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Impact of various progestins with or without transdermal testosterone on gonadotropin levels for non-invasive hormonal male contraception: a randomized clinical trial

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SUMMARY

Although several progestins have been tested for hormonal male contraception, the effects of dosage and nature of various progestins on gonadotropin suppression combined with and without additional testosterone has not been performed in a comparative trial. The aim of this study was to evaluate the differential impact of four oral or transdermal progestins on the suppression of gonadotropins in healthy men: oral: cyproterone acetate (CPA), levonorgestrel (LNG), norethisterone acetate (NETA), and transdermal: Nestorone® (NES), all in combination with transdermal testosterone (T). Randomized clinical trial testing was performed with four progestins at two doses each. After a 2-week progestin-only treatment, transdermal T was added for further 4 weeks and was followed by a 3-week recovery period. Progestin-dose per day: CPA 10 mg/20 mg, NES 2 mg/3 mg/dose e.g. 200/300 μg/day absorbed, NETA 5 mg/10 mg, LNG 120 μg/240 μg. From an andrology outpatient clinic, 56 healthy men aged 18–50 years, with body mass index $\leq$33 kg $\times$ m^{-2} were included in the study. Serum concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were studied. Secondary outcome measure included were serum testosterone concentrations, sperm concentrations, and safety parameters. Intergroup comparisons demonstrated that CPA and LNG had the strongest effect on LH/FSH suppression. Nevertheless, every substance showed significant inhibitory effects on gonadotropin secretion, especially in combination with transdermal T. A decrease in hematocrit and insulin sensitivity as well as cholesterol subfractions and triglycerides was uniformly seen for every group. The combination of oral or transdermal progestins with a transdermal testosterone preparation is able to suppress gonadotropins. Further dose titration studies with sperm suppression as an end-point should be conducted to determine the lowest effective dose for hormonal male contraception.

INTRODUCTION

Gonadotropins and testosterone (T) play pivotal roles in the maintenance of normal spermatogenesis. To achieve a hormonal form of male contraception, suppression of gonadotropins is mandatory (Srinath *et al.*, 1983; Nieschlag *et al.*, 2003; Aaltonen *et al.*, 2007). It has been estimated that both gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), should be suppressed to at least 0.5 IU/mL to reach a marked and clinically meaningful suppression of spermatogenesis (McLachlan *et al.* 2004). This goal can be reached by exogenous administration of androgens, albeit resulting in azoospermia/oligozoospermia in only 50% of healthy men (Paulsen *et al.*, 1982; WHO, 1990, Cummings & Bremner, 1994). Thus, to facilitate a feasible method of hormonal contraception in men, several combinations of androgen preparations with different progestins have been tested in clinical trials (Liu *et al.*, 2008; Nieschlag, 2010). In order to maintain androgenicity (including libido, bone mass, mental effects,

hematopoiesis) when using a progestin, testosterone should be substituted by an androgen preparation (Nieschlag, 2011). However, the most effective antigonadotropic dose of progestins has not been determined using comparative methods.

It is known that synthetic progestins are able to inhibit secretion of gonadotropins based on their binding to androgen receptors or directly via binding to the progesterone receptor (WHO 1972–1983, 1992, 1993; Paulsen *et al.*, 1982; Knuth *et al.*, 1989). Therefore, a differential impact of the various available progestins on gonadotropin suppression is very likely, as each substance has its unique binding profile to various receptors (Nieschlag *et al.*, 2003). Nevertheless, these differences have not been elucidated in a comparative setting. In addition, genetic polymorphisms at the steroid receptor level might also explain the variable responses of men to regimen of hormonal male contraception (Nieschlag, 2011; Piotrowska *et al.*, 2016).

Progestins available today, either marketed or investigational, cover the spectrum from partly androgenic, neutral to antiandrogenic. Hence, progestins with different binding and transactivation properties may act differentially on the hypothalamic–pituitary production and secretion of LH and FSH. In addition, if administered transdermally rather than orally or injected, they may have a different effect on gonadotropin suppression in men. In summary, it remains unclear why different progestins have differential impact on gonadotropin production. In addition, marked interindividual variation of medication effects is known (Nieschlag *et al.*, 2003).

Nestorone (NES), a transdermally available progestin with no androgenic action (Kumar *et al.*, 2000; Sitruk-Ware *et al.*, 2003), has been tested successfully in combination with a transdermally applied testosterone gel (Mahabadi *et al.*, 2009; Ilani *et al.*, 2012; Roth *et al.*, 2013, 2014).

In this study, progestins with different androgenic or antiandrogenic properties were used, at two doses, alone and in combination with T gel, to evaluate and compare their effect on the suppression of gonadotropins.

STUDY OBJECTIVE, DESIGN, SUBJECTS, AND METHODS

Objective and design

The primary objective of this study was to assess the effects of various progestins in different doses to suppress secretion of both gonadotropins: follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (see Fig. 1 for study design). The study was intended to demonstrate putative differences in suppression of gonadotropin secretion by various progestins in direct comparison. We did not have a priori hypothesis favor an androgenic or less androgenic progestin, but tested progestins known to be very active when used in women.

Four progestins were used: cyproterone acetate (CPA), the most potent antiandrogenic progestin, nestorone (NES), a non-oral non-androgenic progestin, norethisterone acetate (NETA), a progestin with slight androgenic effects, and levonorgestrel (LNG), the progestin with the highest androgenic potency in bioassays (Kumar *et al.*, 2000; Sitruk-Ware *et al.*, 2003; overview: Nieschlag *et al.*, 2003; Sitruk-Ware, 2004). The study was conducted in two phases. In the first phase (two weeks), progestins alone were administered in two different doses each. In the second phase (four weeks), the same progestin dose was combined with transdermal testosterone gel. Thereafter, hormone applications were ceased and patients were followed up during a 3-week wash-out period (Fig. 1).

