Ann Anesth Crit Care . In Press(In Press):e80198. doi: 10.5812/aacc.80198.

Published online 2019 April 13. **Research Article**

Mivacurium Sensitivity at the Adductor Pollicis and Hand Grip Muscles: Differences Between Males and Females

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Received 2018 June 05; **Revised** 2019 February 14; **Accepted** 2019 March 13.

Abstract

Background: Males lose more handgrip strength (HGS) than females when adductor pollicis (AP) TOF ratio decreases. The reason is unclear.
Objectives: The primary aim of this study was to explore gender-related differences in neuromuscular sensitivity to mivacurium. As a secondary aim, clearance of mivacurium was determined.
Methods: In 10 healthy males and 10 healthy females, constant-rate infusions of mivacurium were administered to obtain three different levels of stable neuromuscular block (normalized acceleromyography AP TOF ratio 80, 60, and 40%) in each study subject. Arterial blood samples were collected to determine mivacurium plasma concentrations. The HGS was measured every five minutes. A Hill equation was fitted to data on mivacurium concentration versus normalized AP TOF ratio and HGS to determine drug concentrations associated with 50% maximum effect (C50 AP TOF ratio and C50 HGS). Differences within and between genders were tested with the parametric *t*-test. Clearance of mivacurium was calculated at each block level as the ratio between drug infusion rate and concentration. Gender-related differences in relationships between AP TOF ratio and HGS, mivacurium infusion rates, and mivacurium plasma concentrations were determined with linear mixed-models.
Results: The C50 AP TOF ratio was significantly greater than C50 HGS in males, yet not in females. Mivacurium infusion rates, needed to maintain stable neuromuscular blocks, were significantly greater in males, while clearance was similar between genders. Males lost significantly more HGS with decreasing AP TOF ratio than females, both in absolute (kg) and relative (percentage of baseline) terms.
Conclusions: In males, yet not in females, the AP was significantly less sensitive to the effect of mivacurium than the muscles involved in the handgrip function. This finding explains why handgrip strength decreases more in males than females with decreasing AP TOF ratio during the mivacurium block.

Keywords: Neuromuscular Block, Mivacurium, NM Relaxants, Pharmacodynamics, Gender

1. Background

In a previous study on healthy human volunteers, the authors reported that males and females respond differently to mivacurium. As mivacurium dose was increased, to render similar decreases in adductor pollicis (AP) train-of-four (TOF) ratio in both genders, the reduction in actual handgrip strength (HGS) was greater in males. Males also lost the ability to clench teeth, swallow, and raise their head > 5 seconds at a higher TOF ratio than females (1). In that study, all measurements were recorded under conditions, where stable AP TOF ratios were meticulously established. Consequently, although gender-related differences in pharmacokinetics of mivacurium may exist, they cannot explain the findings. In the present investigation, the authors sought to compare the pharmacodynamics of mivacurium in males and females, in a population where gender-related differences in the relationships between AP TOF ratio and HGS were confirmed.

2. Objectives

The specific aims were to determine C50-values (2) for AP TOF ratio and HGS, i.e. drug concentrations at steady state conditions associated with 50% maximum effect (a measure of drug sensitivity), and γ, the slope of the mivacurium drug concentration versus effect curve. The researchers hypothesized that the relationship between C50 AP TOF ratio and C50 HGS was different between males and females.

3. Methods

The study was an interventional clinical trial on healthy volunteers.

3.1. Approvals

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and approval was obtained from the Institutional Review Board at the University of California in San Francisco (University of California protocol record #IRB 11-07970). This investigation adhered to the applicable equator guidelines and was registered with ClinicalTrials.gov (NCT01906528, July 24, 2013).

3.2. Enrolment

Ten males (29 ± 7 years old, 76 ± 7 kg, and BMI: 23.1 ± 2) and 10 females (26 ± 4 years old, 63 ± 9 kg, and BMI: 23.3 ± 2) were included after an in-depth interview and written consent was obtained from each study subject. Inclusion criteria were being healthy, age of 18 to 40 years old, and normal airway exam. Exclusion criteria were BMI > 25, smoking, medication interfering with neuromuscular blocking drugs, history with anesthesia-related problems, acid reflux, current upper airway infection, positive urine pregnancy test, or breast feeding.

