# Diploma Thesis

## *"Influence of gender on the in vivo pharmacokinetics of propofol"*


Submitted by

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Under the supervision of

**Doz. Dr. Ashraf A. Dahaba**


Ort, Datum: Graz, am Unterschrift:

# Diplomarbeit

## *„Der geschlechtsspezifische Einfluss auf die in vivo pharmakokinetik von Propofol“*


eingereicht von

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unter der Anleitung von

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# Abstract

## Background

Females and males have different body compositions. The body fat percentage is larger and the body water content is smaller in females. Furthermore, these differences are age-dependent, with body fat increasing in both genders with age. Body fat composition may affect the volume of distribution of many drugs. For lipophilic drugs such as opioids and benzodiazepines, the volume of distribution per kg body weight generally will be higher in females than in males.

## Materials and Methods

Serial arterial blood samples (approximately 4 ml) will be withdrawn before induction ($Pt_0$) and at 1, 3, 5, 7, 10, 15, 20, 25, 30, 60, 90 and 120 min following propofol administration. During the blood samples acquisition part of the study, the levels of anesthesia were maintained using sevoflurane inhalational anesthesia to avoid additional propofol administration. Within minutes of samples acquisition, plasma was isolated by 2500 g centrifugation for 10 min. Blood samples was immediately extracted, acidified and stored frozen to prevent degradation by adding buffer to 1 ml plasma, carefully mixed and subsequently stored at $-20^{o}C$.

## Results

There were no significant differences between the two gender regarding the propofol plasma concentrations, mean arterial pressure, estimated blood losses, fluid replacements, remifentanil doses during the 120 min blood sample acquisition part of the study.

## Conclusion

In conclusion, we demonstrated that no significant differences in propofol plasma concentrations between males and females. Given our observed results of no clear real differences between the 2 genders, larger studies are not warranted to explore the potential differences between genders that may contribute to our understanding of these differences.

# Zusammenfassung


## Hintergrund

Frauen und Männer haben unterschiedliche Körperzusammensetzung. Beim weiblichen Geschlecht ist der Körperfettanteil größer und der Körperwassergehalt kleiner als bei Männer. Darüber hinaus sind diese Unterschiede altersabhängig. Mit zunehmendem Alter erhöht sich bei beiden Geschlechtern der Körperfettanteil. Die Körperfettzusammensetzung kann die Verteilung der vielen Medikamente beeinflussen. Für lipophile Medikamente wie Opioide und Benzodiazepine, wird das Verteilungsvolumen pro kg Körpergewicht in der Regel bei Frauen höher als bei Männern.

## Material und Methoden

Fortlaufende arterielle Blutproben (ca. 4ml) werden vor der Anästhesieeinleitung (Pt0) entnommen. Im Anschluss an die Propofolgabe werden die Blutproben bei 1, 3, 5, 7, 10, 15, 20, 25, 30, 60, 90 und 120 Minute erneut entnommen. Während der Blutprobenanschaffung wurden die Stufen der Anästhesie mit Sevofluran Inhalationsanästhesie aufrechterhalten um die zusätzliche Propofolgabe zu vermeiden.

Innerhalb von Minuten nach Erwerb der Proben wurde Plasma durch 2500 g Zentrifugation für 10 min isoliert. Die Blutproben wurden sofort extrahiert, angesäuert und tiefgefroren aufbewahrt. Der Abbau von Proben wurde durch die Zugabe von Puffer auf 1 ml Plasma, sorgfältige Mischung und anschließende Lagerung bei -20 ° C verhindert.

## Ergebnisse

Es gab keine signifikanten Unterschiede zwischen den beiden Geschlechter in Bezug auf die Propofol-Plasmakonzentration, mittleren arteriellen Druck, geschätzte Blutverluste, Flüssigkeitsersatz und Remifentanildosen während der 120-minutigen Blutproben- Anschaffungsteil der Studie.

## Schlussfolgerung

Zusammenfassend haben wir gezeigt, dass es keine signifikanten Unterschiede in Propofol-Plasmakonzentrationen zwischen Männer und Frauen gibt. Angesichts unserer beobachteten Ergebnisse mit nicht eindeutig realen Unterschieden zwischen den 2 Geschlechtern, sind größere Studien nicht gerechtfertigt, um die potenziellen Unterschiede zwischen den Geschlechtern, die zu unserem Verständnis dieser Unterschiede beitragen können zu erkunden.

# Index

# 1. Review of Literature

## 1.1 Gender differences in pharmacokinetics

### 1.1.1 Drug distribution

Females and males have different body compositions. The body fat percentage is larger and the body water content is smaller in females. Furthermore, these differences are age-dependent, with body fat increasing in both genders with age. Body fat composition may affect the volume of distribution of many drugs. For lipophilic drugs such as opioids and benzodiazepines, the volume of distribution per kg body weight generally will be higher in females than in males. Conversely, the volume of distribution for water-soluble drugs such as muscle relaxants may be lower in females than in males. Thus, the same dose per kg body weight will result in a lower initial plasma concentration of lipophilic drugs in females, whereas the initial concentration of water-soluble drugs will be higher. As the central volume of distribution is the most important pharmacokinetic factor determining the initial drug concentration after single-dose administration, these gender differences may have an impact on the optimal dosage when the drug is given as single dose. In contrast, the volume of distribution has no direct influence on the drug concentration at steady state. [1]

Volume of distribution for a drug is defined as the ratio of the plasma concentration and the amount of drug in the body. The practical use of this concept is that it determines the amount of drug that has to be given to reach an initial target concentration. For many drugs, a bolus dose is given to achieve the immediate target concentration. As most anesthetic drugs have several pharmacokinetic compartments, it is not possible to reach steady state immediately by giving a bolus dose corresponding to the volume of distribution of the central compartment. [1]