The choice of progestins and doses followed previous experience for CPA, NETA, and LNG which had been administered orally to men in separate studies of hormonal male contraception:

1 Cyproterone acetate (CPA): This progestin is a pregnane derivative with potent antigonadotropic action and a potent antiandrogenic effect (Nieschlag *et al.*, 2003). Doses of 10 mg/day and 20 mg/day were chosen on the basis of previous experience with 12.5 mg/day and 25 mg/day in combination with testosterone preparations used in men for hormonal male contraception (Meriggiola *et al.*, 1998, 2003).
2 Norethisterone acetate (NETA): This progestin is an estrane derivative with a partial androgenic effect. Doses of 5 or

Treatment groups

Group	Progestin	Dose	Route	*n*
1	Cyproterone acetate	10 mg/day	Oral	7
2		20 mg/day		7
3	Nestorone	2 mg/day	Transdermal	7
4		3 mg/day		7
5	Norethisterone acetate	5 mg/day	Oral	7
6		10 mg/day		7
7	Levonorgestrel	120 µg/day	Oral	7
8		240 µg/day		7

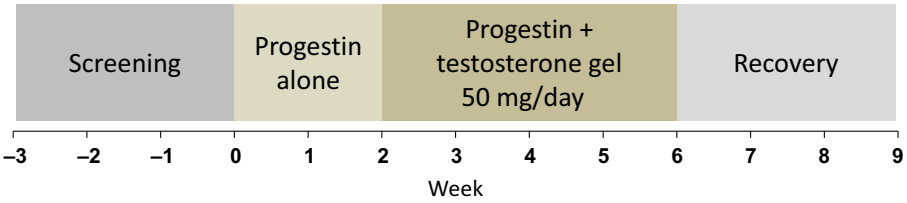


Figure 1 Design of the trial: progestins were administered alone for the first two weeks during the first phase of treatment. In the second phase of treatment, progestin treatment was continued for four additional weeks and 50 mg testosterone gel applied daily were added. The total duration of treatment was 6 weeks, followed by a recovery period of 3 weeks. For the transdermal administration, 10% of the steroid was absorbed and the actual doses tested correspond for nestorone to 200 and 300 µg/day and for testosterone to 5 mg/day. [Colour figure can be viewed at wileyonlinelibrary.com].

10 mg/day were selected based on previously published data using 10 mg/day in combination with injectable testosterone undecanoate in a hormonal male contraception trial (Kamischke *et al.*, 2002).

3 Levonorgestrel (LNG): This progestin is derived from testosterone and belongs to the gonane group of progestins. It is one of the most androgenic progestins used in female contraception. Doses of 120 μg/day or 240 μg/day were given in this study, based on experience in previous trials where 250 μg/day were successfully combined with testosterone (Büchter *et al.*, 1999; Kamischke *et al.*, 2000). Other studies used 125 μg, 250 μg, and also 500 μg of LNG in combination with intramuscular testosterone enanthate (Bebb *et al.*, 1996). In addition, a trial using either 31.25 μg or 62.5 μg of LNG in combination with weekly intramuscular injections of 100 mg of testosterone enanthate is reported (Anawalt *et al.*, 2005).

4 Nestorone (NES): This progestin is under development for non-oral contraception in women. It is derived from the 19-norprogesterone group and has very high progestational and antiovulatory potencies in classic bioassays (Kumar *et al.*, 2000). NES does not bind to the androgen receptor and does not exert any androgenic action. NES has been used in women in various long-acting contraceptive systems because it is not active when given orally. In this study, a NES gel formulation, containing either 2 mg/g of gel or 3 mg/g of gel, was used. The dose for NES was chosen based on experience in women (Kumar *et al.*, 2000; Sitruk-Ware *et al.*, 2003). In 80% of the women receiving NES gel at a dose of 1.2 mg/day, a high degree of antigonadotropic and antiovulatory activity was demonstrated (Sitruk-Ware *et al.*, 2003). The male subjects in this study received 1 g of gel per day, which contained either 2 or 3 mg of NES. Based on previous trials, we reasonably assumed a resorption dose of NES of 200 or 300 μg/day (i.e. 10–12% of the NES in gel). In a NES/E2 gel study in women at three doses of NES (1.5, 3, and 4.5 mg/day via gel), the AUC24 h (area under the curve for 24 h) was compared with the AUC24 h after intravenous bolus injection with NES (200 μg bolus: 100% absorption). The ratio between Gel AUC and IV AUC was 0.109 which is equivalent to 11% absorption of drug NES from both low- and medium-dose transdermal gel (Brache *et al.*, 2015). Thus, it is justified to assume a 10–12% absorption rate for NES in gel also in this trial. It is also known that alcoholic gel-based steroid formulations lead to ~10% absorption of steroids. As this has been shown for testosterone, we assumed, in addition to the evidence mentioned above, the same for NES (Steidle *et al.*, 2003). This assumption is corroborated by a previous study: serum NES levels significantly ($p < 0.0001$) increased with application of increasing amounts of NES gel (Mahabadi *et al.*, 2009). In that paper, serum levels of NES corresponded linearly to the dose. Doses for NES lower than in the other trials were chosen to describe the extent of such lower doses on gonadotropin secretion.

5 Testosterone gel is a transdermal preparation used for testosterone substitution in hypogonadal men. The sealed unit dose packages contain 5 g gel delivering 50 mg of testosterone per day. Subjects used one package per day in the morning. This preparation has been demonstrated to provide stable testosterone concentrations within the normal range for one day (Wang *et al.*, 2000; Snyder *et al.*, 2016). Transdermal testosterone gel in combination with DMPA had been used for trials in male contraception, but at a higher dose of 10 g gel/day (Page *et al.*, 2006; Amory *et al.*, 2007).

The same progestin given at the same two doses were administered in phase 2 of the trial in combination with transdermal testosterone gel for 4 weeks in order to maintain or increase the suppression of gonadotropins while providing normal androgenic function in men.

Serum levels of FSH and LH were assessed during treatment with progestin given alone and after combined progestin and transdermal testosterone treatment.

