Valle Alvarez (2011) - Efecto de una Dosis de 50 mg de Enantato de Noretisterona y 5 mg de Valerato de Estradiol en los Niveles de Testosterona Total en Hombres Mexicanos Sanos [Effect of a Dose of 50 mg of Norethisterone Enanthate and 5 mg of Estradiol Valerate on Total Testosterone Levels in Healthy Mexican Men]

Citation

- Valle Alvarez, Doris del Cisne (2011). Efecto de una Dosis de 50 mg de Enantato de Noretisterona y 5 mg de Valerato de Estradiol en los Niveles de Testosterona Total en Hombres Mexicanos Sanos. [Effect of a Dose of 50 mg of Norethisterone Enanthate and 5 mg of Estradiol Valerate on Total Testosterone Levels in Healthy Mexican Men.] México City, México: El Instituto Politécnico Nacional. [Masters thesis] [130 pages] [Google Scholar] [URL] [PDF]

English Translated

NATIONAL POLYTECHNIC INSTITUTE

HIGHER SCHOOL OF MEDICINE

POSTGRADUATE STUDIES AND RESEARCH SECTION

"Effect of a Dose of 50 mg of Norethisterone Enanthate and 5 mg of Estradiol Valerate on Total Testosterone Levels in Healthy Mexican Men"

THESIS

TO OBTAIN THE DEGREE OF:

MASTER IN HEALTH SCIENCES

PRESENTED BY:

B.Q.F.DORIS DEL CISNE VALLE ALVAREZ

THESIS SUPERVISORS:

D. en C. FRANCISCO JAVIER FLORES MURRIETA

M EN F. MARÍA TERESA DE JESÚS FRANCISCO DOCE

May 2011

SIP-14-BIS

INSTITUTO POLITÉCNICO NACIONAL

SECRETARÍA DE INVESTIGACIÓN Y POSGRADO

ACTA DE REVISIÓN DE TESIS

En la Ciudad de **México, D.F.** siendo las **10:00** horas del día **11** del mes de **Mayo** del **2011** se reunieron los miembros de la Comisión Revisora de Tesis, designada por el Colegio de Profesores de Estudios de Posgrado e Investigación de **ESM** para examinar la tesis titulada:

"Efecto de una Dosis de 50 mg de Enantato de Noretisterona y 5 mg de Valerato de Estradiol en los Niveles de Testosterona Total en Hombres Mexicanos Sanos"

Presentada por el alumno:

Valle	**Álvarez**	**Doris del Cisne**
Apellido paterno	Apellido materno	Nombre(s)

Con registro:

B	0	9	1	9	1	5

aspirante de:

Maestría en Ciencias de la Salud

Después de intercambiar opiniones los miembros de la Comisión manifestaron ***APROBAR LA TESIS***, en virtud de que satisface los requisitos señalados por las disposiciones reglamentarias vigentes.

LA COMISIÓN REVISORA

Directores de tesis

Dr. Francisco Javier Flores Murrieta

M. en F. Maria Teresa de Jesús Francisco Doce

Dra. Maria Esther Ocharan Hernández

Dr. Juan Gerardo Reyes García

M. en C. Rosalba Alonso Campero

PRESIDENTE DEL COLEGIO DE PROFESORES

Dr. Eleazar Lara Padilla

ESCUELA SUPERIOR DE MEDICINA
I.P.N.
SECCIÓN DE ESTUDIOS DE POSGRADO
E INVESTIGACIÓN
CONTROL ESCOLAR

INSTITUTO POLITÉCNICO NACIONAL

SECRETARÍA DE INVESTIGACIÓN Y POSGRADO

CARTA CESIÓN DE DERECHOS

En la Ciudad de México el día 30 del mes Mayo del año 2011, el que suscribe Doris del Cisne Valle Alvarez alumna del Programa de Maestría en Ciencias de la Salud con número de registro B091915 adscrito a La Escuela Superior De Medicina, manifiesta que es autor intelectual del presente trabajo de Tesis bajo la dirección de el Dr. Francisco Javier Flores Murrieta y M. en F. Maria Teresa Francisco Doce y cede los derechos del trabajo intitulado "Efecto de una Dosis de 50 mg de Enantato de Noretisterona y 5 mg de Valerato de Estradiol en los Niveles de Testosterona Total en Hombres Mexicanos Sanos", al Instituto Politécnico Nacional para su difusión, con fines académicos y de investigación.

Los usuarios de la información no deben reproducir el contenido textual, gráficas o datos del trabajo sin el permiso expreso del autor y director del trabajo. Este puede ser obtenido escribiendo a la siguiente dirección dorisvallea@hotmail.com. Si el permiso se otorga, el usuario deberá dar el agradecimiento correspondiente y citar la fuente del mismo.

Doris del Cisne Valle Alvarez

Nombre y firma

THANKS

To the National Polytechnic Institute and in particular to the teachers and people in charge of the Master's in Health Sciences: for allowing me to acquire and share their knowledge and experiences.

To the members of my Thesis Tutorial Committee: for their time and guidance to bring this work to fruition.

To the Q.F.B. Juan Ángeles Uribe: for your support and advice forever.

To my colleagues and friends in my work and academic environment, for their friendship and generous guidance.

DEDICATION.

To God, to whom I owe everything I have.

To my husband and son: Israel Daniel and Diego Israel, for their love, understanding and support throughout the master's degree, especially for lending me the time that often belonged to them.

To my Parents: Lauro and Mariana de Jesús.

To my brothers: Tatiana and Vladimir.

For his unconditional love for his family, for always reminding me that the desire to improve and perseverance are the means to achieve personal and professional goals.

This work was carried out at the Asociación Mexicana para la Investigación Clínica A.C. (AMIC) and at the A. F. Center for Technological Studies (CAFET) under the direction of Dr. Francisco Javier Flores Murrieta and M. in F. María Teresa de Jesús Francisco Doce.

Contents

1. ABBREVIATIONS.

AE: Adverse event

ALT: Alanine aminotransferase

AMIC: Asociación Mexicana para la Investigación Clínica, A. C.

AST: Aspartate aminotransferase

AUC_{0-t}: Area under the plasma concentration curve – time from administration to the last sampling time with quantifiable concentration

$AUC_{0-\infty}$: Area under the plasma concentration curve – time from administration to time extrapolated to infinity

BMI: Body Mass Index

BUN: Blood urea nitrogen

CAFET: Centro A.F. de Estudios Tecnológicos, S. A.

cAMP: 3',5' cyclic adenosine monophosphate

Cmax: Maximum concentration

COSTART: Coding Symbols for a Thesaurus of Adverse Reaction Terms

df: Degrees of freedom

E2: Estradiol

ERC: Ethics and Research Committee

EV: Estradiol valerate

GGT: Gamma glutamyl-transpeptidase

GnRH: Gonadotropin Releasing Hormone

HBV: Hepatitis B virus

HIV: Human Immunodeficiency Virus

IC: Informed consent

Ke: Elimination constant

MRT: Mean residence time

NET: Norethisterone

NETE: Norethisterone enanthate

SH: Secretary of Health

T: Total testosterone

tmax: Time at which Cmax is reached

TS: Taking of samples

t½: Elimination half-life

WHO: World Health Organization

2. GLOSSARY

Adverse Event: Any unfortunate medical occurrence in a patient or clinical research subject who was administered a medication and which may or may not have a causal relationship with this treatment.

Antagonist: A drug that prevents or reverses the effect of a natural substance in the body or another drug.

Analysis of Variance (ANADEVA): Set of statistical techniques to know the way in which the mean value of a variable is affected by different types of data classifications. With the analysis of variance, estimates of the effect of a treatment can be adjusted according to other factors such as sex, age, etc.

Arcuate nucleus: The arcuate nucleus or arcuate, is located at the base of the hypothalamus where the GnRH-producing neurons are found, its function in the production of peptides such as kisspeptin has been established.

Area under the curve: The area formed under the curve resulting from plotting the drug concentration in plasma vs. time.

Azoospermia: Absence of sperm in the ejaculated semen.

Bioavailability: The proportion of the drug that is absorbed into the general circulation after administration of a drug and the time it takes to do so.

Cmax: Maximum plasma drug concentration after administration of a single dose.

Contraceptive method: Those that are used to prevent the reproductive capacity of an individual or a couple temporarily or permanently.

Elimination constant (Ke): A constant that represents the fraction of drug eliminated per unit of time (usually in h^{-1})

Estradiol Valerate: Synthetic estrogen pro-drug of human 17β-estradiol.

Ethics and Research Committee: An independent body made up of medical, scientific and non-scientific members, whose responsibility is to ensure the protection of the rights, safety and well-being of the human subjects involved in a trial through, among other things, the review, approval and continuous supervision of the protocol of a trial and its amendments, as well as the material and methods to be used in obtaining the informed consent of the subjects participating in the trial.

Gonadotropins: Gonadotropins or gonadotropins are a series of hormones secreted by the pituitary gland (pituitary gland), and are involved in the regulation of reproduction. There are three gonadotropins: luteinizing hormone (abbreviated LH), follicle-stimulating hormone (abbreviated FSH), and human chorionic gonadotropin (abbreviated HCG).

Half-life: The amount of time it takes for the drug concentration in plasma to decrease by half.

Mean residence time: Mean time for intact molecules to transit through the body and involves all kinetic processes, including release "in vivo" from the pharmaceutical form, absorption and all disposal processes.

Norethisterone enanthate: A progestogen derived from 19-nortestosterone.

Oligozoospermia: It refers to the low quality of semen in terms of the amount of sperm.

Pharmacodynamics: It is the relationship between drug concentrations at the site of action and the resulting effect, including the time and intensity of therapeutic and adverse effects.

Spermatogenesis: Differentiation of spermatids until sperm formation.

Therapeutic drug monitoring: It is the determination of drug concentrations in plasma to optimize drug therapy in patients making an effective and safe therapeutic regimen.

Total testosterone: Total amount of testosterone in the blood, includes bioavailable testosterone and testosterone bound to the sex hormone transporting globulin.

3. LIST OF FIGURES AND TABLES

FIGURES Page

TABLES

4–5. ABSTRACT

This clinical research was a clinical trial; analytic, open-label, prospective and longitudinal, which primary objective was to determine the effect of a single dose of 50 mg of norethisterone enanthate (NETE) and 5 mg of estradiol valerate (EV), in total testosterone (T) plasma concentrations from 32 healthy male Mexican subjects. All volunteers received a single dose of the combination on study day 0; blood samples were drawn on days -2, -1, 0, 1, 2, 3, 4, 5, 6, 7, 10, 14, 18, 22, 26, and 30 days with the purpose of measuring total testosterone plasma concentrations on days 0, 14, and 30; estradiol plasma concentrations on days -2, -1, and 0; and the bioavailability of NET and E2 on every sample from days 0 to 30. The incidence of adverse events was also registered. Total testosterone concentrations before investigational product administration were (mean ± SD) 5.03 ± 1.30 ng/mL, these values were reduced to 0.61 ± 0.55 ng/mL 14 days after the dose, and recovered to 3.75 ± 1.16 ng/mL for the day 30 of the study ($p<0.05$). These values demonstrated that NETE and EV dose suppress T plasma concentrations in these subjects.

Norethisterone pharmacokinetics parameters were Cmáx = 523.08 ± 487.31 pg/mL, $AUC_{0\text{-}30d}$ = 6459.95 ± 2982.69 day*pg/mL, tmax = 7.06 ± 4.85 days, and Ke = 0.0461 ± 0.0162. Estradiol pharmacokinetic parameters were: Cmax = 368.31 ± 146.74 pg/mL, $AUC_{0\text{-}30d}$ = 2,392.75 ± 655.12 day*pg/mL, tmax = 2.5 ± 1.16 days, and Ke = 0.1707 ± 0.0774. Study subjects presented 9 non-serious adverse events during the study, from which only one had a relation (uncertain) to the investigational product.

6. INTRODUCTION

6.1. MALE CONTRACEPTION

Despite significant advances in female contraception for more than 50 years, the world's population continues to grow rapidly. Overpopulation continues to make an important contribution to human degradation and suffering in the world. More than half of pregnancies are unintended and 10% of these end in abortions. Furthermore, unwanted pregnancies result in unwanted children who suffer disproportionately from poverty and neglect[1].

Men were key elements in the demographic transition to smaller families in many developed countries, using condoms and the intercourse method before the widespread use of the rest of the contraceptives in the 1960s; however, in the last generation, female guidance is at the forefront of family planning; This is given by the real threat to the health of women with successive pregnancies. But men have been excluded from many family planning programs either deliberately or by omission[2].

On the other hand, the rights of women reviewed at the III Nairobi Conference "Safe Motherhood", which culminated in 1985, advocate for the reproductive and sexual health rights of women but with greater male participation. In many societies, men take a dominant role in fertility and family planning decision-making. However, there is a large gap between positive attitudes and the practice of male contraception, which has been called "the forgotten half of family planning"[2]. The truth is that currently men only have methods such as the condom, vasectomy and interrupted intercourse, despite the fact that obtaining a new and effective method of male contraception has been identified as a high priority by the WHO[3].

6.2. ACCEPTABILITY OF MALE CONTRACEPTION

It could be thought that one of the reasons why research in male contraception has not advanced is due to the lack of acceptability that such methods would have. In this regard, there are surveys applied in 4 continents where the majority of men express their desire to use a contraceptive method if it were available on the market[4,5].

Additionally, some multinational and multiethnic studies of hormone-based contraceptives show that the majority of men and their female partners would like to use contraceptive methods[6,7]. 98% of women with stable relationships would like to leave the responsibility of contraception on their partners[6,7]. The acceptability of contraceptive methods however is also influenced by economic, cultural, and religious status, for example; higher income and education are associated with a greater desire to use contraceptives[8]. Most men and their female partners accept the idea and the fact of practicing male contraception. In general, a majority of men feel that the responsibility for contraception falls heavily on women[6] and are receptive to male contraceptive methods[8]. While 71–97% of women believe that male contraception is a good idea[7] and that it is time to introduce a new method to the market[9].

In 2006, an acceptability study of an injectable contraceptive regimen of norethisterone enanthate and testosterone undecanoate was carried out in men[10]. The study lasted 72 weeks and 90 men with an average age of 28 years were evaluated. Most of them had a stable relationship and had no children. 92% of the respondents answered that men and women should share responsibilities in contraception and 75% of them answered that they would use a hormonal contraceptive if it was available. At the end of the treatment phase (48 weeks), 66% of the participants said they would like to use this method, and no one answered the method as unacceptable. No changes in sexual function and mood were detected among men who received the injections with the combination[10].

6.3. CURRENTLY AVAILABLE MALE CONTRACEPTIVE METHODS.

6.3.1. CONDOM

Barrier methods have been used for centuries to prevent pregnancy. The condom is the most effective way to prevent the spread of HIV during sexual contact. Condoms are currently used by approximately 20% of couples in the United States[11,12]. The United Nations reported that only 5% of women of reproductive age leave contraception in the hands of condom use[13]. Condoms are associated with few adverse events, however they have limited contraceptive efficacy, this is mainly due to the inconsistency of their use[14,15]. Additionally, many men do not like to use the condom, because they feel that sexual pleasure decreases[16]. Despite good condom use, rupture and slippage are not uncommon and occur in 2-8% of cases[17-19].

Since 1920, condoms have been made of latex, however this material can cause allergies in both men and women, skin irritation and even death[20,21]. In order to avoid these allergies, polyurethane condoms were created, which entered the market in the 90's. Clinical studies have shown that these condoms have a higher percentage of rupture and slippage compared to latex[18,19,22,23]. However, the effectiveness ranges of the two types of condoms are similar[17,22].

6.3.2. VASECTOMY

Vasectomy is a simple and safe outpatient surgery performed under local anesthesia in which the vas deferens are cut and tied off through a small scrotal incision. Approximately 500,000 vasectomies are performed annually in the United States[24] where approximately 10% of couples use it as a method of

contraception[11,12]. Around the world, some 40 million men have performed this procedure 25 constituting 5% of active contraception[13].

Vasectomy is highly effective with a failure rate of less than 1% and a low incidence of complications[26-28]. Acute pain, blood loss, and infections at the surgery site are very rare[29]. Approximately 6% of men who have undergone vasectomy experience some degree of chronic post-surgical discomfort[30-32]. In a study carried out with these men, most of them felt that the discomfort subsided with the reversal of the procedure called vasovasostomy[33].

From 3 to 5% of men who underwent vasectomy, require reversal, the reason in most cases is that they remarry[34,35]. Vasectomy cannot be considered as a reversible contraceptive method and is the most appropriate for those men who do not want to have more children. Among the disadvantages of vasectomy are the waiting time after surgery, necessary to reach azoospermia, the risk of chronic testicular discomfort, and the inability of surgery to return fertility when desired[9].

6.4. PHYSIOLOGY OF MALE HORMONAL CONTRACEPTION.

To understand the mechanism of action of male hormonal contraceptives, the physiology of testicular function and spermatogenesis and the involvement of gonadotropins and androgens, estrogens and progestogens will be briefly reviewed, an overview below.

There is a ligand called kisspeptin or metastin which is expressed in the brain and is the ligand for receptors called GPR549 that are located in GnRH secreting neurons. (See Figure 1)

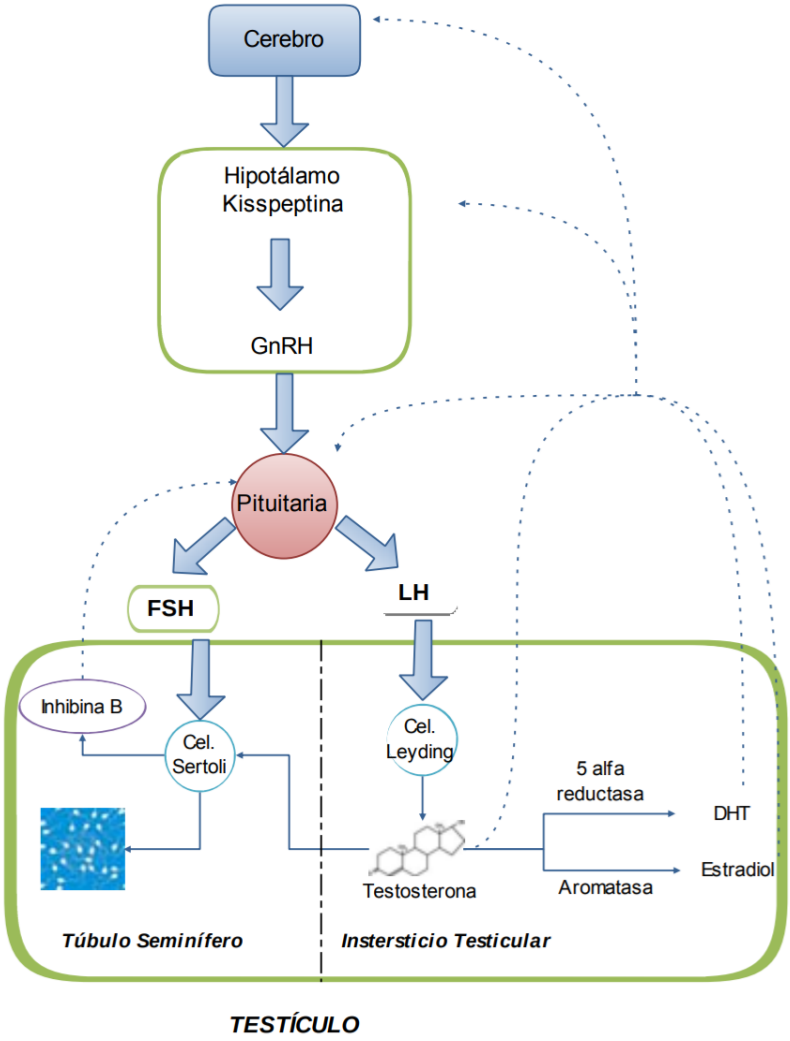


Figure 1. Diagram of the natural state of the reproductive axis. ***Taken from Page ST, Amory JK, Bremner WJ. Advances in male contraception. Endocr Rev 2008; 29 (4): 465-93***

The negative feedback of testosterone and estradiol on GnRH secretion is mediated through inhibition of kisspeptin production in the arcuate nucleus of the hypothalamus. Testosterone clearly inhibits kisspeptin transcription and GnRH and gonadotropin secretion. Part of this inhibition is estrogen dependent[36-39], but estradiol appears to play an important role in negative steroid feedback in men, particularly in decreasing LH production[40-44]. The hypothalamus synthesizes a decapeptide, gonadotropin-releasing hormone (GnRH), and secretes it in pulses every 90 to 120 minutes into the blood of the portal-hypothalamic-pituitary system. After reaching the anterior pituitary gland, GnRH binds to gonadotropic cells and stimulates the release of gonadotropins such as luteinizing hormone (LH) and, to a lesser extent, follicle stimulating hormone (FSH) into the general circulation. LH is taken up by Leyding cells where it binds to specific membrane receptors. The LH receptor is a G-protein-coupled receptor that has seven trans-membrane domains with a cytoplasmic region rich in serine and threonine itself that contains a 350–400 amino acid extracellular domain for hormone binding. Binding of LH to the receptor leads to the activation of adenylyl cyclase and the generation of cAMP and other messengers, which ultimately leads to androgen secretion. In turn, androgen elevation inhibits LH secretion from the

adenohypophysis through direct action on the pituitary and through an inhibitory effect on the hypothalamus. Both the hypothalamus and the pituitary have receptors for androgens and estrogens.

Pure androgens such as dihydrotestosterone (DHT) reduce the LH pulse while estradiol reduces the LH pulse width. However, the main inhibitory effect of androgens in the hypothalamus seems to be mediated mainly by estradiol, which can be produced locally through the aromatization of testosterone[45]. Testosterone, under normal physiological conditions in young men, is produced daily from 4–6 mg of testosterone[46-48] with a circadian rhythm; the highest level of secretion is early in the morning, and the lowest levels are found in the circulation during the mid-afternoon[49].

On the other hand, GnRH stimulation also causes gonadotropic cells to secrete FSH into the systemic circulation, this glycoprotein hormone binds with specific receptors on Sertoli cells and stimulates the production of androgen-binding protein. FSH is necessary for the initiation of spermatogenesis, however full sperm maturation appears to require not only FSH, but also testosterone. In fact, the main action of FSH on spermatogenesis may be through stimulation of the production of androgen-binding protein, which allows maintaining a high intratubular concentration of testosterone[33].

6.5. MALE HORMONAL CONTRACEPTIVE METHODS.

Currently, male hormonal contraception research is based on the suppression of gonadotropins, the interruption of spermatogenesis, and the supplementation of testosterone solely in order to maintain male sexual function, bone mineralization, and prevention of muscle wasting[3].

One of the research lines for hormonal contraceptives is the chronic administration of testosterone, which suppresses sperm production in men[50]. (Figure 2)

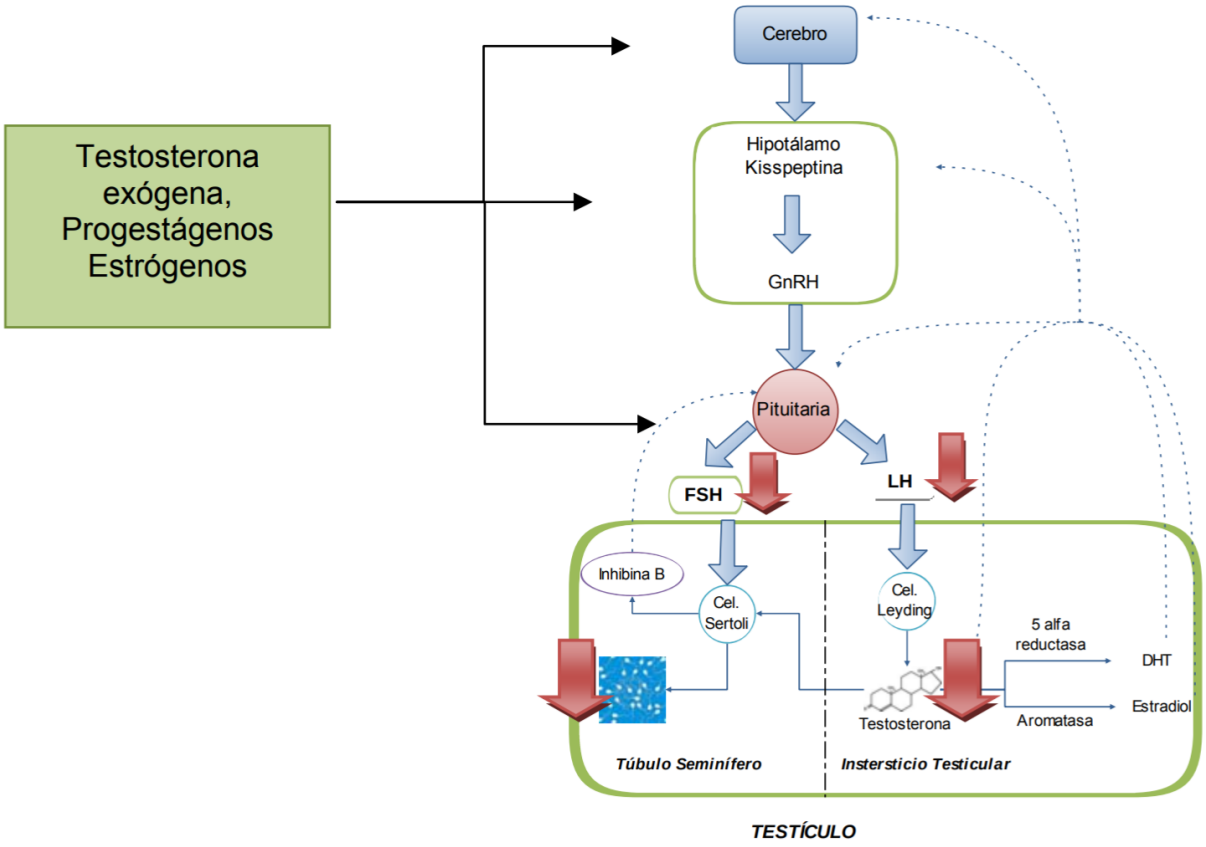


Figure 2. Diagram of the impact of hormonal contraceptives on the reproductive axis. ***Taken from Page ST, Amory JK, Bremner WJ. Advances in male contraception. Endocr Rev 2008; 29 (4): 465-93***

However, only with testosterone, the complete suppression of sperm production has not been achieved, for which it has been associated with progestogens or GnRH antagonists.

When using male hormonal contraceptives, there is a 2 to 3 month delay in reaching the full effect of contraception. This delay is similar to that seen with vasectomy, but is longer than the period required for female oral contraceptives to be effective. In addition, there are ethnic differences in the response of sperm concentrations to male contraceptive regimens. For example, volunteers studied in Asia showed ranges of azoospermia between 90 and 100% with regimens of testosterone alone, while Caucasian men showed ranges of azoospermia close to 60% with the same treatment[5,50-52]. The explanation for this difference is unknown, although it does not appear to be due to ranges of testosterone clearance and this complicates the extrapolation of ranges of suppression of sperm concentrations between populations[9].

At the moment, several studies have been carried out testing the association of androgens with progestogens; a review of randomized controlled trials of male hormonal contraception on bases such as CENTRAL, MEDLINE, EMBASE, POPLINE, and LILACS (as of March 2006[53], shows 30 clinical studies, in which the proportion of men achieving azoospermia varied widely. Few important differences emerged Of these studies, some of them show promising efficacy in terms of percentage of azoospermia. However, no male hormonal contraceptive is ready for clinical use. Most of the clinical studies were exploratory, so their statistical power to detect differences was limited and the imprecise results. Furthermore, the

definition of oligospermia has been imprecise and inconsistent. The authors of this review indicate that having studies with adequate statistical power would help[53].

There are several clinical studies carried out with Norethisterone Enanthate (NETE), in order to evaluate its efficacy as a male contraceptive at doses of 200–400 mg alone or together with testosterone, these doses of NETE have been shown to suppress the levels of FSH, LH and sperm concentration[3,10,53-55].

The association of 200 mg of norethisterone enanthate together with 750 and 1000 mg of testosterone undecanoate (TU) has been studied. Serum gonadotropin levels and sperm concentration were more consistently suppressed in the 1000 mg TU + 200 mg NETE group. The study concludes by indicating that despite a certain accumulation of testosterone, the aforementioned association administered every 8 weeks could become an effective contraceptive in the future, since it demonstrated suppression of gonadotropins and spermatogenesis[54].

Another clinical study was conducted to determine the most appropriate dosing range of 200 mg norethisterone enanthate plus 1000 mg testosterone undecanoate for male contraception administered intramuscularly, in terms of gonadotropins, sperm suppression, and prostate effects. The study was conducted in 50 healthy men who were randomized to five groups (10 subjects per treatment group) with different dosing intervals (group 1: every 8 weeks, group 2: every 12 weeks, group 3: every 6 weeks for 12 weeks and then every 12 weeks, and group 4: every 6 weeks for 12 weeks and after that 1000 mg of testosterone undecan plus placebo every 12 weeks and; group 5: placebo plus placebo every 6 weeks for 12 weeks and then every 12 weeks weeks, the study lasted 48 weeks. Efficacy and safety variables included spermatobioscopy, physical examinations, and prostate ultrasound. Study results concluded that the administration of norethisterone enanthate and testosterone undecanoate administered at 8-week intervals represents an effective male hormonal contraception regimen[56].

6.6. USE OF NORETHISTERONE ENANTHATE AND ESTRADIOL VALERATE IN MALE CONTRACEPTION.

A preclinical study was carried out in monkeys (macaca radiata), in which it was reported that a combination between NETE and estradiol valerate (EV) is superior to NETE alone, in the rapid blockade of testicular function, also showed that testosterone can be included together with NETE and EV in monthly injections without compromising the effectiveness of the combination to block spermatogenesis[57].

