Changes in heart rate during third molar surgery

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SUMMARY

Objective
Anxiety is an undesirable psychological phenomenon with multiple negative influences on patient compliance, treatment comfort, surgical conditions and postoperative outcome. In oral surgery patients are usually anxious, but the exact pattern is unknown. Therefore, the aim of this study was to assess the intensity and course of anxiety during oral surgery.

Method & material
In 48 patients (25±6 years old) a third molar was surgically removed under local anesthesia. During treatment, heart rate was continuously monitored as a measurement of anxiety. Preoperative anxiety was scored with a Dutch version of the Modified Dental Anxiety Score (MDAS). Patient's anxiety level was analyzed by assessing the impact of individual events on heart rate. The events studied were the waiting room, sitting down in the dental chair, application of local anesthesia, application of surgical drapes, time-out procedure, incision, alveolotomy, removal of the third molar, suturing and the end of the procedure. The heart rate during these events were compared to determine if any changes in heart rate occurred during the whole treatment process.

Results
A significant increase in heart rate was observed when sitting down in the dental chair and when applicating the surgical drapes ($p<0.005$). A decrease in heart rate was measured after the onset of the administration of additional anesthesia ($p<0.005$). The lowest heart rate values were recorded in the waiting room (median 91.0 bpm), during application of anesthesia (88.5 bpm), during suturing (95.0 bpm) and at the end of the procedure (90.5 bpm). The highest values were obtained during the time-out procedure (104.5 bpm), incision (105.4 bpm) and the alveolotomy (102.0 bpm; $p<0.005$). Women revealed to have a higher mean heart rate than men ($p<0.05$). No correlation was found between preoperative heart rate values and preoperative MDAS score.

Conclusion
The intensity and course of anxiety has a specific pattern during oral surgery treatment, in which the most stressful period is experienced before the third molar actually is removed.

SAMENVATTING

Doel

Stress is een ongewenst psychologisch fenomeen met een negatieve invloed op het comfort en mede werken van de patiënt tijdens een chirurgische behandeling en het postoperatieve herstel. Rondom een kaakchirurgische behandeling is het gebruikelijk dat patiënten in meer of mindere mate stress ervaren; het beloop van dit stresspatroon is echter onbekend. Het doel van dit onderzoek was inzicht te verkrijgen in de intensiteit en het tijdsbeloop van stress tijdens het operatief verwijderen van een verstandskies.

Methode & materiaal

Bij 48 patiënten (leeftijd 25±6 jaar) werd een derde molaar in de onderkaak chirurgisch verwijderd onder lokale anesthesie. Tijdens de behandeling werd de hartfrequentie continu gemeten als maat voor stress. Preoperatieve stress werd gescoord met de Modified Dental Anxiety Scale (MDAS). Het stressniveau van patiënten werd geanalyseerd door de veranderingen in hartfrequentie tijdens verschillende meetmomenten van de behandeling te registreren, namelijk het plaats nemen in de wachtkamer, het plaats nemen in de tandartsstoel, de toediending van lokale anesthesie, het afdekken met steriele doeken, de time-out procedure, de incisie, de alveolotomie, het verwijderen van de derde molaar, het hechten van de wond en het einde van de procedure. Daarnaast werden de hartfrequenties tijdens de meetmomenten met elkaar vergeleken om te bepalen of veranderingen in hartfrequentie optraden gedurende het gehele behandelproces.

Resultaten

Het gaan zitten in de tandartsstoel en het afdekken met steriele lakens veroorzaakt bij patiënten een significante stijging in hartfrequentie ($p<0{,}005$). Een afname in hartfrequentie trad op tijdens het toedienen van additionele lokale verdoving ($p<0{,}005$). De laagste hartfrequenties werden geobserveerd tijdens het verblijf in de wachtkamer (mediaan 91,0 bpm), tijdens het toedienen van lokale anesthesie (mediaan 88,5 bpm), tijdens het hechten (mediaan 95,0 bpm) en tijdens het einde van de procedure (mediaan 90,5 bpm), terwijl de hoogste hartfrequenties werden gemeten tijdens de time-out procedure (mediaan 104,5 bpm), incisie (mediaan 105,4 bpm) en alveolotomie (mediaan 102,0 bpm; $p<0{,}005$). Daarnaast hadden vrouwen een significant hogere gemiddelde hartfrequentie dan mannen ($p<0{,}05$). Er werd geen correlatie gevonden tussen de preoperatieve hartfrequentie en de preoperatieve MDAS-score.

Conclusie

De intensiteit en het tijdsbeloop van stress tijdens het operatief verwijderen van een verstandskies laat een specifiek patroon zien, waarbij de meest stressvolle periode de periode voor het daadwerkelijk verwijderen van de derde molaar is.