A possible confounding parameter is the potential variation in bioavailability of the various progestins and also of transdermal testosterone. Moreover, intraindividual and interindividual differences in absorption have been described for testosterone gel (Swerdloff *et al.*, 2015).

The secondary objectives were to determine the effects of treatment on serum levels of total testosterone and safety parameters (hematocrit, PSA, insulin sensitivity, inflammation, lipid parameters).

Although the study was neither designed nor powered to assess effects on spermatogenesis, changes in sperm concentrations were monitored and are reported for ethical and safety reasons. This study was not blinded.

Subjects

Fifty-six healthy male volunteers of Caucasian origin were recruited by responses to a press advertisement. Subjects were randomly assigned to one of the eight treatment groups as shown in Fig. 1. The inclusion criteria described men aged between 18 and 50 years with normal mental and physical health, a body mass index (BMI) between 18 and 33 kg/m^2, and normal reproductive hormone levels (FSH, LH, testosterone) based on local reference ranges and normal sperm concentrations according to WHO guidelines (WHO 2010). Volunteers were excluded if they had prostate, testicle, kidney, or liver disease in their medical history; resting systolic blood pressure >140 mmHg or resting diastolic blood pressure >90 mmHG or a history of thromboembolism; abuse of anabolic steroids or similar substances, androgens, drugs or alcohol; dermatitis or skin disorders. Each subject gave informed written consent and had to be willing to use a reliable form of contraception in a heterosexual relationship during treatment. The study was approved by the Population Council IRB and the local Ethics Committee and the State Medical Board of Westphalia/Lippe, Germany, and was carried out according to the declaration of Helsinki. Pre-Registration followed standards (EudraCT Number: 2005-002409-21, BfArM VorlageNr 4030824).

Resting blood pressure and heart rate

Blood pressure (BP) and resting pulse were measured by trained physicians using a standardized oscillometric device (Omron M5 Professional, Omron Medical Technics, Mannheim, Germany) with a cuff size appropriate to individual phenotype. Measurements were taken after the subject had rested for at least 5 min in a quiet room. Three measures of BP were taken each at 5-min intervals and the mean of the last two measures was recorded at each time point.

Biochemical analysis

All venous blood samples were obtained between 0800 and 1200 h after a 30-min rest and overnight fasting. Serum or plasma were separated at 800 g. Samples were snap frozen and immediately stored at −20 °C.

Serum concentrations of gonadotropins and testosterone were checked at baseline and at weeks 2, 3, 4, 5, 6, 7, 8, and 9.

Serum testosterone levels were measured by a commercial ELISA kit (DRG Instruments GmbH, Marburg, Germany), This immunoassay for testosterone is calibrated quarterly against standards using liquid chromatography–mass spectroscopy (LCMS-MS); the immunoassays regularly pass this quality check and reproduce the results of mass spectroscopy with an accuracy of <10% in the range for serum testosterone concentrations between 5 and 20 nmol/L. It is highly reproducible in itself and the CVs (intra- and interassay) are generated in our laboratory. Intraassay CVs were below 5%, mean interassay CVs below 10%.

Gonadotropins (LH and FSH) as well as prostate-specific antigen (PSA) was determined with highly specific time-resolved fluoro-immunoassays (Autodelfia, Freiburg, Germany), the limits of detection for both LH and FSH being 0.02 IU/L. Mean intra- and interassay CVs were below 2% and 5%, respectively.

Red blood cells count was performed on a Sysmex SE 9500 system (Sysmex Europe, Hamburg, Germany). Plasma glucose was measured in fasting condition. Serum levels of insulin were assessed by a solid-phase, two-site chemoluminescent enzyme-labeled immunometric assay (Immulite Insulin Diagnostic Products Corporation, Los Angeles, USA). The intraassay CV was 3.8% and the interassay CV was 4.8%. From this, QUICKI = Quantitative Insulin Sensitivity Check Index (1/(Log Insulin+Log Glucose)) was calculated; lower values indicate decreased insulin sensitivity.

High-resolution C-reactive protein (hsCRP) was determined nephelometrically on a BNII analyzer with an ultrasensitive method (Dade Behring, Bad Schwalbach, Germany). The lower limit of detection was 0.02 ng/mL; the upper normal value was 0.5 ng/mL. A Hitachi 917 autoanalyzer was used for the quantification of serum concentrations of triglycerides and cholesterol with enzymatic tests, of HDL and LDL cholesterol with homogenous assays and of Lp(a) with (latex-enhanced) turbidimetric immunoassays (Hitachi/Roche Diagnostics, Mannheim, Germany). Imprecision was below 5%.

All safety parameters were measured at baseline, weeks 2, 6, and at wash-out (week 9).

This trial was not powered or designed to assess effects on spermatogenesis. However, determination of spermatozoa is important also within this trial and the report of these data is nevertheless mandatory from a safety standpoint. Semen analyses are always performed in double in our laboratory and according to WHO standards after at least 2 days of abstinence (WHO 2010).

Statistics

Study size was determined to detect differences in gonadotropin levels of at least 20% between the two doses of the same progestin at a power of 90% and alpha-error of 0.05. This power level and the alpha-error also apply for comparisons of differences between the various progestins regarding suppression of gonadotropin production. The study design was also set a priori to combine the two doses of each progestin to compare safety parameters.

The study has an exploratory, not a confirmatory nature. Thus, we refrained from general corrections of p values in multiple comparisons. However, the reader should be aware that a p value of 0.05 still holds the risk of 5% of erroneously rejecting the null hypothesis. Thus, all post hoc tests included a correction according to Bonferroni. Such an approach is supportable from a statistical point of view (Bender & Lange, 2001). The non-parametric Wilcoxon-matched pair analysis was used to evaluate results for a single dose in the first two weeks of progestin-alone application (Fig. 1).