Based on this preclinical study, a clinical study[58] was conducted in which the combination of NETE and EV was administered in 4 healthy men at doses of 200 mg/2 mg respectively. The dose used was not arbitrary, it was taken based on the previous preclinical study as well as the recommended dose for the suppression of pituitary gonadotropins in women. The study lasted 180 days and a total of 7 injections were administered (one injection every 25 days) although in the last 4 injections (on days 100, 125, 150 and 175) the EV was excluded and only the NETE was administered. . During the first 3 weeks of treatment, the four volunteers complained of loss of libido and sexual drive, therefore and to maintain the accessory function of the glands, an oral supplementation of 40 mg of testosterone undecanoate (TU) was administered from the day 30 of treatment on alternate days. This dose is very small and does not appear to affect the suppression of pituitary gonadotropins and spermatogenesis, but it did improve the loss of libido in the volunteers. No other clinical problems were presented except for volunteer No. 3 who presented signs of gynecomastia during a routine clinical examination on day 75. This event was the reason for the removal of EV from the fifth injection.

Among the results of this study, a drop in testosterone on day 0 (5.33 ± 1.4 ng/mL) was observed vs. non-detectable values on day 3. Serum FSH fell on Day 0 (5.39 ± 1.4 mIU/mL vs. Day 3 (0.89 ± 0.2 mIU/mL, p <0.01). The suppression of the levels of these two hormones continued until day 15 and by day 25 their levels (FSH: 4.0 ± 1 mIU/mL; T 2.0 ± 2.8 ng/mL) tended to return to normal. By day 54 of treatment the four volunteers presented a marked and reproducible drop in sperm count and motility. An idea of the fertilization potential of the sperm was obtained by calculating the fertility index, the product of the sperm motility count (millions/mL) and the mobility range (range 0–5). The fertility index fell from a pre-treatment value of 268 ± 55.1 for the 4 volunteers to <0.2–12.6 for on treatment day 54, and <0.2–5.0 for treatment day 105.

The use of potent and specific aromatase inhibitors in monkeys and men has shown that oestrogens rather than testosterone could be the true regulators of gonadotropin feedback in the male primate[57,59]. The use of many variables in the investigation was unavoidable. However, the fact that the results of this study agree with the results obtained in a long and well-controlled preclinical study is reliable. Although EV and NETE were used in a ratio of 1:100 in this study, the article indicates that it would be feasible based on the previous preclinical study, to reduce this ratio in the future without compromising efficacy and that once spermatogenesis were blocked, the effect could be maintained by administering NETE alone. Given that 2 of the 4 subjects reached azoospermia[57], the researchers of this study believe that a continuous azoospermia could be achieved in all volunteers by manipulating the dose and the frequency of administration. Previous data indicate that combination therapies such as these are also useful in correcting precocious puberty. Its use as an orchidectomy replacement treatment in some types of prostatic hypertrophy needs to be verified. This study suggests that there is no pharmacokinetic interaction between the two components when they are administered together in an oily solution. The use of an adequate delivery system such as the injection once a month could ensure greater acceptability and compliance by men to this mode of contraception[58].

6.7. PHYSICAL-CHEMICAL PROPERTIES AND PHARMACOKINETIC INFORMATION OF ESTRADIOL VALERATE AND NORETHISTERONE ENANTHATE.

Estradiol valerate[60]

Chemical name: (17β)-estra-1,3,5(10)-triene-3,17-diol pentanoate. 17β estradiol valerate

Molecular mass: 356.50

Description: White crystalline powder

Solubility: Soluble in castor oil, methanol, benzyl benzoate and dioxane; slightly soluble in sesame oil and peanut oil; practically insoluble in water

Condensed formula: C23H3203

Structural formula: It is shown in Figure 3

ESTRADIOL VALERATE

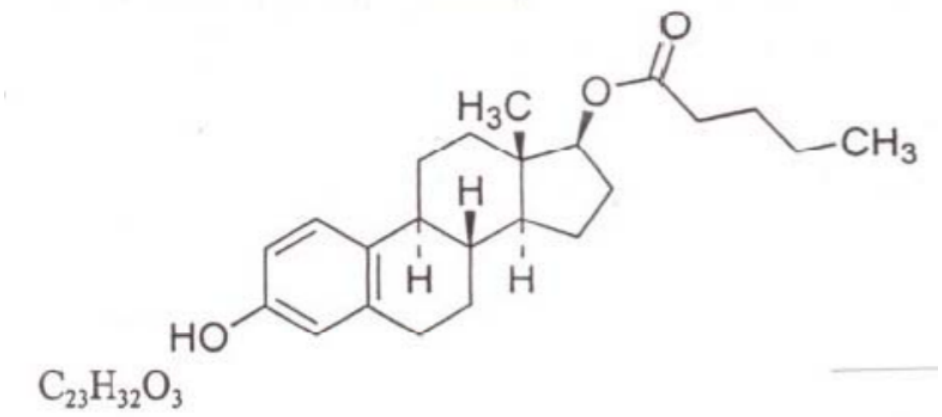


Figure 3. Structural formula of estradiol valerate

PHYSICAL-CHEMICAL PROPERTIES OF NORETHISTERONE ENANTHATE

Norethisterone[60]

Chemical name: 17-Hydroxy-19-nor-17α-pregna-4-en-20-yn-3-one

Description: White or light yellow crystalline powder

Solubility: Easily soluble in pyridine; slightly soluble in acetone and chloroform; slightly soluble in alcohol; almost insoluble in water and vegetable oils

Condensed formula: C20H2602

Structural formula: It is shown in Figure 4

NORETHISTERONE

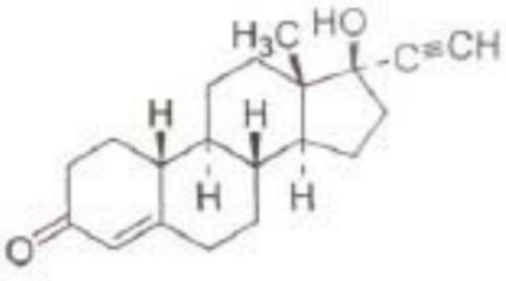


Figure 4. Structural formula of norethisterone

Pharmacokinetics of Norethisterone and Estradiol in women[61,62]

The pharmacologically active components of norethisterone (NET) and estradiol (E2) are completely bioavailable after intramuscular (IM) injection of norethisterone enanthate and estradiol valerate. After IM injection of 50 mg NETE in combination with 5 mg EV, peak plasma E2 concentrations are reached in a

range of 852 and 1,570 pmol/L on average, within 2 days and maximum plasma concentrations of NET are in the range of 4.7 to 10.1 nmol/L approximately 4.1 to 4.8 days after intramuscular injection.

Because the elimination half-life of estradiol is considerably shorter than that of norethisterone (which in turn is due to different rates of release of esters from the depot), the second part of the injection period is dominated. by the progestin component. Both components are completely metabolized. For several years the "in vivo" transformation of norethisterone into ethinylestradiol has been reported but it had not been quantitatively determined. Recent research has confirmed that norethisterone and norethisterone acetate are partially metabolized to ethinyl estradiol. Each milligram of norethisterone or norethisterone acetate administered orally in humans is converted to an equivalent dose of approximately 4 or 6 µg respectively of ethinylestradiol administered orally.

Since the estrogenicity of norethisterone has always been assumed and experienced in clinical practice, the recent discovery of its metabolic characteristics does not imply any change in the existing recommendations for use.

The biotransformation of estradiol follows the same pathway as the endogenous hormone. Estradiol is metabolized mainly in the liver, but also outside it, for example; in the intestine, kidney, skeletal muscle, and target organs. Plasma and liver esterases rapidly break down estradiol valerate to estradiol and valerenic acid. The subsequent metabolism of valerianic acid by beta-oxidation results in CO_2 and water as end products. Estradiol undergoes various hydroxylation steps and its metabolites as well as the unmodified part are finally conjugated. The intermediate products of its metabolism are estrone and estriol, which have a lower estrogenic activity by themselves of less magnitude than that of estradiol.

The proportions of these three estrogens (estradiol, estrone and estriol) mimic the physiological situation. In a study using radioactively labeled estradiol valerate, about 20% of the radioactive substance present in plasma was unconjugated steroids, 17% glucuronidated steroids, and 33% sulfated steroids. Around 30% of the substances were not extracted from the aqueous phase and therefore represent highly polar metabolites.

In plasma, estradiol is mainly bound to proteins. 37% is bound to SHBG (steroidal hormone binding globulin) and 61% to albumin.

The apparent volume of distribution of estradiol after a single intravenous administration is approximately 1 L/kg.

The total serum clearance of estradiol after a single intravenous administration shows a high variability between 10 and 30 mL/min/kg.

A certain proportion of estradiol metabolites is excreted in the bile and undergoes enterohepatic circulation.

Ultimately, estradiol metabolites are primarily eliminated as sulfates and glucuronides in the urine.

Norethisterone and its metabolites are excreted in approximately equal parts in faeces and urine. Excretion of estradiol metabolites is predominantly urinary. At least 85% of the doses of both substances were excreted within 28 days after injection.

Regarding pharmacokinetics and biotransformation, no interaction of norethisterone enanthate and estradiol valerate is to be expected as metabolism overload is unlikely due to slow release rates and consecutive low serum concentrations. of the active substances.

NETE is completely metabolized, mainly in the liver, by enzymatic hydrolysis into norethisterone and heptanoic acid.

In female plasma, 96% of norethisterone is bound to proteins. The respective percentages of binding to SHBG and albumin are approximately 35% and 60%, provided that SHBG levels are within normal limits.

Elimination of estradiol metabolites occurs in 70% via the kidney and 30% via the bile.

PHARMACOKINETIC PARAMETERS IN WOMEN[63].

Estradiol valerate:

- Bioavailability: 100%
- tmax: 2 days
- Cmax: 852 and 1,570 pmol/L (after IM injection of 50 mg NETE in combination with 5 mg EV)
- Protein binding: 37% is bound to SHBG and 61% to albumin
- Clearance: 10 to 30 mL/min/kg
- Volume of distribution: 1 L/kg

Norethisterone enanthate:[64,65]

- Bioavailability: Approximately 44% in the first 7 days, 48% between the 8th and 21st and the remaining 8% from the 22nd to the 30th
- tmax: 5 days
- Cmax: 4.6 ± 1.3 ng/mL (NET)
    - 1.2 ± 0.5 ng/mL (NETE)
- Protein binding: 60% (albumin) and 35% (SHBG). Administration with estrogens increases the proportion bound to SHBG.

7. JUSTIFICATION

Obtaining a male contraceptive method is a goal that has not yet been achieved in the world. One of the drawbacks to establishing a male hormonal contraceptive method is the fact that no product inhibits sperm production immediately and a period prior to achieving azoospermia is always necessary. The shorter the period necessary to achieve azoospermia, the contraceptive efficacy would also be achieved in less time.

In a previous clinical study, it was shown that including EV at the beginning of contraceptive therapy with NETE helps to lower total testosterone more rapidly and therefore decreases the time required to reach azoospermia. However, within the conclusions of this study, the researchers reported adverse events after the fourth dose and found it necessary to carry out studies with a larger population and fewer variables under evaluation.

The combination of 50 mg of NETE and 5 mg of EV is widely used as a contraceptive in women. Using it in men would allow administering a product whose safety and efficacy has been widely proven and

evaluating the impact on total testosterone levels by increasing the EV dose from 2 to 5 mg and decreasing the NETE dose from 200 to 50 mg, in search of the dose that allows to achieve the highest efficacy with the lowest risk of adverse events. None of the previous studies studied the bioavailability of NETE together with EV in men, knowing it would help to better understand the pharmacodynamics of the combination.

The present work quantifies total testosterone as the only efficacy evaluation because we will first focus our attention on total testosterone concentrations as an indicator of testicular function and on the bioavailability of the combination and, according to the results obtained, could define future studies in the line of male contraception, which would necessarily include efficacy variables such as sperm count and motility, dose escalation, multi-dose studies, etc.

The effect of an intramuscularly administered dose of 50 mg of NETE and 5 mg of EV on the total testosterone values of healthy Mexican men is currently unknown.

Which leads to the following research question:

How much do the total testosterone levels decrease in healthy Mexican men after intramuscular administration of a dose of 50 mg of NETE and 5 mg of EV?

8. HYPOTHESIS

The administration of 50 mg of NETE and 5 mg of EV decreases the total T to non-detectable values, in the 14 days after the administration of the investigational product in Mexican men.

9. OBJECTIVES

9.1. GENERAL OBJECTIVE:

To analyze the suppression of total testosterone in healthy Mexican men, after an intramuscular dose of 50 mg of NETE and 5 mg of EV.

9.2. PARTICULAR OBJECTIVES:

- To describe the bioavailability (Cmax and AUC) of a dose of 50 mg of NETE and 5 mg of EV in healthy Mexican male subjects.
- Evaluate the return to normal values of total testosterone in a period of 30 days after a dose of 50 mg of NETE and 5 mg of EV.
- To evaluate the tolerability of the administration of an intramuscular dose of 50 mg of NETE and 5 mg of EV in healthy Mexican male subjects.

10. MATERIAL AND METHODS

10.1. TYPE OF STUDY.

This work was a clinical, analytical, open, prospective, and longitudinal trial. This is a study classified as "Risk greater than the minimum" in accordance with the Regulations of the General Health Law on Research for Health. Art. 17.

10.2. SELECTION CRITERIA

10.2.1. INCLUSION CRITERIA

a) Literate subjects of the masculine gender.

b) Subjects between 18 and 55 years of age.

c) Healthy subjects based on the results of a complete medical history, clinical laboratory studies, chest radiography, and 12-lead electrocardiogram.

d) Subjects with a weight proportional to their height or with a deviation of up to ± 10% from the ideal weight, according to the determination of the Body Mass Index (18 to 27.5 kg/m^2).

e) Subjects whose laboratory values are not outside ± 10% of the normal range, unless in the opinion of the Principal Clinical Investigator, this abnormality has no clinical significance.

f) Subjects who have normal vital sign values at the screening visit, unless they present an abnormality and this, in the opinion of the Principal Clinical Investigator, has no clinical significance. Normal blood pressure values (sitting) are between 90 and 139 mm Hg for systolic and between 60 and 89 mm Hg for diastolic; normal pulse between 55 and 100 beats per minute, respiratory rate from 14 to 24 breaths per minute and temperature (oral) from 35.5 to 37.5 ° C.

g) Subjects who have been fully informed of the possible risks and benefits of their participation; who have voluntarily agreed to participate in the study in writing, by signing the Letter of Informed Consent.

h) Subjects with availability to participate during the entire study.

10.2.2. EXCLUSION CRITERIA

a) Subjects with a history of hypersensitivity to the product under investigation or to any related compound.

b) Subjects who suffer or have a history of conditions that may put them at risk in the study and/or that may alter the results of the study, in the opinion of the Principal Clinical Investigator.

c) Subjects who require any medication during the course of the study.

d) Subjects who have been exposed to agents known as inducers or inhibitors of hepatic enzyme systems in the 30 days prior to the start of the study.

e) Subjects who have taken potentially toxic medications within 30 days prior to the start of the study.

f) Subjects who have been administered any medication, within the 14 days or 7 half-lives prior to the start of the study.

g) Subjects who have been hospitalized for any reason or who have been seriously ill within 60 days prior to the start of the study.

h) Subjects who have participated in a clinical trial, obtaining blood samples, within the 60 days prior to the start of the study.

i) Subjects who have donated or lost a significant amount (approximately 450 mL or more) of blood, within the 60 days prior to the start of the study.

j) Subjects who have ingested alcohol in the 48 h prior to the start of the study.

k) Subjects with a history in the last 6 months of drug use or alcohol abuse.

10.2.3. ELIMINATION CRITERIA

a) Subjects who present adverse events that interfere with their participation.

b) Subjects who withdraw their informed consent.

All subjects met the inclusion criteria and did not incur any exclusion or elimination criteria.

10.3. TABLE OF VARIABLES

Table 1. Study Variables

Variable name and type	**Conceptual definition**	**Operational definition**	**Measurement scale**
Independent NETE and EV	Combined hormonal contraceptive.	Norethisterone enanthate 50 mg and estradiol valerate 5 mg	Qualitative, nominal (Yes/No)
Dependent 1 Total testosterone (T)	Main androgen in male reproductive function that allows evaluating testicular function.	Quantification of total T on days 0, 14, and 30	Quantitative, continuous [ng/mL]
Dependent 2 AUC	**AUC:** Area under the plasma concentration vs. time curve.	**AUC:** Area under the curve obtained from plasma concentrations vs. time of NET and E2 on days: 1, 2, 3, 4, 5, 6, 7, 10, 14, 18, 22, and 26	Quantitative, continuous NET: day*pg/mL E2: day*pg/mL
NET and E2 Cmax	**Cmax:** Maximum concentration reached by the drug in a time.	**Cmax:** Maximum concentration reached by NET and E2 during days 1, 2, 3, 4, 5, 6, 7, 10, 18, 22, and 26	NET: pg/mL E2: pg/mL
Dependent 3 Adverse events	Any unfortunate medical occurrence in the subject to whom the drug was administered, with or without a causal	Any unfortunate medical occurrence reported by the subject or identified from day -2 of the study to day 30.	Qualitative Nominal, Multiple [No, Yes, Name]

	relationship to the treatment		

10.4. SAMPLE SIZE

Considering that there are no statistical data in the previous clinical study that allow them to be taken into account for a sample calculation, and in the case of a phase I study, the sample studied for convenience was 32 subjects.

10.5. STATISTIC ANALYSIS

COMPUTER PROGRAMS.

To perform the statistical calculations and the pharmacokinetic parameters of this study, the following programs were used: WinNonlin® Professional version 5.0.1. (March 2011) Microsoft ® Office Excel 2007, SPSS® Statistics version 17.0 and Statgraphics ®Plus version 5.0. The statistical analysis comprised the following:

a) Descriptive statistics of the demographic characteristics (arithmetic mean, standard deviation, minimum and maximum value) of age, and BMI.

b) Descriptive statistics of plasma testosterone concentrations vs. time.

c) Average plasma profile vs total Testosterone time.

d) Descriptive statistics of plasma concentrations of norethisterone vs. time.

e) Mean plasma profile vs. norethisterone time (arithmetic and semi-logarithmic scale).

f) Descriptive statistics of plasma estradiol concentrations vs. time.

g) Average plasma estradiol profile vs. time (arithmetic and semi-logarithmic scale).

h) Calculation of pharmacokinetic parameters by a non-compartmental method. (AUC_{0-t}, $AUC_{0-\infty}$, tmax, Ke, t½, MRT)

i) Descriptive statistics of the pharmacokinetic parameters of norethisterone and estradiol

j) List of adverse events.

10.6. OPERATIONAL DESCRIPTION OF THE STUDY

Table 2. Schedule of Activities

	Selection	Clinical Stage															Follow up
Activities	-90 d	-2	-1	0	1	2	3	4	5	6	7	10	14	18	22	26	30	30 ± 3 d

IC signature	x																	
Clinical evaluation	x	x															x	
Blood measurements	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Clinical laboratory tests	x																	
12-lead electrocardiogram	x																	
Chest x-ray	x																	
Dose administration				x														
TS for E2 quantification		x	x	x														
TS for quantification of T				x									x				x	
TS for pharmacokinetic study				x	x	x	x	x	x	x	x	x	x	x	x	x	x	
AE evaluation		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
End of the clinical stage																	x	
Report of volunteers for follow-up																		x

10.6.1. SELECTION AND STUDY ACTIVITIES

After a candidate for the study gave written consent by signing the informed consent letter authorized by the Ethics and Research Committee (ERC), the subject was included in the selection process.

The selection process and the total number of evaluations did not exceed 90 days prior to the start of the study. The study comprised two stages, the selection stage in which the corresponding clinical and paraclinical evaluations were carried out to determine the health status of the volunteers and the second, the clinical stage in which blood samples were taken to quantify endogenous E2. on days -2, -1, and 0 prior to dosing. Subsequently, they were administered the product under investigation and blood samples were taken for the pharmacokinetic study of NET, E2, and quantification of total testosterone.

The day of blood sampling to determine pre-dose endogenous estradiol levels on day -2 was considered the start of the study. On the day of initiation of the study and the day of administration of the investigational product, the subjects presented to the AMIC at least one hour before the blood sample collection. The rest of the visits for blood sampling were made from 0800 to 0900 hours.

30 ± 3 calendar days after the last clinical evaluation, a telephone follow-up was carried out to confirm the good health of the subjects.

10.6.2. CLINICAL EVALUATIONS

a. INTERROGATION AND PHYSICAL EXAM

A medical history was taken that consisted of an evaluation of the current state, questioning of relevant antecedents and physical examination during the selection process.

On the start day of the study on day -2 and at the end of the study, a clinical evaluation was performed that included questioning on the current state of health and physical examination.

b. SOMATOMETRY

The subject's height was measured during the selection process. The subject's weight was measured during the selection process, on the start of the study day -2 and at the end of the study. During the selection process, the body mass index was calculated using the following formula:

$$BMI = kg\ (weight) / m^2\ (height)$$

c. MEASUREMENT OF VITAL SIGNS:

The vital signs were measured:

- During the selection process
- The day of study start day -2
- On day -1 predose
- In the hour prior to the administration of the assigned treatment dose (Day 0)
- And at 1, 2, 3, 4, 5, 6, 7, 10, 14, 18, 22, 26, and 30 days after the administration of the dose

Vital sign measurements included blood pressure, pulse, respiratory rate, and oral temperature. These values were within the normal range.

The times for measurement of vital signs were between 0730 and 0900 hours of each day that the volunteer attended the AMIC (except in the selection stage and on days -2 and 0) prior to taking his biological sample.

No incidents or noncompliance were reported in the clinical evaluations.

10.6.3. DIAGNOSTIC TESTS

During the selection process, the following diagnostic tests were performed:

- 12-lead electrocardiogram at rest.
- Chest X-ray.
- Clinical laboratory tests, which included at least:
    - Hematology: Erythrocytes, hemoglobin, hematocrit, total leukocyte count with differential and platelet count.
    - Blood chemistry: Glucose, blood urea nitrogen (BUN), creatinine, total protein, albumin, globulin, albumin/globulin ratio, total bilirubin, alkaline phosphatase, lactic dehydrogenase, gamma glutamyl-transpeptidase (GGT), aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), uric acid, triglycerides, and total cholesterol.

- General Urine Exam: Specific gravity, pH, proteins, ketones, glucose, occult blood, and microscopic examination of the sediment.
- Serology: Detection of viral hepatitis B and HIV.

10.6.4. PREVIOUS AND CONCOMITANT TREATMENT

During the study the subjects were not authorized to take any other treatment that is not prescribed by the investigator. In the case of requiring any medication during the study, the subjects had to consult it previously with the Investigator, who verified that there was no drug interaction with the study product. Only one subject ingested bismuth subsalicylate 262 mg (unit dose) tablets to counteract dyspepsia in the 14 days prior to the administration of the investigational product, this drug does not modify the kinetics of the investigational product or its quantification in the analysis, so the subject was included in the study.

Due to the presence of adverse events, some of the study subjects received treatment, which was considered as concomitant therapy, the detail is found in Table 3:

Table 3. Concomitant therapy

Subject	Treatment	Daily dose	Duration	Indication
03	Nifuroxazide capsules	200 mg each 8 hours	4 days	Diarrhea
05	Omeprazole capsules	20 mg each 24 hours	15 days	Gastritis
12	Caladryl lotion	10 mL each 6 hours	7 days	Contact dermatitis
14	Norfloxacin pills	400 mg each 12 hours	7 days	Urinary tract infection
37	Paracetamol tablets	500 mg each 8 hours	2 days	Pharyngitis
44	Amoxicillin tablets	500 mg each 8 hours	7 days	Laryngitis
44	Celecoxib capsules	200 mg each 12 hours	3 days	Laryngitis
50	LM6 tablets	1 each 8 hours	5 days	AE

These concomitant treatments are not known to modify the kinetics of the investigational product or its quantification in the analysis, so the subjects were authorized to continue in the study.

10.6.5. DETECTION OF ADVERSE EVENTS

The subjects were instructed to report to the medical personnel of the research site, any signs or symptoms that they present; Furthermore, the physicians participating in the study conducted interrogations aimed at detecting signs or symptoms that appear in the subjects during the conduct of the study. The detail of adverse events is found in Table 16.

10.6.6. ADMINISTRATION OF THE PRODUCT UNDER INVESTIGATION

One dose of the investigational product was injected deep intramuscularly into the upper outer quadrant of the right gluteus. The administration of the product under investigation was carried out from 0800 to 0835. The product under investigation was stored at a storage temperature ranging between 19 and 26 ° C and relative humidity between 27 and 45%.

10.6.7. OBTAINING THE SAMPLES

Venous blood samples were drawn in the following scheme:

- 6 mL for the quantification of endogenous estradiol on days -2 and -1.
- 18 mL for post-dose pharmacokinetic study on days 1, 2, 3, 4, 5, 6, 7, 10, 18, 22, and 26.
- 24 mL for determination of endogenous estradiol, total testosterone and post-dose pharmacokinetic study on days 0, 14, and 30.

Samples were obtained by venipuncture and the samples were deposited in tubes with heparin. The samples were obtained at a time established between 0800 and 0900. During the study, there were lags in the time for obtaining samples, the detail is as follows:

Table 4. Lags in the time of obtaining biological samples

Subject	Gap
19	24-hour delay in TS. Day 18
31	24 hour and 29 minute delay in TS. Day 18
41	He did not attend his visit. TS was not performed. Day 4
	31 hours and 27 minutes delay in TS. Day 26
47	He did not attend his visit. TS was not performed. Day 18
59	7 hours and 16 minutes delay in TS. Day 30

TS = Taking of samples

10.6.8. SAMPLE PROCESSING

Blood samples were kept refrigerated (2 to 8 ° C) for a maximum of 60 minutes before centrifugation. They were centrifuged at 3000 ± 200 rpm, at 4 ± 2 ° C for 15 minutes. The plasma obtained was separated from the globular package and was deposited in cryo tubes. The tubes were immediately stored at -70 ± 5 ° C until shipment to the analytical unit.

10.6.9. ANALYTICAL METHOD

Total Testosterone. The method of quantification of total testosterone was by chemiluminescence.

Estradiol The method for quantifying estradiol in plasma by radioimmunoassay was previously validated. The method was specific for estradiol and did not cross-react with norethisterone and testosterone. The

method was selective to different plasma sources. The limit of quantification (LC) was 20 pg/mL and the limit of detection (LD) was 0.365 pg/mL. E2 stability was at least 137 days in samples stored at -70 ° C, 3 freeze-thaw cycles, and on the bench after being thawed and kept at room temperature for at least 5 hours.

Norethisterone. A commercial method (Immunometrics) was validated for the quantification of Norethisterone (NET) in plasma by Radioimmunoassay (RIA), the limit of quantification was 93.75 pmol/L and the limit of detection was 84.2 pmol/L. The method was specific for NET and did not show any interference with estradiol. The NET was stable at a freeze-thaw cycle, on the bench it was 5 hours and long-term 38 days at -70 ° C.

11. RESULTS

11.1. DEMOGRAPHIC CHARACTERISTICS

32 male subjects with an average age of 29.16 years with an age range between 19 and 53 years were studied. The average body mass index was 23.86 kg/m^2 with a range of 18.69 to 27.48 kg/m^2. Descriptive statistics (mean, standard deviation, minimum, and maximum) of the following demographic characteristics were calculated: age, height, body mass index (BMI) and weight of the 32 subjects at the time they entered the study, which are shown in Table 5:

Table 5. Descriptive statistics of demographic characteristics; n = 32

Demographic characteristics	Minimum	Maximum	Mean	SD
Age (years)	19.00	53.00	29.16	7.64
Height (cm)	154.00	183.00	169.22	7.45
Weight (kg)	54.60	86.50	68.30	8.94
BMI (kg/m^2)	18.69	27.48	23.86	2.34

11.2. PLASMA CONCENTRATIONS OF TESTOSTERONE VS TIME.

Descriptive statistics (mean and standard deviation) of total testosterone plasma concentrations were performed on days 0, 14, and 30 of the study. The details are in Table 6.

Table 6. Descriptive statistics of total T ng/mL Day 0, 14 and 30; n = 32

Day	Mean	SD	Maximum	Minimum	%C.V.
Day 0	5.03	1.30	7.8	2.9	25.79
Day 14	0.61	0.55	2.8	0.2	90.88
Day 30	3.75	1.16	6.1	1.1	31.00

Day 0 shows a mean total testosterone plasma concentration of 5.03 ± 1.30 ng/mL. A 4.4 ng/mL decrease was observed for day 14, with the average total testosterone concentration being 0.61 ± 0.55

ng/mL on this day. On the 30th day of the study, the total testosterone concentration increased by 3.14 ng/mL with an average of 3.75 ± 1.16 ng/mL.

To confirm that the differences observed are statistically significant, the plasma concentrations of total testosterone obtained were subjected to an ANADEVA test for related samples, obtaining with 95% confidence that the combination of 50 mg of norethisterone enanthate and 5 mg of valerate of estradiol caused a statistically significant decrease in total testosterone concentrations of the 32 healthy male volunteers studied ($p<0.05$).

Throughout the investigation it was seen that more detailed information could be obtained if the total T was quantified in the biological samples obtained on days 1, 2, 3, 4, 5, 6, 7, 10, 14, 18, 22, 26, and 30. For logistical reasons, it was not possible to have all the biological samples from all the 32 subjects, only a subsample of 13 subjects was available, in which the total T was quantified. The descriptive statistics of these concentrations are found in Table 7.