On average, females have lower body weight than males. Thus, it is important to give doses on a mg $kg^{-1}$ basis. In the latter case, the mean body weight should ideally

be the same in females and in males in order to justify direct comparisons of initial drug concentrations. [1]

### 1.1.2 Drug elimination

Another component of gender-related differences in drug disposition is the use of contraceptive pills, pregnancy, menstrual cycle and menopause, and the associated effects of hormonal changes. Some evidence indicates that the clearance of drugs in general may be higher on day 15 than on day one in the menstrual cycle in females not taking oral contraceptives [2]
However, for anesthetic drugs, no such differences have been revealed. Very limited information is available regarding the menopause and the disposition of anesthetic drugs. [1]

### 1.1.3 Drug concentration at the effect site

The ultimate pharmacokinetic parameter to be discussed is the plasma effect-site equilibration time. This parameter actually links pharmacokinetics to pharmacodynamics. Plasma effect-site equilibration time is the temporal dissociation between the serum (central compartment) concentration and the apparent effect site (effect compartment) concentration of a drug. This kinetic-dynamic dissociation can be quantified by a rate constant, ke0.[1]

The plasma effect-site equilibration time can be determined by measuring serum concentrations and a pharmacodynamic outcome simultaneously. For a drug like propofol, the dynamic outcome may be time to maximum Bispectral index (BIS) depression.[3,4]

Drugs with a high ke0 such as thiopental have a rapid equilibration, while drugs with a low ke0 have slower equilibration. Little is known about gender differences of plasma effect-site equilibration time, but a recent study on morphine indicates that gender may also play a role in this context .[1,5]

## 1.2 Other examples of gender based effects

### 1.2.1 Atracurium

In a study in 41 otherwise healthy patients undergoing minor surgery, atracurium clearance was lower and elimination half-life was longer in females than in males.[6] Although statistically significant, an absolute difference of 1.9 min is expected to be clinically insignificant compared with the mean elimination half-life of 20 min. In another study, the same authors examined the effect of gender on the pharmacodynamics of atracurium in 21 female and 17 male patients undergoing minor surgery.[7] The concentrations that produced 50% neuromuscular blockade and the highest concentrations that failed to provoke any effect did not differ between females and males. This is consistent with the results in another study on 10 patients, in which no gender difference in sensitivity to atracurium was found, measured as per cent twitch depression after injection of atracurium 276 μg $kg^{-1}$ body weight.[8] As the number of subjects in this study was very low, there is a high risk for a false negative result.[1]

### 1.2.2 Cisatracurium

Possible gender effects on the disposition of cisatracurium have been studied by a population pharmacokinetic/pharmacodynamic approach where data from 241 patients in eight prospectively designed phase I–III trials were pooled and analyzed.[9]
The results showed that gender as a variable produced small, but statistically significant changes in some of the pharmacokinetic parameters. These changes were not associated with any clinically significant alterations in the predicted onset or recovery profile for cisatracurium, and they therefore do not warrant any gender-specific dose recommendations.[1]
It is reasonably well documented that females require less muscle relaxants. The mechanism is most likely pharmacokinetic and explained by gender differences in the volume of distribution. One may speculate that as the properties related to distribution in the body are quite similar for all muscle relaxants, it might well be the case that significant gender differences will be found also for the other drugs when adequately designed and sufficiently powered studies are carried out.[1]

### 1.2.3 Vecuronium

An early and small study in 10 patients .[10] showed no gender differences in sensitivity to vecuronium, measured as per cent twitch depression after injection of vecuronium 56 μg $kg^{-1}$ body weight. However, subsequent and larger studies have come to opposing conclusions. In a study of 40 patients undergoing routine surgery, females required 22% less vecuronium to achieve the same neuromuscular blockade as males.[11]

Similarly, in another study in 60 patients undergoing elective plastic surgery, the mean percentage depression of T1 (using train-of-four monitoring) was 43% greater for females at each dose of vecuronium. The dose–response curve for females was shifted to the left with $ED_{50}$ and $ED_{90}$ of 18 and 34 μg $kg^{-1}$, respectively, in females, and 24 and 45 μg $kg^{-1}$, respectively, in males .[12] Moreover, the clinical duration was significantly longer in females than in males (37 and 27 min, respectively) after a dose of 0.08 mg $kg^{-1}$. A further study .[13] including 80 patients undergoing elective surgery found that the intubating conditions after 60 s were better for females than for males when given the same dose of vecuronium. No differences in the time to full relaxation or in the duration of relaxation were found in this study.[1]

Most studies indicate that the required dose of vecuronium is on average 20–30% lower in females than in males. The explanation for the gender difference in sensitivity to vecuronium appears to be pharmacokinetic.[14]

At equal doses, vecuronium plasma concentrations were significantly lower in males, as a result of a larger central volume of distribution (54 vs. 40 ml $kg^{-1}$) as well as a larger steady-state volume of distribution (201 vs. 165 ml $kg^{-1}$). No gender differences in clearance were observed.[1]

### 1.2.4 Rocuronium

Gender differences in the dose–response relationship and in the time course of the effect of rocuronium have been studied in 60 adult patients scheduled for elective plastic surgery.[15]

The dose–response curve for females was shifted to the left with $ED_{50}$, $ED_{90}$ and $ED_{95}$ values of 128, 252 and 274 μg $kg^{-1}$, respectively, in females, and 178, 358 and 386 μg $kg^{-1}$, respectively, in males. The neuromuscular block was significantly prolonged in females, with the duration of peak effect, clinical duration and total duration being 11.8, 18.5 and 46.8 min, respectively, in females, and 6.5, 12.5 and 35.6 min, respectively, in males. The results from this study thus clearly suggest that females are more sensitive than males, requiring approximately 30% less rocuronium to achieve the same degree of neuromuscular block, and imply that the rocuronium dose should be reduced in females compared with males. .[1]