Efficacy of gonadotropin suppression over the whole treatment period of 6 weeks (including testosterone supplementation starting at week 2 and lasting to week 6, see Fig. 1) was measured by an index of gonadotropin suppression, calculated as follows:

A = serum concentration of gonadotropin (LH or FSH) at baseline (means of screening and week 0 values were calculated to avoid regression to the mean effects).

B = serum concentration of potentially suppressed gonadotropin (LH or FSH) at week 2 (end of progestin-alone phase) in percent compared to baseline.

C = area under the curve for serum concentrations of the potentially suppressed gonadotropin (LH or FSH) for treatment with progestin plus testosterone from week 2 to 3, week 3 to 4, week 4 to 5, and week 5 to 6, thus an area under the curve generated from four time intervals.

This resulted in a *Suppression Index (SuI) = Log ((B/A)*C).*

For a conservative approach, all values of serum concentrations of gonadotropins below the detection limit were set to the detection limit of 0.02 IU/L. This also avoids the situation of Log(0) which cannot be calculated.

Complete non-reaction of gonadotropins results in a value of SuI = 2.602, irrespective of the baseline concentration.

The SuI takes the speed and degree of initial suppression (B) and the continuity in combination of the respective progestin with testosterone into account (C) as well as the baseline concentration (A). There is an indication in literature that the baseline gonadotropins can affect the suppression of sperm counts in a meta-analysis of trials for male contraception (Liu *et al.*, 2008).

For example, in case of a higher baseline concentration of a gonadotropin in comparison with a lower one (such as FSH = 10 IU/L vs. FSH = 2 IU/L) and otherwise similar suppression rates, the SuI is lower for the subject presenting the higher baseline FSH level, indicating a more pronounced suppressive effect of the regimen. Intergroup differences were calculated by the non-parametric Mann–Whitney test. This method of assessing the suppression of gonadotropin concentrations differs from previously published methods where the suppression was considered effective if suppression of serum LH and FSH concentrations reached 0.5 IU/liter or less after treatment (e.g. Mahabadi *et al.*, 2009). The advantage of using our method allows comparison between different groups of treatments irrespective of baseline values of the FSH or LH within a trial of short duration. In addition, it creates a continuous variable that reflects the treatment effects over the whole study time. Nevertheless, suppression of gonadotropin secretion at least at one time point below the previously described threshold of 0.5 IU/mL was also used and results are compared by chi-square tests.

The QUICKI (Quantitative Insulin Sensitivity Check Index) describes changes in insulin sensitivity and was determined as previously described (Katz *et al.*, 2000).

Computations were performed using the statistical software package SPSS (Chicago, IL, USA, release 22.0) and GRAPH PAD PRISM (San Diego, CA, USA, release 5.0).

RESULTS

Adherence to the protocol was very good with >95% compliance, as documented using a subject study drug diary and counting of study drug containers which were to be kept by the subjects and brought back to the center at the next visit.

Suppression of gonadotropins and statistical evaluations in various regimens over time is presented in Fig. 2. Differences in LH and FSH concentrations between week 0 (baseline) and week 2 were significant in those subjects treated with CPA; suppression from baseline to week 2 was not significant for other progestins (Fig. 2). Suppression from baseline to week 6 was highly significant for every progestin.

Serum concentrations of gonadotropins at week 2 (progestin-alone phase) as displayed in Fig. 2 were also compared non-parametrically between progestin groups. For LH: CPA vs. NES ($p = 0.021$), CPA vs. NETA ($p = 0.18$), CPA vs. LNG ($p = 0.65$), for FSH: CPA vs. NES ($p = 0.073$), CPA vs. NETA ($p = 0.91$), CPA vs. LNG ($p = 0.13$).

In addition, serum concentrations of gonadotropins at the end of the suppression phase (week 6) as displayed in Fig. 2 were compared non-parametrically between progestin groups: For LH: CPA vs. NES ($p = 0.02$), CPA vs. NETA ($p = 0.04$), CPA vs. LNG ($p = 0.08$), for FSH: CPA vs. NES ($p = 0.02$), CPA vs. NETA ($p = 0.07$), CPA vs. LNG ($p = 0.08$).

In addition, the SuI was lowest in both gonadotropins in CPA-treated subjects (combined doses). Table 1 summarizes the gonadotropin profile over the whole treatment period, with and without testosterone administration. Results are presented by the SuI as a comparison of CPA vs. other progestins. As compared with CPA, none of the progestins showed a higher suppressive effect on both gonadotropins also when an index of suppression of LH or FSH below 0.5 IU/L at least at one time was used (Table 1).

Safety parameters such as serum concentrations of total testosterone, hemoglobin content, hematocrit, high-resolution C-reactive protein (CRP) and QUICKI, prostate-specific antigen (PSA) and body mass index (BMI) are displayed in Fig. 3 and Table 3, statistical analyses over time are presented. The phase of progestin-alone resulted in a marked decrease of testosterone, hemoglobin content, hematocrit, and also insulin sensitivity. This effect was attenuated during the second phase including additional testosterone substitution.

Figure 4 demonstrates the overall effects on suppression of spermatogenesis. Substance-specific parameters on suppression of sperm concentrations are demonstrated in Table 2 along with statistical evaluations. A threshold of 3 million spermatozoa/mL was chosen as appropriate to indicate a clinically relevant suppression of spermatogenesis given the short duration of exposure to the treatments.

Other safety parameters (lipoprotein subfractions, liver enzymes, and concentrations of interleukin-6) did not change significantly during treatment and are not shown in detail.

Resting blood pressure values and resting heart rate did not change significantly throughout the trial (data not shown).

Adverse events

There were 12 non-serious adverse events (AE) reported by nine subjects during the trial, 10 of which were mild, two moderate, and zero severe adverse events. In detail, all symptoms were reported in phase 2 of the trial when the progestin was combined with testosterone:

1 CPA (with T): sweating at night (possible), common cold (unlikely), elevated SGOT, elevated CPK (not related), lymphangitis (unlikely)
2 NETA (with T): 2 × sweating at night (possible), axillary eczema (possible), laryngitis (possible)
3 NES (with T): one increased aggressiveness, one increased anger, one lower libido (all possible)

Indeed, all these subjects had also received 50 mg of transdermal testosterone when adverse events were reported (events may have been associated with the androgen or the combination).