Table 7. Descriptive statistics of total testosterone; (ng/mL); (Days 0, 1, 2, 3, 4, 5, 6, 7, 10, 14, 18, 22, 26, and 30); (Subsample)

Day	n	Maximum (ng/mL)	Minimum (ng/mL)	Mean (ng/mL)	SD (ng/mL)	Mean difference (ng/mL)	%CV
0	13	6.11	2.54	3.89	0.93	–	24.03%
1	13	6.31	1.26	3.41	1.51	-0.48	44.29%
2	13	5.90	0.47	2.26	1.70	-1.15	75.10%
3	13	3.03	0.20	1.10	0.91	-1.16	82.76%
4	12*	1.20	0.17	0.49	0.28	-0.61	55.97%
5	13	0.71	0.19	0.38	0.16	-0.11	41.60%
6	13	0.86	0.12	0.38	0.20	0.00	52.67%
7	13	0.52	0.13	0.30	0.13	-0.07	41.48%
10	13	0.65	0.12	0.32	0.17	0.02	52.18%
14	13	1.64	0.19	0.51	0.38	0.19	74.35%
18	12*	3.58	0.23	1.24	1.13	0.72	91.30%
22	13	4.83	0.24	1.96	1.35	0.72	68.89%
26	13	5.20	0.35	2.80	1.19	0.84	42.59%
30	13	5.93	0.57	3.16	1.33	0.36	41.92%

*Two of the study subjects did not attend their sampling on day 4 and 18.

The lowest levels of plasma total testosterone concentration were observed on day 7 with an average of 0.30 ± 0.13 ng/mL.

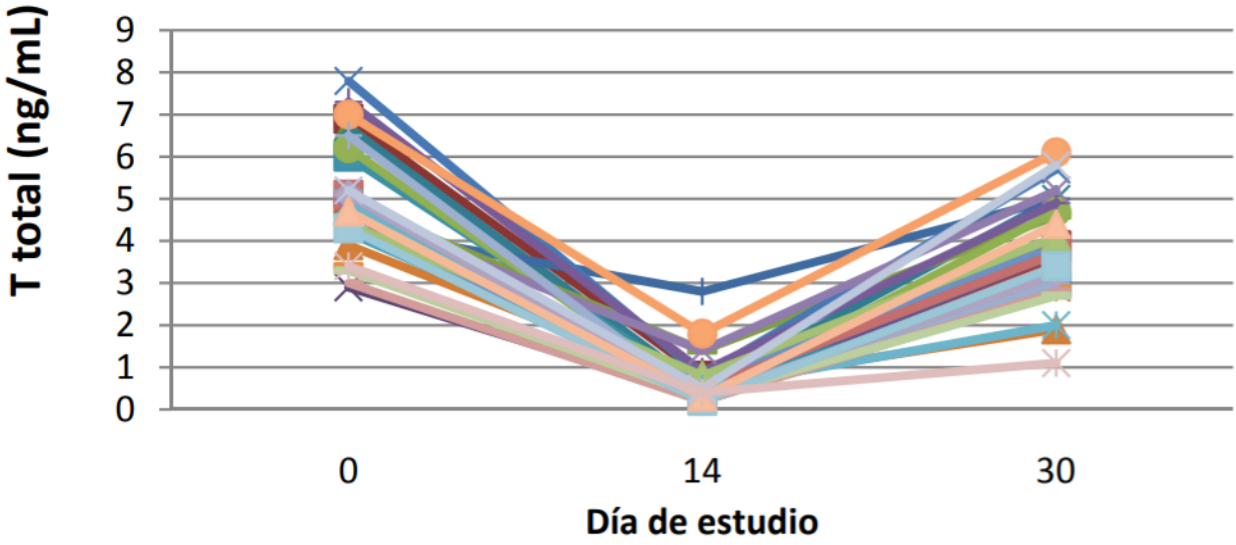
The average plasma concentration of total testosterone on day 0 is 3.89 ± 0.93 ng/mL, from that average the suppression of plasma concentrations of total testosterone begins. A gradual suppression is observed ranging from 0.48 ng/mL from day 0 to day 1, 1.15 ng/mL from day 1 to day 2, 1.16 ng/mL from day 2 to day 3, 0.61 ng/mL from day 3 to day 4, 0.11 ng/mL from day 4 to day 5. It is observed that between day 5 and 6 there is no suppression of testosterone concentrations, and by day 7 they are suppressed by an additional 0.07 ng/mL. Starting on day 7, the recovery of total testosterone plasma concentrations begins, which increase by 0.02 ng/mL on day 10, 0.19 ng/mL for day 14, 0.72 ng/mL for day 18 and 22 respectively, 0.84 ng/mL by day 26 (maximum recovery) and by 0.36 ng/mL by day 30. We can observe that the suppression is faster than the recovery of plasma concentrations of total testosterone.

To confirm that the differences observed are statistically significant, the plasma concentrations of total testosterone obtained were subjected to an ANADEVA test for related samples, obtaining with 95% confidence that the combination of 50 mg of norethisterone enanthate and 5 mg of valerate of estradiol caused a statistically significant decrease in total testosterone concentrations of the 13 healthy male volunteers studied in this sub-sample ($p<0.05$).

11.3. PLASMA PROFILES OF TESTOSTERONE VS TIME

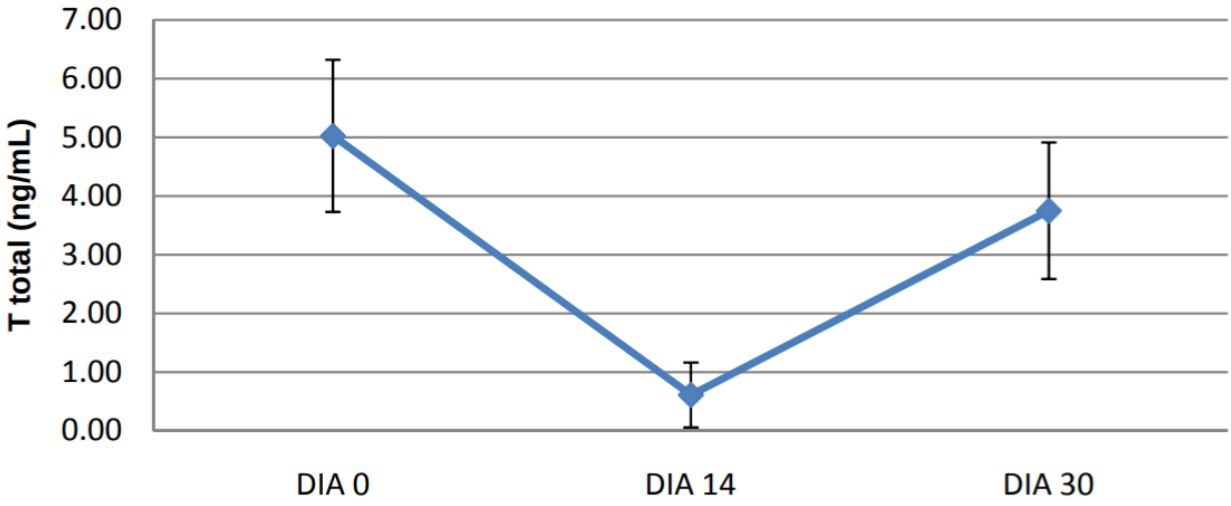
The total testosterone plasma profiles of each of the study subjects were performed for days 0, 14, and 30, which are presented accumulated in Figure 5.

Figure 5. Total testosterone plasma profile; n = 32



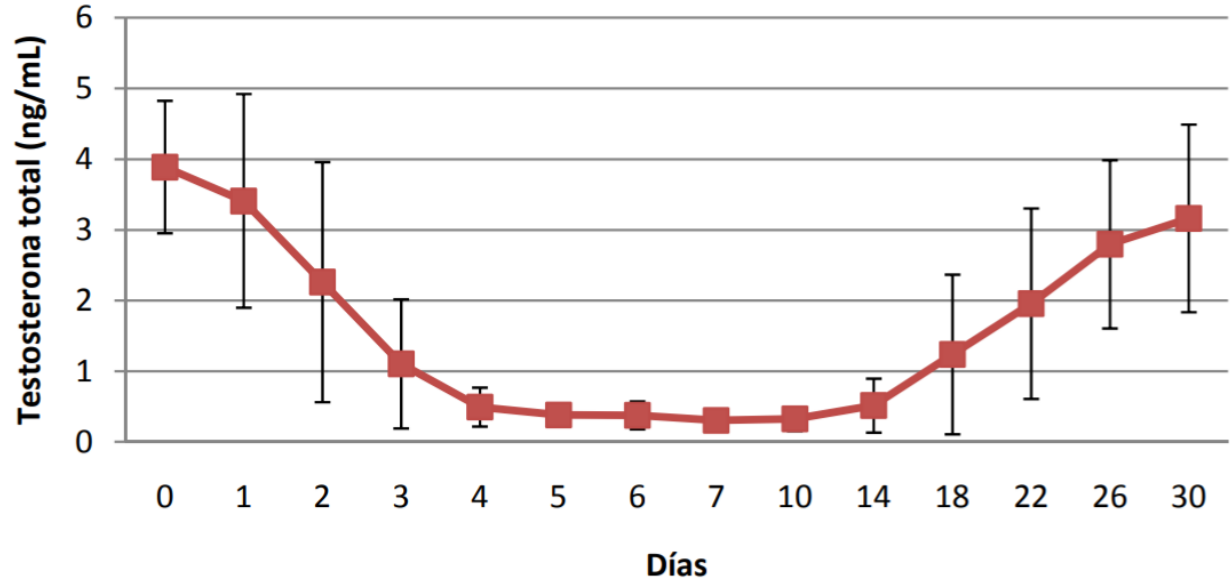
The mean plasma profiles (± SD) of the plasma concentrations of total testosterone were also carried out, which are presented in Figures 6.

Figure 6. Average plasma profile ± SD of total Testosterone; n = 32 subjects



Likewise, the average plasma profiles of the total testosterone concentrations in the subsample of 13 subjects shown in Figure 7 were performed.

Figure 7. Average plasma profile of total testosterone ± SD; (Subsample); n = 13



11.4. PLASMA CONCENTRATIONS OF NORETHISTERONE VS TIME.

Descriptive statistics (Table 8) of the plasma concentrations of norethisterone in pg/mL reached on study days 0, 1, 2, 3, 4, 5, 6, 7, 10, 14, 18, 22, 26, 28, and 30 were performed.

Table 8. Descriptive statistics of plasma concentrations of norethisterone (pg/mL) vs time; n = 32 subjects

Day	N	Minimum	Maximum	Mean	SD	CV (%)

0	32	0.00	0.00	0.00	0.00	0.00%
1	32	65.50	517.60	226.12	116.77	51.64%
2	32	93.50	818.80	281.74	163.98	58.20%
3	32	101.60	1,258.30	300.08	222.90	74.28%
4	31*	104.30	1,830.60	378.57	383.77	101.37%
5	32	129.50	1,261.90	351.99	296.43	84.22%
6	32	104.40	1,167.30	314.28	201.86	64.23%
7	32	100.90	1,437.10	302.31	234.43	77.55%
10	32	85.50	1,644.80	347.00	319.67	92.12%
14	32	88.70	562.50	235.20	101.99	43.36%
18	31*	74.70	318.20	156.24	57.02	36.50%
22	32	55.70	269.80	134.10	53.97	40.25%
26	32	39.90	221.10	115.54	45.69	39.54%
30	32	39.20	189.70	100.31	37.05	36.94%

* Two of the study subjects did not attend their sampling on day 4 and 18.

In Annex 1 the plasma concentrations of norethisterone vs sampling time per subject are presented.

11.5. PLASMA PROFILES OF NORETHISTERONE VS TIME

Average plasma profiles (± SD) of norethisterone concentrations were performed, which are presented in Figures 8, 9, and 10 on a semi-log scale.

Figure 8. Average plasma profile of norethisterone; n = 32 subjects

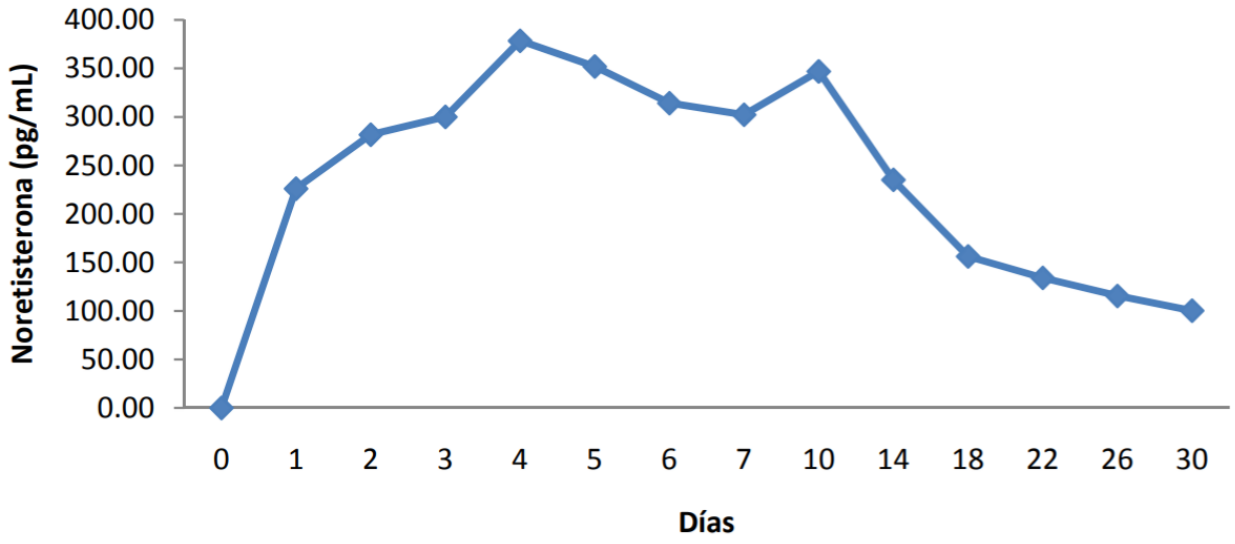


Figure 9. Mean ± SD plasma profile of norethisterone; n = 32 subjects

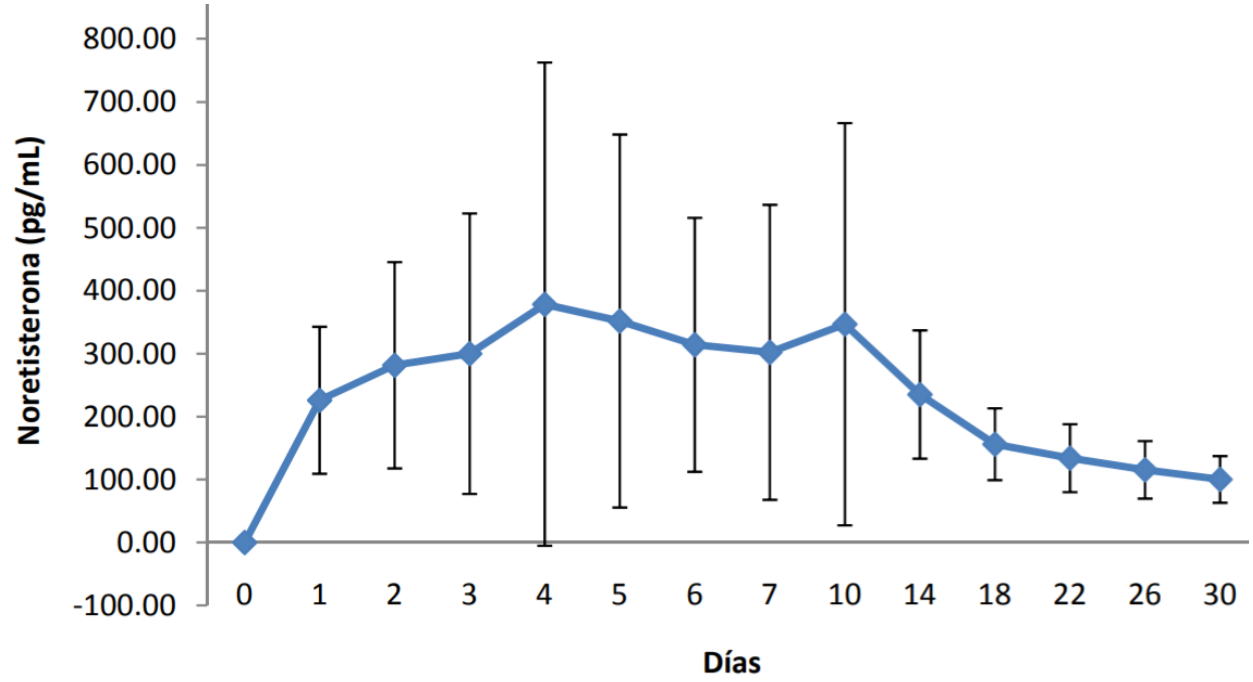
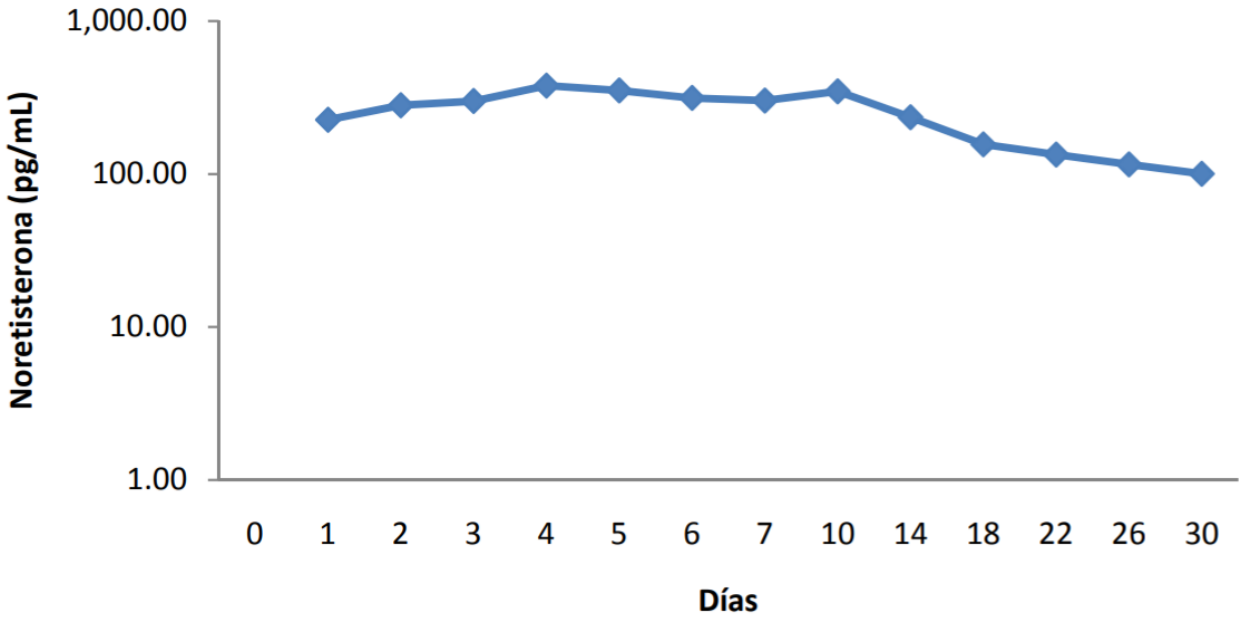


Figure 10. Average plasma profile of norethisterone on a semilogarithmic scale; n = 32 subjects



The average plasmatic profiles of norethisterone show high variability, for which a statistical analysis was carried out to evaluate if there are extreme values; this analysis is shown in section 11.11. The individual plasma profiles of norethisterone are presented in Annex 1.

11.6. PLASMA CONCENTRATIONS OF ESTRADIOL VS TIME

Descriptive statistics (Table 9) of plasma estradiol concentrations in pg/mL reached on days: -2, -1, 0, 1, 2, 3, 4, 5, 6, 7, 10, 14, 18, 22, 26, 28, and 30 of study.

Table 9. Descriptive statistics of plasma estradiol concentrations (pg/mL) vs. time; n = 32 subjects

Day	N	Minimum	Maximum	Mean	SD	CV (%)
-2	32	0.00	73.10	25.21	16.97	67.30%
-1	32	0.00	51.60	20.65	15.93	77.12%
0	32	0.00	45.10	26.16	13.02	49.77%
1	32	61.90	649.70	281.25	156.67	55.71%
2	32	105.20	538.90	289.62	121.53	41.96%
3	32	124.00	762.80	305.02	120.20	39.41%
4	31*	163.70	400.00	243.74	61.71	25.32%
5	32	90.80	300.40	184.65	58.91	31.90%
6	32	73.80	407.50	155.42	66.33	42.68%
7	32	61.10	270.90	115.26	43.74	37.95%

10	32	10.00	148.90	62.42	35.40	56.71%
14	32	0.00	172.90	30.89	34.93	113.08%
18	31*	0.00	71.80	17.49	18.37	105.00%
22	32	0.00	49.60	15.31	15.44	100.87%
26	32	0.00	75.50	19.87	18.85	94.90%
30	32	0.00	97.80	27.27	24.49	89.82%

* Two of the study subjects did not attend their sampling on day 4 and 18.

For the calculation of the descriptive statistics of E2, data below the limits of quantification (20 pg/mL) were considered as half[66] of the limit of quantification (10 pg/mL) and data below 10 pg/mL were they were considered non-quantifiable, in order to have a better characterization of $AUC_{0\text{-}30d}$ and given that the sensitivity of the analytical method excluded the data in the last sampling times.

In Annex 1 the plasma concentrations of estradiol vs sampling time per subject are presented.

11.7. PLASMA PROFILES OF ESTRADIOL VS TIME

The mean comparative plasma profiles (± SD) of estradiol concentrations were made, which are presented in Figures 11 and 12 and on a semi-log scale in Figure 13.

Figure 11. Average plasma estradiol profile; n = 32 subjects

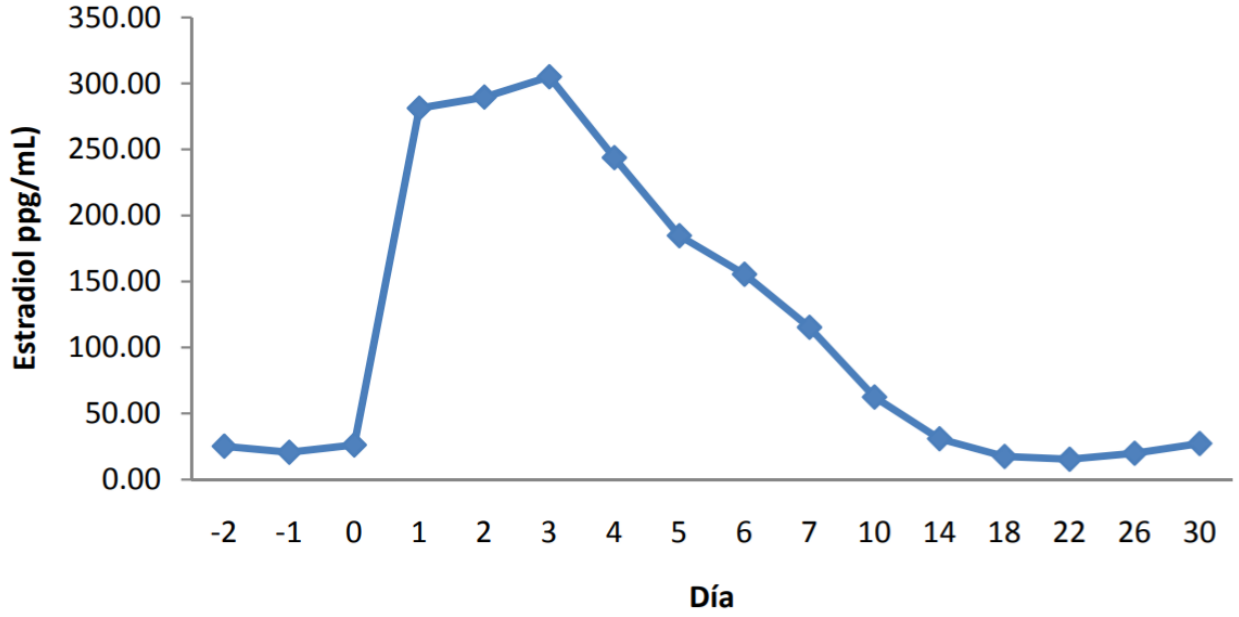


Figure 12. Mean plasma profile ± SD of estradiol; n = 32 subjects

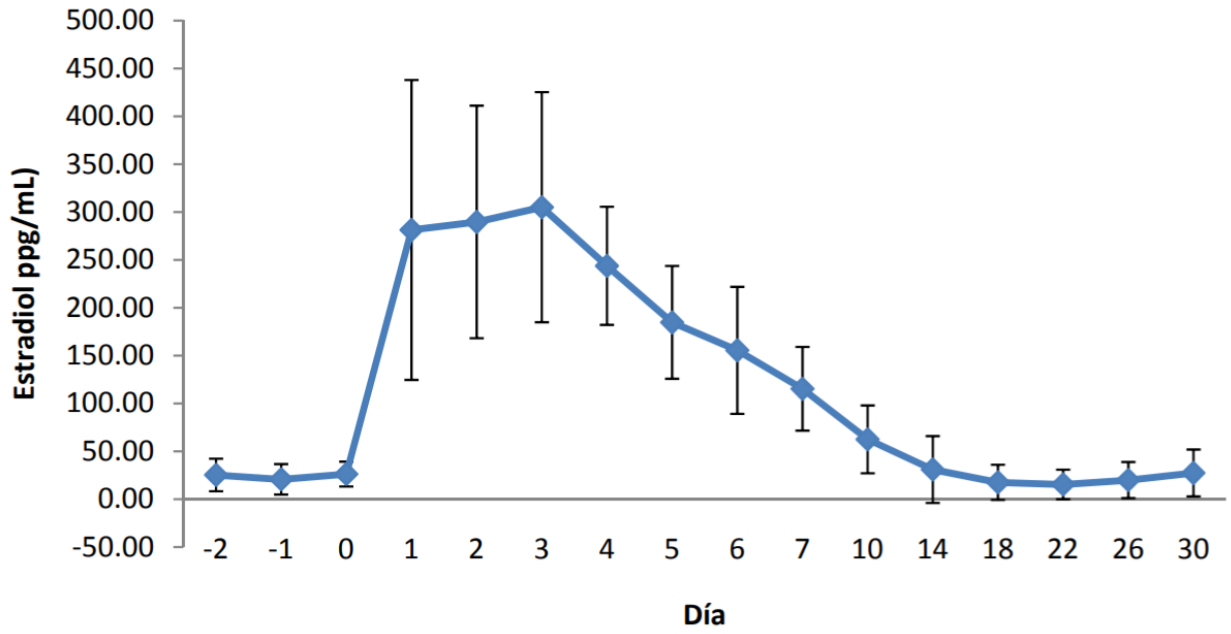
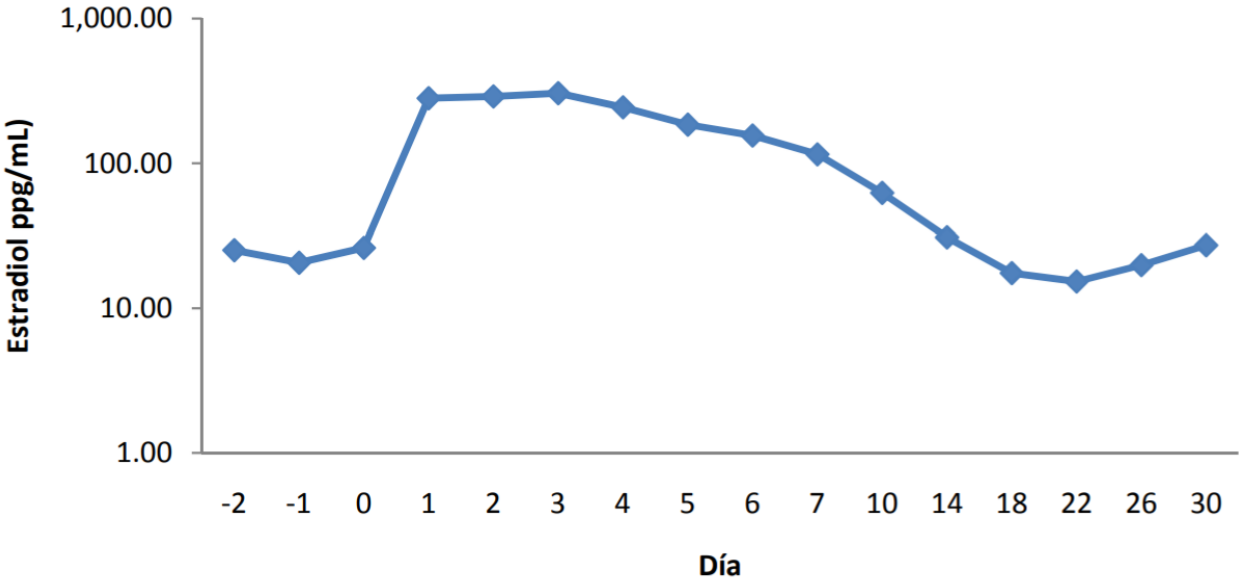


Figure 13. Average plasma estradiol profile on a semi-logarithmic scale; n = 32 subjects



The individual plasma estradiol profiles are presented in Annex 1.

11.8. PHARMACOKINETIC PARAMETERS

The calculation of the individual pharmacokinetic parameters was carried out under a non-compartmental method, with the WinNonlin Professional software (version 5.0.1.).