3.3. Administration of Mivacurium

Mivacurium, as the sole drug, was administered together with lactated Ringer's solution via the antecubital vein in the left arm, using a Harvard infusion pump (Harvard Clinical Technology, Inc. Natick, MA). The initial infusion rates were 1.5 (males) and 1.2 (females) $\mu g.kg^{-1}.min^{-1}$ (3). The infusion rate was changed (± 10% to 50%) twice to obtain three levels of stable plasma concentrations.

3.4. Train-of-Four (TOF) Ratio Measurements

The TOF ratio was measured every 15 seconds at the left AP muscle using acceleromyography ("TOF Watch SX", Merck Co., Whitehouse Station, NJ). A hand adapter (Merck Co.) applying approximately 75 g preload to the thumb was used to enhance repeatability of the measurements (4). The signal from the monitor was analyzed using the TOFMON software (Merck Co., Whitehouse Station, NJ). A continuous moving average of four consecutive TOF ratios was the value taken. The TOF ratio was considered stable if the difference in average TOF ratios was < 3% over a period of at least 10 minutes of continuous nerve stimulation.

The researchers believed that the volunteers would not tolerate supramaximal nerve stimulation during each study. The stimulating current was maintained at ≥ 15 mA above the threshold for detection of the twitch response in order to obtain reliable recordings (5). The intensity of nerve stimulation was transiently increased by 5 mA every 15 minutes in order to ensure the stability of the TOF response. Hand skin temperature of > 320°C was maintained with blankets.

Stable AP TOF ratios were targeted at three different levels: AP TOF ratio approximately 0.8 (superficial), 0.6 (moderate), and 0.4 (deep). In order to compensate for baseline TOF ratios different from 100%, all values were normalized by dividing each uncorrected TOF ratio with that subjects' baseline value.

3.5. Recording of Handgrip Strength

Every five minutes, the handgrip strength of the right hand was measured twice with a calibrated dynamometer (Jamar 5030J1, Sammons Preston, Chicago, IL) (6). The average HGS-value at each level of stable neuromuscular block was used in the pharmacodynamic analysis. All volunteers familiarized themselves with the equipment before initiation of each study.

3.6. Volunteer Safety Management

Two trained anesthesiologists were present at all times during experiments, and electrocardiogram, heart rate, non-invasive blood pressure, and arterial oxygen saturation were monitored. Emergency equipment and drugs were readily available.

As a safety measure during each study, and the ability of the volunteer to clench teeth and swallow was tested. Inability to clench the teeth is an important predictor of reduction in upper airway patency (3, 7), and was tested by asking the volunteers to retain a wooden tongue depressor placed between the incisors, while the investigator attempted to pull it out with the thumb and index finger (3). Swallowing was tested by asking the volunteer to grade subjectively if it was harder than normal to clear saliva from the throat, and by checking the ability to drink water via a straw. Volunteers were considered unable to swallow if it was observed that the study subject could not elevate the larynx (8).

In order to detect rapid functional changes during non-steady state plasma concentrations of mivacurium, all tests were performed every five minutes. Baseline evaluations were obtained before commencement of the mivacurium infusion. All assessments were performed in the supine position with the upper body elevated 15 degrees.

The following criteria indicated that the mivacurium infusion had to be reduced or terminated immediately: The volunteer asked to discontinue; signs of airway obstruction (stridor); complete loss of ability to swallow; or arterial oxygen saturation below 90%.

3.7. Outcome Measures

3.7.1. Primary: Pharmacodynamics

Differences between C50 AP TOF ratio and C50 HGS, and between γ AP TOF ratio and γ HGS, within and between genders.

3.7.2. Secondary: Clearance of Mivacurium

Gender-related differences in body clearance of mivacurium.

