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Commentary

The elusive minimum threshold concentration of levonorgestrel for contraceptive efficacy

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Progestins, either alone or in combination with ethinyl estradiol (EE), are a critical component of hormonal contraceptives. Levonorgestrel (LNG) has been extensively studied over decades and is considered to be a safe and highly effective progestin [1]. However, the minimum threshold level of circulating LNG required for contraceptive efficacy is still uncertain.

Plasma LNG concentration of 0.3–0.4 ng/mL is often mentioned as a threshold level below which contraceptive effectiveness declines. This is based on early studies of Norplant® (Wyeth, Pfizer, NY), a subdermal contraceptive implant. Several weeks after insertion of the implant, mean plasma LNG levels stabilized between 0.3 and 0.4 ng/mL and declined slowly to a mean level of 0.28 ng/mL after 5 years of use; by the eighth year, the levels were around 0.22 ng/mL (range, 0.02–0.35 ng/mL) [2,3]. At 5 years of use, the gross cumulative pregnancy rate was 2.6 per 100 woman-years, but after 5 years, the pregnancy rate increased with declining LNG levels. Mean (±standard deviation) LNG concentrations associated with the occurrence of an unwanted pregnancy were reported to be 0.21±0.06 ng/mL [4].

Subsequently, an implant system containing two LNG-releasing covered rods was developed [Norplant 2, now marketed under the name Jadelle® (Bayer, Leverkusen, Germany)]. These rods were shown to have a similar release rate as Norplant [5]. When plasma levels of LNG were compared in women utilizing either Norplant or Norplant 2, no significant difference was found between the two formulations [6]. In a study of 199 Jadelle users, mean serum LNG levels declined from 0.43 ng/mL at 1 month of use to 0.28 ng/mL at 5 years of use. Thereafter, the levels declined to levels of 0.22 ng/mL after 7 years. Five of the women in the study got pregnant, and two of them were in their fifth year of Jadelle use at conception; their last serum LNG levels were 0.14 ng/mL. The other three pregnant women were in their seventh year of use, and their last levels were 0.14, 0.16 and 0.18 ng/mL. Based on these data, it appears that pregnancy risk increased when serum LNG levels fell to or below 0.18 ng/mL. This suggests high contraceptive effectiveness with lower drug concentrations than previously reported. Admittedly, this latter study reported serum levels that are typically lower than plasma levels owing to differences in LNG binding proteins [7,8].

The above studies highlight the inconsistency in 'threshold' concentration linked to hormonal contraceptive effectiveness. The objective of this commentary is to closely examine the accuracy and usefulness of an LNG 'threshold' concentration. We discuss various potential sources of the inconsistency and subsequently present suggestions to minimize the inconsistency as well as redefine the useful LNG 'threshold' concentration.

1. The minimum threshold plasma concentration of LNG is likely overestimated

The purported threshold mean LNG value of 0.3–0.4 ng/mL was determined by radioimmunoassay (RIA) with a preceding

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diethyl ether extraction step [9]. Although the organic solvent extraction step removed all water-soluble conjugated (sulfated and glucuronidated) metabolites of LNG, a substantial number of unconjugated LNG metabolites remained [10], which lowered the assay specificity. As a result of this low specificity, the true threshold value would be expected to be lower than the reported 0.3–0.4 ng/mL range. Furthermore, the reported interassay coefficients of variation (CVs) were 23–25% for LNG concentrations of 0.25–0.31 ng/mL in low-level quality control LNG pools. These CVs are well above the generally accepted limit of interassay precision, which is <15%.