The pharmacokinetic parameters calculated for both estradiol and norethisterone were:

- Maximum plasma concentration (Cmax)
- Area under the curve from zero to the last sampling time with non-zero concentration (AUC_{0-t})
- Area under the curve from zero to infinity ($AUC_{0-\infty}$)
- Time to reach maximum concentration (tmax)
- Elimination constant of terminal phase (Ke)
- Elimination half-life (t½)
- Mean time of residence (MRT)

Additionally, Percent of the area under the extrapolated curve (% AUC extrap) was calculated. The pharmacokinetic parameters for each of the volunteers can be found in detail in Annex 2.

11.9. STATISTICAL ANALYSIS OF THE PHARMACOKINETIC PARAMETERS OF NORETHISTERONE

Descriptive statistics of the pharmacokinetic parameters of plasma concentrations of norethisterone were performed, which are shown in Table 10.

Table 10. Descriptive statistics of the pharmacokinetic parameters of norethisterone; n = 32 subjects

Parameter	**Mean**	**SD**	**CV (%)**
Cmax (pg/mL)	523.08	487.31	93.16%
$AUC0_{-30}$ (day*pg/mL)	6,459.95	2,982.69	46.17%
$AUC_{0-\infty}$ (day*pg/mL)	8,898.71	3,490.80	39.23%
Ke (1/day)	0.0461	0.0162	35.24%
t½ (day)	16.53	5.01	30.30%
tmax (day)	7.06	4.85	68.61%
Extrapolated AUC (%)	27.96	10.47	37.45%
MRT (day)	24.47	7.09	28.99%

11.10. STATISTICAL ANALYSIS OF THE PHARMACOKINETIC PARAMETERS OF ESTRADIOL

Descriptive statistics of the pharmacokinetic parameters of plasma estradiol concentrations were performed, which are shown in Table 11.

Table 11. Descriptive statistics of the pharmacokinetic parameters of estradiol; n = 32 subjects

Parameter	**Mean**	**SD**	**CV (%)**
E2 Cmax (pg/mL)	368.31	146.74	39.84%
E2 AUC_{0-30} (day*pg/mL)	2,392.75	655.12	27.38%
E2 $AUC_{0-\infty}$ (day*pg/mL)	2,610.20	774.15	29.66%

E2 Ke (1/day)	0.1707	0.0774	45.34%
E2 t½ (day)	4.92	2.15	43.70%
E2 tmax (day)	2.50	1.16	46.56%
E2 extrapolated AUC (%)	7.66	6.19	80.85%
E2 MRT(day)	9.67	3.04	31.46%

11.11. EXTREME DATA

Considering the high coefficient of variation of norethisterone, mainly in the parameters of Cmax and AUC_{0-t}, 3 subjects were identified as extreme values (44.50 and 56). The criterion to determine an extreme value was one that is outside ± 2 SD. To evaluate the impact of these extreme values on the coefficient of variation, the data of these 3 subjects was eliminated and the descriptive statistics of the pharmacokinetic parameters of NET and E2 were recalculated, obtaining the results shown in Table 12 and 13:

Table 12. Descriptive statistics of the NET pharmacokinetic parameters; n = 29 subjects

Parameter	**Mean**	**SD**	**CV (%)**
NET Cmax (pg/mL)	401.37	313.60	78.13%
NET AUC_{0-30} (day*pg/mL)	5820.33	2105.51	36.18%
NET $AUC_{0-\infty}$ (day*pg/mL)	8200.68	2561.21	31.23%
NET Ke (1/day)	0.0463	0.0171	36.88%
NET t½ (day)	16.62	5.26	31.64%
NET tmax (day)	7.17	5.00	69.72%
NET extrapolated AUC (%)	28.91	10.53	36.42%
NET MRT (day)	25.09	7.15	28.49%

Table 13. Descriptive statistics of the pharmacokinetic parameters of E2; n = 29 subjects

Parameter	**Mean**	**SD**	**CV (%)**
E2 Cmax (pg/mL)	353.08	144.79	41.01%
E2 AUC_{0-30} (day*pg/mL)	2293.19	589.89	25.72%
E2 $AUC_{0-\infty}$ (day*pg/mL)	2501.80	717.29	28.67%
E2 Ke (1/day)	0.1766	0.0785	44.45%
E2 t½ (day)	4.77	2.15	45.08%

E2 tmax (day)	2.59	1.15	44.47%
E2 extrapolated AUC (%)	7.59	6.36	83.68%
E2 MRT(day)	9.59	3.17	33.08%

After eliminating the extreme values, the coefficient of variability of the NET decreased, for the Cmax from 93.16% to 78.13%, for the $AUC_{0\text{-}30d}$ from 46.17% to 36.18%, the $AUC_{0\text{-}\infty}$ from 39.23% to 31.23%.

11.12. ADVERSE EVENTS:

During the conduct of the study, adverse events occurred, the intensity of which was evaluated as mild, moderate and severe. The relationship with the study drug was evaluated using the Naranjo Algorithm, the detail in Table 14.

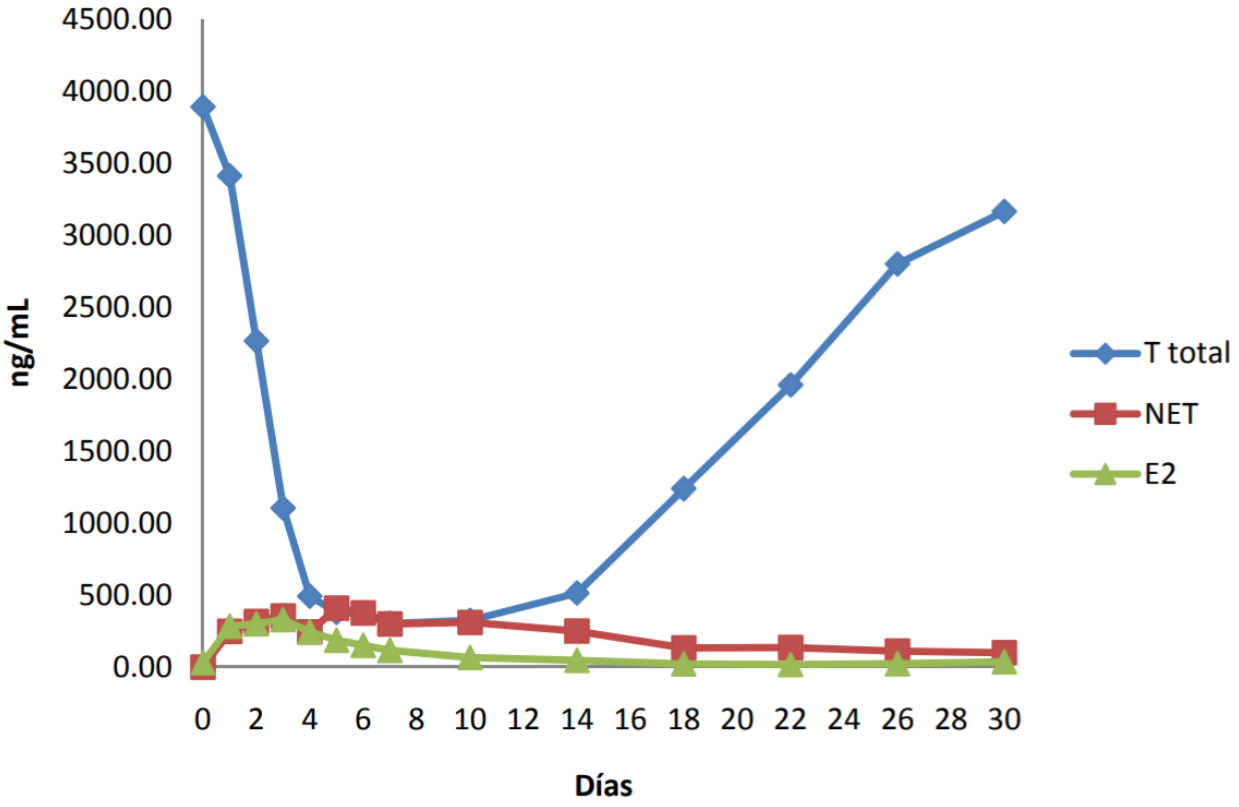
Table 14. Adverse Events.

No.	**Adverse event**	**Duration**	**Intensity**	**Relationship to study medication**
1	Diarrhea	2 days	Mild	Unrelated
2	Gastritis	16 days	Mild	Unrelated
3	Erectile dysfunction	37 days	Moderate	Dubious
4	Contact dermatitis	10 days	Mild	Unrelated
5	Urinary tract infection	11 days	Mild	Unrelated
6	Pharyngitis	5 days	Mild	Unrelated
7	Pharyngitis	3 days	Mild	Unrelated
8	Laryngitis	11 days	Moderate	Unrelated
9	Rhinitis	7 days	Mild	Unrelated

12. DISCUSSION

In this study it was observed that when modifying the ratio of the combination of NETE and EV from 1:100 (previous study) 57 to 1:10 (present study), and administering it in healthy Mexican men, the levels of T total were suppressed at concentrations of 0.61 ± 0.55 ng/mL by study day 14, thus achieving a suppression of total T. This is explained by the action of EV on GPR54 receptors in GnRH secreting neurons, and the action of NETE on progestin receptors found in the brain, hypothalamus and pituitary. The action of NETE and EV prevents the production of GnRH, LH, FSH and testosterone. Throughout the investigation it was seen that more detailed information could be obtained if the total T was quantified in the biological samples obtained on days 1, 2, 3, 4, 5, 6, 7, 10, 14, 18, 22, 26, and 30. For logistical reasons, it was not possible to have all the biological samples from all the 32 subjects, only a subsample of 13 subjects was available, in which the total T was quantified. This allowed obtaining a comparative graph of NET concentrations. E2 and total T in plasma (Figure 14):

Figure 14. Comparative graph NET, E2 and total T



This graph allows us to see that the day in which the lowest value of total T was presented was Day 7 (0.30 ± 0.13 ng/mL), at no time were undetectable values of total T reached, unlike the previous study in which, undetectable values were presented from Day 3. However, reaching undetectable values would not be the most important objective considering that in the previous study, there was a need to supplement testosterone in order to maintain its basic functions and avoid adverse events . Thus, to achieve azoospermia and a contraceptive effect, “undetectable” values are not necessarily required but rather a sufficient total T range to achieve azoospermia and avoid adverse events.

The maximum concentration of NET was presented on average on day 7, coinciding with the day in which the T values were at the minimum, which would confirm the direct relationship between NET and total T suppression. Regarding E2, the maximum concentration in plasma occurred on average on day 5, coinciding with the days in which total T was gradually and intensely suppressed; from day 5 and 6 the suppression of total T was stopped, although to continue in a lesser amount until day 7.

The graph shows an abrupt drop in total T values (day 1 to day 5) during the days in which E2 concentrations reached tmax, suggesting that effectively the administration of EV improves the suppression speed of Total T, which could be useful in the case of a contraceptive scheme in which the first doses of the contraceptive include minimal amounts of E2, and once the minimum necessary levels of total T have been reached, then the contraceptive effect is maintained only with NET .

In relation to the bioavailability and pharmacokinetic parameters of NET and E2, the data obtained in this study are unique, since there is no study reported that shows the pharmacokinetics after intramuscular

administration of the combination of NET together with E2 in men. The average concentrations of NET and E2 on the days of analysis generally allow a typical plasma profile with absorption area, Cmax and elimination to be obtained.

However, the coefficients of variability (% CV) of NET reached 93.16% for Cmax and 68.61% for tmax, higher than 45% and 56% respectively, reported in NET pharmacokinetic studies in women67, $AUC_{0\text{-}30d}$ NET did not present a % CV as high as the two previous pharmacokinetic parameters (46.17%). The elimination of the 3 extreme subjects allowed the reduction of the % of the coefficient of variability in Cmax from 93.16% to 78.13%, which confirms that these subjects are extreme and influential.

On the other hand, the coefficients of variability (% CV) of E2 reached 39.84% for Cmax, 46.56% for tmax, and 27.38% for $AUC_{0\text{-}30d}$, unlike that observed with NET, the pharmacokinetic parameters of E2 are very similar to the% CV reported in E2 pharmacokinetic studies in women[67] (AUC = 57%, Cmax = 41%, tmax = 44%).

Previous research in women has reported high inter-subject variability with respect to plasma steroid levels[68], the reasons for the variability have not been fully elucidated but it may be due to the first step effect as suggested by Back[69].

Some of the factors that could also affect variability were evaluated. All the subjects met the selection criteria and all the samples were taken at a similar time of the day, the lags in the time of taking some samples (Table 3) in the first place do not correspond to subjects with extreme values and on the other side were presented on days in which the drugs were already in the elimination stage.

3 subjects with extreme values (44, 50, and 56) were identified, these subjects did not present any clinical reason that could justify the extreme values in their NET concentrations. Except that two of these three subjects ingested concomitant medication for the treatment of adverse events (Table 2). However, the days in which the subjects ingested the concomitant medication do not correspond to the days in which they presented extreme values and on the other hand, it was not found in the literature that there is any interaction between the concomitant medication that the subjects ingested (amoxicillin, celecoxib, LM6) with the metabolism of NET or E2.

Obesity (BMI >30 kg/m^2) has been reported to affect normal gonadotropin levels in adults[70]. Although 12 of the 32 subjects have a BMI greater than 25 kg/m^2, which is considered overweight, it is not considered that this may have impacted on the concentration and effect of NET and E2 in plasma.

Regarding adverse events, none were related to the investigational product except for one (erectile dysfunction), which was classified as a doubtful relationship. Although this event could be related to the administered investigational product, erectile dysfunction integrates medical, psychological, and social aspects[71] the fact of participating in a research study and receiving a hormonal product that includes estrogen predisposes the subject in a certain way to think that their sexuality may be affected, in any case it would be necessary to carry out future clinical trials that include more objective evaluations of this type of effects.

13. CONCLUSIONS

13.1. The suppression of total T in healthy Mexican men after a dose of 50 mg of NETE and 5 mg of EV was presented as follows: total T levels on day 0 prior to the administration of the combination, were 5.03 ± 1.30 ng/mL, these values were suppressed to 0.61 ± 0.55 ng/mL for study day 14, after the

administration of the investigational product and recovered to 3.75 ± 1.16 ng/mL for day 30 study ($p<0.05$). The maximum concentration of NET was presented on average on day 7, coinciding with the day in which the T values were at the minimum, which would confirm the direct relationship between NET and total T suppression. Regarding E2, the maximum concentration in plasma occurred on average on day 5, coinciding with the days in which total T was gradually and intensely suppressed; from day 5 and 6 the suppression of total T was stopped, although to continue in a lesser amount until day 7.

13.2. The mean Cmax of NET was 401.37 ± 313.60 pg/mL, $AUC_{0\text{-}30d}$ was 5,820.33 ± 2,105.51 day * pg/mL, and tmax was 7.17 ± 5 days. The coefficient of variation of the AUC of NET (36.18%) shows that the amount of NET that entered the body of the subjects studied was similar, however the coefficient of variation of the Cmax of NET (78.13%) shows us that the speed at the that the NET was absorbed was very different.

13.3. The mean Cmax of E2 was 353.08 ± 144.79 pg/mL, $AUC_{0\text{-}30d}$ was 2293.19 ± 589.89 day * pg/mL, and the tmax was 2.59 ± 1.15 days. The amount of E2 that entered the body is similar in all the subjects studied (coefficient of variation of $AUC_{0\text{-}30d}$ = 25.72%) and the speed at which E2 entered is different, although not in the measure of the NET (coefficient of variation of Cmax = 41.01%).

13.4. There were 9 non-serious adverse events, of which only one had a doubtful relationship with the drug.

14. PERSPECTIVES

Based on the results of this work, it would be useful and interesting to conduct controlled studies with arms that include NET + E2, with a control group with only NET and possibly another group with placebo, and take as efficacy variables, FSH, LH, T total and sperm count and motility, in addition to conducting surveys that evaluate the impact of these medications on the libido of the participants. It would also be necessary to investigate dose escalation and contraceptive schemes that initially include one or a maximum of two doses of EV followed by periodic doses and standards of NETE. The use of a suitable delivery system such as the once-a-month injectable could ensure greater acceptability and compliance by men to this mode of contraception.

Combinations like these are also useful for correcting precocious puberty. Its use as an orchidectomy replacement treatment in some types of prostatic hypertrophy would need to be verified.

15. LITERATURE

1. Trussell J, Wynn LL. Reducing unintended pregnancy in the United States. *Contraception* 2008;77(1):1–5.

2. González Labrador I, Miyar Pieiga E. Consideraciones sobre planificación familiar: métodos anticonceptivos. *Revista Cubana de Medicina General Integral* 2001;17:367–78.

3. Weber RF, Dohle GR. Male contraception: mechanical, hormonal and non-hormonal methods. *World J Urol* 2003;21(5):338–40.

4. Heinemann K, Saad F, Wiesemes M, Heinemann LA. Expectations toward a novel male fertility control method and potential user types: results of a multinational survey. *J Androl* 2005;26(2):155–62.

5. Handelsman DJ, Farley TM, Peregoudov A, Waites GM. Factors in nonuniform induction of azoospermia by testosterone enanthate in normal men. World Health Organization Task Force on Methods for the Regulation of Male Fertility. *Fertil Steril* 1995;63(1):125–33.

6. Martin CW, Anderson RA, Cheng L, Ho PC, van der Spuy Z, Smith KB, et al. Potential impact of hormonal male contraception: cross-cultural implications for development of novel preparations. *Hum Reprod* 2000;15(3):637–45.

7. Glasier AF, Anakwe R, Everington D, Martin CW, van der Spuy Z, Cheng L, et al. Would women trust their partners to use a male pill? *Hum Reprod* 2000;15(3):646–9.

8. Heinemann K SF, Wiesemes M, White S, Heinemann L Attitudes toward male fertility control: results of a multinational survey on four continents. *Hum Reprod* 2005;20:549–56.

9. Page ST, Amory JK, Bremner WJ. Advances in male contraception. *Endocr Rev* 2008;29(4):465–93.

10. Meriggiola MC, Cerpolini S, Bremner WJ, Mbizvo MT, Vogelsong KM, Martorana G, et al. Acceptability of an injectable male contraceptive regimen of norethisterone enanthate and testosterone undecanoate for men. *Hum Reprod* 2006;21(8):2033–40.

11. Mosher WD, Martinez GM, Chandra A, Abma JC, Willson SJ. Use of contraception and use of family planning services in the United States: 1982-2002. *Adv Data* 2004(350):1–36.

12. Piccinino LJ MW. Trends in contraceptive use in the United States. *Fam Plann Perspect* 1998;30:4–10, 46.

13. United Nations Department of Economic and Social Affairs PD. World contraceptive use 2005. 2005.

14. Fu H, Darroch JE, Haas T, Ranjit N. Contraceptive failure rates: new estimates from the 1995 National Survey of Family Growth. *Fam Plann Perspect* 1999;31(2):56–63.

15. Trussell J. Contraceptive failure in the United States. *Contraception* 2004;70(2):89–96.

16. Grady WR, Klepinger DH, Billy JO, Tanfer K. Condom characteristics: the perceptions and preferences of men in the United States. *Fam Plann Perspect* 1993;25(2):67–73.

17. Potter WD, de Villemeur M. Clinical breakage, slippage and acceptability of a new commercial polyurethane condom: a randomized, controlled study. *Contraception* 2003;68(1):39–45.

18. Steiner MJ, Dominik R, Rountree RW, Nanda K, Dorflinger LJ. Contraceptive effectiveness of a polyurethane condom and a latex condom: a randomized controlled trial. *Obstet Gynecol* 2003;101(3):539–47.

19. Walsh TL, Frezieres RG, Peacock K, Nelson AL, Clark VA, Bernstein L. Evaluation of the efficacy of a nonlatex condom: results from a randomized, controlled clinical trial. *Perspect Sex Reprod Health* 2003;35(2):79–86.

20. Levy DA, Khouader S, Leynadier F. Allergy to latex condoms. *Allergy* 1998;53(11):1107–8.

21. Turjanmaa K. Allergy to natural rubber latex: a growing problem. *Ann Med* 1994;26(4):297–300.

22. Gallo MF, Grimes DA, Schulz KF. Nonlatex vs. latex male condoms for contraception: a systematic review of randomized controlled trials. *Contraception* 2003;68(5):319–26.

23. Frezieres RG, Walsh TL, Nelson AL, Clark VA, Coulson AH. Evaluation of the efficacy of a polyurethane condom: results from a randomized, controlled clinical trial. *Fam Plann Perspect* 1999;31(2):81–7.

24. Magnani RJ, Haws JM, Morgan GT, Gargiullo PM, Pollack AE, Koonin LM. Vasectomy in the United States, 1991 and 1995. *Am J Public Health* 1999;89(1):92–4.

25. Peterson HB, Curtis KM. Clinical practice. Long-acting methods of contraception. *N Engl J Med* 2005;353(20):2169–75.

26. Jamieson DJ, Costello C, Trussell J, Hillis SD, Marchbanks PA, Peterson HB. The risk of pregnancy after vasectomy. *Obstet Gynecol* 2004;103(5 Pt 1):848–50.

27. Philp T, Guillebaud J, Budd D. Complications of vasectomy: review of 16,000 patients. *Br J Urol* 1984;56(6):745–8.

28. Schmidt SS. Vasectomy by section, luminal fulguration and fascial interposition: results from 6248 cases. *Br J Urol* 1995;76(3):373–4; discussion 75.

29. Kumar V, Kaza RM, Singh I, Singhal S, Kumaran V. An evaluation of the no-scalpel vasectomy technique. *BJU Int* 1999;83(3):283–4.

30. Awsare NS, Krishnan J, Boustead GB, Hanbury DC, McNicholas TA. Complications of vasectomy. *Ann R Coll Surg Engl* 2005;87(6):406–10.

31. Morris C, Mishra K, Kirkman RJ. A study to assess the prevalence of chronic testicular pain in post-vasectomy men compared to nonvasectomised men. *J Fam Plann Reprod Health Care* 2002;28(3):142–4.

32. Schwingl PJ, Guess HA. Safety and effectiveness of vasectomy. *Fertil Steril* 2000;73(5):923–36.

33. Myers SA, Mershon CE, Fuchs EF. Vasectomy reversal for treatment of the post-vasectomy pain syndrome. *J Urol* 1997;157(2):518–20.

34. Jequier AM. Vasectomy related infertility: a major and costly medical problem. *Hum Reprod* 1998;13(7):1757–9.

35. Potts JM, Pasqualotto FF, Nelson D, Thomas AJ, Jr., Agarwal A. Patient characteristics associated with vasectomy reversal. *J Urol* 1999;161(6):1835–9.

36. Kerrigan JR, Veldhuis JD, Rogol AD. Androgen-receptor blockade enhances pulsatile luteinizing hormone production in late pubertal males: evidence for a hypothalamic site of physiologic androgen feedback action. *Pediatr Res* 1994;35(1):102–6.

37. Knuth UA, Hano R, Nieschlag E. Effect of flutamide or cyproterone acetate on pituitary and testicular hormones in normal men. *J Clin Endocrinol Metab* 1984;59(5):963–9.

38. Veldhuis JD, Urban RJ, Dufau ML. Evidence that androgen negative feedback regulates hypothalamic gonadotropin-releasing hormone impulse strength and the burst-like secretion of biologically active luteinizing hormone in men. *J Clin Endocrinol Metab* 1992;74(6):1227–35.

39. Vermeulen A, Deslypere JP. Long-term transdermal dihydrotestosterone therapy: effects on pituitary gonadal axis and plasma lipoproteins. *Maturitas* 1985;7(3):281–7.

40. Finkelstein JS, Whitcomb RW, O'Dea LS, Longcope C, Schoenfeld DA, Crowley WF, Jr. Sex steroid control of gonadotropin secretion in the human male. I. Effects of testosterone administration in normal and gonadotropin-releasing hormone-deficient men. *J Clin Endocrinol Metab* 1991;73(3):609–20.

41. Hayes FJ, Seminara SB, Decruz S, Boepple PA, Crowley WF, Jr. Aromatase inhibition in the human male reveals a hypothalamic site of estrogen feedback. *J Clin Endocrinol Metab* 2000;85(9):3027–35.

42. Raven G, de Jong FH, Kaufman JM, de Ronde W. In men, peripheral estradiol levels directly reflect the action of estrogens at the hypothalamo-pituitary level to inhibit gonadotropin secretion. *J Clin Endocrinol Metab* 2006;91(9):3324–8.

43. Rochira V, Zirilli L, Genazzani AD, Balestrieri A, Aranda C, Fabre B, et al. Hypothalamic-pituitary-gonadal axis in two men with aromatase deficiency: evidence that circulating estrogens are required at the hypothalamic level for the integrity of gonadotropin negative feedback. *Eur J Endocrinol* 2006;155(4):513–22.

44. Bagatell CJ, Dahl KD, Bremner WJ. The direct pituitary effect of testosterone to inhibit gonadotropin secretion in men is partially mediated by aromatization to estradiol. *J Androl* 1994;15(1):15–21.

45. Greenspan F-GD. Endocrinología básica y clínica.

46. Anderson RA, Wallace AM, Wu FC. Comparison between testosterone enanthate-induced azoospermia and oligozoospermia in a male contraceptive study. III. Higher 5 alpha-reductase activity in oligozoospermic men administered supraphysiological doses of testosterone. *J Clin Endocrinol Metab* 1996;81(3):902–8.

47. Horton R. Sex steroid production and secretion in the male. *Andrologia* 1978;10(3):183–94.

48. Vierhapper H, Nowotny P, Waldhausl W. Determination of testosterone production rates in men and women using stable isotope/dilution and mass spectrometry. *J Clin Endocrinol Metab* 1997;82(5):1492–6.

49. Leymarie P, Roger M, Castanier M, Scholler R. Circadian variations of plasma testosterone and estrogens in normal men. A study by frequent sampling. *J Steroid Biochem* 1974;5(2):167–71.

50. Organization WH. Contraceptive efficacy of testosterone- induced azoospermia and oligospermia in normal men. *Fertil Steril* 1996;65:821–29.

51. Handelsman DJ. Editorial: hormonal male contraception--lessons from the East when the Western market fails. *J Clin Endocrinol Metab* 2003;88(2):559–61.

52. Wang C, Wang XH, Nelson AL, Lee KK, Cui YG, Tong JS, et al. Levonorgestrel implants enhanced the suppression of spermatogenesis by testosterone implants: comparison between Chinese and non-Chinese men. *J Clin Endocrinol Metab* 2006;91(2):460–70.

53. Grimes DA, Lopez LM, Gallo MF, Halpern V, Nanda K, Schulz KF. Steroid hormones for contraception in men. *Cochrane Database Syst Rev* 2007(2):CD004316.

54. Qoubaitary A, Meriggiola C, Ng CM, Lumbreras L, Cerpolini S, Pelusi G, et al. Pharmacokinetics of testosterone undecanoate injected alone or in combination with norethisterone enanthate in healthy men. *J Androl* 2006;27(6):853–67.

55. Vogelsong KM. Métodos hormonales de regulación de la fertilidad masculina. *Boletín Médico de IPPF Septiembre* 2006;40(3):4.

56. Meriggiola MC CA, Saad F, D'Emidio L, Morselli L, Bertaccini A et al. . Norethisterone enanthate plus testosterone undecanoate for male contraception: Effects of various injection intervals on spermatogenesis, reproductive hormones, testis, and prostate. *The Journal of Clinical Endocrinology & Metabolism* 2005;90(1):2005–14.

57. Shetty G, Krishnamurthy H, Krishnamurthy HN, Ramachandra SG, Moudgal NR. Use of norethisterone and estradiol in mini doses as a contraceptive in the male. Efficacy studies in the adult male bonnet monkey (Macaca radiata). *Contraception* 1997;56(4):257–65.

58. K. M. Prasanna Kumar HK, H. N. Krishnamurthy, G. Shetty and N. R. Moudgal Once-a-month injection of norethisterone enanthate and estradiol valerate combination suppresses pituitary FSH/LH secretion and testicular function in man. *Current Science* 2002;82(5):550–53.

59. Trunet PF, Mueller P, Bhatnagar AS, Dickes I, Monnet G, White G. Open dose-finding study of a new potent and selective nonsteroidal aromatase inhibitor, CGS 20 267, in healthy male subjects. *J Clin Endocrinol Metab* 1993;77(2):319–23.

60. Salud Sd. Farmacopea de los Estados Unidos Mexicanos. In: México SdS, editor. Septima Edición ed. Tomo I, 2000:766,67, 888,89.

61. www.shering.com.ar AeM.

62. PLM. T. Diccionario de especialidades farmacéuticas. In: PLM E, editor. *Diccionario de especialidades farmacéuticas*., 2010:1790, 2287-90, 543-4,912-3.

63. Bulent T NV, Fener N, Guner H, Yildirim M, Darney PD. Effects of a monthly injectable steroidal contraceptives, Mesigyna, on menstrual pattern, lipoproteins, and coagulation parameters. Gazi University School of Medicine; Dept. of Obstetrics and Gynecology, Ankara, Turkey.

64. Sang GW, Liu XH. Pharmacokinetics of norethindrone oenanthate in 5 women after a single dose of norethindrone oenanthate and estradiol valerate. *Zhongguo Yao Li Xue Bao* 1986;7(3):255–9.

65. Sang GW LX. Pharmacokinetics of norethindrone oenanthate 200 mg after intramuscular injection in 25 Chinese woman. *Act Pharmacol Sin* 1991 1991:12:184.

66. Beal SL. Ways to fit a PK model with some data below the quantification limit. *J Pharmacokinet Pharmacodyn* 2001;28(5):481–504.

67. Brody SA, Turkes A, Goldzieher JW. Pharmacokinetics of three bioequivalent norethindrone/mestranol-50 micrograms and three norethindrone/ethinyl estradiol-35 micrograms OC formulations: are "low-dose" pills really lower? *Contraception* 1989;40(3):269–84.