In conclusion, it is reasonably well documented that females require less vecuronium and rocuronium than males. This may also well be true for pancuronium, but is not as clearly demonstrated as for vecuronium and rocuronium. The mechanism is most likely pharmacokinetic and explained by gender differences in the volume of distribution. One may speculate that as the properties related to distribution in the body are quite similar for all muscle relaxants, it might well be the case that significant gender differences will be found also for the other drugs when adequately designed and sufficiently powered studies are carried out.[1]

# 2. Propofol

## 2.1 Intravenous Anesthetics

Rapidly acting and short-acting intravenous agents are commonly used today for the induction of anesthesia. The most commonly used drug is propofol, which has replaced thiopental as the primary agent used for this purpose. Other agents include methohexithal and ketamine. High doses of opioid analgesics, such as fentanyl, can also be used intravenously for the induction and maintenance of anesthesia. The disadvantages associated with all of these are the irrevocability of intravenous administration of a potent drug and the consequent dangers of overdosing the patient. Furthermore, while quite safe in the hands of specialists who are prepared to deal with side effects and anesthesia accidents, the intravenous anesthetics are very dangerous when used on an occasional basis by the inexperienced practitioner who falls prey to the temptations of convenience. [16]

Propofol is the most commonly used parenteral anesthetics in the U.S. Fospropofol is a Prodrug from that is converted to propofol *in vivo*.[17]

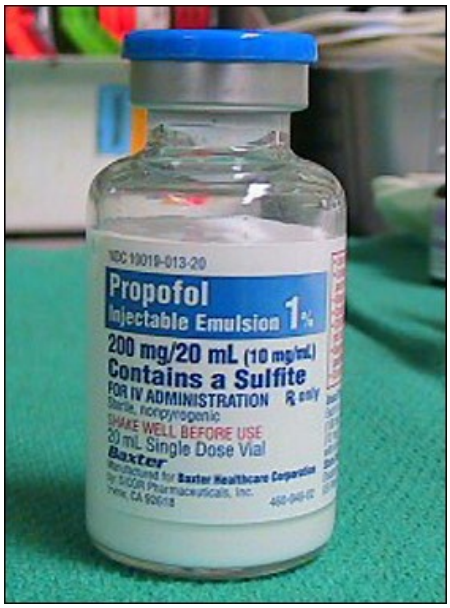



**Image 1: Propofol injectable Emulsion 1%**

## 2.2 Chemistry and Formulations

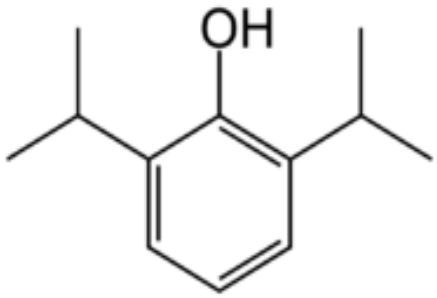



**Image 2: 2,6 diisopropylphenol**

The active ingredient in propofol, 2,6-diisopropylphenol , is an oil at room temperature and insoluble in aqueous solutions. Propofol is formulated for IV administration as a 1% (10 mg/mL) emulsion in 10% soybean oil, 2.25 % glycerol and 1.2% purified egg phosphatide. In the U.S., disodium EDTA( 0.05 mg/ mL) or sodium metabisulfite ( 0.25 mg/mL) is added to inhibit bacterial growth. Nevertheless, significant bacterial contamination of open containers has been associated with serious patient infections; propofol should be administered within 4 hours of its removal from sterile packaging; unused drug should be discarded.[17]

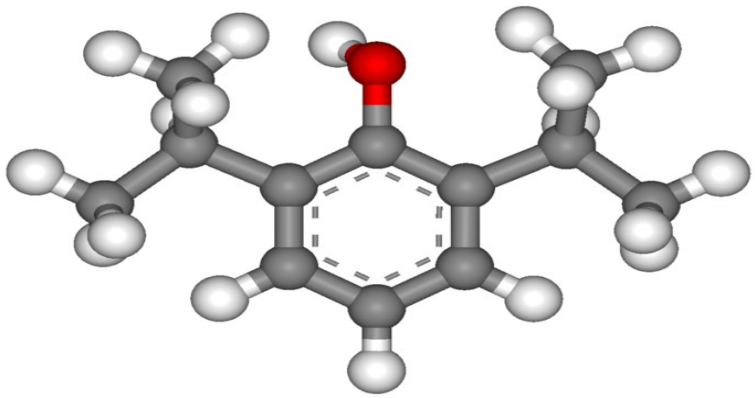


**Image 3: 2,6 diisopropylphenol**

The lipid emulsion formulation of propofol is associated with significant pain on injection and hyperlipidemia. A new aqueous formulation of propofol, fospropofol, which is not associated with these adverse effects, has recently been approved for use for sedation in patients undergoing diagnostic procedures. Fospropofol, which itself is inactive, is a phosphate ester prodrug of propofol that is hydrolyzed by endothelial alkaline phosphatases to yied propofol, phosphate, and formaldehyde. The formaldehyde is rapidly converted to formic acid, which then is metabolized by tetrahydrofolate dehydrogenase to CO2 and water.[17, 18]

