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Influence of Changes in the Concentration of Sex Hormone-binding Globulin in Human Serum on the Protein Binding of the Contraceptive Steroids Levonorgestrel, 3-Keto-desogestrel and Gestodene

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The serum protein binding of levonorgestrel, gestodene and 3-keto-desogestrel has been determined during several clinical studies with different oral contraceptive formulations and one *in vitro* study. The results of these studies were combined in order to assess the relation between changes in the concentration of sex hormone-binding globulin (SHBG) and the effect on the free fraction of the progestins as well as on their distribution with respect to the binding proteins albumin and SHBG. Although marked differences in protein binding were seen for the three progestins at low concentrations of SHBG, these differences became less pronounced at high levels of SHBG which were reached during established oral contraceptive therapy. A nonlinear relation could be shown for either the free or the protein-bound fraction of the progestins and the concentration of SHBG in the serum, respectively.

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INTRODUCTION

Levonorgestrel (LNG), gestodene (GEST) and desogestrel (DESO) are synthetic progestins which are used together with ethinylestradiol (EE_2) in a number of combination oral contraceptives. While LNG and GEST become completely bioavailable after oral administration, DESO is subject to a substantial first-pass effect and its major metabolite, 3-keto-desogestrel (KDG), is the pharmacologically active progestin. It has been well established that progestins of the 19-nor testosterone series are highly bound to serum proteins, in particular to sex hormone-binding globulin (SHBG) [1–4]. The free fraction of these progestins as well as their distribution in serum with respect to the binding proteins albumin and SHBG have been studied during treatment with various oral contraceptive formulations [5–13]. However, each of these studies provide different and non-comprehensive information on the protein binding of the particular progestin. Limiting factors are firstly, the range of SHBG concentrations which was achieved during treatment, secondly, the number of women included, thirdly, the duration of treatment and fourthly, the restriction to a particular contraceptive formulation which was administered during the study.

In order to reach a better and more general understanding of the relation between the serum levels of SHBG and the concomitant protein binding of the synthetic progestins, it would be of advantage to look at a wide range of SHBG serum levels in a large collective of women who received different contraceptive formulations over an extended period of time. Furthermore, it would be desirable to determine the protein binding over a wider range of serum concentrations for each progestin.

For that purpose, protein binding data and the corresponding SHBG concentrations which had been obtained during several clinical trials with contraceptive formulations containing either LNG, GEST or DESO as progestogenic component were analyzed and will be discussed in the present communication.

MATERIALS AND METHODS

Origin of serum samples

Previously performed clinical trials. Serum samples were collected from healthy young women who

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Table 1. Summary of the clinical studies during which serum samples were obtained for the determination of the protein binding of the progestins LNG, GEST and KDG

Oral contraceptive	Progestin	Composition progestin/EE_2 (mg/mg)	No. of women	No. of samples	Duration of treatment	Ref.
LNG	LNG	0.15	12	83	1 cycle	[8]
Microgynon®	LNG	0.15/0.03	9	131	3 cycles	[9]
Triquilar® (tri-step)	LNG	0.05/0.03; 0.075/0.04; 0.125/0.03	8	16	15–28 cycles	Study II
GEST	GEST	0.075	12	144	1 cycle	[10]
Femovan®	GEST	0.075/0.03	18	18	Single dose	[12]
Femovan®	GEST	0.075/0.03	40	240	3 cycles	[11]
Femovan®	GEST	0.075/0.03	37	37	*ca* 11 cycles	[7]
Milvane® (tri-step)	GEST	0.05/0.03; 0.07/0.04; 0.1/0.03	10	120	1 cycle	[14]
Marvelon®	DESO	0.15/0.03	18	18	Single dose	[12]
Marvelon®	DESO	0.15/0.03	22	44	1–9 years	Study I
Marvelon®	DESO	0.15/0.03	43	258	3 cycles	[13]

participated in clinical trials with different oral contraceptive formulations (Table 1). Details on design and outcome of these studies have been described elsewhere [7–14]. The serum samples were obtained from each subject on several days during a treatment cycle either immediately prior to the following drug intake, or representative individual serum pools were formed on the days of blood sampling. The samples were used for the determination of the protein binding of the progestins as well as for the analysis of SHBG. Although the results of some of these studies have already been published, individual data on protein binding and SHBG levels are presented for the first time.