68. Fotherby K, Hamawi A, Howard G, Bye PG, Elder M. Pharmacokinetics of different doses of norethisterone oenanthate. *Contraception* 1984;29(4):325–33.

69. Back DJ, Breckenridge AM, Crawford FE, MacIver M, Orme ML, Rowe PH, et al. An investigation of the pharmacokinetics of ethynylestradiol in women using radioimmunoassay. *Contraception* 1979;20(3):263–73.

70. Rosenfield RL, Bordini B. Evidence that obesity and androgens have independent and opposing effects on gonadotropin production from puberty to maturity. *Brain Res* 2010;1364:186–97.

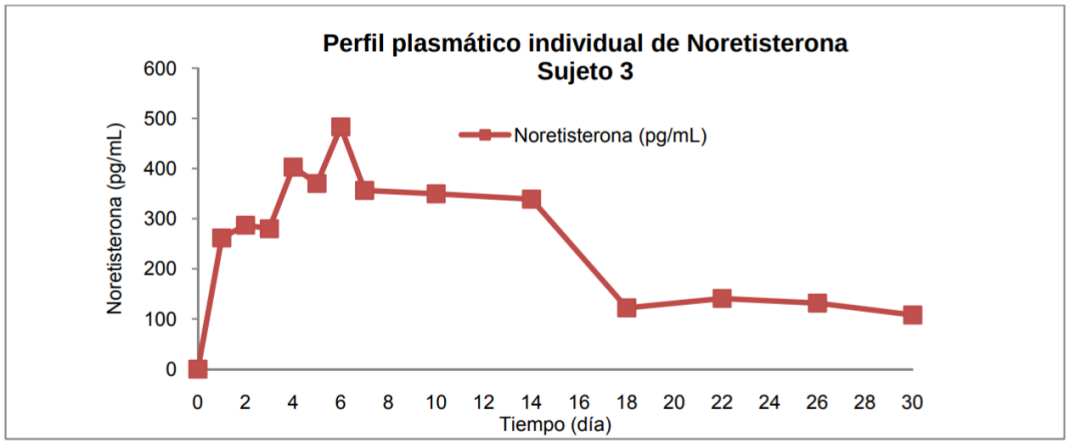
71. Borras-Valls JJ, Gonzalez-Correales R. Specific aspects of erectile dysfunction in sexology. *Int J Impot Res* 2004;16 Suppl 2:S3–6.

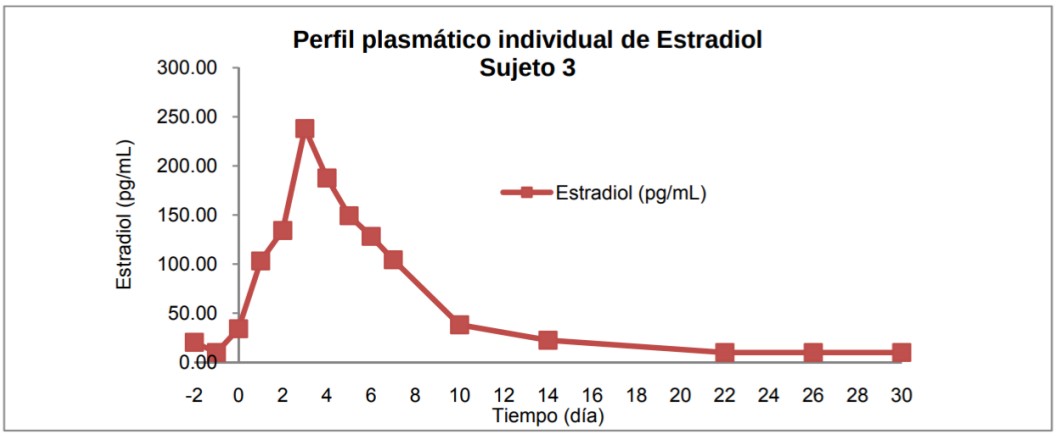
16. ANNEXES

16.1. APPENDIX 1

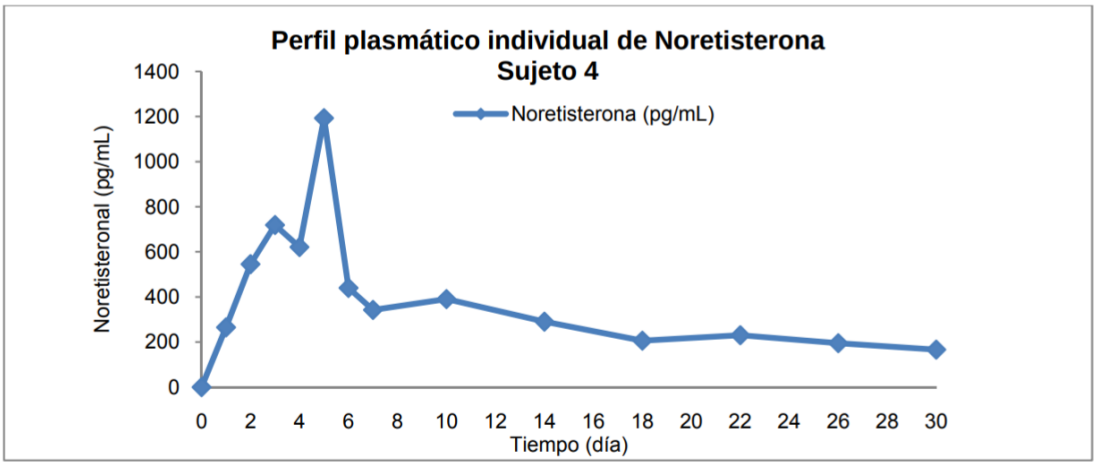
Data on plasma norethisterone and estradiol concentrations vs. time per subject and individual plasma profiles.

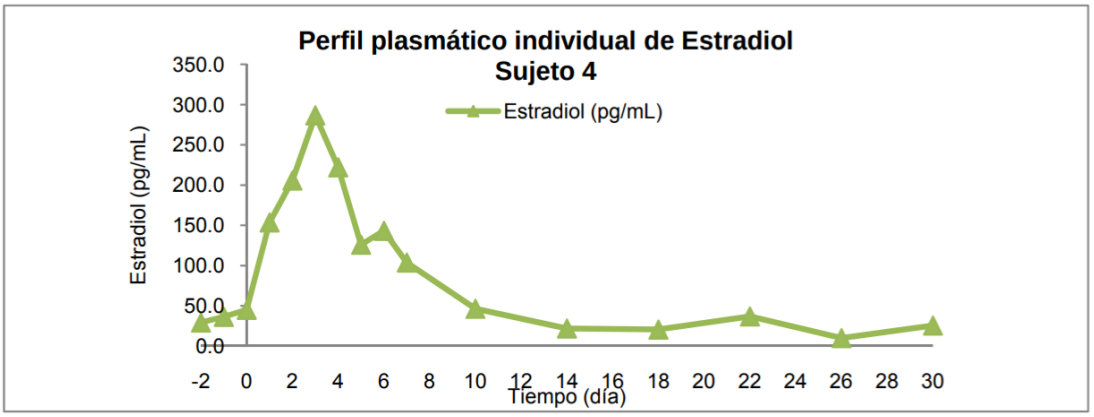
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
3	-2			20.40	20.4
3	-1			10.00	14.2
3	0	0.00		34.20	34.2
3	1	261.60		103.20	103.2
3	2	287.00		134.30	134.3
3	3	279.90		238.10	238.1
3	4	402.70		187.70	187.7
3	5	370.10		149.30	149.3
3	6	483.00		128.30	128.3
3	7	356.30		104.40	104.4
3	10	349.60		38.20	38.2
3	14	338.80		22.50	22.5
3	18	122.10		0.00	4.7
3	22	140.90		10.00	17.3
3	26	131.70		10.00	16.3
3	30	107.90		10.00	14.8



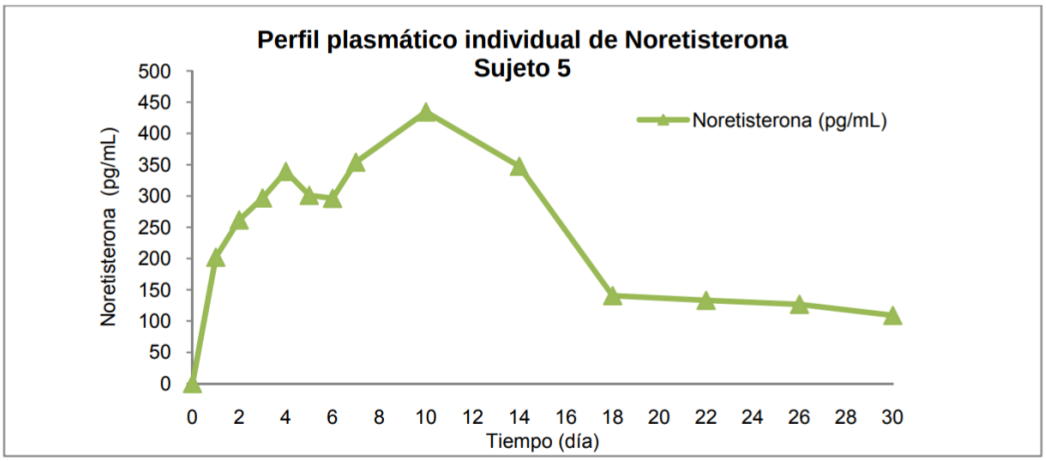


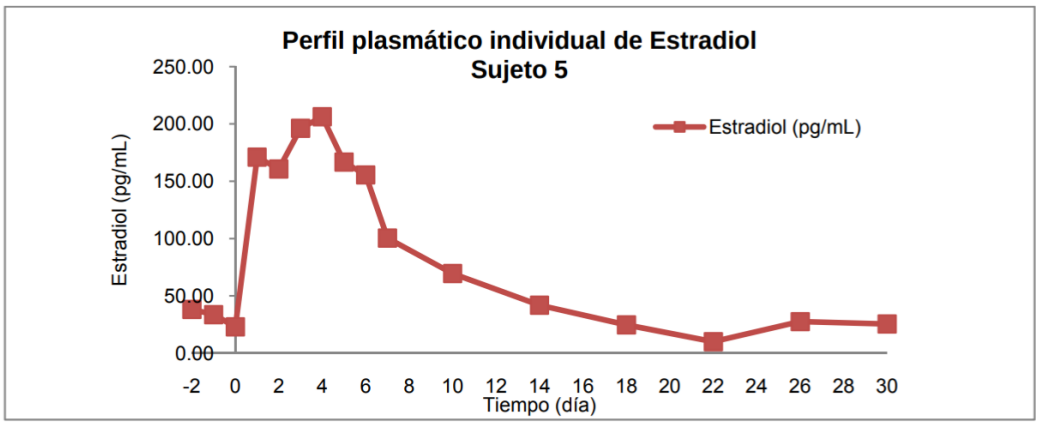
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
4	-2			29.30	29.3
4	-1			36.40	36.4
4	0	0.00		45.10	45.1
4	1	265.30		153.60	153.6
4	2	545.30		205.80	205.8
4	3	719.10		286.70	286.7
4	4	621.20		222.20	222.2
4	5	1,193.00		125.90	125.9
4	6	439.90		143.20	143.2
4	7	342.40		103.80	103.8
4	10	390.50		46.40	46.4
4	14	290.50		21.90	21.9
4	18	206.00		20.60	20.6
4	22	230.20		36.90	36.9
4	26	194.90		10.00	15.0
4	30	166.40		25.60	25.6



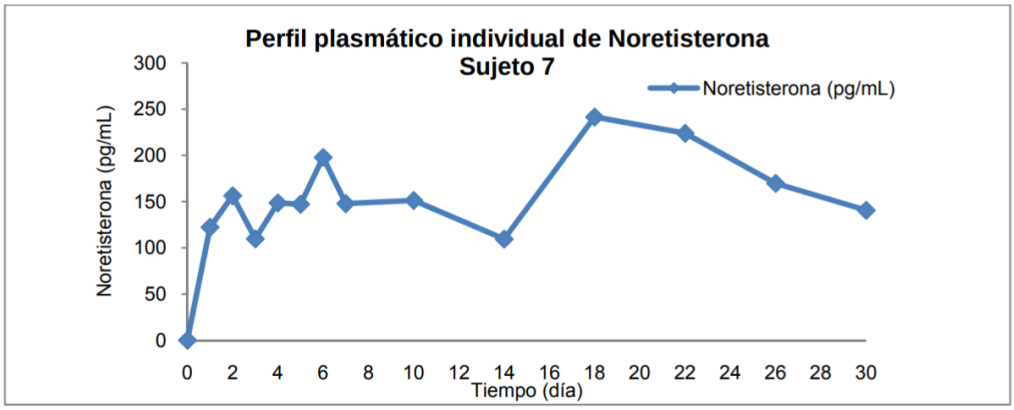


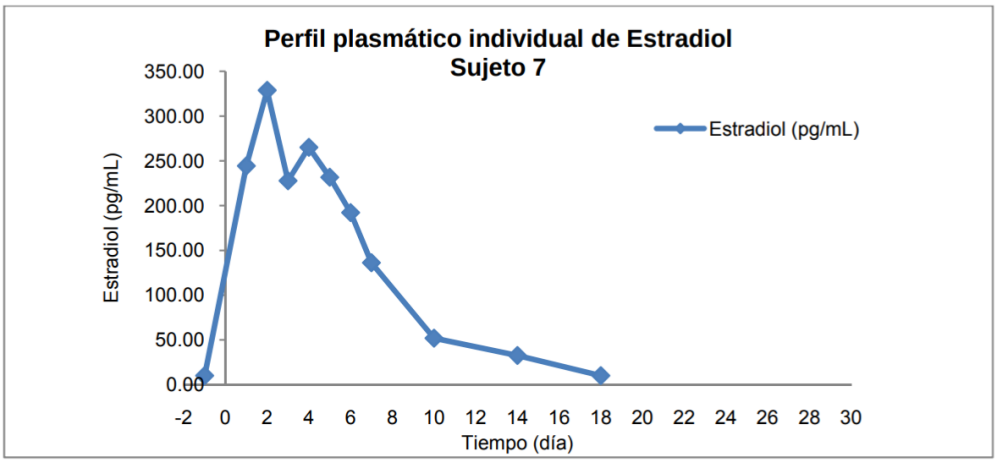
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
5	-2			38.20	38.2
5	-1			33.60	33.6
5	0	0.00		23.00	23.0
5	1	202.00		171.00	171.0
5	2	261.70		160.70	160.7
5	3	296.80		196.20	196.2
5	4	339.20		206.30	206.3
5	5	301.00		166.70	166.7
5	6	296.20		155.50	155.5
5	7	354.00		100.40	100.4
5	10	434.40		69.50	69.5
5	14	347.50		41.80	41.8
5	18	140.60		24.70	24.7
5	22	133.00		10.00	14.8
5	26	126.70		27.50	27.5
5	30	109.00		25.50	25.5



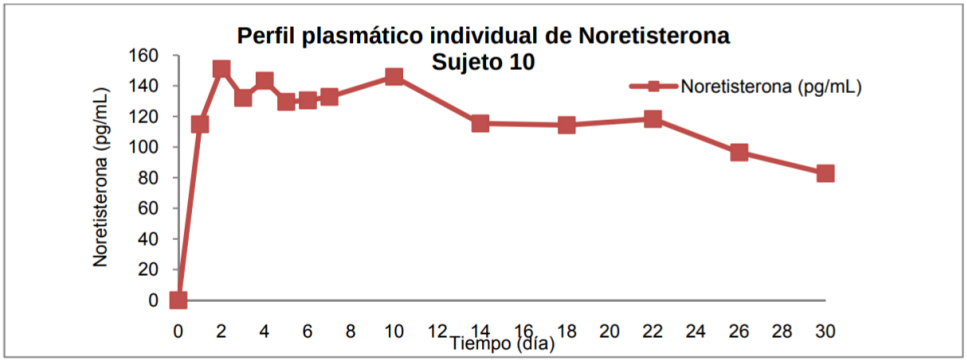


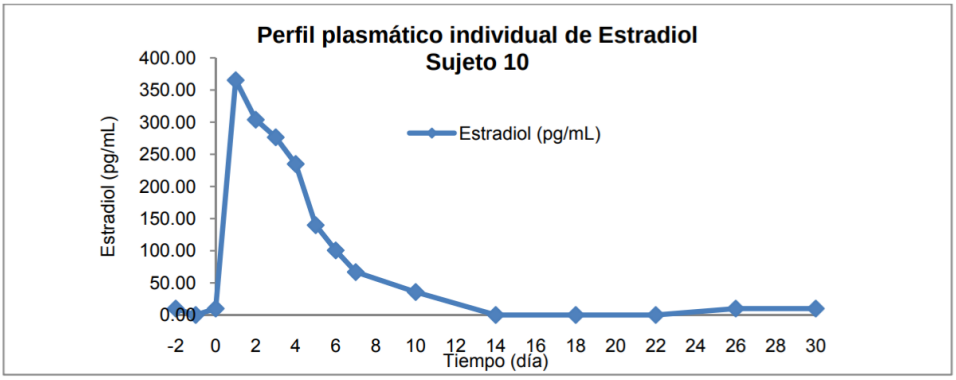
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
7	-2			0.00	1.3
7	-1			10.00	17.3
7	0	0.00		0.00	7.2
7	1	122.30		244.40	244.4
7	2	156.30		328.90	328.9
7	3	109.70		227.80	227.8
7	4	148.60		265.10	265.1
7	5	147.10		231.70	231.7
7	6	197.80		192.20	192.2
7	7	147.80		136.20	136.2
7	10	151.20		51.90	51.9
7	14	109.40		32.60	32.6
7	18	241.50		10.00	13.9
7	22	223.90		0.00	4.6
7	26	169.70		0.00	7.6
7	30	140.60		0.00	4.0



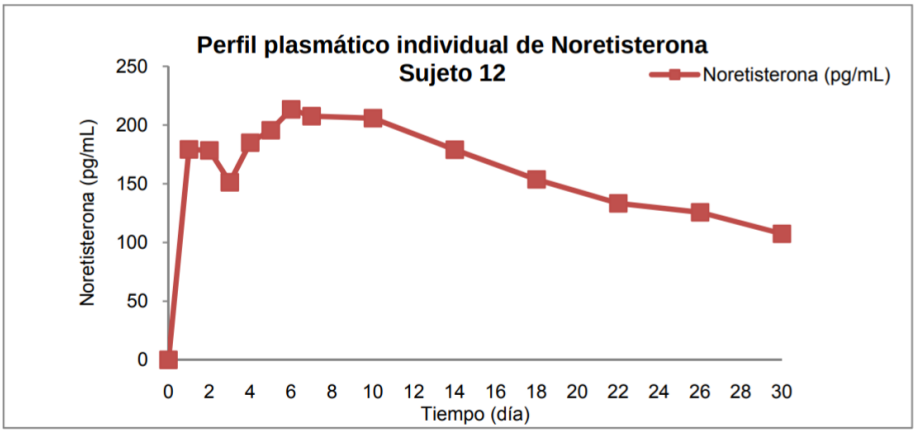


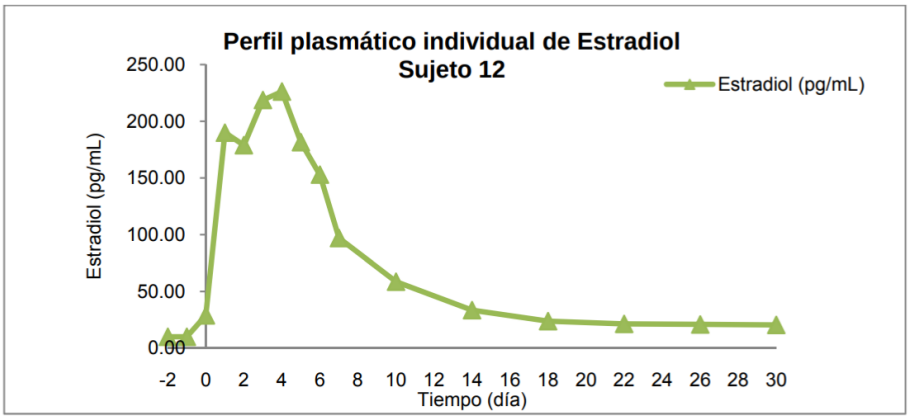
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
10	-2			10.00	11.6
10	-1			0.00	1.9
10	0	0.00		10.00	11.4
10	1	114.90		365.40	365.4
10	2	151.10		303.90	303.9
10	3	132.10		276.50	276.5
10	4	143.30		235.00	235.0
10	5	129.50		139.80	139.8
10	6	130.50		100.60	100.6
10	7	132.80		66.80	66.8
10	10	145.90		35.80	35.8
10	14	115.40		0.00	4.7
10	18	114.30		0.00	7.7
10	22	118.30		0.00	7.0
10	26	96.50		10.00	16.2
10	30	82.80		10.00	13.5



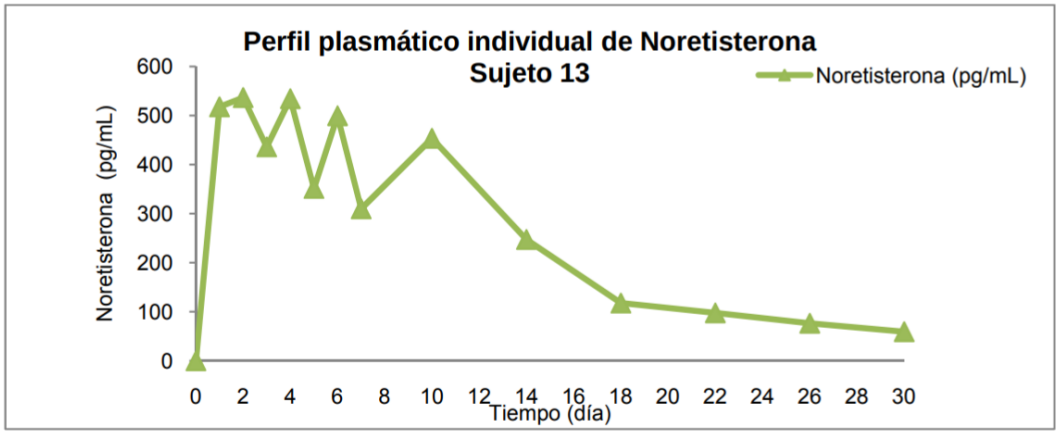


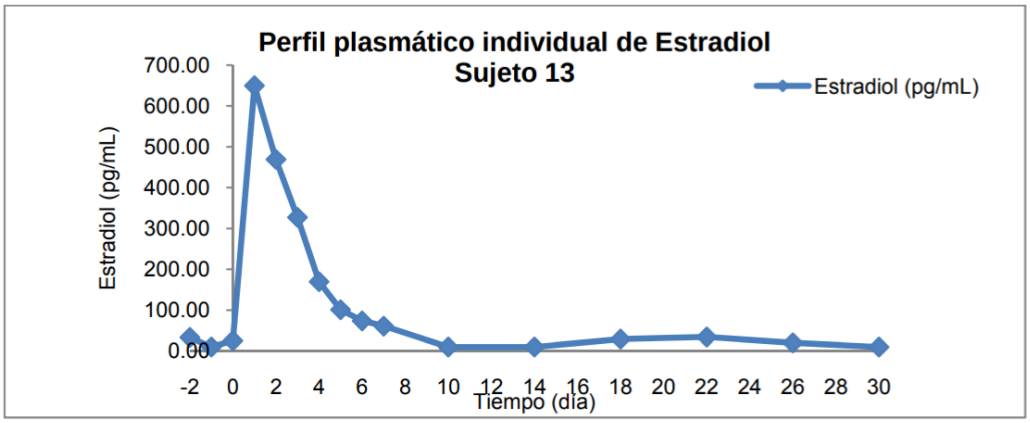
Subject	Time (day)	NET (pg/mL)	E2_10 (pg/mL)	E2 (pg/mL)
12	-2		10.00	16.4
12	-1		10.00	15.2
12	0	0.00	28.90	28.9
12	1	179.30	189.90	189.9
12	2	178.40	178.90	178.9
12	3	151.30	218.70	218.7
12	4	185.00	226.20	226.2
12	5	195.60	181.50	181.5
12	6	213.40	152.90	152.9
12	7	207.60	97.00	97.0
12	10	205.90	58.60	58.6
12	14	179.00	33.50	33.5
12	18	153.70	23.80	23.8
12	22	133.30	21.40	21.4
12	26	125.70	20.90	20.9
12	30	107.40	20.50	20.5



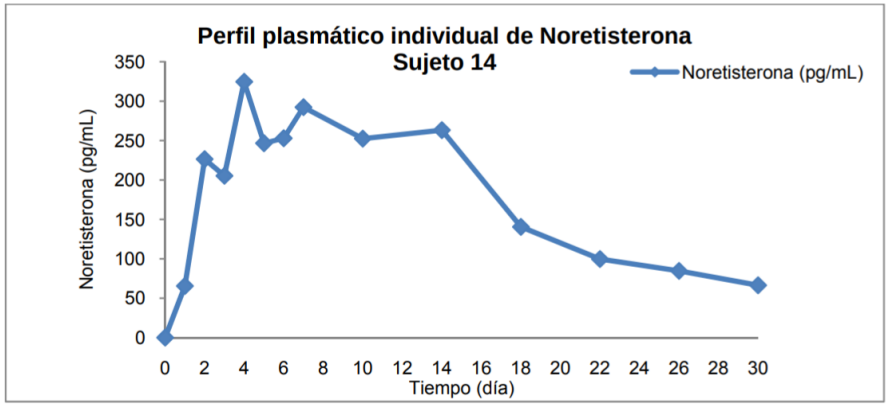


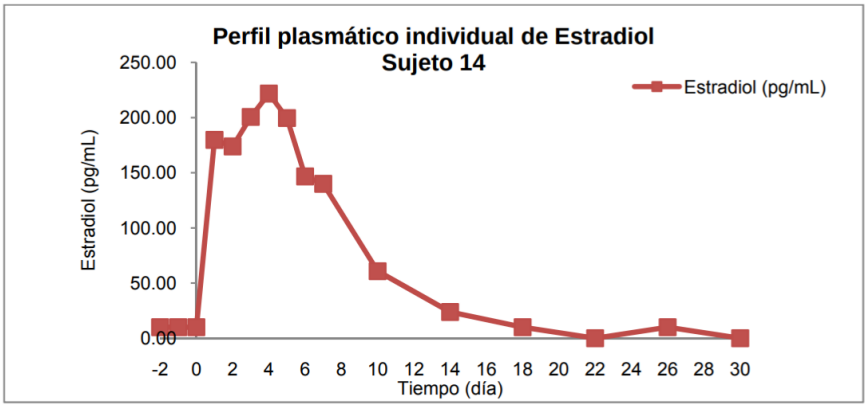
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
13	-2			33.50	33.5
13	-1			10.00	14.8
13	0	0.00		25.40	25.4
13	1	517.60		649.70	649.7
13	2	536.50		469.10	469.1
13	3	436.00		327.30	327.3
13	4	534.20		169.70	169.7
13	5	351.30		101.20	101.2
13	6	499.30		73.80	73.8
13	7	309.80		61.10	61.1
13	10	453.50		10.00	14.7
13	14	247.20		10.00	16.1
13	18	117.70		29.40	29.4
13	22	97.60		34.50	34.5
13	26	76.60		20.20	20.2
13	30	59.30		10.00	19.8



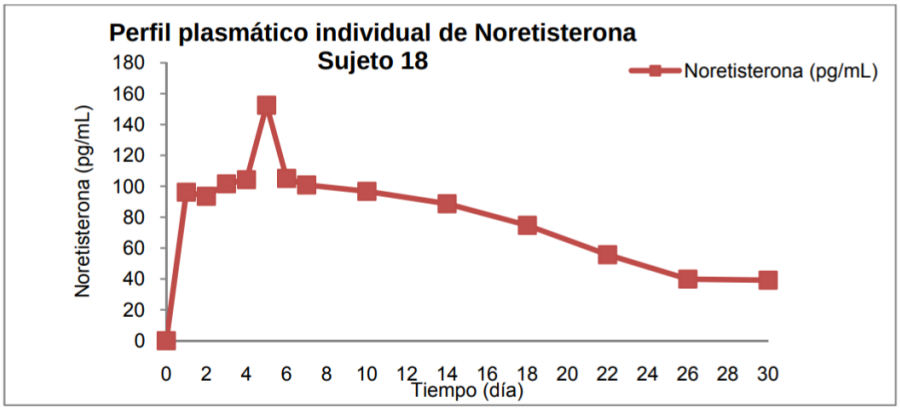


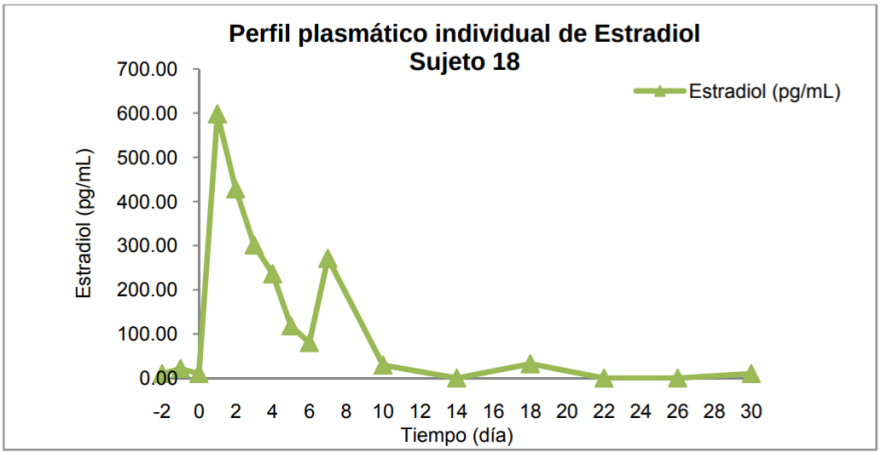
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
14	-2			10.00	17.5
14	-1			10.00	18.1
14	0	0.00		10.00	18.7
14	1	65.50		179.80	179.8
14	2	226.50		173.90	173.9
14	3	205.30		200.60	200.6
14	4	324.70		221.80	221.8
14	5	246.70		199.60	199.6
14	6	253.00		146.70	146.7
14	7	292.40		140.00	140.0
14	10	252.50		60.80	60.8
14	14	263.30		23.80	23.8
14	18	140.50		10.00	11.2
14	22	99.70		0.00	5.7
14	26	84.70		10.00	10.6
14	30	66.40		0.00	5.0