## 2.3 Dosage and Clinical Use

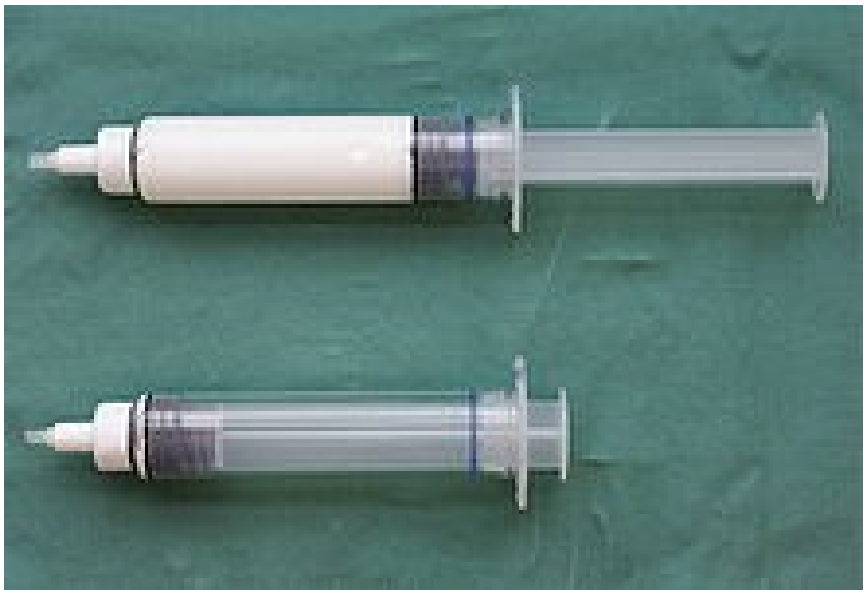


**Image 4: In lipid emulsion dissolved Propofol**

As with most other inductions agents, propofol must be used with great caution in patients with hypovolemia or shock and in the elderly. Propofol does not trigger malignant hyperthermia crises and can be used to induce and maintain anesthesia in susceptible individuals. Propofol is sub-anesthetic does shows antiemetic activity due

to its anti-dopaminergic activity. It also relieves pruritis induced by spinal injection of opioids.[16]

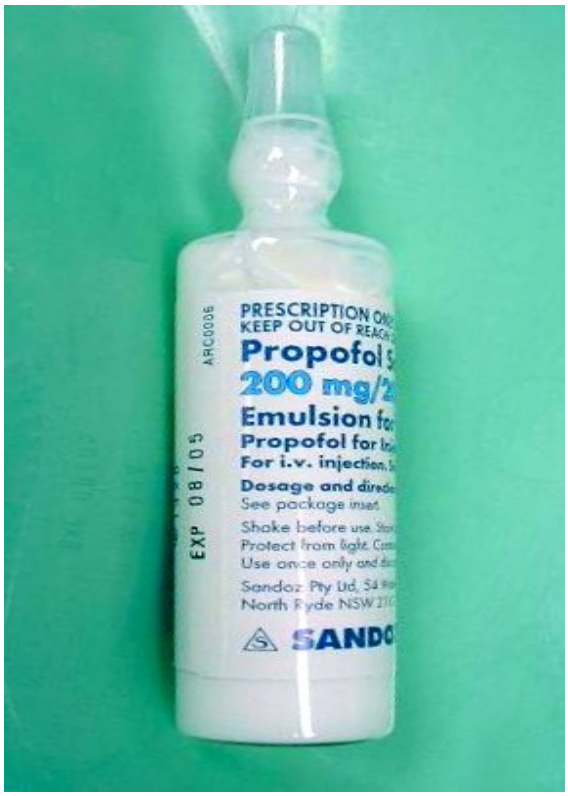



**Image 5: 20 ml ampoule of 1% propofol emulsion by SANDOZ**

The Induction dose of propofol (DIPRIVAN, others) in healthy adult is 2-2.5 mg/kg and it has an onset and duration of anesthesia similar to thiopental. As with barbiturates, dosages should be reduced in the elderly and in the presence of other sedatives and increased in young children. Because of its reasonably short elimination t ½, propofol often is used for maintenance of anesthesia as well as for induction. For short procedures, small boluses (10-50% of the induction dose) every 5 minutes or as needed are effective. An infusion of propofol produces a more stable drug level (100-300 µg/kg per minute) and is better suited for longer-term anesthetic maintenance. Infusion rates should be tailored to patient response and the levels of other hypnotics. Sedating doses of propofol are 20-50% of those required for general anesthesia. However, even at these lower doses, caregivers should be vigilant and prepared for all of the side effects of propofol discussed below, particularly airway obstruction and apnea. Fospropofol produces dose-dependent sedation and can be administered on otherwise healthy individuals at 2-8 mg/kg intravenously (delivered either as a bolus or

by a short infusion over 5-10 min). The optimum dose for sedation is ~ 6.5 mg/kg. This results in a loss of consciousness in ~ 10 minutes. The duration of the sedative effect is ~ 45 min. .[17]

## 2.4 Pharmacokinetics and Metabolism

Intravenous administration of propofol, like that of thiopental, results in rapid distribution of the drug into the vessel-rich group of tissues, including brain. Unconsciousness is induced in 15 to 30 seconds, depending on the speed of injection. Propofol can be irritating to the blood vessel at the site of injection; this can be minimized by prior administration of intravenous lidocaine or a slower rate of injection (and thus a slower onset of anesthesia). The termination of action is due to redistribution of the drug out of the brain into less-well-perfused tissues such as muscle and fat, such that the patient will wake up in 5 to 10 minutes. When it is given as a bolus for induction, the redistribution an elimination half-lives are approximately 5 minutes and 3 hours, respectively. When it is given as an infusion or in repeated boluses, the half-lives may vary greatly. .[17]