New clinical trials. In study I, 22 women (aged between 19 and 38 years) were included. They had been taking the DESO-containing preparation (Marvelon®, Organon, Oss) for at least 1 to 2 years and, in 8 cases, for more than 5 years. Women who, within 1 week prior to the start of the trial, had used systemic or local medication, which could have interfered with the aim of the trial were excluded. No restrictions were made with respect to smoking or any physical activity during the trial. A blood sample for the determination of SHBG and the serum protein binding of KDG was taken on the 2nd and 21st day of a treatment cycle prior to drug intake. The purpose of this study was to assess the serum protein binding of KDG in long-term users and to compare the results with those obtained in a previously performed study [7]. The nature and purpose of the study was explained and written informed consent was given by each participant.

In study II, 8 healthy Japanese women (aged between 22 and 40 years) were included in a phase-III study. They had been taking the LNG-containing tri-step preparation (Triquilar®, Schering AG) for a period of 15 to 28 cycles. A blood sample for the determination of SHBG and the serum protein binding of LNG was taken 2 and 24 h after drug intake on day 21 of the last treatment cycle, respectively. One of the purposes of this study was to assess the serum protein binding of LNG in long-term users.

In vitro study

Serum samples were obtained from 15 women at the time of parturition. The samples were drawn for routine biochemical-clinical analyses at a local maternity hospital. Remains of these samples were kindly donated to us. Individual samples ($n = 5$ for each progestin) were spiked with either ^{3}H-labeled LNG, GEST or KDG and the *in vitro* serum protein binding of the progestin as well as the concentration of SHBG was determined in each sample.

Serum protein binding of the progestins

An ultrafiltration method (MPS-1, Amicon, Danvers, U.S.A.) was used for protein binding analysis. The details of this method have been described elsewhere [7]. In short, 250 μl of serum were spiked with ^{3}H-labeled steroid (*ca* 5×10^4 cpm) and, after an equilibration period of 1 h at 37°C, the sample was centrifuged in a prewarmed (37°C) fixed angle rotor for 15 min at 1500 *g*. Aliquots (50 μl) of the serum sample and the ultrafiltrate were taken and the total radioactivity was measured in a liquid scintillation counter. The unbound fraction of the progestins was calculated from the ratio of the radioactivity measured in the ultrafiltrate and the serum and was not corrected for nonspecific binding (LNG *ca* 14%, GEST *ca* 16% and KDG *ca* 27%). The free fraction of each progestin was determined in native and heat-treated serum according to a published procedure. The results were used for the calculation of the distribution of the steroids with respect to albumin and SHBG [5].

The following radiolabeled steroids (Isotope Chemistry, Schering AG) were purified immediately prior to use by HPLC on a reversed-phase system: [15, 16-^{3}H]LNG (1.81 TBq/mmol), [9, 11-^{3}H]GEST (2.05 TBq/mmol), [15, 16-^{3}H]KDG (1.67 TBq/mmol).

Table 2. Free and protein-bound fractions (mean ± SD; n = 5) of the progestins LNG, GEST and KDG in serum samples obtained from pregnant women; concentration of SHBG in the same samples

Progestin	Free (%)	Albumin-bound (%)	SHBG-bound (%)	SHBG (nmol/l)
LNG	0.5 ± 0.2	20.4 ± 8.6	79.1 ± 8.8	634 ± 185
GEST	1.2 ± 0.1	25.0 ± 3.3	73.8 ± 3.4	488 ± 76
KDG	0.9 ± 0.1	40.6 ± 6.6	58.5 ± 6.6	576 ± 198

The radiochemical purity achieved was at least 99% for each steroid.

Analysis of SHBG

The SHBG concentrations in the serum samples were measured radioimmunologically with a commercially available assay (Diagnostic Products Corp., Los Angeles, U.S.A.). Assay quality was assessed by the inclusion of two quality control sera in each batch of samples.