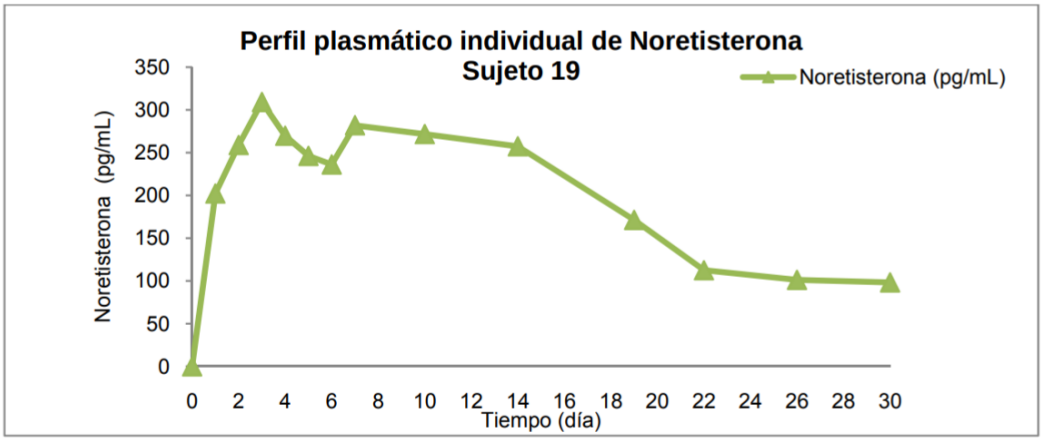


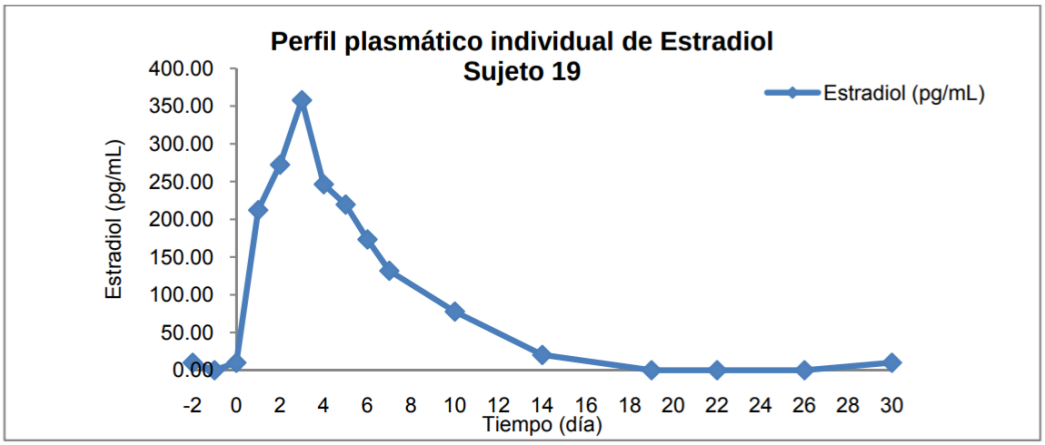
Subject	Time (day)	NET (pg/mL)	E2_10 (pg/mL)	E2 (pg/mL)
18	-2		10.00	17.7
18	-1		21.50	21.5
18	0	0.00	10.00	16.8
18	1	96.20	598.20	598.2
18	2	93.50	428.10	428.1
18	3	101.60	300.90	300.9
18	4	104.30	236.10	236.1
18	5	152.50	118.10	118.1
18	6	105.10	80.30	80.3
18	7	100.90	270.90	270.9
18	10	96.70	29.30	29.3
18	14	88.70	0.00	2.6
18	18	74.70	32.20	32.2
18	22	55.70	0.00	6.7
18	26	39.90	0.00	8.6
18	30	39.20	10.00	16.9



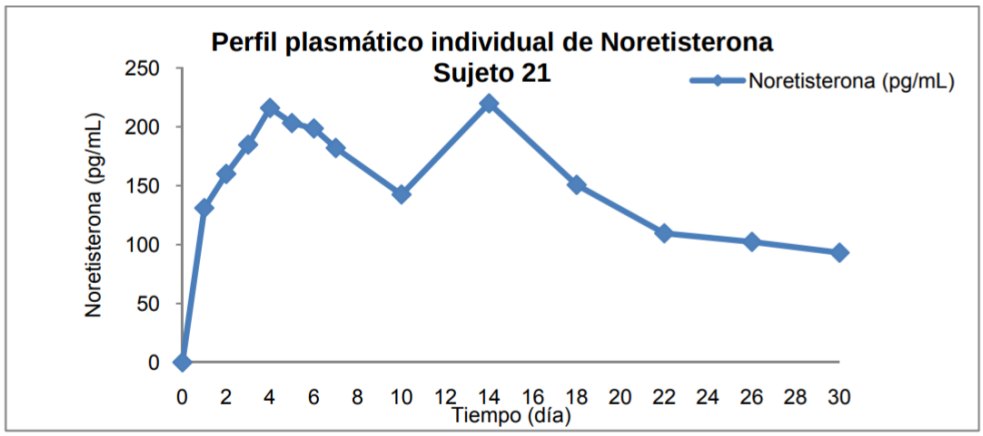


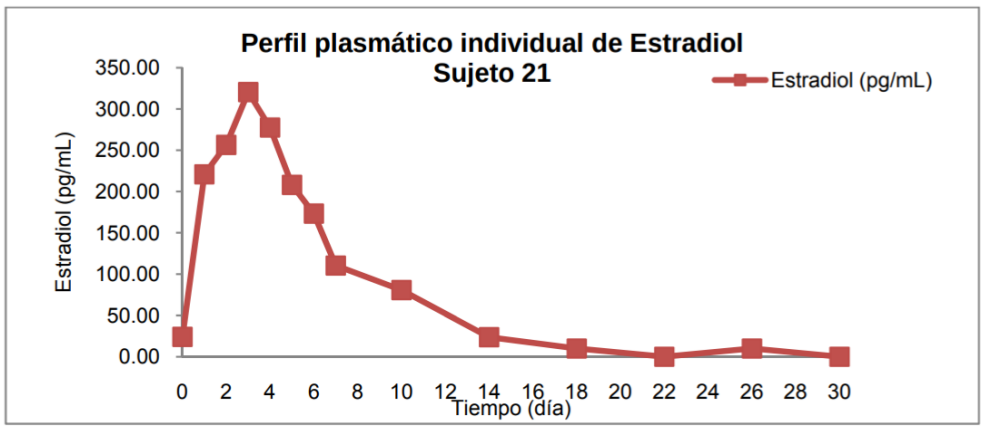
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
19	-2			10.00	14.4
19	-1			0.00	8.2
19	0	0.00		10.00	18.8
19	1	202.20		212.20	212.2
19	2	258.90		272.40	272.4
19	3	309.20		357.90	357.9
19	4	269.70		246.40	246.4
19	5	246.20		219.60	219.6
19	6	236.10		173.40	173.4
19	7	282.00		131.80	131.8
19	10	271.70		77.70	77.7
19	14	257.40		20.30	20.3
19	19	171.30		0.00	6.2
19	22	112.40		0.00	0.2
19	26	101.20		0.00	6.4
19	30	98.20		10.00	16.6



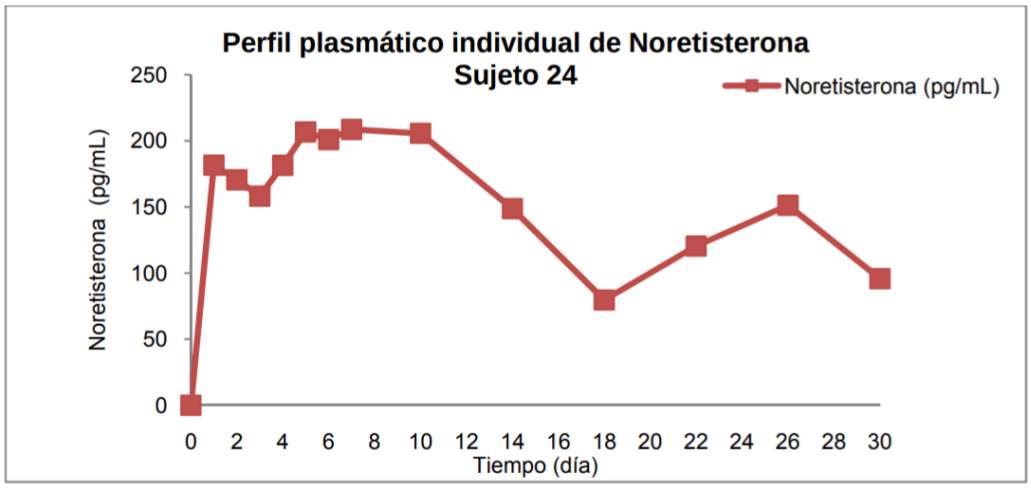


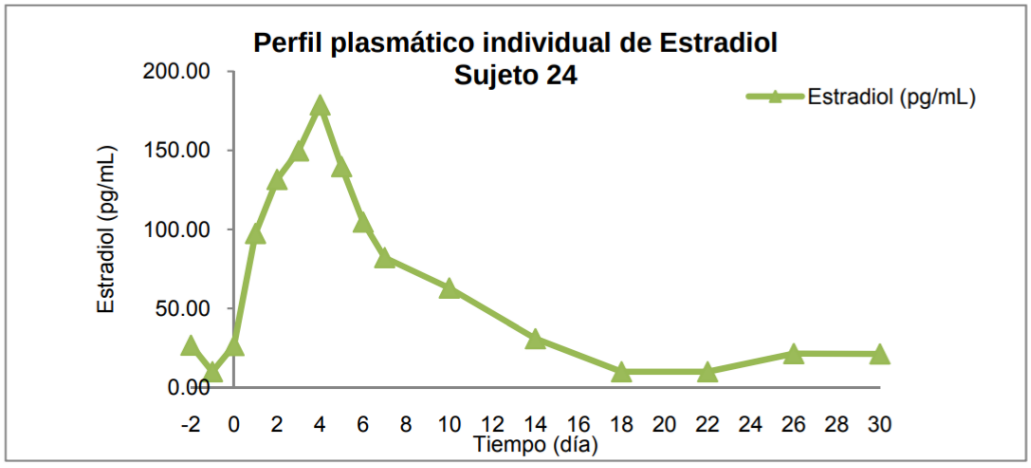
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
21	-2			25.00	25.0
21	-1			28.50	28.5
21	0	0.00		24.10	24.1
21	1	131.00		220.80	220.8
21	2	160.00		256.50	256.5
21	3	184.80		320.50	320.5
21	4	216.00		277.50	277.5
21	5	203.20		208.00	208.0
21	6	198.60		173.20	173.2
21	7	182.00		110.30	110.3
21	10	142.50		80.60	80.6
21	14	219.90		23.70	23.7
21	18	150.70		10.00	19.1
21	22	109.60		0.00	9.5
21	26	102.30		10.00	12.8
21	30	93.10		0.00	7.2



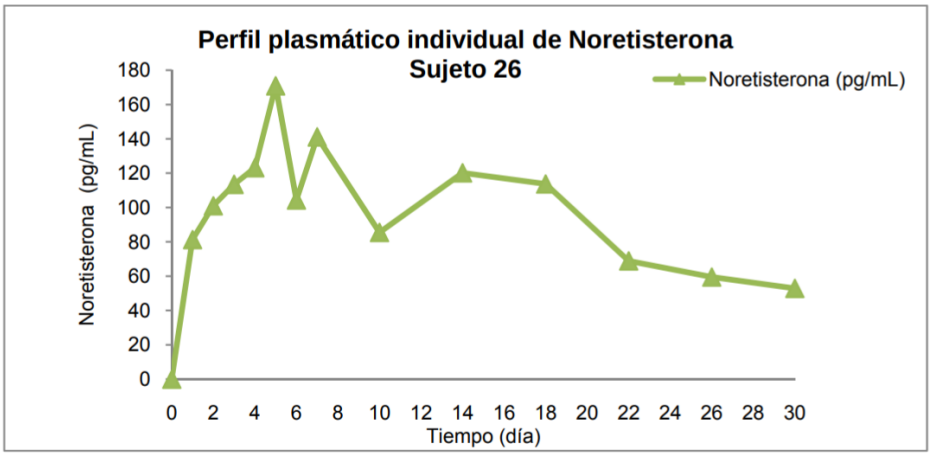


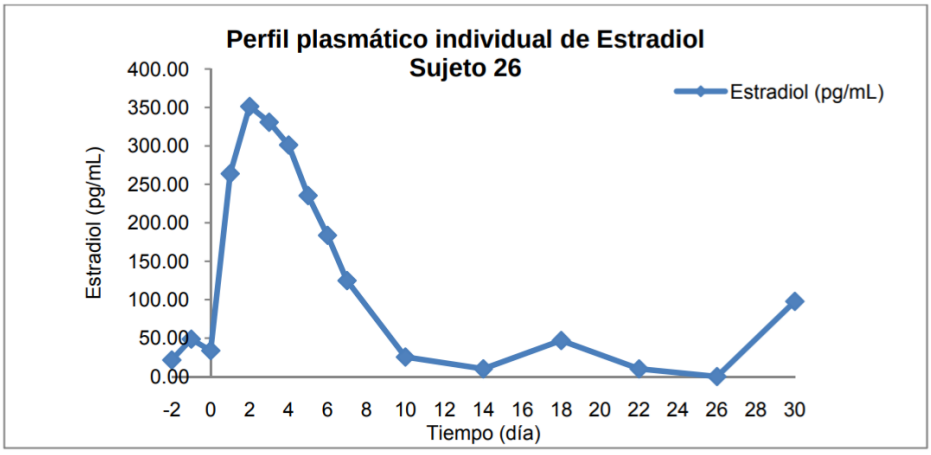
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
24	-2			26.80	26.8
24	-1			10.00	14.3
24	0	0.00		26.60	26.6
24	1	181.60		97.40	97.4
24	2	170.50		131.60	131.6
24	3	158.20		149.70	149.7
24	4	181.50		178.80	178.8
24	5	206.80		139.70	139.7
24	6	201.00		104.60	104.6
24	7	208.70		82.10	82.1
24	10	205.70		62.90	62.9
24	14	148.80		30.90	30.9
24	18	79.60		10.00	18.9
24	22	120.40		10.00	17.4
24	26	151.30		21.50	21.5
24	30	95.80		21.30	21.3



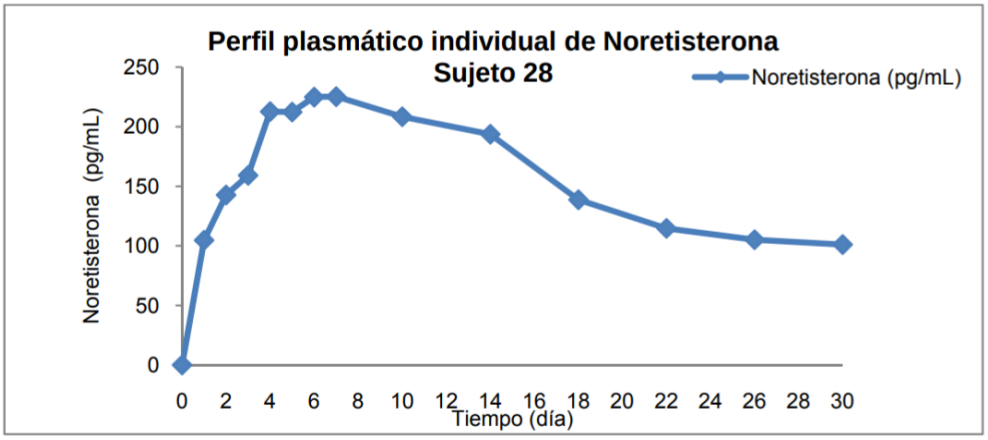


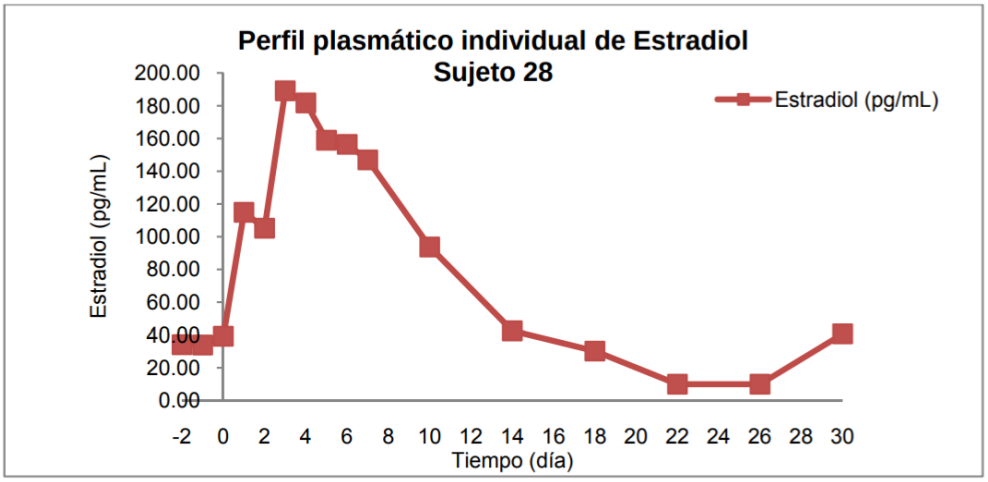
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
26	-2			21.50	21.5
26	-1			48.70	48.7
26	0	0.00		33.70	33.7
26	1	81.30		263.90	263.9
26	2	101.00		351.40	351.4
26	3	113.50		330.90	330.9
26	4	123.20		301.30	301.3
26	5	170.90		235.40	235.4
26	6	104.40		183.70	183.7
26	7	141.10		124.80	124.8
26	10	85.50		25.40	25.4
26	14	120.20		10.00	15.9
26	18	113.70		46.90	46.9
26	22	68.90		10.00	11.6
26	26	59.50		0.00	8.4
26	30	52.90		97.80	97.8



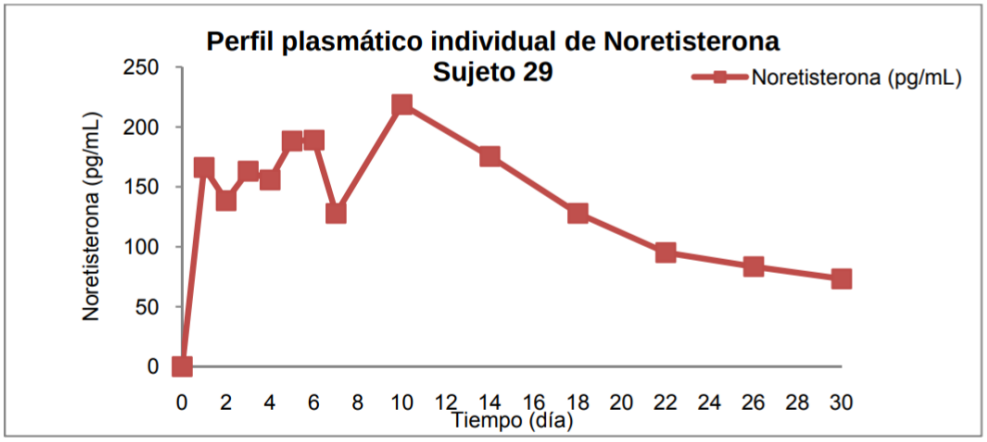


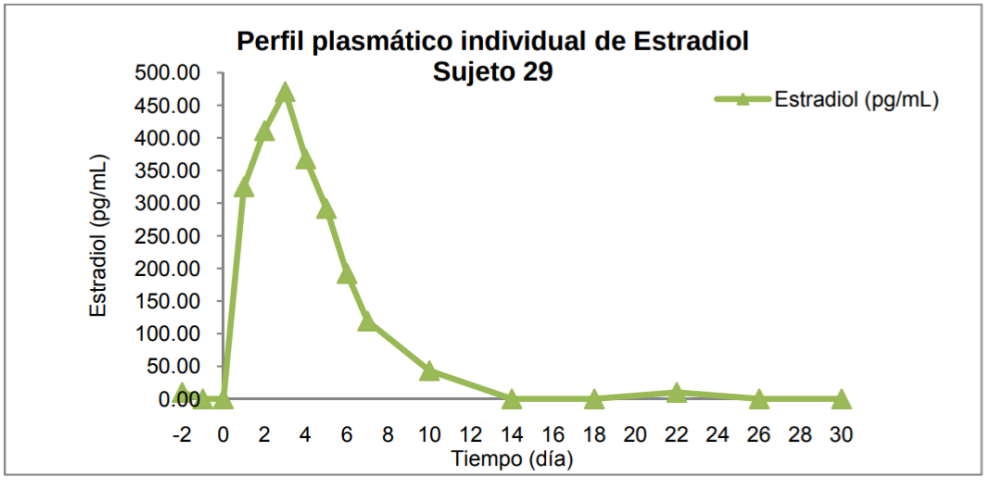
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
28	-2			34.10	34.1
28	-1			33.90	33.9
28	0	0.00		39.20	39.2
28	1	104.70		114.90	114.9
28	2	142.70		105.20	105.2
28	3	159.20		189.10	189.1
28	4	212.70		181.70	181.7
28	5	212.30		158.90	158.9
28	6	224.90		156.40	156.4
28	7	225.30		146.90	146.9
28	10	208.40		93.70	93.7
28	14	193.70		42.50	42.5
28	18	138.70		30.20	30.2
28	22	114.70		10.00	17.6
28	26	105.20		10.00	18.6
28	30	101.10		40.70	40.7



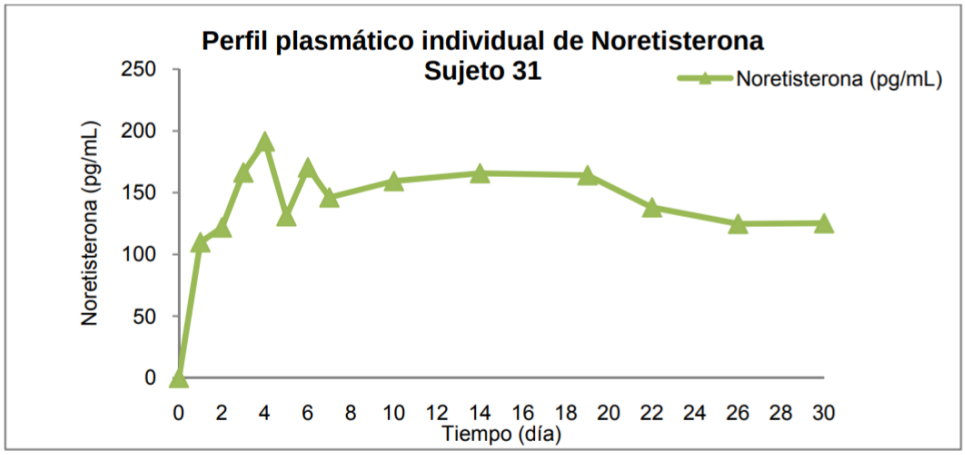


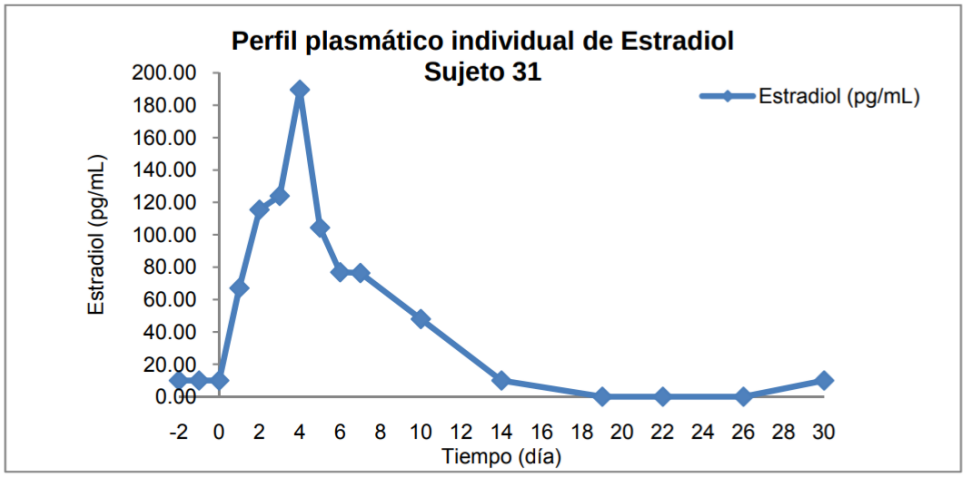
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
29	-2			10.00	12.3
29	-1			0.00	4.7
29	0	0.00		0.00	4.5
29	1	166.20		325.10	325.1
29	2	138.40		411.00	411.0
29	3	163.10		471.00	471.0
29	4	155.80		367.90	367.9
29	5	188.20		291.70	291.7
29	6	189.00		192.20	192.2
29	7	127.80		119.20	119.2
29	10	218.70		43.20	43.2
29	14	175.40		0.00	6.3
29	18	127.90		0.00	2.0
29	22	95.10		10.00	17.5
29	26	83.40		0.00	0.0
29	30	73.20		0.00	3.4



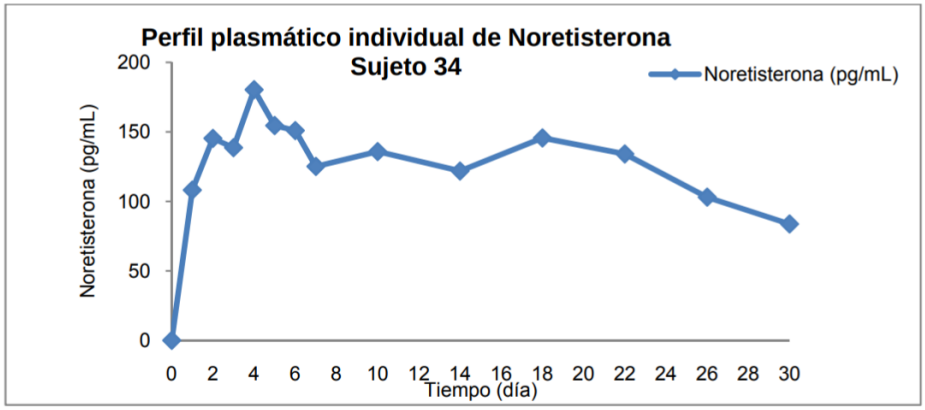


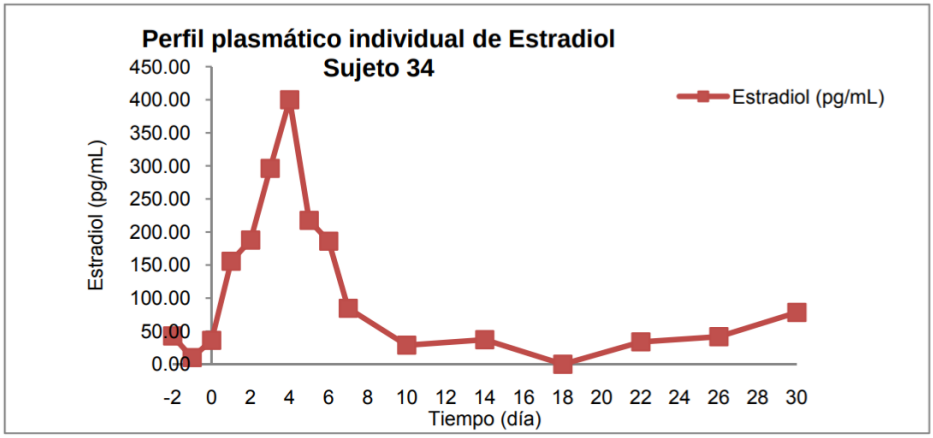
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
31	-2			10.00	10.9
31	-1			10.00	17.3
31	0	0.00		10.00	17.5
31	1	109.80		67.10	67.1
31	2	121.70		115.50	115.5
31	3	166.30		124.00	124.0
31	4	191.50		189.60	189.6
31	5	130.90		104.40	104.4
31	6	170.40		76.90	76.9
31	7	146.10		76.40	76.4
31	10	159.30		48.00	48.0
31	14	165.70		10.00	10.1
31	19	164.10		0.00	7.2
31	22	138.00		0.00	3.1
31	26	124.70		0.00	8.5
31	30	125.20		10.00	13.4