3.8. Mivacurium Analyses

3.8.1. Drug Concentration

At each level of stable neuromuscular block, arterial blood samples were collected via a catheter inserted in the left radial artery to determine mivacurium plasma concentrations. Duplicate samples, 10 minutes apart were collected to confirm stable plasma concentrations, and the average value was reported. Overall, 5 mL of blood was transferred to an EDTA-vacutainer containing 1.25 mg of phospholine iodide (prevents mivacurium degradation). Samples were centrifuged and plasma was frozen at -80 degrees for later analysis with mass spectrometry (6). The assay is sensitive to 5 $ng.mL^{-1}$ with a coefficient of variation of < 3.2% in the concentration range between 5 and 400 $ng.mL^{-1}$.

3.8.2. Pharmacodynamic Analysis

C50 is the blood or plasma concentration at steady state conditions, associated with 50% of maximum drug effect, and it has been a long tradition in anesthesia literature to use this variable as a measure of drug sensitivity (2). Using an iterative process (Solver function in Excel), a Hill equation (Effect (Effect = $C^{\gamma}/C^{\gamma} + C^{\gamma}50$) was fitted to normalize AP TOF ratio and HGS values versus corresponding mivacurium plasma concentrations obtained at stable neuromuscular blocks, in order to estimate C50 AP TOF ratio and C50 HGS in each study subject. The slope factor of the concentration-effect curve (γAP TOF ratio and γHGS) was estimated in the same process.

3.8.3. Drug Clearance

The plasma clearance of mivacurium was calculated at each level of stable neuromuscular block as steady state mivacurium infusion rate ($\mu g.kg^{-1}.min^{-1}$) divided by the mivacurium plasma concentration ($\mu g.mL^{-1}$).

3.9. Power Analysis

The researchers assumed that the same number of study subjects were needed in the present as in the previous investigation (1). In that study, the power analysis was based on data from a publication in 2010 (9), where a difference of 20% in loss of HGS between males and females was observed. The interindividual standard deviations (SD) were 7% (males) and 14% (females). A 5% error margin was added to compensate for the small number of study subjects (males 7 + 5% and females 14 + 5%). With these assumptions, the power analysis showed that 20 study subjects had to be enrolled to detect a 20% difference with > 80% probability, assuming an α error of < 0.05.

3.10. Statistical Analysis

Groupwise comparisons were tested using the parametric *t*-test. The relationships between AP TOF ratio and HGS, mivacurium infusion rates, and mivacurium concentrations were tested with least squares linear regression. The regressions were analyzed with a mixed-model that accommodates dependencies in the observations and compensates completely for repeated measurements. This model used AP TOF ratio as a continuous variable and included HGS, mivacurium infusion rates and concentrations, gender, and the TOF ratio × gender interaction (interaction term) as fixed effects, and individuals as random effects. The significance level was set at $P < 0.05$. All statistical analyses were made using JMP version 11.0 (SAS Institute, Cary, NC).

4. Results

Body weight was significantly greater in males than females ($P < 0.001$) yet age and BMI were similar ($P < 0.85$ and < 0.95). All study subjects completed the study. Eight males and two females lost the ability to clench teeth, while two males lost the ability to swallow at the deepest level of neuromuscular block. Breathing problems or signs of aspiration were not observed, and oxygen saturation was never < 95% in any individual. Blurry vision occurred as the first sign of neuromuscular blockade, and was the last to disappear after conclusion of the experiment.

Slopes of the relationships between AP TOF ratio and HGS were different between genders both in absolute (loss of HGS in kg from baseline / % reduction in normalized AP TOF ratio) and relative (% loss of HGS from baseline / % reduction in normalized AP TOF ratio) terms (interaction term: $P < 0.001$ and $P = 0.003$, respectively), confirming the researchers' previous findings (1) that the loss of handgrip strength with decreasing AP TOF ratio is greater in males than females (Table 1). The relationships between AP TOF ratio and mivacurium infusion rate and concentration did not differ between genders (Table 1).