INDEX

INTRODUCTION

Anxiety in general

Anxiety is characterized by the interaction of cognitive, physiological and behavioral components. At the cognitive level, anxiety is a feeling of apprehension of a possible future threat. Anxiety can be conceptualized as a biological warning system to react mentally and physically to potentially dangerous situations. To be able to respond to the threatening situation, the body prepares itself for fight or flight. An individual increases his or her muscle tension and activates the sympathetic and, to a lesser degree, the parasympathetic nervous systems. Physiological manifestations include symptoms of arousal, most obviously as an increased heart rate, blood pressure, sweat gland activity, and respiration due to autonomic stimulation. Behavioral features of anxiety may include avoidance of the possible threat.[1]

Anxiety and medical interventions

Anxious behavior on specific stimuli can be interpreted as a physiological mechanism of adaptation in unknown situations. In surgical procedures, multiple negative effects of such a state of mind have nearly always been proved by multiple studies within the last 50 years. Moderately to highly anxious patients suffer from significantly more intense postoperative pain and show higher psychological co-morbidity and incidence of posttraumatic stress reactions.[2,3]

Furthermore, anxiety can have a pain-increasing effect during treatment due to a decreased pain threshold.[4] Additionally, anxious patients do not only perceive more severe pain, but also a longer duration of pain during the procedure and during the postoperative period.[5] Among the many variables that influence the pain reaction threshold of a patient, anticipation and anxiety appear to be the most important.[6]

Numerous procedures provoke anxiety. In most cases of acute procedure anxiety, the anxiety tends to peak prior to the procedure and decrease immediately after the procedure. This pattern has been observed in studies of sigmoidoscopy screening for colorectal cancer, colposcopy, percutaneous coronary intervention (PTCA) and cardiac surgery.[7-10] Especially surgical treatment of diseases of the facial and oral region is linked with specific and intensive fear.[4,11-13]

Anxiety in dental procedures

Most people experience some apprehension or anxiety when attending a dentist for treatment.[14-19] In a study from Stouthard and Hoogstraten, 40% of patients indicated that they were anxious about dental treatment, and 10% claimed to be extremely anxious.[19] An estimated 2.4% of the adult population in the US meets criteria for DSM-IV-TR dental phobia.[20] Only 14.2% of the people in a Dutch sample experienced no fear or apprehension while visiting the dentist.[19] Numerous studies have demonstrated that patients with dental anxiety have significantly worse oral health status compared to non-anxious individuals.[21-23] People with high levels of dental anxiety often report that their anxiety has a large impact on daily life in terms of limited functioning and oral health-related problems.[3,24-26]

A visit to the dental clinic not only provokes feelings of anxiety but induces also an increase in blood pressure and heart rate. The latter is due to continuous adaptation of blood pressure and heart rate to internal and external stimuli. Heart rate is directly affected by the autonomic nervous system and can increase with hormonal epinephrine, produced by the adrenal medulla. During dental treatment, the serum levels of epinephrine may increase. Pain and anxiety reactions stimulate the adrenal medulla to release endogenous epinephrine.[27] For example, when patients know that dental treatment is scheduled, this information may induce an increase in blood pressure and heart rate. Twenty-four hours before a scheduled checkup the mean heart rate of women is increased and immediately prior to the procedure the heart rate increases further.[12] During dental treatment the most pronounce increase in heart rate was observed immediately before the injection of local anesthetic. During the actual injection, the heart rate decreased.[28] Similar cardiovascular reactions were observed before and during the injection of isotonic saline and even during a pseudoinjection, whereby the syringe was placed in the mouth but the needle did not touch the mucosa.[29,30] This suggests that patients' anxiety mainly contributes to an increased heart rate and this effect is to a lesser extent due to the use of vasoconstrictors used in local anesthetics.

Fear-provoking stimuli in the dental setting

Various stimuli in the dental office have the potential of provoking patients' anxiety. Quite a large number of studies have been conducted to identify the potential anxiety-provoking stimuli present in the dental setting. For example, the action of sitting down in the dental chair increased the heart rate considerably by 12 beats per minute.[31] Barash and Kleinknecht & Bernstein found that while sitting in the waiting area the anxious patients statistically will move more often than other patients.[32,33]

The individual changes in heart rate and blood pressure are affected by pain and individual factors. Patients' anxiety may be affected by these individual factors such as age, gender, educational status and personality. Studies have shown that the younger population appears to have higher dental anxiety levels.[22,34,35] In general, women report higher anxiety than men.[34,36,37] Some studies have reported that individuals of lower socioeconomic status and with less education have higher anxiety,[22] whereas others have reported greater dental anxiety in those with more education.[38-40] Furthermore, patients with high trait anxiety showed an increased dental anxiety.[37,41] The relationship between the intensity of dental anxiety and treatment experience received has been controversial. Thomson et al indicated that dental anxiety was greatest among people who had never visited a dentist and lowest among those who routinely visited them for preventive care.[34] Kaakko et al reported that students who had never received dental injections showed higher anxiety scores for dental injections than did students who had previously experienced them.[42] However, another report indicated that subjects who had previously experienced tooth extraction showed higher anxiety before oral surgery than did subjects without such experience.[43]