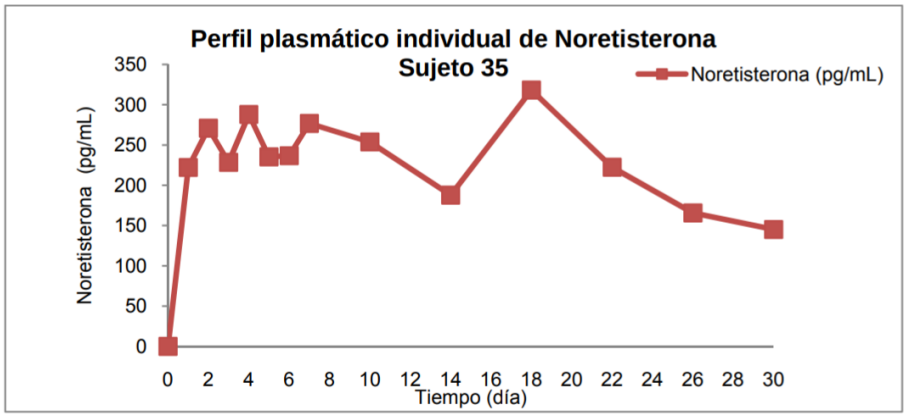


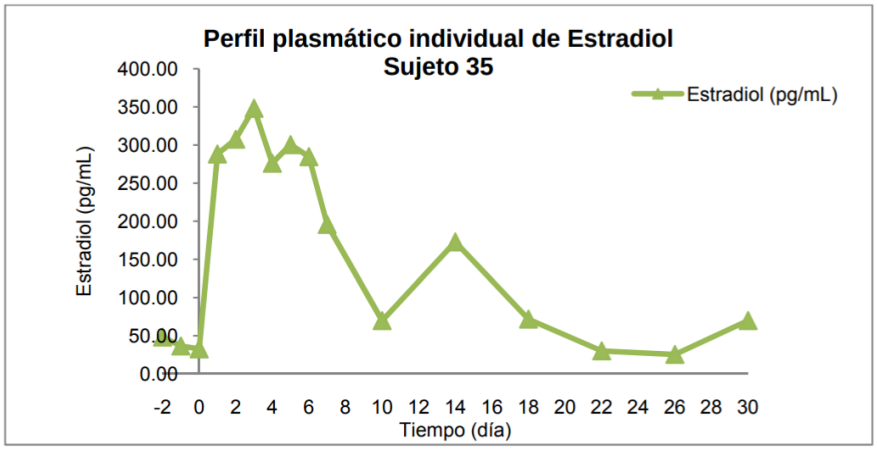
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
34	-2			42.80	42.8
34	-1			10.00	17.2
34	0	0.00		36.10	36.1
34	1	108.10		155.70	155.7
34	2	145.30		187.80	187.8
34	3	138.60		296.20	296.2
34	4	180.30		400.00	400.0
34	5	154.50		217.60	217.6
34	6	150.90		186.10	186.1
34	7	125.10		84.60	84.6
34	10	135.80		28.80	28.8
34	14	121.80		37.10	37.1
34	18	145.60		0.00	4.1
34	22	134.10		33.80	33.8
34	26	103.00		41.60	41.6
34	30	83.70		78.40	78.4



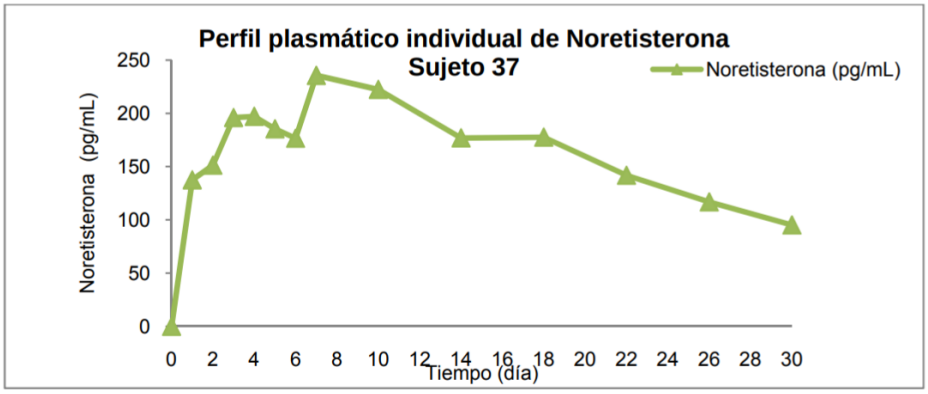


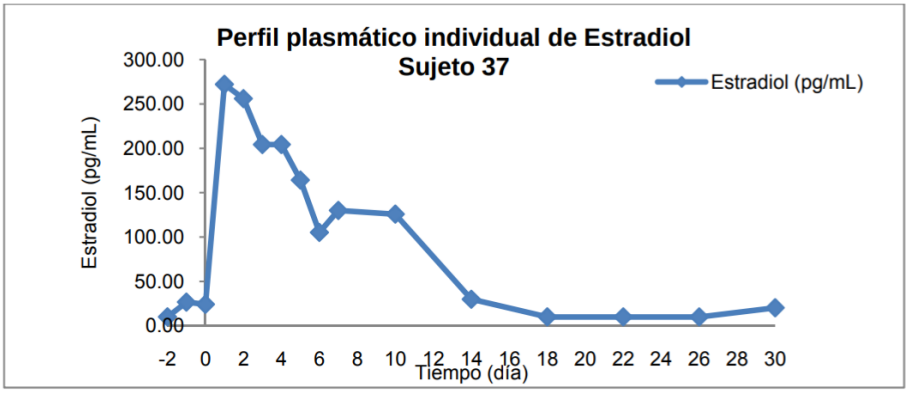
Subject	Time (day)	NET (pg/mL)	E2_10 (pg/mL)	E2 (pg/mL)
35	-2		48.20	48.2
35	-1		36.50	36.5
35	0	0.00	32.80	32.8
35	1	222.10	288.20	288.2
35	2	270.90	307.60	307.6
35	3	228.50	348.20	348.2
35	4	287.90	276.00	276.0
35	5	235.30	300.40	300.4
35	6	236.70	284.70	284.7
35	7	276.70	195.80	195.8
35	10	253.80	69.80	69.8
35	14	187.70	172.90	172.9
35	18	318.20	71.80	71.8
35	22	222.40	30.10	30.1
35	26	165.70	25.50	25.5
35	30	145.10	69.90	69.9



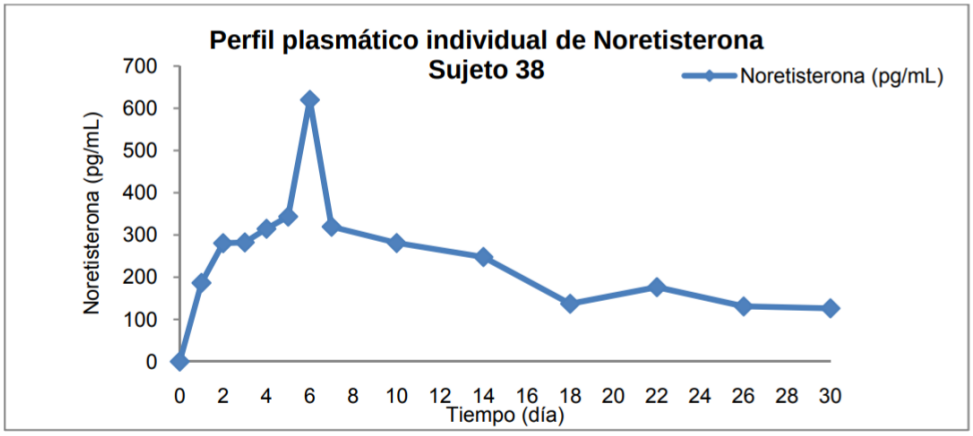


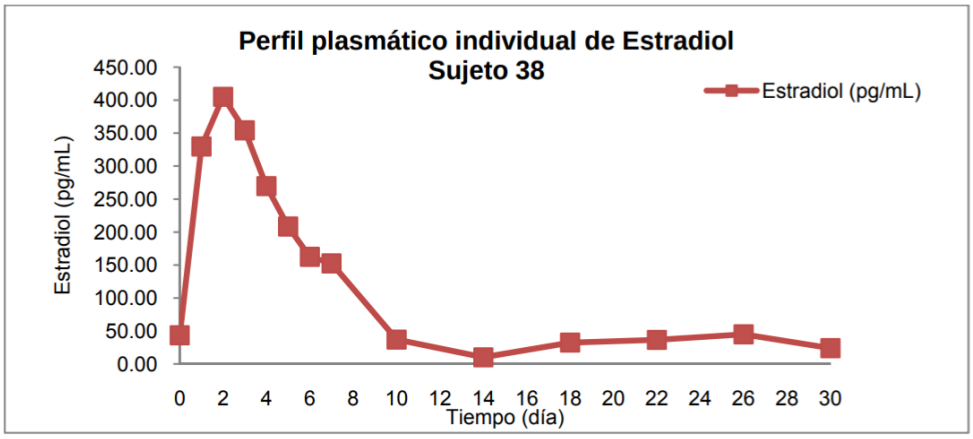
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
37	-2			10.00	15.5
37	-1			26.70	26.7
37	0	0.00		24.20	24.2
37	1	137.50		272.10	272.1
37	2	151.30		256.00	256.0
37	3	196.00		204.20	204.2
37	4	197.10		204.30	204.3
37	5	185.40		164.20	164.2
37	6	176.60		105.20	105.2
37	7	235.60		130.00	130.0
37	10	222.40		125.80	125.8
37	14	176.90		29.90	29.9
37	18	177.60		10.00	14.3
37	22	141.80		10.00	17.1
37	26	116.80		10.00	12.8
37	30	95.20		20.30	20.3



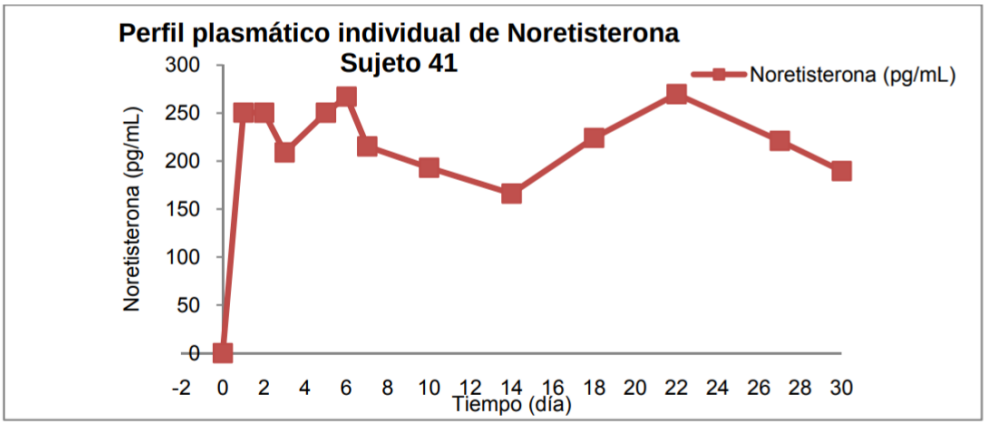


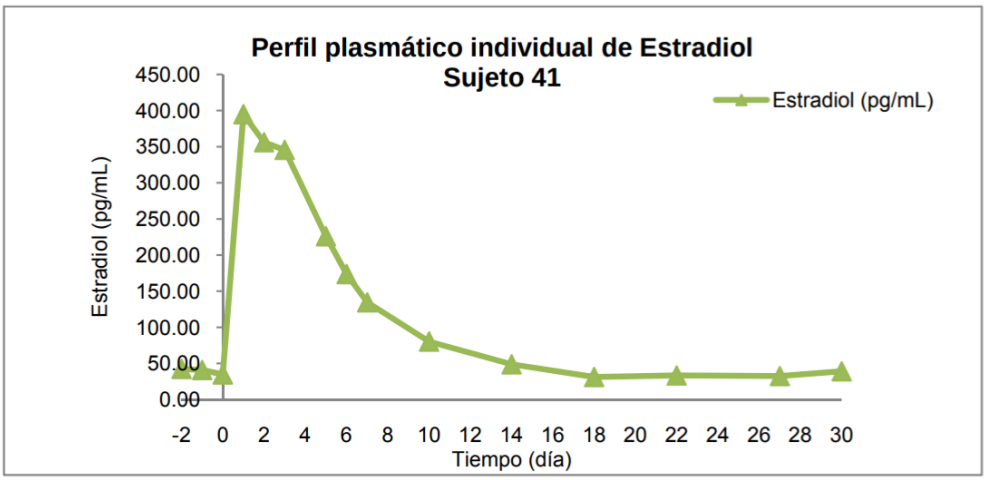
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
38	-2			30.60	30.6
38	-1			26.30	26.3
38	0	0.00		43.40	43.4
38	1	186.40		329.80	329.8
38	2	280.40		405.10	405.1
38	3	282.40		354.30	354.3
38	4	314.60		269.60	269.6
38	5	343.50		208.40	208.4
38	6	620.10		162.40	162.4
38	7	319.60		152.40	152.4
38	10	281.00		36.90	36.9
38	14	247.80		10.00	12.0
38	18	137.00		32.30	32.3
38	22	176.20		36.40	36.4
38	26	130.70		44.80	44.8
38	30	126.10		23.90	23.9



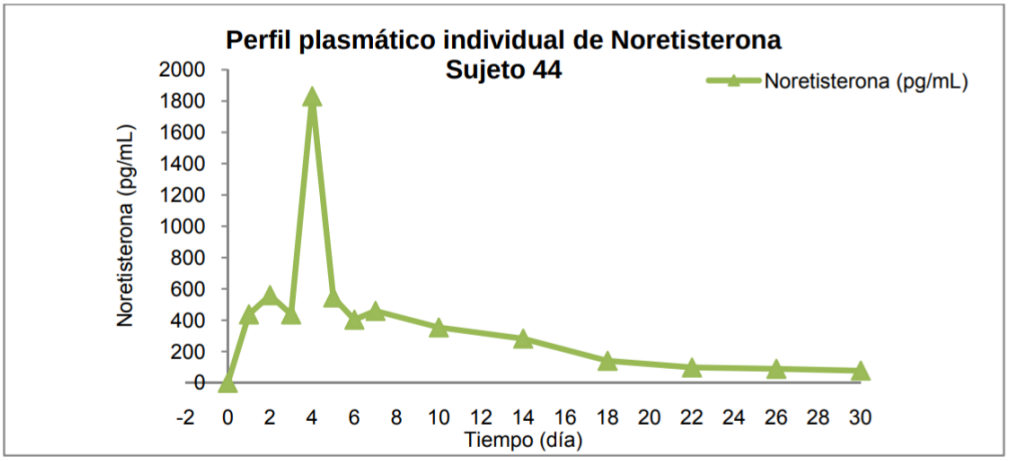


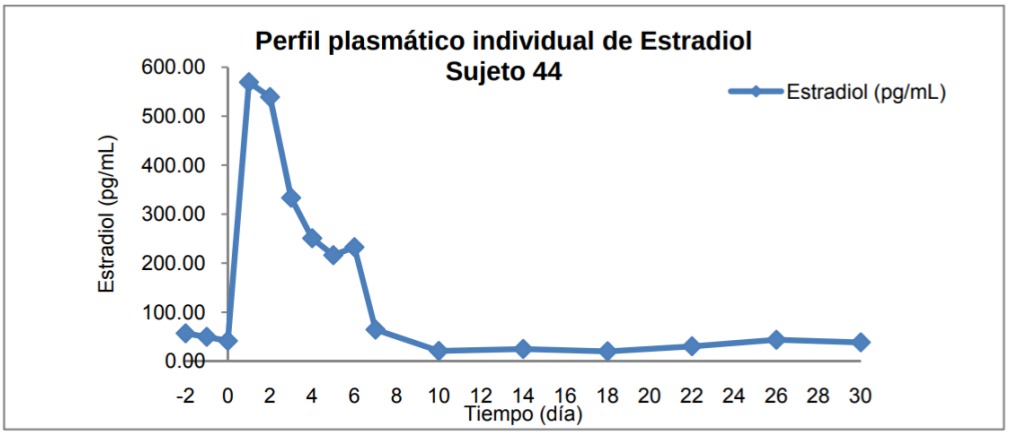
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
41	-2			42.50	42.5
41	-1			41.00	41.0
41	0	0.00		34.80	34.8
41	1	250.50		394.90	394.9
41	2	250.40		356.10	356.1
41	3	209.00		345.50	345.5
41	4	No sample		No sample	No sample
41	5	250.40		226.00	226.0
41	6	267.20		173.70	173.7
41	7	215.30		134.50	134.5
41	10	193.10		80.40	80.4
41	14	166.20		48.80	48.8
41	18	224.20		31.30	31.3
41	22	269.80		33.40	33.4
41	27	221.10		32.60	32.6
41	30	189.70		39.20	39.2



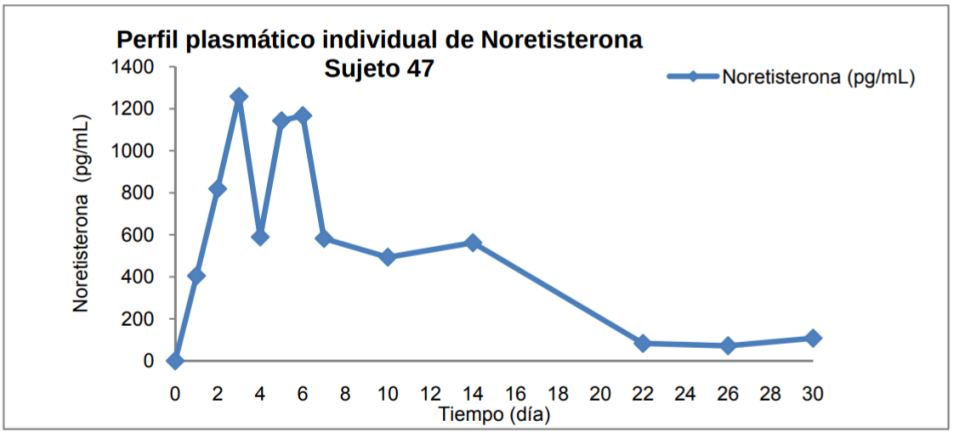


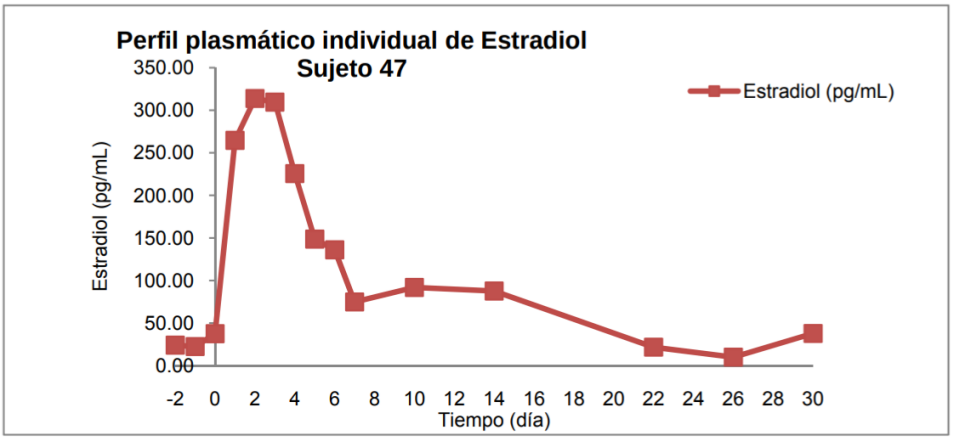
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
44	-2			56.80	56.8
44	-1			49.50	49.5
44	0	0.00		41.30	41.3
44	1	437.50		569.20	569.2
44	2	559.90		538.90	538.9
44	3	437.60		333.30	333.3
44	4	1,830.60		250.70	250.7
44	5	545.80		216.20	216.2
44	6	403.20		232.50	232.5
44	7	459.40		64.40	64.4
44	10	353.80		20.90	20.9
44	14	282.40		24.80	24.8
44	18	140.30		20.10	20.1
44	22	98.10		30.30	30.3
44	26	89.70		43.60	43.6
44	30	77.80		38.30	38.3



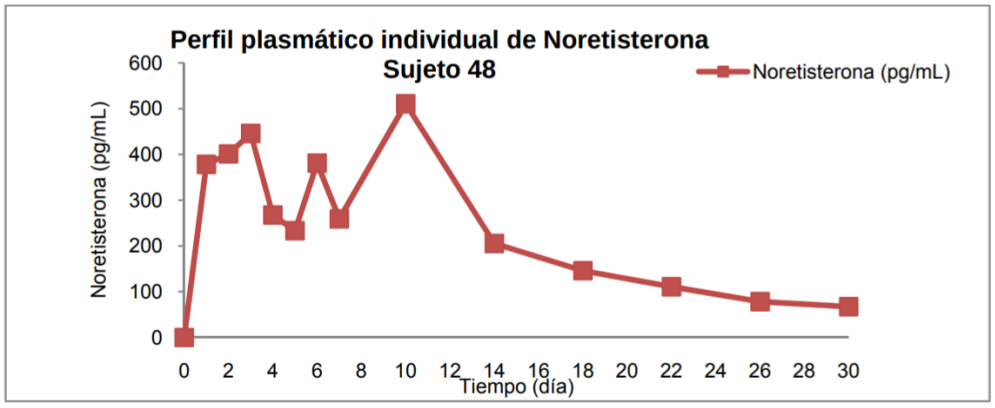


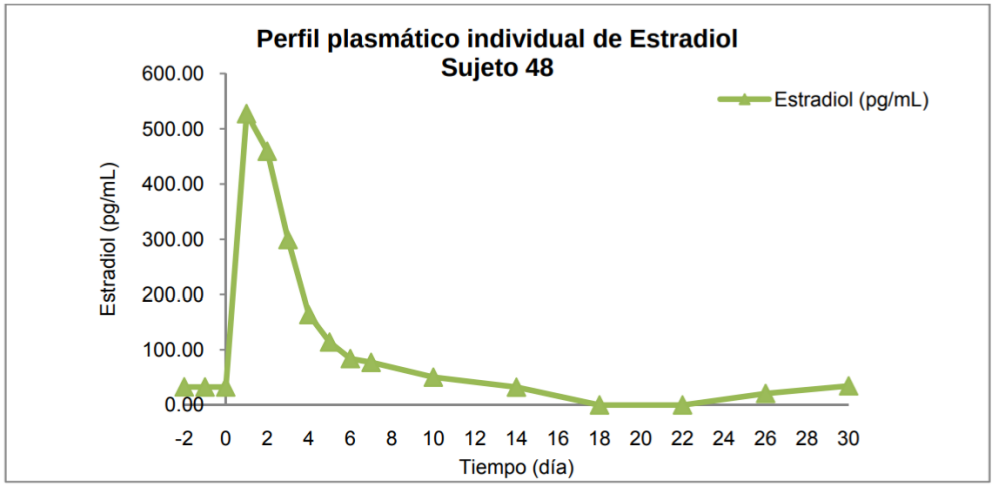
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
47	-2			24.10	24.1
47	-1			22.40	22.4
47	0	0.00		37.50	37.5
47	1	404.70		264.60	264.6
47	2	818.80		313.80	313.8
47	3	1,258.30		309.50	309.5
47	4	589.60		225.60	225.6
47	5	1,143.00		148.60	148.6
47	6	1,167.30		136.10	136.1
47	7	582.50		74.80	74.8
47	10	493.30		91.80	91.8
47	14	562.50		87.70	87.7
		No		No	No
47	18	sample		sample	sample
47	22	83.00		21.70	21.7
47	26	72.00		10.00	19.4
47	30	107.90		37.80	37.8



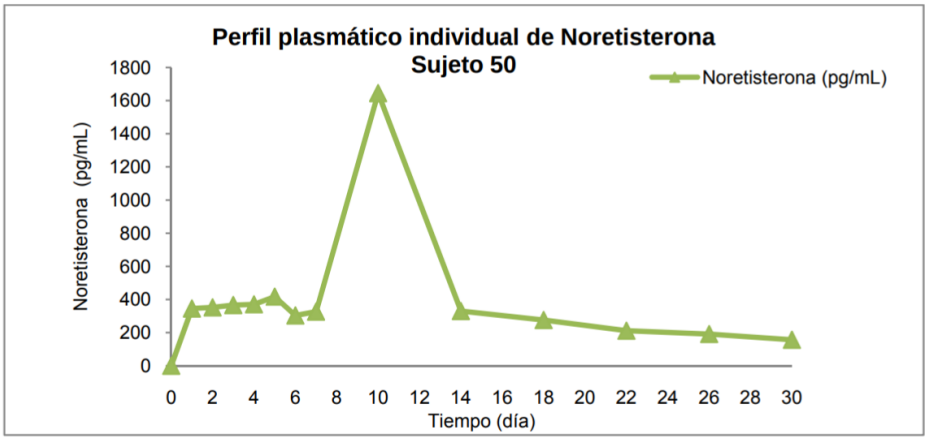


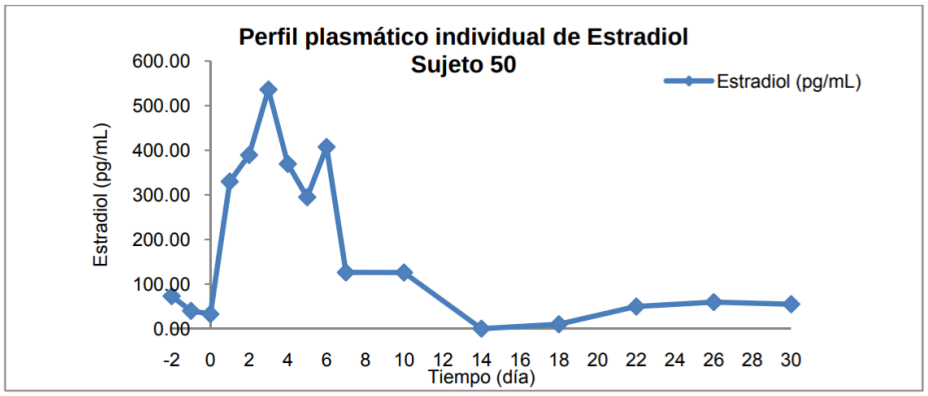
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
48	-2			32.80	32.8
48	-1			32.60	32.6
48	0	0.00		32.60	32.6
48	1	378.40		527.50	527.5
48	2	401.10		459.40	459.4
48	3	445.90		299.40	299.4
48	4	267.60		163.70	163.7
48	5	233.30		114.00	114.0
48	6	381.10		84.00	84.0
48	7	259.10		77.20	77.2
48	10	510.80		50.40	50.4
48	14	205.30		32.30	32.3
48	18	146.00		0.00	1.9
48	22	111.00		0.00	9.3
48	26	78.40		20.90	20.9
48	30	67.30		34.60	34.6



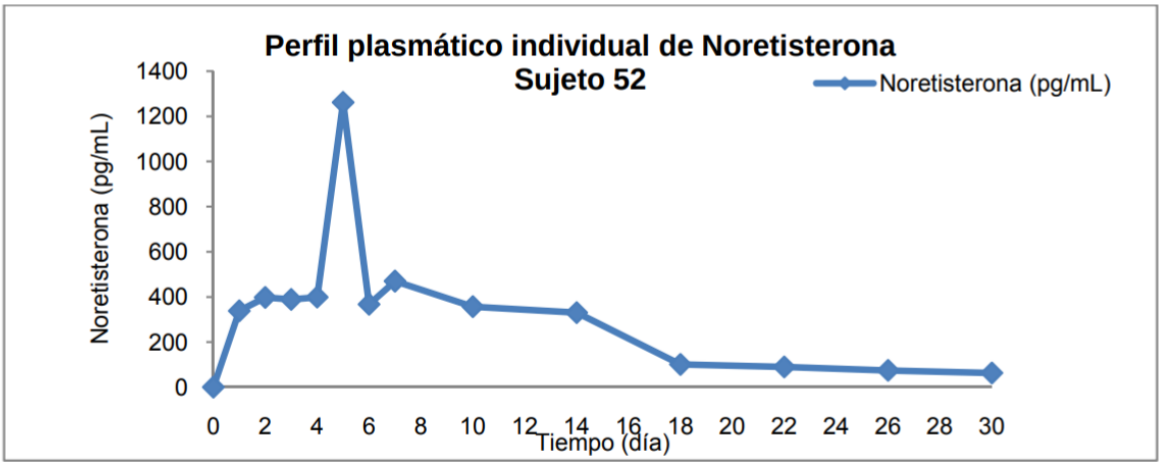


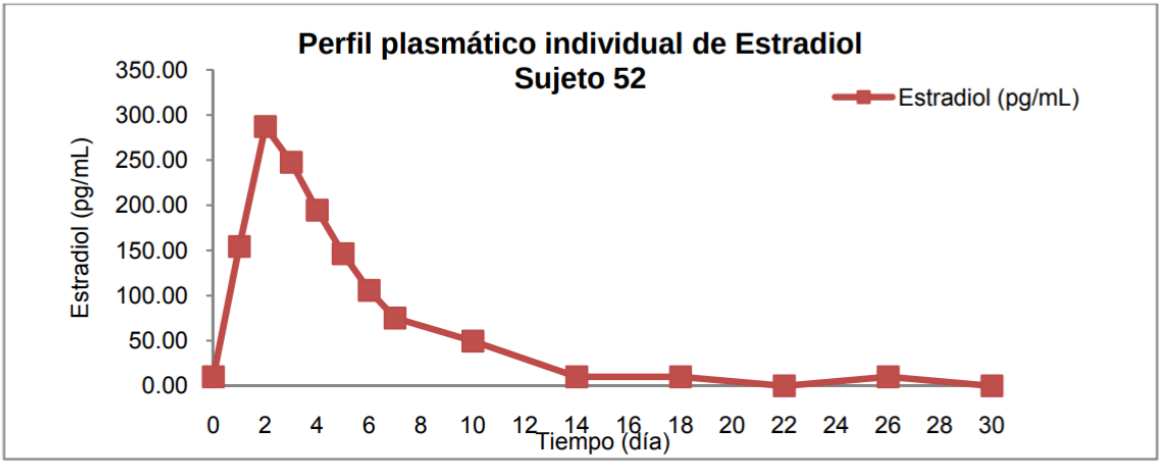
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
50	-2			73.10	73.1
50	-1			39.90	39.9
50	0	0.00		32.70	32.7
50	1	345.40		330.00	330.0
50	2	352.30		389.30	389.3
50	3	366.50		536.20	536.2
50	4	370.90		369.40	369.4
50	5	417.30		294.90	294.9
50	6	303.60		407.50	407.5
50	7	326.60		126.10	126.1
50	10	1,644.80		125.90	125.9
50	14	330.40		0.00	3.8
50	18	276.10		10.00	18.7
50	22	211.70		49.60	49.6
50	26	191.70		59.70	59.7
50	30	157.30		54.80	54.8