Data evaluation

The relation between the concentration of SHBG ([SHBG]) in the serum samples and the fraction of the progestin bound to SHBG can be expressed by an exponential function which approaches a constant baseline at high SHBG levels:

$$\% \text{ SHBG-bound} = A \cdot e^{(-B \cdot [\text{SHBG}])} + C$$

For positive values of A, B and C this is a monotonically decreasing function with a lower asymptote C. A similar expression can be used for the relation of SHBG levels in the serum and the free and the albumin-bound fractions of the progestin, respectively:

$$\% \text{ albumin-bound (or free)} = A \cdot (1 - e^{(-B \cdot [\text{SHBG}])})$$

For positive values of A and B, this is a monotonically increasing function with an upper asymptote A. Values for the parameters A, B and C were calculated from the experimental data by nonlinear regression.

RESULTS

Clinical trials

Study I. The serum protein binding of KDG was determined on the 2nd and 21st day of a treatment cycle in 22 women. On the 2nd day, the free fraction of KDG was 1.0 ± 0.3% and the fractions bound to albumin and SHBG were 37.0 ± 10.7 and 62.0 ± 10.9%, respectively. On the 21st day of the cycle, the free fraction was 0.9 ± 0.1% and the fractions bound to albumin and SHBG were 25.9 ± 4.9 and 73.3 ± 5.0%, respectively. For SHBG, mean concentration values of 244.2 ± 62.6 and 369.0 ± 105.7 nmol/l were determined in the serum samples obtained on days 2 and 21, respectively.

Study II. The serum protein binding of LNG in 8 Japanese women was determined 2 and 24 h after the administration of the oral contraceptive on day 21 of the last treatment cycle and practically identical values were obtained at both time points. The free fraction of LNG was found to be 0.8 ± 0.2 and 0.7 ± 0.2% at 2 and 24 h after drug intake, respectively. The corresponding fractions bound to albumin were 21.7 ± 4.4 and 18.3 ± 2.9%, respectively, and the fractions bound to SHBG were 77.6 ± 4.6 and 81.0 ± 3.0%, respectively. The concentration of SHBG in the serum samples was 165.3 ± 48.4 and 171.8 ± 42.4 nmol/l, respectively.

In vitro study

The serum protein binding of LNG, GEST and KDG was determined in five individual serum samples for each steroid. The results are presented in Table 2.

Comprehensive presentation of all results from clinical and in vitro studies

The results on the serum protein binding of LNG, GEST and KDG from previously performed clinical studies, from the present two clinical studies and the *in vitro* study, were combined and evaluated together. They are presented in Figs 1–3.

Within a concentration range of SHBG of about 30 to 200 nmol/l, the free fraction of LNG was reduced from about 2.5 to about 0.8%. A further increase in the SHBG levels had only minor effects on the free fraction, which approached a lower level of about 0.5%. Within the same range of 30 to 200 nmol SHBG/l, the SHBG-bound fraction of LNG increased from about 20 to about 70 to 80%, with no further change at higher SHBG concentrations. The albumin-bound fraction reached a plateau level of about 20% (Fig. 1).

The free fraction of GEST decreased from about 3 to 0.6%, when the SHBG concentration in the serum was increased from about 30 to 100 nmol/l. There was no further change in the free fraction when SHBG levels went up to 400 nmol/l. Only at the very high SHBG levels which were present in the sera obtained from pregnant women, a slight increase in the free fraction of GEST to about 1% was observed. Within the same range of 30 to 100 nmol SHBG/l, there was a marked increase in the SHBG-bound fraction from 30 to 80%. At higher SHBG concentrations a slight decrease to about 70% was noted for the SHBG-bound fraction. The albumin-bound fraction reached values between 15 and 25% at high SHBG concentrations (Fig. 2).