Propofol is rapidly and metabolized in the liver. About 98% is excreted in the urine: 40% is found as propofol glucuronide or sulfate, while approximately 60% is metabolized by the cytochrome P450-dependent mixed-function oxidase system to 2,6–diisopropyl-1,4-quinol, which is then conjugated to glucuronide or sulfate. This elimination process has a half-life of about 2 to 3 hours. Because of rapid biotransformation and elimination, propofol does not accumulate in the body to any great extent, and therefore, unlike thiopental, it can be used to maintain anesthesia with continuous infusion. At sub-hypnotic doses, propofol can be used for sedation and amnesia.[17]

## 2.5 Pharmacology and Side Effects

### 2.5.1 Nervous System

Propofol is useful for intravenous sedation and for maintenance of anesthesia during surgery. It can produce subjective feeling of well-being and may have abuse potential. It has anticonvulsant properties, but, unlike thiopental, it does not cause hyperalgesia. Propofol reduces brain basal metabolic rate, cerebral blood flow, and intracranial pressure. However, it does not affect cerebrovascular autoregulation and vasomotor response to carbon dioxide. Unconsciousness is induced following an intravenous dose of 1.5 to 2.5 mg/kg. The duration of anesthesia is about 5 to 10 minutes, depending on the dose used. The infusion dose is between 25 and 75 μg/kg/min for sedation and 100 to 200 μg/kg/min for hypnosis. Recovery from propofol is associated with less residual sedation fatigue (“hangover”), and cognitive impairment than with other intravenous anesthetics.[16]

The sedation and hypnotic actions of propofol are mediated by its action on GABA A (Gamma-aminobutyric acid) receptors; agonism at these receptors results in an increased chloride conduction and hyperpolarization of neurons. Propofol suppresses the EEG, and in sufficient doses, can produce burst suppression of the EEG. Propofol decreases CMRO2, cerebral blood flow, and intracranial and intraocular pressures by about the same amount as thiopental. Like thiopental, propofol has been used in patients at risk for cerebral ischemia; however, no human outcome studies have been performed to determine its efficacy as a neuroprotectant. Excitatory phenomena, such as choreiform movements and opisthotonus, have been observed after propofol injection with the same frequency as that seen with thiopental but less than with methohexital. These movements, which are transient, are not associated with seizure activity. Results from studies on the anticonvulsant effects of propofol have been mixed; some data even suggest it has proconvulsant activity when combined with other drugs. However, propofol has been shown to suppress seizure activity in experimental models and has been used for the treatment of status epilepticus in humans.[17, 19]

### 2.5.2 Cardiovascular System

Propofol administration reduces both systolic and diastolic blood pressure because of its direct myocardial depressant effect with associated reduction in cardiac output and systemic vascular resistance. The baroreflex mechanism is also blunted. These effects are potentiated by prior administration of opioid analgesics. When propofol is used for the maintenance of anesthesia, the blood pressure is reduced by about 20%, or even more in elderly patients.[16]

As with thiopental, propofol should be used with caution in patients at risk for or intolerant of decreases in blood pressure; these include patients with significant blood loss and hypovolemia.[17]

### 2.5.3 Respiratory System

Induction of aesthesia with propofol is frequently accompanied by a period of apnea that may last for more than 1 minute, depending on the dose administered. The maintenance of anesthesia with propofol results in a dose-dependent decrease in ventilation (decreased tidal volume and increased respiratory rate) and increased when propofol is administered together with other anesthetic adjuvant such as opioids and benzodiazepines. In contradistinction to inhalation anesthetics, propofol does not inhibit hypoxia-induced pulmonary vasoconstriction. It may offer some bronchodilatation in patients with chronic obstructive pulmonary disease.[16]

### 2.5.4 Other Side Effects

Propofol has no clinically significant effects on hepatic, renal, or endocrine organ systems. Unlike thiopental, propofol does not have an anti-analgesic effect. It has significant anti-emetic action. Propofol elicits pain on injection that can be reduced with lidocaine and the use of larger arm and antecubital veins. Propofol provokes anaphylactoid reactions at about the same low frequency as thiopental; the histamine release ( in the absence of anaphylactic or anaphylactoid reactions) that occurs with thiopental administration is greater than that with propofol. Although propofol does cross placental membranes, it is considered safe for use in pregnant women; like thiopental, propofol only transiently depresses activity in the newborn.[17, 20]

Propofol does not trigger malignant hyperthermia.[17]

A rare but potentially fatal complication, termed propofol infusion syndrome (PRIS), has been described primarily in prolonged, higher-dose infusions of propofol in young or head-injured patients. The syndrome is characterized by metabolic acidosis, hyperlipidemia, rhabdomyolysis , and an enlarged liver. While the precise mechanisms by which PRIS occurs are not clear, alterations in mitochondrial metabolism and electron transport chain function have been described.[17, 21]

The side-effect profile of fospropofol is similar to that of propofol. Fospropofol`s slower onset of sedation ( due to the need for hydrolysis of the prodrug) results in a lower incidence of hypotension, respiratory depression, apnea, and loss of airway patency. Nonetheless, unintended deep levels of sedation can occur with fospropofol, and the drug should therefore be used only by individuals who can maintain an adequate airway and support cardiorespiratory function.[17]

Whether fospropofol can also cause PRIS is not currently know.[17, 18]

A metabolic byproduct of fospropofol is formic acid. This is degraded to CO2 and water by tetrahydrofolate dehydrogenase, an enzyme that requires folate as a co-factor. In patients who have a folate deficiency, there is a theoretical risk of formic acid accumulation; to date, such an adverse event has not been reported.[17]

# 3. Introduction

“There are two major classes of living organisms; male and female. In many cases, they are so different in form and habit that one might well be excused the thought that males and females are different species”.[1, 22]