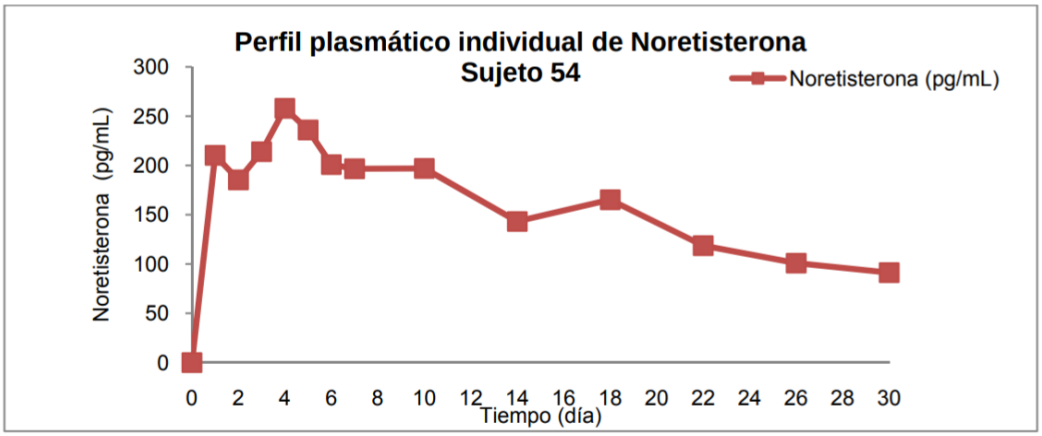


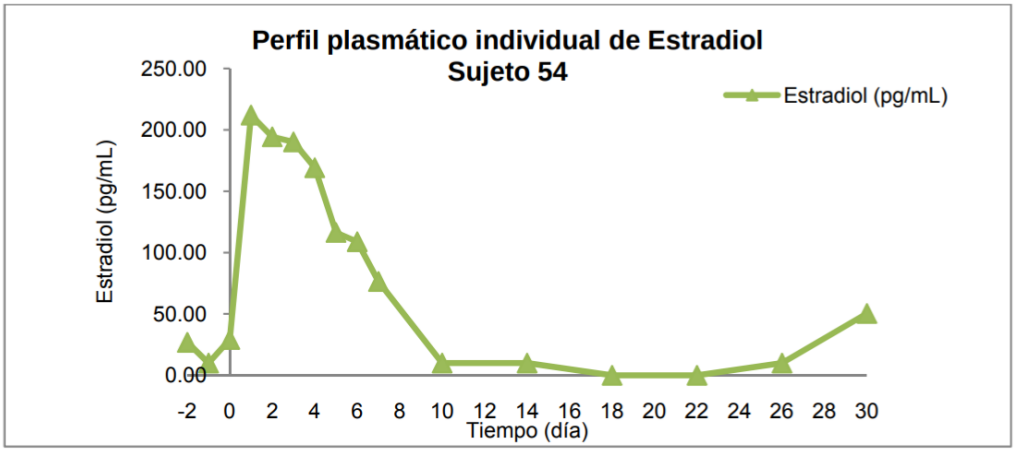
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
52	-2			0.00	6.6
52	-1			10.00	10.2
52	0	0.00		10.00	11.6
52	1	337.90		154.30	154.3
52	2	397.70		287.20	287.2
52	3	388.60		247.60	247.6
52	4	398.80		194.50	194.5
52	5	1,261.90		146.30	146.3
52	6	367.10		105.70	105.7
52	7	469.90		75.10	75.1
52	10	356.70		49.60	49.6
52	14	330.30		10.00	16.1
52	18	100.90		10.00	15.6
52	22	89.60		0.00	1.1
52	26	74.30		10.00	14.2
52	30	63.00		0.00	6.3



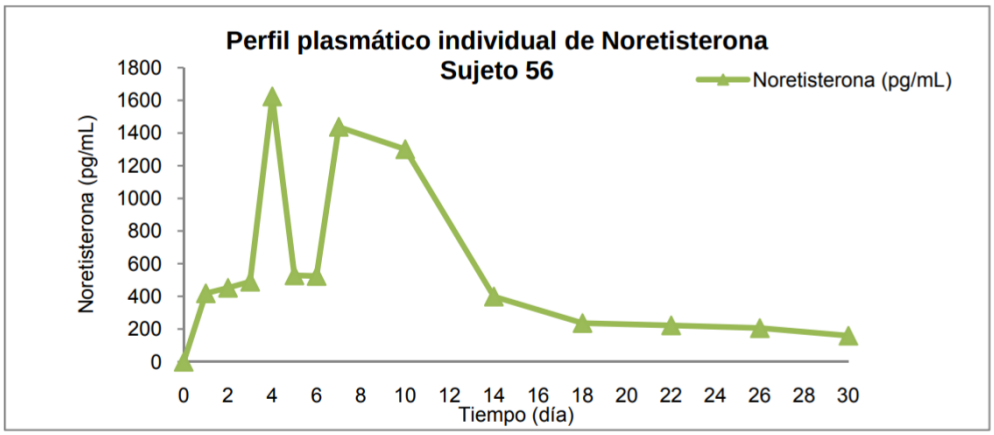


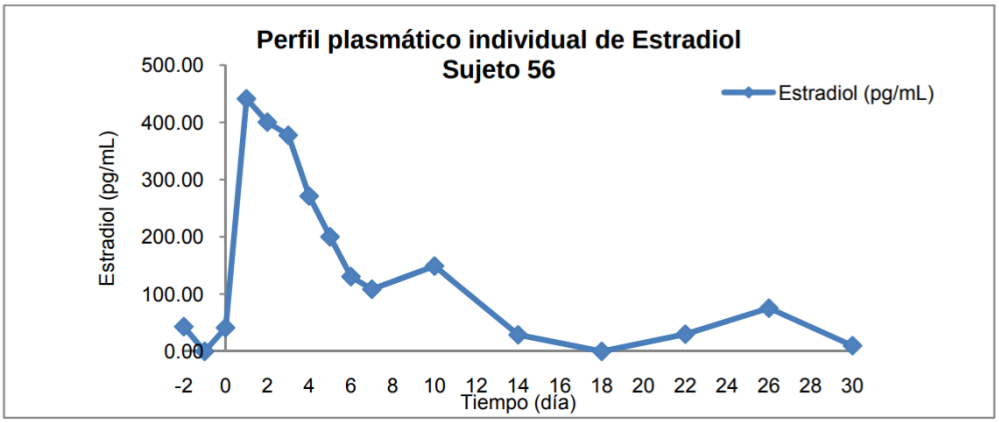
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
54	-2			26.90	26.9
54	-1			10.00	11.5
54	0	0.00		29.20	29.2
54	1	210.30		212.00	212.0
54	2	185.40		194.30	194.3
54	3	214.00		190.10	190.1
54	4	258.00		169.10	169.1
54	5	236.10		116.40	116.4
54	6	200.90		108.80	108.8
54	7	196.70		76.50	76.5
54	10	197.10		10.00	12.0
54	14	143.20		10.00	11.4
54	18	165.20		0.00	5.9
54	22	118.70		0.00	8.1
54	26	100.90		10.00	17.8
54	30	91.30		50.40	50.4



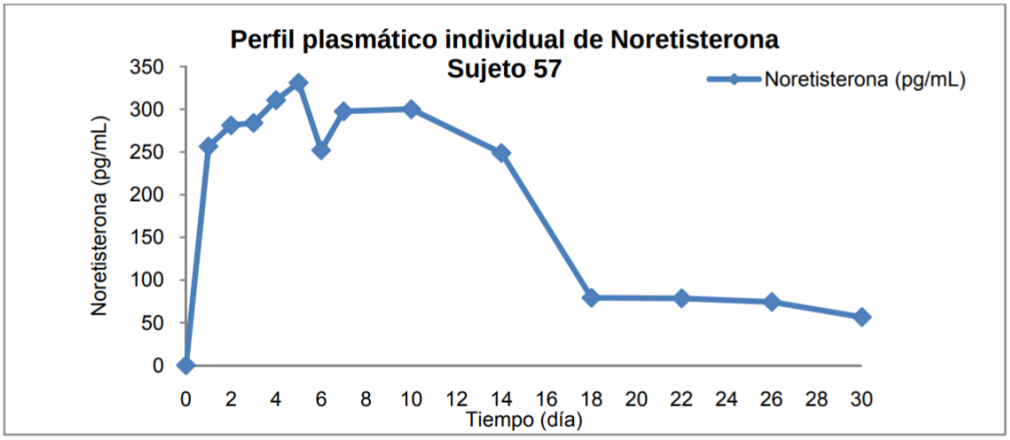


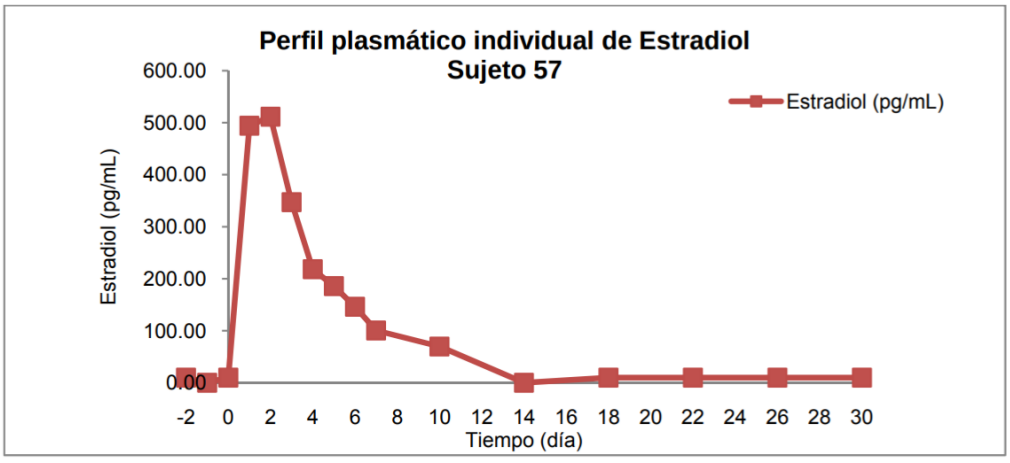
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
56	-2			43.20	43.2
56	-1			0.00	9.8
56	0	0.00		41.30	41.3
56	1	418.90		441.10	441.1
56	2	452.30		400.30	400.3
56	3	490.00		377.40	377.4
56	4	1,623.30		271.10	271.1
56	5	528.80		199.90	199.9
56	6	524.80		130.50	130.5
56	7	1,437.10		108.60	108.6
56	10	1,300.30		148.90	148.9
56	14	399.30		28.90	28.9
56	18	236.80		0.00	6.0
56	22	222.30		30.10	30.1
56	26	206.00		75.50	75.5
56	30	159.20		10.00	19.4



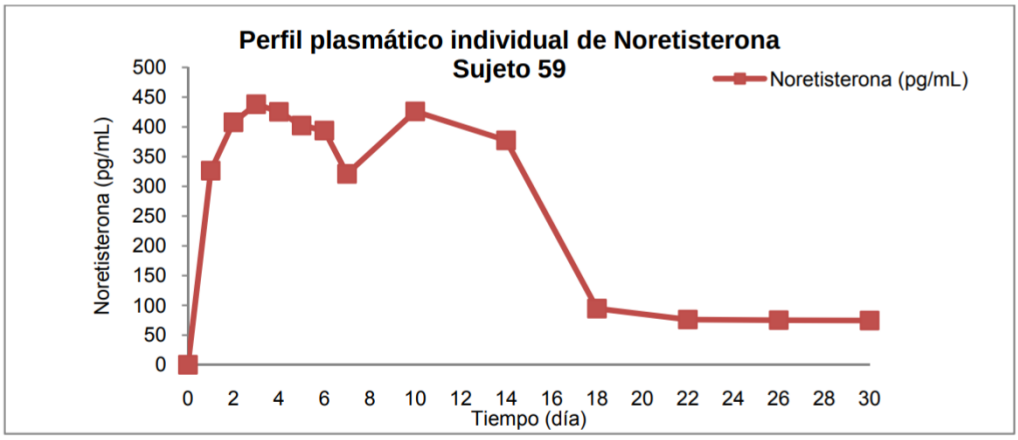


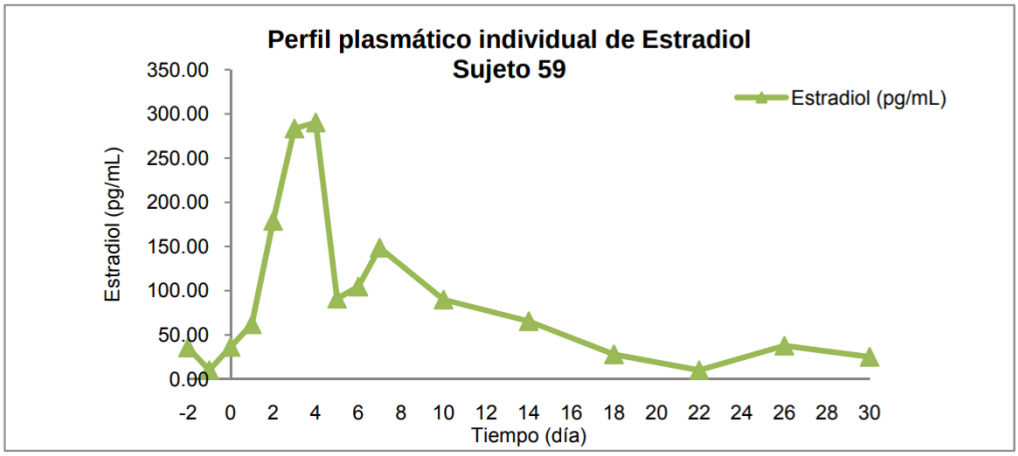
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
57	-2			10.00	17.1
57	-1			0.00	3.8
57	0	0.00		10.00	16.4
57	1	256.50		494.20	494.2
57	2	281.20		511.40	511.4
57	3	284.00		347.20	347.2
57	4	310.80		218.40	218.4
57	5	331.20		185.80	185.8
57	6	252.00		146.20	146.2
57	7	297.60		100.60	100.6
57	10	300.10		69.70	69.7
57	14	248.80		0.00	5.4
57	18	79.10		10.00	18.4
57	22	78.50		10.00	13.2
57	26	74.20		10.00	13.7
57	30	56.50		10.00	14.6



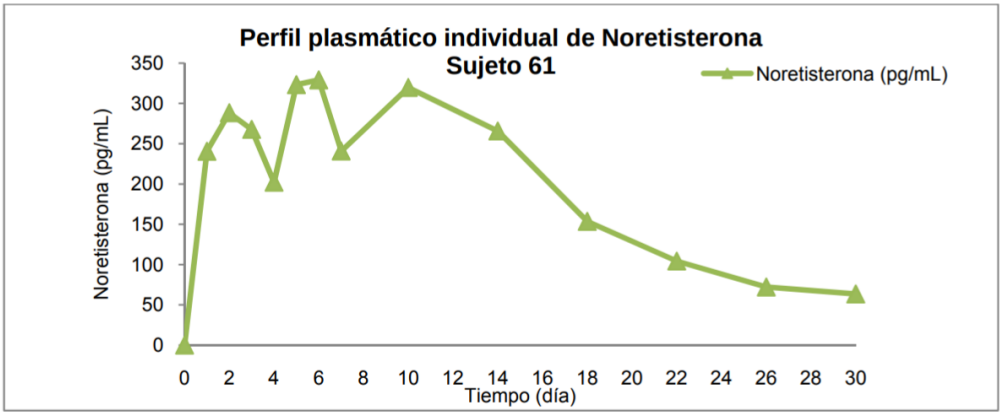


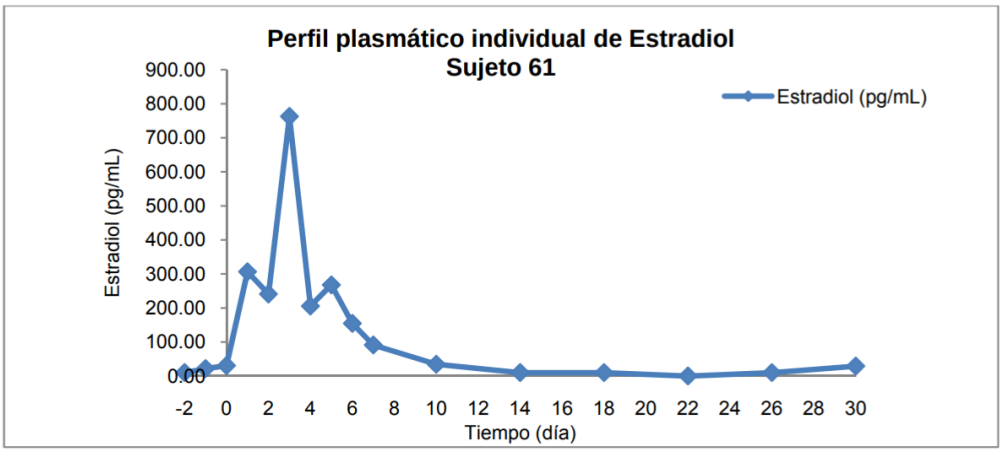
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
59	-2			35.60	35.6
59	-1			10.00	17.8
59	0	0.00		36.40	36.4
59	1	326.50		61.90	61.9
59	2	407.70		178.80	178.8
59	3	438.20		283.50	283.5
59	4	425.40		290.30	290.3
59	5	402.40		90.80	90.8
59	6	393.90		104.60	104.6
59	7	321.00		148.60	148.6
59	10	426.10		89.90	89.9
59	14	377.40		65.50	65.5
59	18	94.50		27.90	27.9
59	22	76.10		10.00	14.8
59	26	75.10		37.70	37.7
59	30	74.50		25.10	25.1



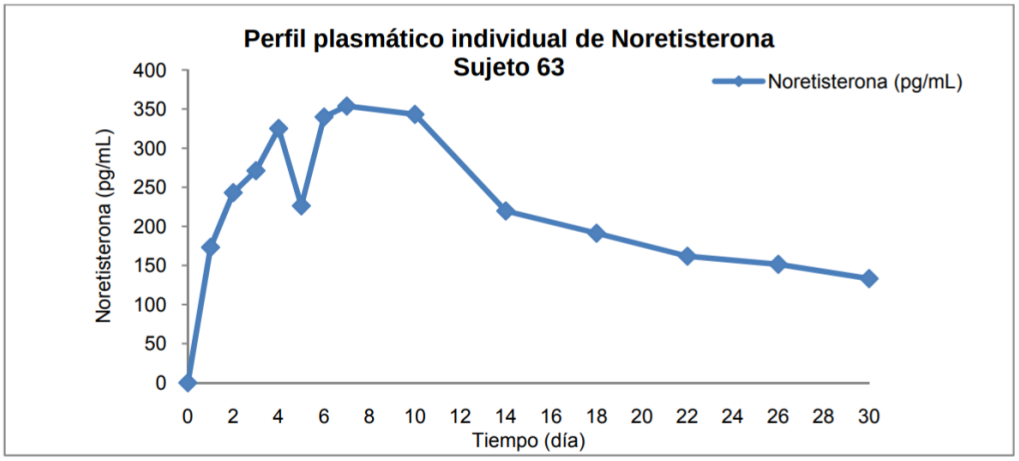


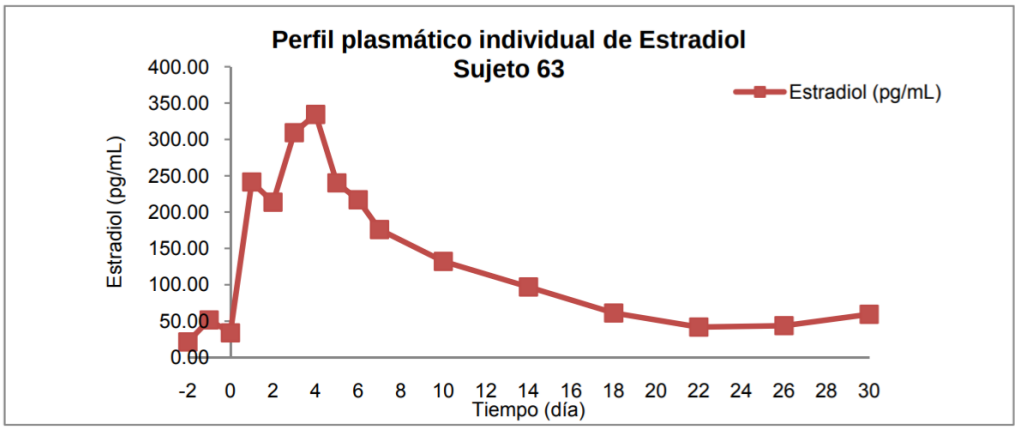
Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
61	-2			10.00	11.8
61	-1			21.70	21.7
61	0	0.00		30.70	30.7
61	1	240.50		306.40	306.4
61	2	288.30		240.90	240.9
61	3	267.70		762.80	762.8
61	4	202.10		205.50	205.5
61	5	323.20		267.70	267.7
61	6	329.10		154.50	154.5
61	7	240.80		91.10	91.1
61	10	319.70		34.40	34.4
61	14	265.70		10.00	17.3
61	18	153.60		10.00	17.9
61	22	104.30		0.00	0.0
61	26	72.10		10.00	16.0
61	30	63.50		29.10	29.1





Subject	Time (day)	NET (pg/mL)		E2_10 (pg/mL)	E2 (pg/mL)
63	-2			21.30	21.3
63	-1			51.60	51.6
63	0	0.00		33.80	33.8
63	1	173.20		241.40	241.4
63	2	243.10		213.60	213.6
63	3	271.20		309.30	309.3
63	4	325.20		334.40	334.4
63	5	226.30		240.20	240.2
63	6	339.90		216.90	216.9
63	7	353.90		176.00	176.0
63	10	343.20		132.10	132.1
63	14	219.70		97.00	97.0
63	18	191.20		61.10	61.1
63	22	161.90		41.70	41.7
63	26	151.50		43.70	43.7
63	30	133.20		59.30	59.3





16.2. APPENDIX 2

INDIVIDUAL PHARMACOKINETIC PARAMETERS OF NORETHISTERONE AND ESTRADIOL

INDIVIDUAL PHARMACOKINETIC PARAMETERS OF NORETHISTERONE

Subject	Cmax	AUC_{0-30}	AUC_{0-inf}	Ke	t½	tmax	Extrapolated	MRT
	(pg/mL)	(day*pg/mL)	(day*pg/mL)	(1/day)	(day)	(day)	AUC (%)	(day)
3	483.00	7,170.30	10,405.13	0.0334	20.78	6.00	31.09	26.87
4	1,193.00	9,854.55	13,956.18	0.0406	17.09	5.00	29.39	24.28
5	434.40	7,134.50	11,516.38	0.0249	27.87	10.00	38.05	34.30
7	241.50	4,965.80	7,925.77	0.0475	14.59	18.00	37.35	29.15
10	151.10	3,521.25	5,377.77	0.0446	15.54	2.00	34.52	27.37
12	213.40	4,820.45	8,333.83	0.0306	22.67	6.00	42.16	34.41
13	536.50	7,356.75	8,388.56	0.0575	12.06	2.00	12.30	14.46
14	324.70	5,275.85	6,377.20	0.0603	11.50	4.00	17.27	18.17
18	152.50	2,307.85	2,999.20	0.0567	12.22	5.00	23.05	20.63
19	309.20	5,875.35	8,003.49	0.0461	15.02	3.00	26.59	22.88
21	219.90	4,472.55	6,932.62	0.0378	18.32	14.00	35.49	28.77
24	208.70	4,428.95	7,758.54	0.0288	24.09	7.00	42.92	35.55
26	170.90	2,830.75	4,432.25	0.0330	20.98	5.00	36.13	30.33
28	225.30	4,647.90	6,648.56	0.0505	13.72	7.00	30.09	24.38
29	218.70	4,095.35	5,717.11	0.0451	15.36	10.00	28.37	24.16
31	191.50	4,374.60	9,321.29	0.0253	27.39	4.00	53.07	43.85
34	180.30	3,788.60	5,209.21	0.0589	11.76	4.00	27.27	23.28
35	318.20	6,789.30	9,507.45	0.0534	12.98	18.00	28.59	24.12
37	235.60	4,936.30	6,780.71	0.0516	13.43	7.00	27.20	23.45
38	620.10	6,669.10	9,684.54	0.0418	16.58	6.00	31.14	25.36
41	269.80	6,520.32	10,918.39	0.0431	16.07	22.00	40.28	30.57
44	1,830.60	8,969.30	10,643.82	0.0465	14.92	4.00	15.73	15.88
47	1,258.30	12,683.75	13,639.10	0.1129	6.14	3.00	7.00	11.42
48	510.80	6,710.80	7,786.80	0.0625	11.08	10.00	13.82	15.67
50	1,644.80	12,920.20	16,441.06	0.0447	15.51	10.00	21.42	20.71
52	1,261.90	7,846.65	9,277.57	0.0440	15.74	5.00	15.42	16.39
54	258.00	4,682.55	6,563.58	0.0485	14.28	4.00	28.66	23.74
56	1,623.30	16,039.35	19,854.05	0.0417	16.61	4.00	19.21	18.96
57	331.20	5,396.65	6,771.10	0.0411	16.86	5.00	20.30	19.65
59	438.20	7,191.29	9,785.08	0.0287	24.13	3.00	26.51	24.98
61	329.10	5,761.25	6,784.97	0.0620	11.17	6.00	15.09	16.83
63	353.90	6,680.40	11,017.45	0.0307	22.57	7.00	39.37	32.57

INDIVIDUAL PHARMACOKINETIC PARAMETERS OF ESTRADIOL

Subject	Cmax	AUC_{0-30}	AUC_{0-inf}	Ke	t½	tmax	Extrapolated	MRT
	(pg/mL)	(day*pg/mL)	(day*pg/mL)	(1/day)	(day)	(day)	AUC (%)	(day)
3	238.10	1,555.50	1,646.23	0.1102	6.29	3.00	5.51	8.84
4	286.70	1,938.75	2,190.96	0.1015	6.83	3.00	11.51	11.62
5	206.30	1,978.95	2,149.89	0.1492	4.65	4.00	7.95	10.78
7	328.90	2,094.55	2,143.13	0.2058	3.37	2.00	2.27	5.59
10	365.40	2,019.90	2,127.16	0.0932	7.44	1.00	5.04	7.50
12	226.20	2,001.05	2,201.64	0.1022	6.78	4.00	9.11	10.99
13	649.70	2,357.10	2,422.07	0.1539	4.50	1.00	2.68	6.68
14	221.80	1,815.40	1,857.32	0.2385	2.91	4.00	2.26	6.84
18	598.20	2,851.65	2,949.63	0.1021	6.79	1.00	3.32	7.21
19	357.90	2,305.45	2,342.36	0.2709	2.56	3.00	1.58	6.83
21	320.50	2,166.05	2,204.38	0.2609	2.66	3.00	1.74	6.28
24	178.80	1,531.65	1,624.31	0.2299	3.02	4.00	5.70	9.99
26	351.40	2,700.75	2,977.26	0.3537	1.96	2.00	9.29	11.47
28	189.10	1,999.75	2,230.53	0.1764	3.93	3.00	10.35	11.58
29	471.00	2,681.30	2,746.72	0.1528	4.53	3.00	2.38	5.37
31	189.60	1,183.30	1,217.09	0.2960	2.34	4.00	2.78	8.29
34	400.00	2,480.05	3,206.16	0.1080	6.42	4.00	22.65	16.39
35	348.20	3,798.40	4,494.90	0.1004	6.91	3.00	15.50	14.16
37	272.10	2,198.60	2,294.03	0.2127	3.26	1.00	4.16	8.17
38	405.10	2,727.05	2,883.59	0.1527	4.54	2.00	5.43	9.23
41	394.90	3,008.78	3,341.20	0.1179	5.88	1.00	9.95	10.91
44	569.20	2,915.20	3,366.61	0.0848	8.17	1.00	13.41	11.57
47	313.80	2,662.07	2,872.02	0.1800	3.85	2.00	7.31	10.29
48	527.50	2,489.90	3,032.02	0.0638	10.86	1.00	17.88	13.76
50	536.20	3,895.10	4,256.63	0.1516	4.57	3.00	8.49	10.57
52	287.20	1,604.40	1,639.63	0.2838	2.44	2.00	2.15	6.17
54	212.00	1,454.10	1,942.73	0.1031	6.72	1.00	25.15	15.51
56	441.10	3,255.30	3,351.02	0.1045	6.64	1.00	2.86	9.14
57	511.40	2,652.75	2,698.19	0.2201	3.15	2.00	1.68	5.80
59	290.30	2,263.97	2,408.59	0.1736	3.99	4.00	6.00	10.88
61	762.80	2,473.95	2,566.25	0.3153	2.20	3.00	3.60	6.49
63	334.40	3,507.30	4,142.16	0.0934	7.42	4.00	15.33	14.59