Drop-outs: three subjects concluded the study prematurely because of reasons not related to the study drug. However, all three were in the LNG-groups (one subject receiving 120 μg/day and two subjects receiving LNG 240 μg/day.

DISCUSSION

This is, to the best of our knowledge, the first published study comparing different progestins simultaneously as single medication and in combination with testosterone to elucidate their potential for hormonal male contraception; the study was designed for a non-invasive treatment regimen, that is, transdermal and/or oral application of study drugs. We generally strongly suggest the promotion of non-invasive regimens for hormonal male contraception as a timely approach as many men who participated in previous trials disliked the intramuscular application pathways (Nieschlag, 2010).

The novel characteristic of this study is the comparative design involving various progestins at different dosages. The combination of orally or transdermally applied progestins administered with a transdermal testosterone preparation is effective for gonadotropin suppression and, therefore, will most likely be useful for regimens of hormonal male contraception to suppress spermatogenesis. Such a demand has been identified previously (Aaltonen *et al.*, 2007; Nieschlag, 2010, 2011). Suppressive effects on gonadotropin secretion exhibited by the progestin alone were enhanced by the addition of transdermal testosterone (Table 1, Fig. 2).

We observed a lack of dose-related effects of the progestins on gonadotropin levels. One explanation could be that the progestin receptors involved in the mechanism of suppressing gonadotropin secretion are already saturated with the lower doses of the respective progestin. Saturation processes of receptors might also not be dose equivalent. In addition, absorption and bioavailability between subjects might be different.

It was shown previously that a combination treatment with levonorgestrel (LNG, 500 μg/day, orally) and testosterone enanthate (TE, 100 mg/week, im) resulted in a more pronounced suppression of sperm counts than did treatment with TE alone (Bebb *et al.*, 1996). In addition, the onset of spermatogenic suppression was faster in men who received T + LNG compared to men receiving treatment with T alone. Thus, it was

Figure 2 Serum concentrations of gonadotropins (LH left, FSH right, log2-Y-axes) during treatment with various progestins in various doses (CPA = cyproterone acetate. NES = nestorone. NETA = norethisterone acetate. LNG = levonorgestrel) in medians (interquartile ranges not shown for legibility). The previously used threshold of 0.5 IU/L (Mahabadi *et al.*, 2009) is indicated in red to allow a better comparison between groups. Wilcoxon tests for paired samples were calculated separately, when the progestin was either administered without testosterone (for phase 1, baseline vs. week 2) or with testosterone gel (50 mg/day) (phase 2 of the trial: baseline vs. week 6) and respective indicators are given (n.s. = not significant; *$p < 0.05$, ***$p < 0.001$). For further analyses, also see Results and Table 1. [Colour figure can be viewed at wileyonlinelibrary.com].

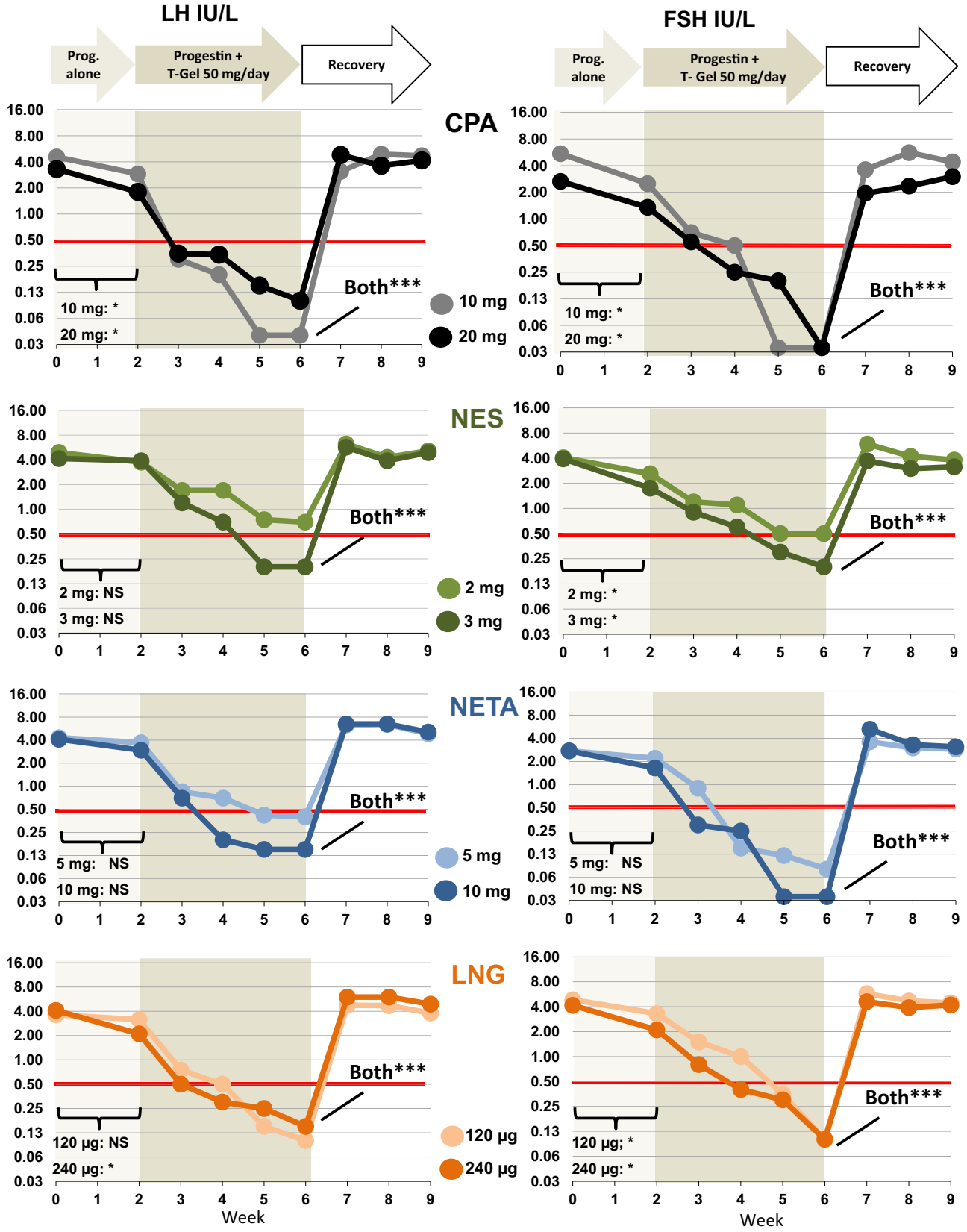
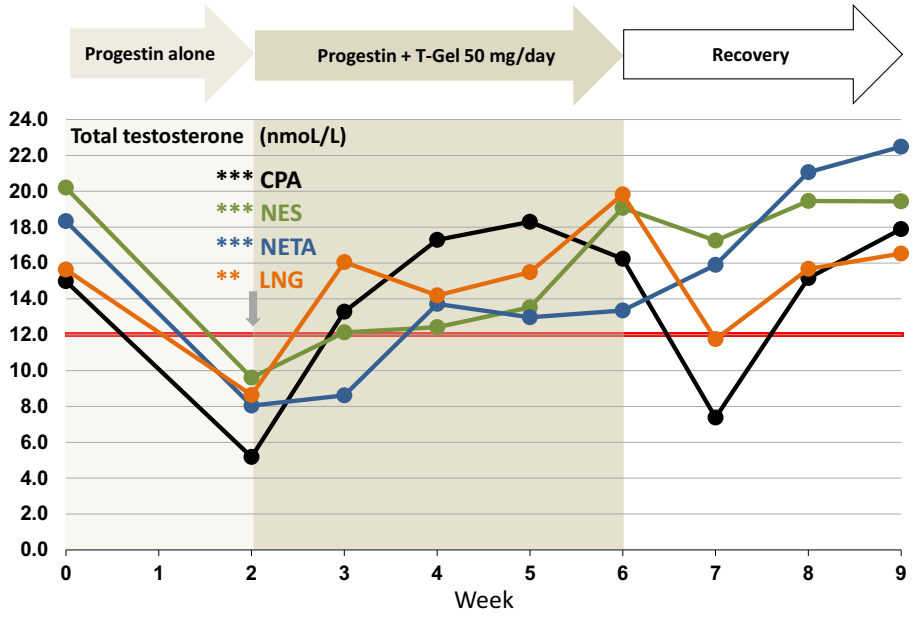