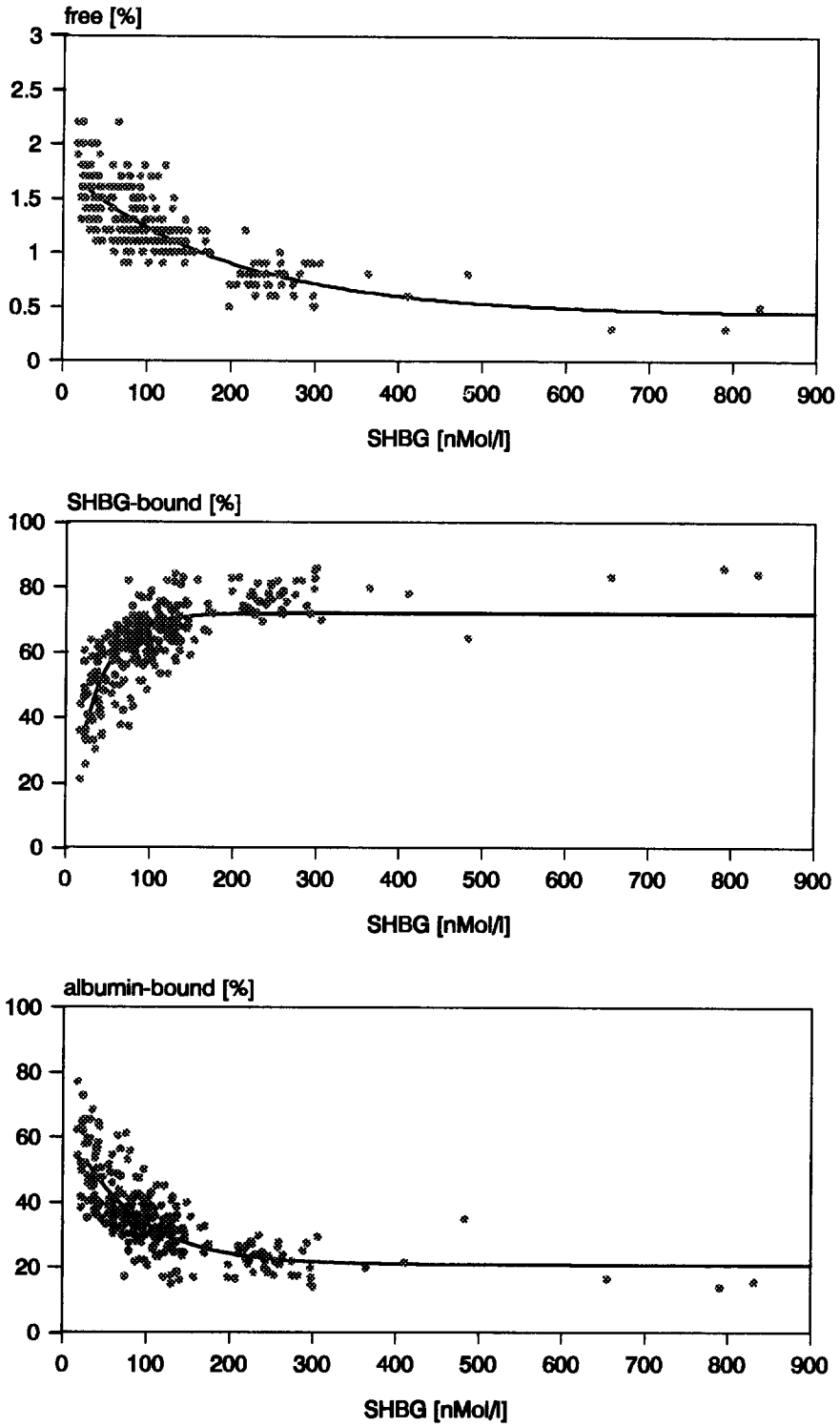

Fig. 1. Relation between the serum protein binding of LNG and the concentration of SHBG in the serum. Each data point represents the mean of two independent measurements; free fraction (top), SHBG-bound fraction (middle) and albumin-bound fraction (bottom). The fitted curve is also shown.

