preceding period and a delayed response tyrosine at T = 0 was assumed to be at steady state, and thus equal to the ratio of input rate and efflux rate disregarding the at that time present amount of endogenous cortisol. The baseline values for the tyrosine, Tyr0, were thus calculated by Scientist® as the input divided by the Kdis. The input was estimated separately for the control day (Inputcontr) and the HC day (Input). (cartoon 3)

Cartoon 3.

Equation 7.
Tyrosine' = Input - Tyrosine * (Kdis + (KdisHC * HC^n / (EC_{50}^n + HC^n)))
At T = 0: Tyrosine = Input / Kdis.
On the control day Input must be read Inputcontr.

Statistics
Comparisons between parameters were done with the Wilcoxon sign test. A value of p = 0.05 was used to show statistically significant difference. No power calculation was done in order to find the number of subjects necessary to show a certain effect, as this was a hypothesis testing study.
**PK/PD modelling of cortisol effects on lymphocytes and tyrosine in man**

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**RESULTS**

Although the circumstances on the test days were rather demanding for the experimental subjects, all procedures went uneventful. The order of the polynomial function necessary to describe the HC data on the control day varied for the 7 subjects from 6 to 9. The coefficients of correlation as indicators of the goodness of fit were similar under these conditions and not statistically significantly different (p>0.05) from those on the HC day. The pharmacokinetic parameters for HC as estimated on the HC day by using equation 4 are given in table 1 together with the correlation coefficients for both

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**Figure 1.** Representative fits of hydrocortisone plasma concentrations on the experimental (solid squares) and on control day (open circles). (subj. 3)
days. The measured concentrations and the fitted curves of hydrocortisone on the control and HC day of a representative subject (subj. 3) are shown in figure 1. Tyrosine (Tyr0) at the start of each day was not based on the actually measured baseline value but assumed to be at steady state, and thus equal to the ratio of input rate and efflux rate disregarding the at that time present amount of endogenous cortisol. Calculating Tyr0, taking into account the assumed effect of cortisol, did not lead to better fits. It actually resulted in an under estimation of the measured tyrosine concentrations at T = 0, in EC₅₀ values that became unphysiologically high, and made it impossible to fit the tyrosine of the control day and the HC day with common parameters. In table 2 the pharmacodynamic parameters for the effect of hydrocortisone on lymphocyte counts are given. With the same parameters for both days the goodness of fit was similar (p>0.05). Representative of these curves is presented for subject 3 in figure 2. In table 3 the pharmacodynamic parameters for the effect of hydrocortisone on plasma tyrosine concentrations are given. With the same parameters for both days the goodness of fit was less than on the HC day (p<0.02). Representative of these curves is presented for subject 3 in figure 3. The EC₅₀ for the effect on plasma tyrosine differed from IC₅₀ value for the effect on lymphocyte counts (p = 0.02). For the effect on tyrosine the Input differed from the Inputcontr (p = 0.01), i.e. the baseline values were different. The mean measured baseline value for tyrosine was 12.4 mg/L on the HC day and 11.3 mg/L on the control day (p = 0.15). The mean time of the first samples on the HC and the placebo day was 6:29 am and 6:49 am respectively (p = 0.18). The estimated baseline value for the lymphocytes at the HC day (E0) differed not from the control day (EOpl) (p = 0.46). The measured effect points and the fitted effect-time curves of the lymphocytes and the tyrosine concentrations on the control and HC day of a representative subject (subj. 3) are shown respectively in figure 2 and figure 3. The steady-state concentration-effect relationships for both effects, calculated with mean parameter values from all subjects, are shown in figure 4. Comparing the goodness of fit for the effect on lymphocyte counts and tyrosine concentrations on both days, the correlation for the tyrosine concentration effect curve on the HC day and the correlation for the control days were similar.
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(p>0.05); the correlations for the tyrosine concentration curves of the different subjects on the HC day were less than for the concentration-effect curves for the lymphocytopenic effect (p<0.05).

Figure 2. Representative fits of lymphocytopenia on the experimental (solid squares) and on control day (open circles). (subj. 3)
Figure 3. Representative fits of tyrosine variation on the experimental (solid squares) and on control day (open circles). (subj. 3)
Table 1. Estimated pharmacokinetic parameters for HC.

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Figure 4. Steady-state concentration-effect relationships for lymphocyte count (dotted line) and tyrosine plasma concentration (solid line), calculated with mean parameter values from all subjects.
Table 2. Estimated dynamic parameters for HC induced lymphocytopenia.

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<td>153</td>
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<td>n</td>
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<td>1.0</td>
<td>1.0</td>
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*\(p<0.05\) for comparisons with equivalent tyrosine parameter (EC\(_{50}\) and HC).

Table 3. Estimated dynamic parameters for tyrosine lowering effect of HC.

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<td>HC r</td>
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<td>0.7</td>
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*\(p<0.05\) for comparisons between the two test days.

**DISCUSSION**

Although we did not include possible underlying physiologic processes as e.g. modulation of glucocorticoid receptors\(^{17,29,30}\), our models adequately described the dynamic data.