In recent years it has become clear that gender differences exist both in the pharmacokinetics and the pharmacodynamics of drugs related to the practice of anesthesia. Some of these differences are of clinical importance whereas others are mainly of theoretical interest. Differences in pharmacokinetics are more straightforward to study than differences in clinical effects because it is a relatively simple procedure to measure drug concentrations in human plasma. However, isolated pharmacokinetic data are of less value if they are not accompanied by measurements of clinical effects or outcomes.[1]

The gender aspect in pharmacokinetics of anesthetics has attracted little attention, although this is required to decide whether gender-based differences in anesthesia practice are justified and to determine the need for further research. Females have 20-30% greater sensitivity to the muscle relaxant effects of vecuronium, pancuronium and rocuronium. When rapid onset of action or short duration of action is important, gender-modified dosing may be considered. Interestingly males are more sensitive than females to propofol. It may therefore be necessary to decrease the propofol dose by 30-40% in males compared with females in order to achieve similar recovery times. Furthermore, Females are more sensitive than males to opioid receptor agonists such as morphine and a number of kappa (OP2) receptor agonists. On this basis, males will be expected to require 30-40% higher doses of opioid analgesics than females to achieve similar pain relief. On the other hand, females may experience respiratory depression and other adverse effects more easily if they are given the same doses as males.[1]

These examples illustrate that gender should be taken into account as a factor that may be predictive for the dosage of several anesthetic drugs. Moreover, there is an obvious need for more research in this area in order to find the basis for such huge gender differences.[1]

# 4. Patients and Methods

## 4.1 Study design

Our study was registered at EudraCT, trial registration number: 2009-017921-20. A prospective controlled clinical consecutive study will be conducted in conformity with the guidelines of the "Consolidated standards of reporting trials (CONSORT)-statement".[23]

After Medical University of Graz ethics committee approval (approval number 21-329 ex 9/10 on the 5$^{th}$ August 2010 chaired by Prof. Dr. Peter Rehak), all patients who agree to participate in the study will give a written informed consent. Potential participants with body mass index <20-26< kg/m$^2$, or patients on treatment with drugs thought to interfere with neuromuscular transmission will be excluded from the study. Twenty consecutive male and female (10/10), ASA I-III patients, aged 18-65 yr, undergoing general anesthesia for scheduled elective surgery will be recruited in the study.

Anesthesia was induced with propofol 2 mg/kg ($t_0$) followed by cisatracurium 0.1 mg/kg 2 min from propofol administration.

## 4.2 Anesthesia

Oral midazolam 3.75-7.5 mg was the only premedication given 1 h before surgery. Anesthesia was induced with fentanyl 1 μg/kg and propofol 2 mg/kg, Patients were ventilated via a facemask. Patients received a single bolus dose of cisatracurium 100 μg $kg^{-1}$. (2 x $Ed_{95}$ effective $dose_{95}$) for tracheal intubation. Analgesia was maintained with remifentanil 0.1-0.2 μg $kg^{-1}$ $min^{-1}$ infusions. The lungs were ventilated mechanically with 40% oxygen in air and adjusted to maintain 25-35 mm Hg end-tidal carbon dioxide. Patients were warmed using a forced-hot-air-blanket to maintain core temperature above 36°C and skin temperature above 32°C. Ephedrine 10-20 mg bolus doses were administered when hypotension occurred. Mean arterial pressure (MAP) and heart rate (HR) were continuously recorded.

## 4.3 Blood sample acquisition, handling, processing and Propofol Concentration Assay

Serial arterial blood samples (approximately 4 ml) will be withdrawn before induction ($Pt_0$) and at 1, 3, 5, 7, 10, 15, 20, 25, 30, 60, 90 and 120 min following propofol administration. During the blood samples acquisition part of the study, the levels of anesthesia were maintained using sevoflurane inhalational anesthesia to avoid additional propofol administration. Within minutes of samples acquisition, plasma was isolated by 2500 g centrifugation for 10 min. Blood samples was immediately extracted, acidified and stored frozen to prevent degradation by adding buffer to 1 ml plasma, carefully mixed and subsequently stored at –20°C. Internal standard was added to each set of plasma samples that will be used to rectify any variations in recovery and stability among samples.

After all samples were collected, blood samples were assayed in duplicate using high performance liquid chromatography (HPLC). Precision of the assay expressed as Relative Standard Deviation for concentrations less than 400 ng $ml^{-1}$ was 3.9%.

## 4.4 Statistical Analysis

We used repeated measures Analysis of Variance (ANOVA) to compare parameters differences over time (group and time factors) for different propofol plasma concentrations. Data were expressed as means ±SD. $P$ <0.05 was considered statistically significant.

Statistical analyses were performed using Number Crunching Statistical System 2007 (NCSS Inc., Kaysville, UT, USA) and StatXact (Cytel Software Corporation, Cambridge, MA, USA).

# 5. Results

There were no significant differences between the two gender groups regarding mean arterial pressure, estimated blood losses, fluid replacements, and remifentanil doses during the 120 min blood sample acquisition part of the study.

**Table 1: Patients' demographics and blood investigations.**

| | Males | Females | P Value |
|---|---|---|---|
| Age (yr) | 47.3±11 | 49.4±11.1 | 0.6669 |
| Weight (kg) | 84.9±20.8 | 69±12.7 | 0.0609 |
| Height (cm) | 177.4±9.3 | 163.3±5.5 | 0.0009 |

Means±SD.