In the range of about 30 to 150 nmol SHBG/l, the free fraction of KDG decreased from about 2.5 to 1.0%. With increasing SHBG concentrations, a plateau was reached at about 400 nmol/l with a free fraction of about 0.6%. The SHBG-bound fraction of KDG increased from about 20% to a plateau value of 70%. The albumin-bound fraction reached plateau values of about 20 to 30% (Fig. 3).

DISCUSSION

Synthetic progestins of the 19-nor testosterone series, like LNG, GEST and KDG are extensively bound to serum proteins, mainly to albumin and SHBG. Little is known about how changes in circulating binding proteins affect the serum protein binding of contraceptive steroids. The distribution of the

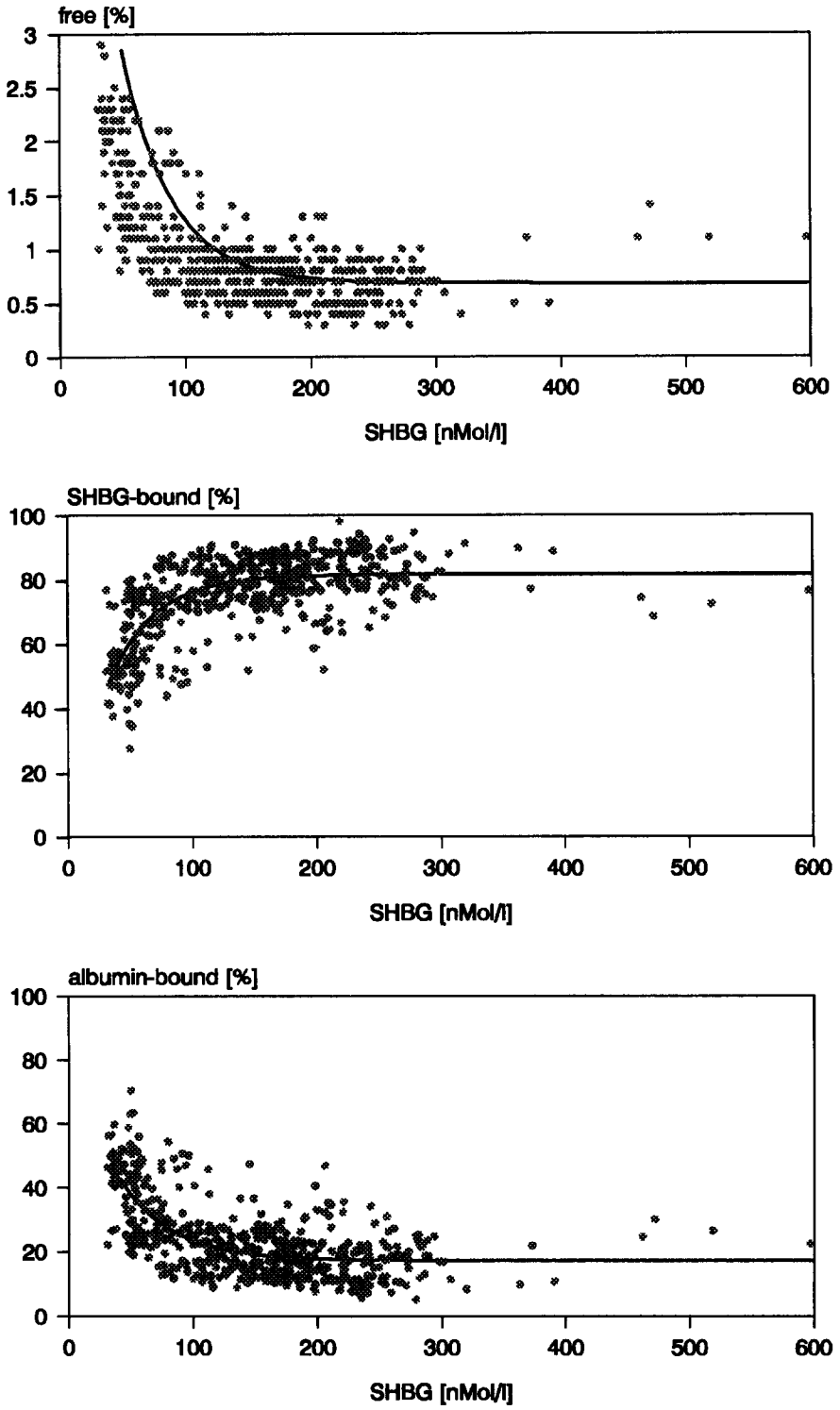

Fig. 2. Relation between the serum protein binding of GEST and the concentration of SHBG in the serum. Each data point represents the mean of two independent measurements; free fraction (top), SHBG-bound fraction (middle) and albumin-bound fraction (bottom). The fitted curve is also shown.