The variations of the lymphocyte counts and the tyrosine plasma concentrations on the control day could be linked to the cortisol concentration. The good fits of
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the two effects on both days using identical effect models with common parameter values, support the concept to ascribe all observed responses to plasma HC concentrations. The concentration-effect relationships suggest that steady state HC blood concentrations above 2000 mg/l give only a minimal increase in the measured responses.

The HC on the control day showed rather variable plasma concentrations which could not be described as well by a polynomial equation as the HC on the test day by a one compartment pharmacokinetic model. Both the spurious concentration time course and the low concentrations in the range of the EC₅₀ values will have contributed to the fact that the fits of the effects on the control day were less good. A placebo was not used on the control day as we did not expect a placebo to influence our hypothesis testing, nor did we expect it to have a large—if any at all—effect on the objective parameters.

The so-called indirect model that we used, which is based on some physiological assumptions, appeared to be capable to describe both a genomic and a presumably non-genomic corticosteroid effect. In the model HC inhibits the input of lymphocytes in the blood and the disappearance of tyrosine from the blood. The output of the lymphocytes, the input of tyrosine, and a HC independent disappearance of tyrosine were assumed to be constant. Since the subjects had not eaten for at least seven hours, it was assumed that only a steady baseline endogenous tyrosine production remained. The less important route of degradation of tyrosine that does not use the amino transferase enzyme, which is influenced by HC, was also thought to stay unchanged. Besides the diurnal variation of lymphocytes, and the lymphopenia observed after exogenous corticosteroids, transient lymphocytopenia is known to occur in stressful situations and with acute infections, as a result of increased corticosteroid plasma levels. If such a response had occurred during our study, then this would have been partly incorporated in our model, as the changed cortisol pattern would have been fitted. Absolute lymphocytosis is often observed in association with viral and other infections, as a part of the immune response.

In contrast to the lymphocyte counts, tyrosine (Tyr0) at the start of each day was not based on the actually measured baseline value as tyrosine measurements showed larger variations than the lymphocytes, as judged from the data on the control day. This was done because tyrosine at T = 0 was assumed to be at steady
state and an inexact tyrosine concentration measurement at T = 0 could have an unacceptably great influence on these rate constants. The assumption that tyrosine at T = 0 was at steady state and thus equal to the ratio of input rate and efflux rate, disregarding the at that time present amount of endogenous cortisol, is justified by considering the low endogenous HC concentrations in the preceding period and a delayed response. Assuming the same HC concentrations at T = 0 to have been present in the preceding period would result in an overestimation. These assumptions were further justified by the fact that calculating Tyr0, taking into account the assumed effect of cortisol, did not lead to better fits. It actually resulted in an under estimation of the measured tyrosine concentrations at T = 0, in EC50 values that became unphysiologically high, and made it impossible to fit the tyrosine of the control day and the HC day with common parameters. Furthermore the first one and a half-hour after dosing the mean curve for all subjects showed very little change in tyrosine concentration both on the control and the HC day.

From this study it is not possible to tell if both effects indeed differ with respect to the mechanism of action of corticosteroids. From studies in rats in which the relation between TAT in liver biopsies and tyrosine plasma concentrations were described, it can be expected that the tyrosine change that we observed also is a DNA mediated effect. Efficiency of TAT induction has been shown in rat to be more closely related to corticosteroid receptor occupancy than plasma corticosteroid concentrations, and to the time required for mRNA and the TAT enzyme to readjust to baseline conditions. From the observed tyrosine data, it appears that tyrosine is lowered rather fast after the HC concentration rise. The maximum tyrosine effect was on average reached eight hours after the HC concentration peak. The lymphocyte effect which also seems to start directly after dosing, though occurs more rapidly than the tyrosine effect, had its nadir on average two hours earlier. The mechanism of lymphocyte count decrease is not known, but has been regarded to be a direct effect.

One would like to know whether the efficacy of corticosteroids can be studied by two effects that supposedly represents the mechanisms of action of these agents and their role in the treatment of diseases. If so, these effects could serve as surrogate markers, and potency differences with other corticosteroids could be studied. Tyrosine can be used as a surrogate marker of presumably indirect effects.
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of corticosteroids. It has been suggested that free blood tyrosine can possibly reflect on the whole body glucocorticoid activity at the cellular level, similar to blood glucose in relation to insulin. Since tyrosine is a marker of metabolism, influenced by protein and glucose uptake, strict fasting must be observed for reliable parameter estimation in the acute studies, while in a chronic follow up, an over-night fast might be sufficient for monitoring effect. This marker can also be used in studies with healthy volunteers. The metabolism and plasma concentration of tyrosine is dependent on several factors. Plasma concentration of tyrosine varies directly with the content of the diet. Insulin, and indirectly glucose, aggravates the reduction in tyrosine plasma concentration, which shows a diurnal rhythm in humans eating a protein free diet with a trough occurring around 3 pm. Tyrosine monitoring can be potentially useful for dosing strategies in patients, especially in case of chronic treatment in which markers of outcome of disease are often difficult to establish.

REFERENCES

Chapter 8


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