Table 1. Mixed-Model Analysis[a]

Relationship	Slope	
	Males	Females
Loss of HGS (kg) vs. AP TOF ratio[b]	0.0080 ± 0.012	-0.0010 ± 0.016
Range	-0.017 - 0.024	-0.0017 - 0.0013
95% CI	0.004 - 0.016	-0.0040 - 0.002
P Value for TOF Ratio × Interaction[c]	< 0.001	
Loss of HGS (%) vs. AP TOF ratio	0.016 ± 0.020	-0.0045 ± 0.020
Range	-0.030 - 0.038	-0.021 - 0.019
95% CI	0.002 - 0.030	-0.018 - 0.000
P Value for TOF Ratio × Interaction[c]	0.003	
Mivacurium infusion rate (μg.kg.min^{-1}) vs. AP TOF ratio	0.024 ± 0.01	0.021 ± 0.01
Range	0.011 - 0.047	0.007 - 0.035
95% CI	0.018 - 0.030	0.015 - 0.027
P Value for TOF Ratio × Interaction[c]	0.81	
Mivacurium plasma concentration (μg.kg^{-1}) vs. AP TOF ratio	0.66 ± 0.27	0.70 ± 0.24
Range	0.44 - 1.12	0.40 - 1.18
95% CI	0.50 - 0.82	0.55 - 0.85
P Value for TOF Ratio × Interaction[c]	0.79	

Abbreviation: 95% CI, 95% confidence interval.
[a]Values are expressed as mean ± SD unless otherwise indicated.
[b]Loss of AP TOF ratio (%), percentage loss of normalized adductor pollicis TOF ratio from baseline; Loss of HGS, absolute loss (kg) or percentage loss (%) of handgrip strength from baseline.
[c]TOF ratio × Interaction, the interaction term, showing the influence of sex on relationship.

4.1. Primary Outcome Measure: Pharmacodynamics

In males C50 AP TOF ratio (54.1 ± 12.0, CI: 44.1 - 62.2) was significantly greater than C50 HGS (38.4 ± 9.1, CI: 31.0 - 44.0) (P = 0.007) (Figure 1). In females, C50 TOF ratio (44.4 ± 9.1, CI: 37.7 - 51.6) was similar to C50 HGS (41.0 ± 6.2, CI: 36.1 - 45.1) (P = 0.35) (Figure 1). Accordingly, the calculated C50 AP TOF ratio - C50 HGS difference was significantly greater in males than females (15.1 ± 10.0, CI: 8.0 - 21.1 versus 3.0 ± 8.1, CI: -2.3 - 8.0, P = 0.01) (Table 2). The C50 AP TOF ratio was marginally greater in males than females (P = 0.07), while C50 HGS was similar between genders (P = 0.45) (Table 2). The γTOF ratio and γHGS were similar within and between genders (Table 2).

4.2. Secondary Outcome Measure: Clearance of Mivacurium

Mivacurium infusion rates were greater in males than females at all levels of stable neuromuscular block (P < 0.001) (Table 3). The gender-related differences in mivacurium plasma concentrations reached a significance level at the superficial and moderate block levels (P = 0.05 and 0.03), yet not at the deep block (P = 0.13) (Table 3). The calculated clearance of mivacurium was similar in males and females at all neuromuscular blocks (P = 0.32 to 0.45) (Table 3).

5. Discussion

5.1. Pharmacodynamics of Mivacurium

In the present study, where the gender-related difference in the relationship between AP TOF ratio and HGS was confirmed (Table 1), differences in the pharmacodynamics of mivacurium between males and females was demonstrated. C50 AP TOF ratio was significantly greater than C50 HGS only in males (Figure 1), and the calculated C50 AP TOF ratio - C50 HGS difference was five times greater in males than females (P = 0.01, Table 2). These findings adequately explain why males lose handgrip strength to a greater extent than females when AP TOF ratio decreases. It is likely that most of the observed gender-related difference is caused by C50 AP TOF ratio being greater in males than females, although not unequivocally shown with the current data (P = 0.07).