steroids with respect to albumin and SHBG is determined by the concentration of these proteins in the serum and by their relative affinity for the contraceptive steroids. The binding to albumin is characterized by a high capacity but low affinity, whereas the binding to SHBG is characterized by a low capacity and high affinity. The synthetic progestins LNG, GEST and KDG possess different relative binding affinities to SHBG. The ranking order of the relative binding affinities (RBA) of these steroids for human SHBG is GEST > LNG > KDG when either testosterone or dihydrotestosterone were used as reference. The relative affinity of GEST was found to be either equal to or about twice that of LNG, whereas the affinity

of KDG to SHBG was only about one-half that of LNG [1–4]. More information on the stability of steroid–SHBG complexes can be gained from the association and dissociation rates of such complexes. For KDG, a much lower rate of association was observed than for LNG and GEST. The half-lives of dissociation of LNG, GEST and KDG from the binding to SHBG at 4°C were determined to be 11.8 min (LNG), 26.4 min (GEST) and 2.6 min (KDG), respectively. The corresponding value for the reference compound dihydrotestosterone was found to be 5.1 min [3]. Thus, except for KDG, the synthetic progestins are liberated more slowly from their binding to SHBG than dihydrotestosterone.

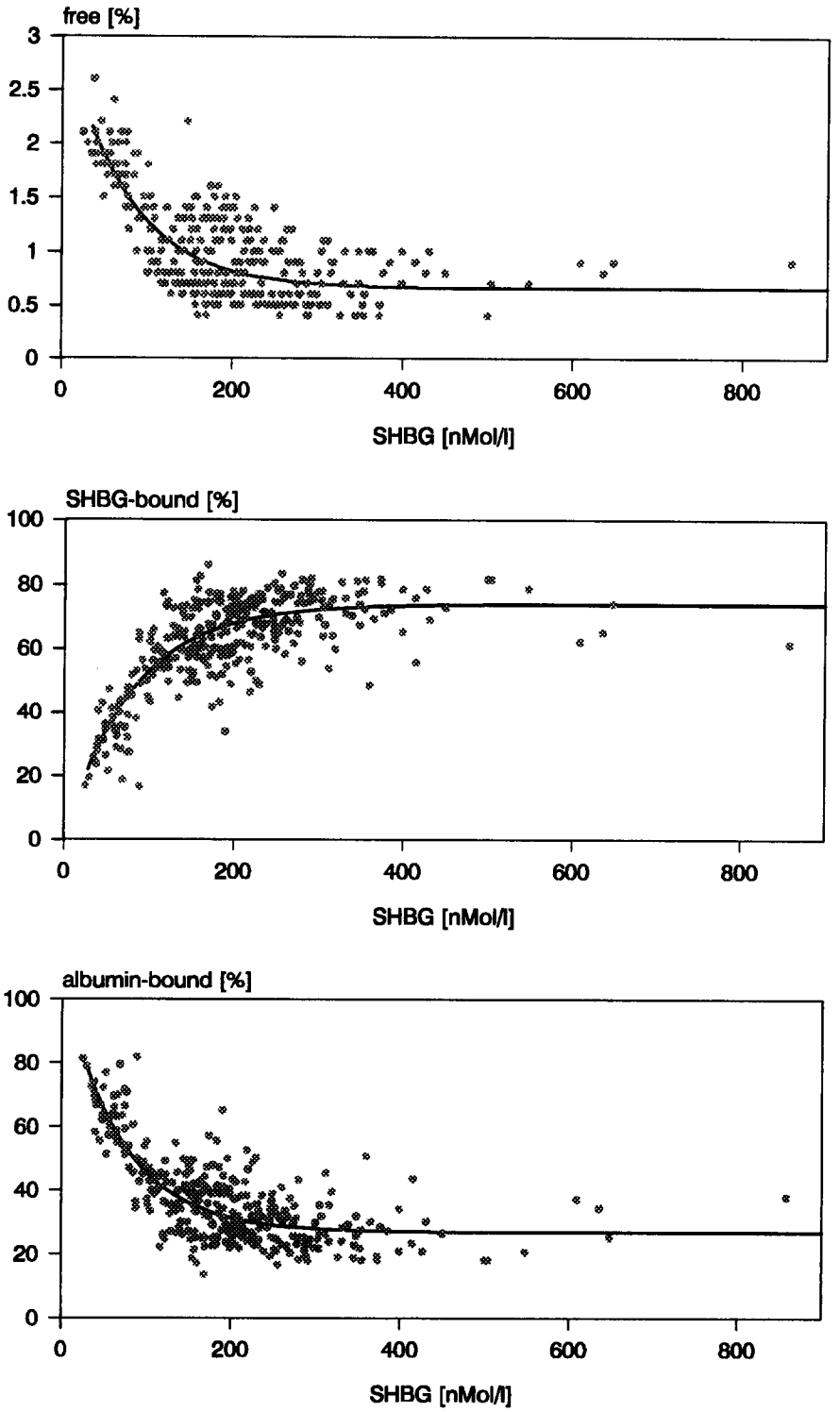