Table 1 Suppression Index (SuI, see Statistics section for details of calculation of SuI). Briefly, the lower the SuI is, the higher is the suppression of the gonadotropin

Group	Progestin treatment	SuI-LH median, IQR	SuI-FSH, median, IQR	*p* (Wilcoxon), comparison to CPA combined	LH <0.5 IU/L (at least one time point)	FSH <0.5 IU/L (at least one time point)	*p* (chi-square), comparison to CPA combined
1	CPA 10 mg/day	1.45, 0.99	1.44, 0.71	**Referent**	7/7	6/7	**Referent**
2	CPA 20 mg/day	1.68, 0.78	1.18, 0.87		6/7	5/7	
1 + 2	**CPA combined**	1.56, 0.75	1.37, 0.79		**13/14**	**11/14**	
3	NES 2 mg/day	2.01, 0.44	1.73, 0.57		3/7	3/7	
4	NES 3 mg/day	1.95, 0.91	1.76, 0.39		3/7	3/7	
3 + 4	**NES combined**	1.97, 0.52	1.74, 0.44	**LH: 0.012 FSH: 0.006**	**6/14**	**6/14**	**LH: 0.006 FSH: 0.06**
5	NETA 5 mg/day	2.24, 0.66	2.03, 0.56		3/7	3/7	
6	NETA 10 mg/day	2.01, 1.06	1.37, 1.35		5/7	5/7	
5 + 6	**NETA combined**	2.08, 0.79	1.80, 1.33	**LH: 0.056 FSH: 0.137**	**8/14**	**8/14**	**LH: 0.038 FSH: 0.21**
7	LNG 120 µg/day	2.05, 0.47	1.92, 0.66		5/6	3/6	
8	LNG 240 µg/day	1.51, 1.01	1.66, 1.28		3/5	2/5	
7 + 8	**LNG combined**	1.79, 0.72	1.78, 0.68	**LH: 0.134 FSH: 0.029**	**8/11**	**5/11**	**LH: 0.21 FSH: 0.098**

Differences in SuI between different dosing regimens of each progestin were not detected in non-parametric Mann–Whitney tests. Differences in suppression of serum LH or FSH at least at one time point (week 2, 3, 4, 5, or 6) below 0.5 IU/L are reported and *p* levels according to chi-square tests are given. IQR, interquartile range. Post hoc tests between dosing groups were not significant and corrected according to Bonferroni. Values in bold have been tested for statistical significance.