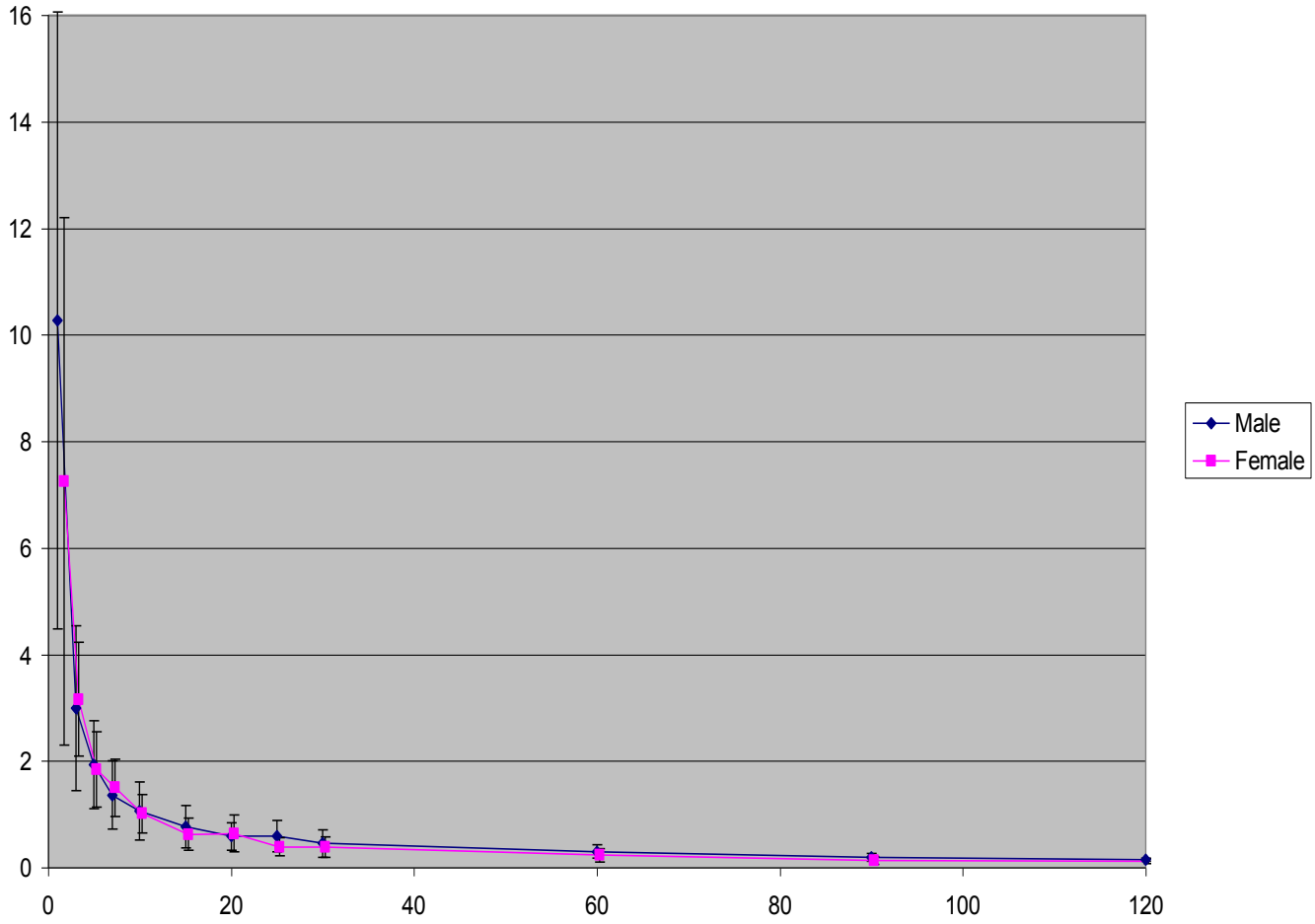
**Table 2 : Propofol plasma concentrations.**

| | Males | Females | P Value |
|---|---|---|---|
| 1 min | 10.3±5.8 | 7.3±5 | 0.4723 |
| 3 min | 3±1.5 | 3,2±1.1 | 0.8235 |
| 5 min | 1.9±0.8 | 1.8±0.7 | 0.8146 |
| 7 min | 1.4±0.6 | 1.5±0.5 | 0.6561 |
| 10 min | 1±0.5 | 1±0.4 | 0.8335 |
| 15 min | 0.8±04 | 0.6±0.3 | 0.4528 |
| 20 min | 0.6±0.3 | 0.6±0.3 | 0.7085 |
| 25 min | 0.6±0.3 | 0.4±0.2 | 0.1178 |
| 30 min | 0.5±0.3 | 0.4±0.2 | 0.5218 |
| 60 min | 0.3±0.1 | 0.2±0.1 | 0.2536 |
| 90 min | 0.2±0.1 | 0.1±0.1 | 0.1376 |
| 120 min | 0.1±0 | 0.1±0 | 0.2889 |

Mean ±SD.

There were no statistically significant differences between the two groups.

**Figure 1. Propofol plasma concentrations of the two groups.**




Means±SD.

# 6. Discussion

Despite the fact that patients in a single geographic region share certain traits both genetic and environmental, thus we tried to identify gender differences in the same population that share clear described factors; such as diet, pollution and physical exercise; accounting for body composition, fat distribution, and skeletal muscle fiber type proportion, which could have resulted in considerably different propofol distributions. Our study was an attempt to evaluate the gender differences in patients sharing these combined ethnic/environmental variables.[24]

A possible explanation of our results regarding the fact that there was no statistically significant difference between males and females in our study was the fact that Propofol, although a lipophilic but it seems the different fat distribution between males and females did not considerably contribute to our study results. Thus there was no statistically significant difference in the propofol plasma concentrations at different time points.