Fig. 3. Relation between the serum protein binding of KDG and the concentration of SHBG in the serum. Each data point represents the mean of two independent measurements; free fraction (top), SHBG-bound fraction (middle) and albumin-bound fraction (bottom). The fitted curve is also shown.

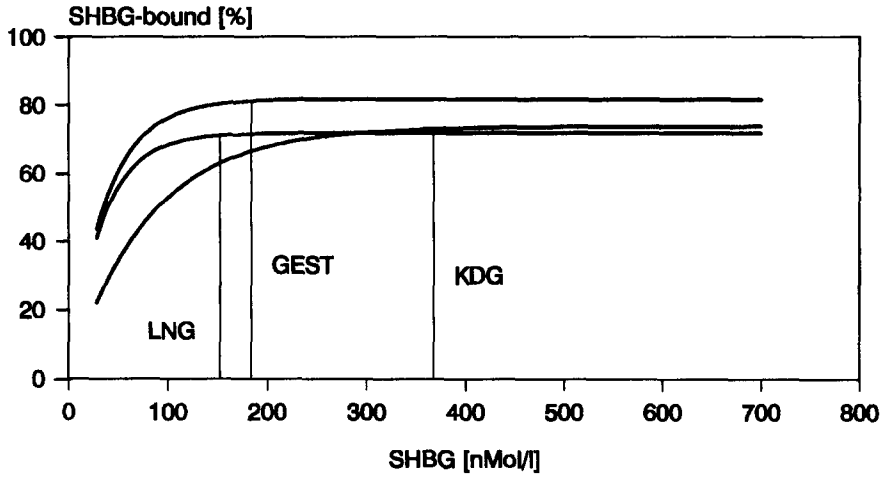

Fig. 4. Presentation of the fitted curves for the SHBG-bound fractions of LNG, GEST and KDG. Those SHBG concentrations where 99% of the plateau values were reached are marked.

Treatment with oral contraceptive steroids affects the concentration of several serum proteins, including SHBG, while there seems to be no influence on the concentration of albumin. The extent of change in the SHBG concentrations depends on the dose of EE_2 administered, the nature of the coadministered progestin and the dose ratio of EE_2 and progestin [15–17]. In the presently reported studies, the repeated oral administration of LNG and GEST caused a decrease in SHBG levels of about 40 and 26% as compared to pretreatment concentrations, respectively. During treatment with combination oral contraceptives containing LNG and EE_2 at a dose ratio of 5 and 4.2, an increase in SHBG by about 50 and 100% was observed, respectively. The GEST or DESO-containing combination oral contraceptives on the other hand, caused increases in SHBG levels of about 200% as compared to pretreatment values. Thus, the protein binding of LNG, GEST and KDG could be studied over a fairly wide range of SHBG concentrations, which was even further extended by the inclusion of serum samples obtained from pregnant women.