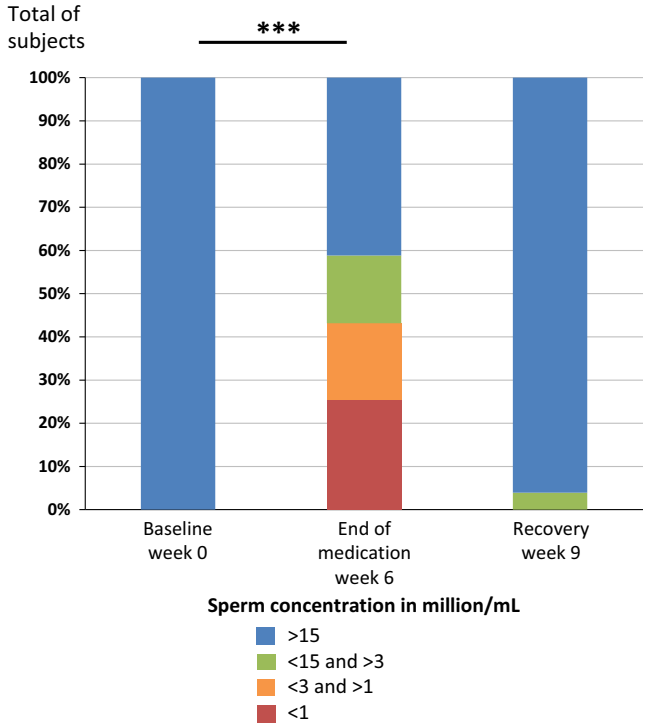
Figure 3 Serum concentrations of total testosterone during treatment with various progestins (CPA = cyproterone acetate. NES = nestorone. NETA = norethisterone acetate. LNG = levonorgestrel) in means. The dose groups have been combined. Error bars are not shown for reasons of legibility. *t*-tests for paired samples were calculated for each progestin group during the initial progestin-alone phase (baseline vs. week 2) and respective indicators are given (**$p < 0.01$, ***$p < 0.001$). A red line indicates the threshold to hypogonadism as defined by guidelines of the European Association of Urology is set at 12 nmol/L (Dohle *et al.*, 2015). [Colour figure can be viewed at wileyonlinelibrary.com].



demonstrated that an advantage of a testosterone-plus-progestin regimen vs. treatment with T alone exists (Anawalt *et al.*, 1999). It had also been previously demonstrated that suppression of gonadotropins to <0.5 IU/L is likely to produce an effect on the suppression of spermatogenesis, but suppression of spermatogenesis can also be seen at higher levels of gonadotropins (McLachlan *et al.* 2004). The effectiveness of a combination of injectable or implanted progestins with androgens has been established in larger trials (Kamischke *et al.*, 2001, 2002; Mommers *et al.*, 2008).

In terms of suppression of gonadotropins in the present trial, the most robust effect was seen for CPA vs. NES and NETA; however, CPA effects were not significantly different in some analyses vs. LNG. This suppressive effect may be related to the antiandrogenic action of CPA maintaining a decrease in androgen activity at intratesticular levels, hence able to suppress spermatogenesis more efficiently (Nieschlag *et al.*, 2003). CPA has been combined with injectable testosterone undecanoate before and was effective in suppression of gonadotropins and spermatogenesis (Meriggiola *et al.*, 2003). As demonstrated in Table 1, CPA was used as a reference in comparison with the other progestins. Comparing either the suppression index or the incidence of gonadotropin levels below 0.5 IU/mL showed CPA in the chosen dose to be more effective than NES and NETA, and levels of significance vs. LNG were not reached in most comparisons. Nevertheless, this might still be compensated by higher

Figure 4 Sperm concentrations at baseline, week 6 (end of medication phase), and week 9 (end of wash-out). The different groups according to concentration per mL are indicated by colors. Chi-square test for comparison of baseline to week 6: the overall difference was highly significant ($p < 0.001$). Putative changes in sperm concentrations were not an end-point of this trial and are reported for ethical and safety reasons. The margin of 3 million spermatozoa/mL is arbitrary and has no direct clinical significance. [Colour figure can be viewed at wileyonlinelibrary.com].



doses of other progestins, also in purely transdermal approaches, for example, using NES and testosterone gel (Ilani *et al.*, 2012; Roth *et al.*, 2013, 2014).

Hence, based on our findings, oral CPA and also oral LNG are promising agents for hormonal male contraception. As a purely transdermal regimen, nestorone gel in combination with testosterone gel has a good potential, and subsequent studies showed its efficacy in sperm suppression (Ilani *et al.*, 2012). The antiandrogenic properties may be a disadvantage of CPA for future development as well as the combination of oral administration with another mode of delivery for testosterone.

For every progestin involved in male contraception, the effective dose has to be titrated. However, we did not show significant differences in the doses tested here for each of the progestin. It was shown in another 6-month study (Ilani *et al.*, 2012) that the new progestin nestorone, although active at microgram levels, requires a dosage higher than the one used here to suppress spermatogenesis. Combining NES gel with testosterone gel may therefore offer a total transdermal regimen for hormonal male contraception.

A clinically relevant topic in hormonal male contraception is also the recovery of spermatogenesis. Although suppression of spermatogenesis and the respective recovery cannot be elucidated within this short-term trial, we saw effects of suppression and regaining of sperm concentrations (Fig. 4). Nevertheless, also in trials of longer duration, recovery of spermatogenesis is reliable after hormonal male contraception, albeit depending on co-factors such as duration of suppression (see a summary of data in: Liu *et al.*, 2006).