Previously, the current researchers showed that males lose the ability to clench teeth, raise head > 5, and swallow at greater AP TOF ratios than females during mivacurium block (1). In addition, reexamination of data from a study on pulmonary function during low-degree mivacurium block (9) showed that males also lost the ability to perform vital capacity breathing at greater AP TOF ratios

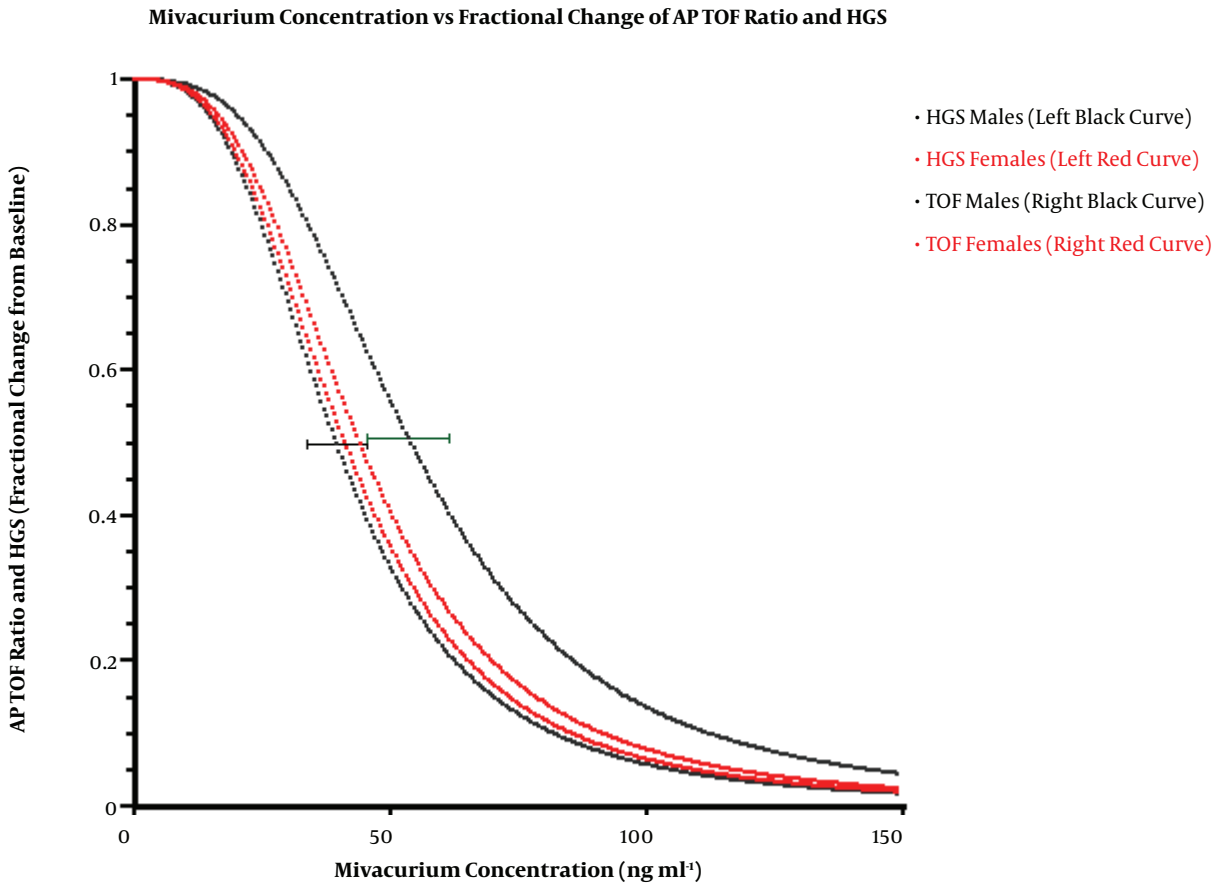

Figure 1. Mivacurium concentrfation vs. fractional change of AP TOF ratio and HGS

than females. These previous findings are consistent with the assumption that in males a significant gap exists, not only between C50 AP TOF ratio and C50 HGS, yet between C50 AP TOF ratio and C50 of several other muscle groups as well. The current authors infer that the observations mirror gender-related differences in the functional relationship between the AP muscle and muscle groups relevant for maintenance of a patent upper airway and normal breathing ability during low-degree neuromuscular block (i.e. AP TOF ratio in the range 40% - 80%).