The three progestins LNG, GEST and KDG showed a similar distribution with respect to the binding proteins over a wide range of SHBG concentrations in the serum. The differences in the relative binding affinity of the progestins to SHBG became evident particularly at low SHBG levels: GEST was predominantly bound to SHBG, LNG was bound to about equal proportions to SHBG and albumin and KDG was predominantly bound to albumin. These distributions correspond well with the ranking order of the RBA values of the three progestins to SHBG [1–4]. Furthermore, changes in the SHBG-bound fraction could be noted already at smaller increments of changes in SHBG and had a more marked effect on GEST and LNG than on KDG. This is illustrated by a comparison of the fitted curves of the SHBG-bound fractions of the three steroids (Fig. 4). Plateau levels of the SHBG-bound fraction were already reached for LNG and GEST at SHBG concentrations around 150 to 180 nmol/l, whereas the corresponding plateau for KDG was only reached at about 350 nmol/l. These findings are also consistent with the already mentioned differences in the association rates of the progestin–SHBG complexes seen *in vitro* [2]. However, although there were marked differences in the distribution of the three progestins with respect to the binding proteins at the outset of treatment (low SHBG levels), these differences became less pronounced during established oral contraceptive therapy, when high SHBG concentrations were reached. The plateau value of the SHBG-bound fraction of GEST was about 80%, while for both LNG and KDG a value of *ca* 70% was observed. The corresponding free fraction was less than 1% for all three progestins. The slightly reduced binding capacity seen with GEST and KDG at very high concentrations of SHBG, may be due to the formation of SHBG polymers which are unable to bind steroid hormones [18].

During a previously performed study, the serum protein binding of KDG was investigated in 28 women who had been taking a preparation containing 0.15 mg DESO amd 0.03 mg EE_2 for a time period of 38 ± 24 months. For KDG, an unbound fraction of $2.5 \pm 0.2\%$ was found and the fractions bound to SHBG and albumin were 31.6 ± 12.0 and $65.9 \pm 11.9\%$, respectively [7]. This finding was in contrast to our own results obtained in a later study [13] as well as to already existing data published by others [6]. Since the possibility of obvious mistakes, such as inadvertent confusion of data, changes in assay methodology etc. could be excluded and no other factors became apparent which might have caused these divergent results, it was thought that duration of treatment might have an influence on the serum protein binding of KDG. In other studies [6, 13], the protein binding of KDG had been investigated in women who had been taking the preparation only up to 3 months, while in our study

duration of treatment was much longer. In order to investigate this possibility, women were included in the present study who had been taking the contraceptive formulation continuously for a time period of at least 1 year. Actually, the majority of participants had been under established therapy for more than 5 years. The outcome of this study, however, gave no indication that a prolonged duration of treatment would lead to a marked redistribution of KDG with respect to the binding proteins. In fact, the distribution of KDG between albumin and SHBG was the same as the one observed during shorter treatment periods. The results of our previous study on the serum protein binding characteristics in women under long-term therapy could not be reproduced. Therefore, other yet unidentified reasons must account for this discrepancy in the results.

In conclusion, it could be shown that a nonlinear relation exists between the serum concentration of SHBG and the free as well as the protein-bound fractions of LNG, GEST and KDG. Since this relation was derived from a large number of data points collected in several clinical studies with different oral contraceptive formulations, it may be of general significance for users of LNG-, GEST- and DESO-containing oral contraceptives. If that was true, it should be possible to predict the free fraction and the distribution of each of the three progestins between albumin and SHBG from a measurement of SHBG only. However, it also became evident from the results of the present study, that there is considerable inter-subject variability in the protein binding of the progestins. Therefore, a clear-cut prediction for a particular individual cannot be derived from the equation of the corresponding fitted curve.

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