Table 2 Safety parameters

Parameter	Group 1+2 (CPA)		Group 3+4 (NES)		Group 5+6 (NETA)		Group 7+8 (LNG)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Hemoglobin g/L								
Week 0	**15.20**	0.28	**15.66**	0.25	**14.92**	0.28	**15.05**	0.26
Week 2	**14.72****	0.25	**15.18***	0.25	**14.51****	0.21	**14.35***	0.28
Week 6	**14.56****	0.27	**15.25***	0.26	**14.49**$^{n.s}$**	0.23	**14.73***	0.27
Wash-out, Week 9	14.60	0.19	15.36	0.25	14.69	0.19	14.87	0.31
Hematocrit %								
Week 0	**44.61**	0.80	**45.78**	0.69	**44.44**	0.64	**44.44**	0.67
Week 2	**42.83****	0.70	**44.18****	0.73	**42.99****	0.55	**42.79***	0.75
Week 6	**42.39****	0.84	**44.45***	0.66	**43.39**$^{n.s}$**	0.50	**43.68**$^{n.s}$**	0.66
Wash-out, Week 9	43.14	0.65	45.286	0.71	44.31	0.50	44.05	0.78
hsCRP ng/mL								
Week 0	**0.12**	0.03	**0.06**	0.02	**0.12**	0.08	**0.09**	0.03
Week 2	**0.15**$^{n.s}$**	0.04	**0.11**$^{n.s}$**	0.03	**0.40**$^{n.s}$**	0.23	**0.09**$^{n.s}$**	0.04
Week 6	**0.09**$^{n.s}$**	0.03	**0.05**$^{n.s}$**	0.01	**0.16***	0.09	**0.11**$^{n.s}$**	0.06
Wash-out, Week 9	0.09	0.03	0.05	0.01	0.36	0.20	0.09	0.03
QUICKI								
Week 0	**0.355**	0.009	**0.354**	0.011	**0.362**	0.011	**0.378**	0.012
Week 2	**0.315****	0.010	**0.297*****	0.007	**0.312****	0.011	**0.316****	0.013
Week 6	**0.337**$^{n.s}$**	0.008	**0.357**$^{n.s}$**	0.010	**0.332****	0.011	**0.334***	0.012
Wash-out Week 9	0.348	0.010	0.339	0.010	0.359	0.010	0.332	0.010
PSA µg/L								
Week 0	0.79	0.10	0.65	0.08	0.80	0.09	0.71	0.08
Wash-out, week 9	0.82	0.07	0.66	0.06	0.87	0.09	0.71	0.09
BMI kg × m^{-2}								
Week 0	26.02	0.74	25.17	0.74	24.47	0.58	24.41	0.81
Wash-out, Week 9	25.99	0.69	25.35	0.70	24.83	0.50	24.52	0.65

Changes from baseline to weeks 2 and 6, respectively, are analyzed by ANOVA for repeated measurements. Levels of significant or non-significant changes are indicated by asterisks (***$p < 0.001$. **$p < 0.01$. *$p < 0.05$. n.s.$p > 0.05$). TT, total testosterone; hsCRP, high-resolution C-reactive Protein; QUICKI, Quantitative Insulin Sensitivity Check Index (1/(Log Insulin+Log Glucose), lower values indicate decreased insulin sensitivity. Note that there is no statistical evaluation of wash-out values vs. baseline values in order to reduce the number of comparisons. Post hoc tests included a correction according to Bonferroni. Values in bold have been tested for statistical significance.

Table 3 Lipid parameters

Parameters	All groups combined								
	Baseline		Week 2		Week 6		*p*	Wash-out, week 9	
	Mean	SEM	Mean	SEM	Mean	SEM		Mean	SEM
Total Chol mg/dL	**186.3**	4.68	**169.7**	4.33	**173.3**	4.57	**<0.001**	**182.4**	5.01
Triglycerides mg/dL	**122.3**	8.57	**94.1**	6.73	**90.9**	5.51	**<0.001**	**112.4**	8.48
HDL Chol mg/dL	**60.8**	2.03	**54.8**	1.69	**54.1**	1.45	**<0.001**	**60.2**	2.43
LDL Chol mg/dL	**101.1**	4.41	**95.9**	4.11	**99.6**	4.46	**0.03**	**99.7**	4.55
Lp(a) mg/dL	**30.3**	5.56	**30.5**	5.39	**26.6**	5.66	**0.107**	**27.4**	4.87

Changes from baseline to weeks 2 and 6 for lipid parameters, respectively, are analyzed by ANOVA for repeated measurements. *p* values for overall models are given. Post hoc tests between progestin groups and weeks 2 vs. 6 revealed no significant results. Note that there is no statistical evaluation of wash-out values vs. baseline values. Chol: Cholesterol, Lp(a): lipoprotein (a). Post hoc tests included a correction according to Bonferroni. Values in bold have been tested for statistical significance.

The 2-week phase of progestin-alone application led to markedly lower concentrations of serum testosterone (Fig. 3). This short phase of hypogonadism resulted in significant decreases in hemoglobin content as well as hematocrit and insulin sensitivity, effects that were attenuated by testosterone substitution in the following weeks, while application of the progestin continued (Table 2). There was a marked decrease in fasting serum concentrations of total cholesterol, LDL cholesterol, and also HDL cholesterol, while levels of lipoprotein (a) remained unaffected (Table 3). Such effects have been described before and should be part of monitoring the effects of regimens for hormonal male contraception. On the basis of these data, it cannot be judged whether this effect could be of clinical relevance in either direction.

As meta-analysis of patients subjected to androgen ablation during treatment for advanced stages of prostate cancer indicates impairment of glucose homeostasis (Shahani *et al.*, 2008), the adverse influence of induced hypogonadism on insulin sensitivity is demonstrated here to occur within 14 days.

Overall, these data may indicate that the short-lived hypogonadism induced by the progestin administered alone is responsible for side-effects rather than the progestin molecule itself. However, it has been shown previously that progestin can have marked effects on hemostasis or inflammation when administered along with testosterone for a longer time period (Zitzmann *et al.*, 2002, 2005). Also, these results have to be seen within the limitations of such trials and cannot be generally applied.

Moreover, the possible positive or negative effects exerted by the different progestins require special attention as has been shown in a non-human primate model that the prostate-stimulating effect of testosterone can be blocked when administered together with norethisterone (Wistuba *et al.*, 2012).

There were no significant differences in suppression of gonadotropins between dosing groups (see Table 1 and Fig. 2). Thus, effects on suppression of gonadotropins point toward the future use of lower doses of progestins in combination with testosterone application, which might also be administered transdermally. It has only been shown for NES that higher doses might be favorable (Mahabadi *et al.*, 2009; Ilani *et al.*, 2012; Roth *et al.*, 2013, 2014).

Altogether, the non-invasive self-administered forms of sex steroid application, such as oral tablets or transdermal gel are effective for suppressing gonadotropin production and spermatogenesis. Doses of progestins might be chosen also at lower levels, as no clear dose–response relationship could be described. Longer term trials are warranted to determine effective doses and pathways of application.

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