## 6.1 Effect of propofol plasma proteins on gender differences

Only the unbound free fraction can exert a pharmacological effect. However changes in plasma protein binding were recently shown to have very little clinical relevance,[25-27] except for a small group of drugs that are extensively protein bound (>96%) with a very large volume of distribution.[28,29]

## 6.2 Effect of hemodynamic parameters on propofol gender differences

The effect of propofol, with its high hepatic extraction ratio.[30] is expected to depend on hemodynamic changes during induction. However in our study, hemodynamic parameters did not seem to play a significant role, as there were no significant differences between the two groups. However cardiac output was not

measured in our study and it is possible that the gender differences was due to differences in cardiac output.[31]

## **6.3** Propofol and gender differences

In a phase IV multicenter study of 2981 patients, females recovered more quickly than males from propofol [32] This was also confirmed in a recent prospective cohort study [33] Moreover, among 274 adult patients given propofol/alfentanil/nitrous oxide anesthesia, females had consistently approximately 40% shorter recovery times than males [34]. The authors discussed the possibility that this observation was partly the result of the lower sensitivity to propofol in females compared with males. They provided support for this hypothesis in a small (n =10) volunteer study showing that males became more deeply sedated (measured by auditory evoked potentials) than females with the same dose of propofol.[35] The authors then re-examined possible gender aspects in a previous volunteer study (n =72) using the bispectral index to determine the sedation level. Again, evidence of a lower sensitivity for propofol was found in females [36] In a study in 32 elderly patients, blood propofol concentrations were approximately 10% lower in females than in males [37] The pharmacokinetic analysis revealed a larger volume of distribution and a higher metabolic clearance in females.

These studies consistently show that females are less sensitive than males to the anesthetic effects of propofol resulting in 40% faster recovery in females at the same propofol dose. One of the studies [38] provides evidence that the gender difference is caused by pharmacodynamic factors, whereas another study [37] in the elderly showed that a pharmacokinetic difference also might exist, at least in this population. This pharmacokinetic differences we did not encounter in our study. This could lead us to conclude that the gender differences in propofol effect are a pure pharmacodynamic effect with no pharmacokinetic contribution to the gender differences in propofol effect. Thus, the precise mechanism for the observed gender difference is not established, and further research is required to clarify this issue. Also, clinical studies designed to demonstrate whether a dose-reduction of propofol in males is safe should be encouraged. [1]

Finally, in case we would have found statistically significant differences in propofol plasma concentrations, which we did not find, this would have necessitated a new pharmacokinetic model especially designed for gender differences, or a change in target concentrations using the same model according to gender.

Given the fact that there is probably already considerable, and often unpredictable, variability even among patients, surely this greatly emphasizes the importance of target controlled infusion (TCI) drug titration to Bispectral Index (BIS) effect, and gives more credence to the current usual practice of “dosing to clinical effect”. [24]

## 6.4 Future Aspects

In general, the base of knowledge on gender differences of anesthetic drugs is small. One explanation is probably that clinical investigators historically have been more or less reluctant to include females in clinical trials because of concerns with potential birth defects, not least caused by discriminating guidelines and regulations from bodies responsible for drug approval procedures.[1]

This shows that gender differences in the pharmacokinetics and pharmacodynamics for some drugs used in the practice of anesthesia do exist. Isolated pharmacokinetic observations could lead to altered clinical practice, although a pharmacokinetic gender difference might be counterbalanced by a pharmacodynamic difference in the opposite direction. If pharmacokinetic differences of relevance for the outcome are demonstrated, anesthesiologists should be aware that in general, differences in the volume of distribution call for a change in the practice of the initial loading of the drug. However, group differences of less than 20–30% are most often not relevant in clinical practice.

By and large, significant gender differences are recognized for the major groups of anesthetic drugs such as propofol, opioids and muscle relaxants. The advanced infusions of the target-controlled infusions (TCI) could be beneficial in that regard. Apparently there is a need for research to solve whether gender differences should be incorporated in the software of these TCI devices.[1]

The emerging knowledge on gender differences relevant for pharmacotherapeutics has changed the attitudes of drug-regulating authorities, which have now issued guidelines and regulations ensuring that females are included appropriately in clinical trials. For example, the Food and Drug Administration (FDA) in the USA issued a guideline on this issue already in 1993. The final rule, proposed in 1998 and effective from July 2000, permits the FDA to delay a proposed clinical investigation or to suspend an ongoing trial if females or males are excluded from participation solely because of a potential risk of reproductive or developmental toxicology from the drug [26] Hopefully, such guidelines and regulations will increase the awareness of the importance of performing clinical studies with both genders adequately represented and of designing specific studies with the primary aim of revealing possible gender differences. In the future, such studies should preferably be carried out before new drugs are launched.[1]

This clearly indicates that gender should be taken into account as a predictive factor for the dosage of several drugs used in the anesthesia practice. There is an obvious need of more research in this field in order to further optimize drug treatment towards a more individualized dosage in anesthesia.[1]

## 6.5 Conclusion

In conclusion, we demonstrated that no significant differences in propofol plasma concentrations between males and females. Given our observed results of no clear real differences between the 2 genders, larger studies are not warranted to explore the potential differences between genders that may contribute to our understanding of these differences.

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# E. List of abbreviations

**ANOVA:** Analysis of Variance

**BIS:** Bispectral Index

**CMRO2:** Cerebral Metabolic Rate of Oxygen

**CONSORT:** Consolidated standards of reporting trials

**ED:** Effective dosis

**EDTA:** Ethylenediaminetetraacetic acid

**EEG:** Electroencephalography

**FDA:** Food and Drug Administration

**GABA:** Gamma-aminobutyric acid

**HPLC:** High performance liquid chromatography

**HR:** Heart rate

**MAP:** Mean arterial pressure

**PRIS:** Propofol infusion syndrome

**SD:** Standard deviation

**TCI:** Target-controlled infusions