5.2. Clearance of Mivacurium

In the authors' previous study (1), potential differences in pharmacokinetics of mivacurium could not explain the finding that males lost relatively more handgrip strength than females with decreasing AP TOF ratio, because measurements were obtained at stable neuromuscular blocks. However, the study design did not rule out the possibility that differences in pharmacokinetics of this drug exist between genders. The authors, therefore, included determination of mivacurium clearance as a secondary aim of the present investigation. The study design allowed calculations of mivacurium clearance at three different neuromuscular blocks in each study subject, and the results suggest that mivacurium clearance is similar in males and females (Table 3). Gender-related differences in other pharmacokinetic variables may exist, yet determination of a complete pharmacokinetic profile of mivacurium was not possible with the study design used in the current investigation.

5.3. Clinical Implications

The current findings may have a bearing on the clinical use of TOF monitoring. When neuromuscular function is assessed with TOF monitoring at the adductor pollicis, several clinically important muscle functions may be relatively more affected by the effect of mivacurium in males than females when TOF ratio is similar in both genders. This fact may make males potentially more vulnerable to the occurrence of postoperative airway and pul-

Table 2. Pharmacodynamic Data for Mivacurium

Variable	Males	Females	P Value (Males vs. Females)
C50 AP TOF ratio ($ng.mL^{-1}$)[a]	54.1 ± 12.0	44.0 ± 9.1	0.07
Range	38.5 - 77.0	27.2 - 60.2	
95% CI	44.1 - 62.2	37.7 - 51.6	
γ AP TOF ratio[a]	4.0 ± 2.1	3.2 ± 1.1	0.38
Range	2.2 - 8.0	1.4 - 5.6	
95% CI	2.4 - 5.2	2.1 - 4.1	
C50 HGS ($ng.mL^{-1}$)[a]	38.4 ± 9.1	41.0 ± 6.2	0.45
Range	29.0 - 60.5	29.5 - 54.1	
95% CI	31.0 - 44.0	36.1 - 45.1	
γ HGS[a]	4.2 ± 2.3	4.0 ± 1.3	0.60
Range	2.4 - 8.1	2.1 - 5.5	
95% CI	2.2 - 5.2	2.2 - 4.2	
C50 AP TOF ratio - C50 HGS difference ($ng.mL^{-1}$)[a]	15.1 ± 10.0	3.0 ± 8.1	0.01
Range	1.2 - 35.1	-13.1 - 12.4	
95% CI	8.0 - 21.1	-2.3 - 8.0	

Abbreviations: AP TOF ratio, normalized TOF ratio at the adductor pollicis muscle; C50, plasma concentration at steady state associated with 50% of maximum effect; HGS, handgrip strength; γ, slope of the drug concentration - effect curve; 95% CI, 95% Confidence Interval.
[a]Values are expressed as mean ± SD.