All the initial RIA methods used to measure LNG did not remove unconjugated metabolites prior to the quantitation step [11,12]. At that time, measuring LNG together with its metabolites was considered acceptable. The rationale for this was that one or more of the LNG metabolites may be biologically active, resulting in a more biologic measurement. Although this may be true, there is presently no evidence to confirm or refute this. It is now generally accepted that, to measure steroids accurately, a chromatographic step that separates LNG from most, if not all, unconjugated metabolites is essential. This has been done in recent years using either RIA or liquid chromatography–tandem mass spectrometry (LC–MS/MS) assay methodology. However, even the chromatographic separation step may not completely resolve the assay differences. A recent report of direct comparison of plasma LNG levels measured by RIA or LC–MS/MS showed, on average, 20–25% higher values by RIA compared to LC–MS/MS [13,14]. The RIA values were higher even with a chromatographic separation step and use of a highly specific antiserum in the RIA. It is not known whether the higher LNG levels obtained by RIA are due to one or more interfering metabolites or to the inherent nature of the assay methodology. Mass spectrometry is a chemical method, whereas RIA can be considered a biologic method because it utilizes an antigen–antibody reaction.

It is now well recognized that mass spectrometry assays will become the ‘gold standard’ for measurement of natural and synthetic steroids. Presently, interlaboratory differences exist in steroids measured by mass spectrometry [15]. This is due to the fact that conditions used to perform the assays often vary from one laboratory to another. Assay conditions that can affect the measurements include calibrator purity, derivative preparation, type of internal standard and even type of instrumentation. The Centers for Disease Control in conjunction with the Endocrine Society is currently pursuing a program to standardize steroid hormone assays. However, this process is very slow and will take years to standardize a significant number of endogenous steroid hormones. It is uncertain whether this can be achieved for synthetic steroids such as those used for hormonal contraception.

2. The minimum threshold for LNG is not comparable across formulation, dosage and routes of administration

First, progestins differ in their plasma exposure profiles when given via different routes. Even if a more accurate and precise threshold value was known for the LNG contraceptive implant, this value cannot be applied to orally dosed LNG. Parenteral routes have more uniform circulating LNG levels as compared with the high C_{max} and low C_{min} values (“peaks” and “troughs”) found with daily oral dosing. In addition, serum levels of progestins obtained following oral administration are affected by hepatic first pass metabolism and enterohepatic recirculation. These processes can cause much higher circulating progestin levels than those obtained by parenteral routes. Hepatic first pass metabolism can be affected by various factors such as food and intestinal disorders.

Second, the formulation and dosage of LNG also affect its levels. In combined oral contraceptive (COC) regimens, the estrogen component EE induces hepatic synthesis of sex hormone binding globulin (SHBG) that binds to LNG with high affinity. In blood, the total [SHBG-bound+albumin-bound+unbound (‘free’)] concentration of LNG will be affected by the concentration of SHBG. In addition, the concentration of SHBG depends on the androgenicity of the progestin. It is well recognized that LNG is an androgenic progestin and suppresses SHBG when delivered alone and the EE-induced increase in SHBG. Thus, apparent threshold levels of LNG in a COC will be affected by its binding affinity for SHBG and the extent to which it suppresses the EE-induced increase in SHBG.

Levels of SHBG are an important determinant of the pharmacologically active ‘free’ LNG concentrations, and therefore alterations of SHBG levels, as a function of formulation, further complicate universal application of the above referenced threshold range. Furthermore, the reported free fraction of LNG is very small (2–3%) and variable; hence, slight fluctuations in SHBG binding could change the free amount by a large magnitude and proportionally affect efficacy. Given the scenarios described above for nonoral and/or COC regimens, studies are needed to identify the minimum therapeutic LNG concentration based on formulation, dosage and route of administration. According to the ‘free’ drug hypothesis, ‘free’ drug in tissues is mainly responsible for biological activity and, at steady state, exists in equilibrium with ‘free’ drug concentrations in blood [16]. Hence, ‘free’ drug concentrations, compared to ‘total’ drug levels, should be more meaningful in predicting drug efficacy.

3. Discordance between drug levels and drug efficacy question the relevance of a threshold value

Typically, blood concentrations of drug, both C_{max} and C_{min}, along with area under the curve are used as surrogate measures of drug effectiveness [17]. However, there are instances where these pharmacokinetic (PK) markers are in discordance with pharmacodynamic outcomes [18]. In recent studies involving the failure of COCs among obese women, either there was discordance between PK and drug efficacy [19] or PK findings were counterintuitive to drug efficacy [20–22]. Although various patient-specific factors such as