Table 3. Mivacurium Infusion Rate, Plasma Concentration, and Calculated Clearance at Three Levels of Stable Neuromuscular Blocks

Block Level	Superficial		Moderate		Deep	
	Males	Females	Males	Females	Males	Females
Mivacurium infusion rate ($\mu g.kg.min^{-1}$)[a]	1.46 ± 0.12	1.09 ± 0.19	1.96 ± 0.28	1.49 ± 0.18	2.29 ± 035	1.87 ± 0.22
Range	1.1 - 1.5	0.6 - 1.2	1.5 - 2.5	1.2 - 1.8	2.0 - 3.0	1.7 - 2.2
95% CI	1.37 - 1.54	0.95 - 1.22	1.75 - 2.16	1.36 - 1.61	2.03 - 2.54	1.71 - 2.02
P Value (males vs. females)	< 0.001		< 0.001		< 0.001	
Mivacurium plasma concentration ($ng.mL^{-1}$)[a]	32.0 ± 6.1	28.2 ± 5.0	46.0 ± 7.8	38.2 ± 6.8	54.8 ± 7.0	51.0 ± 7.1
Range	17.4 - 39.2	21.4 - 36.6	32.4 - 58.1	27.8 - 50.9	40.0 - 65.7	39.0 - 63.3
95% CI	27.6 - 36.3	24.6 - 31.7	40.4 - 51.5	33.3 - 43.0	49.7 - 59.8	45.9 - 56.0
P Value (males vs. females)	0.05		0.03		0.13	
Mivacurium clearance ($mL.kg.min^{-1}$)[a]	43.0 ± 15.0	41.1 ± 10.0	43.1 ± 8.0	40.9 ± 7.0	42.1 ± 5.0	37.8 ± 5.0
Range	30.5 - 84.0	25.9 - 56.7	31.0 - 61.0	29.9 - 54.6	34.0 - 48.0	30.5 - 48.5
95% CI	38.0 - 58.0	34.1 - 46.0	35.0 - 51.0	36.1 - 47.2	37.0 - 47.0	33.5 - 43.5
P Value (males vs. females)	0.45		0.34		0.35	

Abbreviation: 95% CI, 95% Confidence Interval.
[a]Values are expressed as mean ± SD.

monary function-related complications when adequacy of neuromuscular function is judged by standard TOF ratio monitoring (i.e. at the adductor pollicis). Further studies are needed to confirm this assumption. At present, it is unknown if similar gender-related differences exist when TOF-monitoring is performed at other muscles than the adductor pollicis.

5.4. Study Limitations

Because the current study subjects were awake, the researchers employed submaximal nerve stimulation during the experiments. If not conducted with care, submax-

imal nerve stimulation may introduce erroneous results from AP TOF ratio monitoring (5). It is believed that the TOF ratio data are valid because the researchers always ensured that the stimulating current was > 15 mA above threshold for detecting the twitch response (5), and because they waited until the TOF ratio settled at a stable value. The assumption that stable neuromuscular blocks were obtained is supported by similar plasma concentrations of mivacurium in duplicate measurements at each block level. The observation that AP TOF ratio recovered to baseline value in all volunteers after termination of the mivacurium infusion also suggests valid recordings.

The researchers decided to use mivacurium in their experiments due to its rapid clearance from the blood stream, which enabled them to establish stable neuromuscular blocks, much faster than would have been the case with alternative longer-acting drugs (rocuronium or cisatracurium). At present, there is no reason to believe that the results would have become different, had alternative neuromuscular blocking drugs been used. However, this assumption must be verified in future studies.

5.5. Conclusions

This research demonstrated that in males, but not in females, a significant difference in sensitivity to mivacurium exists between the adductor pollicis and the handgrip muscles. This finding explains the previous observation that men lose more handgrip strength than women with decreasing AP TOF ratio.

Footnotes

Authors' Contribution: Tom Heier: protocol, data collection, data analysis, and manuscript. Silke Leonie Bicknell: data collection, data analysis, and manuscript. James E Caldwell: protocol, data analysis, and manuscript. Peter MC Wright: protocol, data analysis, and manuscript. John R Feiner: protocol, data collection, data analysis, and manuscript.

Conflict of Interests: The authors declared no conflict of interests.

Ethical Approval: The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and approval was obtained from the Institutional Review Board at the University of California in San Francisco (University of California Protocol record #IRB 11-07970). This investigation adhered to the applicable equator guidelines and was registered with ClinicalTrials.gov (NCT01906528, July 24, 2013).

Funding/Support: The study funded by departmental funds only.

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