Furthermore, characteristics of the procedure, such as the degree of invasiveness influence the level of anxiety. In a study of Oosterink et al patients rated invasive procedures, such as extraction of a tooth or molar, as more anxiety provoking than non-invasive procedures, such as a dental X-ray or setting (e.g. the waiting room, the dentist).[44] Hermes et al observed that some of the fearful aspects of treatment included fear of complications, pain during treatment and wait-time before treatment.[45]

Anxiety in Oral and maxillofacial surgery

Anxiety assessments indicate a high level of treatment anxiety for oral and maxillofacial treatment situations. Oral and maxillofacial surgery (OMFS) patients' anxiety scores were significantly higher than those in a dental setting.[45] This means that OMFS patients experience a more intense anxiety compared to patients in dental setting. Therefore, the results obtained in dental studies cannot readily be applied in OMFS without any further investigation.

Additionally, the change in intensity of the anxiety throughout the process of third molar removal has not been evaluated thoroughly, although patients' anxiety is likely to vary as the situation changes. Meyer et al and Goldstein et al pointed out that the most marked changes in heart rate were observed just before application of local anesthetics and during the extraction, whereas Alemany-Martinez et al described that the highest heart rate was observed during incision and flap raising.[28,46,47] Moreover, these studies showed variation in heart rate during surgical extraction of the molars, but the differences often did not reach statistical significance. In summary, treatment anxiety is an undesirable psychological phenomenon, whose multiple negative influences on patient compliance and treatment comfort, surgical conditions and postoperative outcome are observable. This is a major issue in the oral surgery which prevents optimal treatment.

Objective

Summarizing, anxiety may fluctuate during oral surgery treatment. A more detailed knowledge of the level and course of anxiety during the treatment process may indicate a pattern. This, in turn may help in finding anxiety reducing interventions to reduce patients stress and subsequent negative stress-related effects. Therefore, the aim of this study was to assess the intensity and course of anxiety during oral and maxillofacial surgery. This was done by measuring real time heart rate changes before, during and after the surgical removal of a third molar and the Modified Dental Anxiety Scale (MDAS).

METHODS AND MATERIALS

Institutional review board

The study was approved by the medical ethical commission (METc) of the Scheper Hospital (METc study number SH2014/1).

Location

This study was performed at the Department of Oral and Maxillofacial Surgery of the Scheper Hospital, Emmen, The Netherlands.

Patients

All included patients were scheduled for removal of a third molar under local anesthesia. All consecutive patients fulfilling the inclusion/exclusion criteria were asked to join the study. Inclusion criteria for these patients were:

- Patients indicated to have a third lower molar extraction
- Surgery performed under local anesthesia
- Surgical technique; use of drill (alveolotomy)
- Age between 18 and 40 years
- Fluency in Dutch language and the ability to complete the questionnaire

Exclusion criteria were:

- Third molar removal in the past 6 months
- Other oral or maxillofacial procedures in the past 6 months
- Use of medication that induce alterations in heart rate (e.g., β-blocker)
- Any medical condition that induce alterations in heart rate (e.g., thyrotoxicosis)
- Implanted pacemaker or Implantable Cardioverter Defibrillator (ICD)

Informed consent was obtained from all patients prior to the study. No other interventions or medical treatment were given to participating patients in comparison to regular patients.

Study design

The study design is an observational study. The studied group consisted of a group of patients who were already having a lower third molar removed. Their heart rate was followed throughout the whole treatment process (e.g. having a third lower molar surgically removed) to determine fluctuations in heart rate over time. Heart rate was recorded continuously immediately before and during the surgical procedure to determine patients' stress level. In addition, preoperative stress level was estimated by using an anxiety questionnaire, the Modified Dental Anxiety Scale (MDAS).

Determination of pre-surgery stress level

Patients' stress level was determined by measuring real-time heart rate throughout the whole treatment procedure and preoperative stress level was determined by using a Dutch version of the Modified Dental Anxiety Scale (MDAS).[35,49-52] The MDAS was filled out by patients after they were waiting in the waiting area. After completing the questionnaire they returned to the waiting room as they waited for the treatment to begin.

Determination of stress level during surgery

Patients' stress level was determined by continuously measuring heart rate throughout the whole treatment procedure.

Heart rate - primary outcome

Heart rate was measured continuously by using the Alpha Mio watch (Mio Alpha, Mio Global Physical Enterprises Inc., China), which can be worn like any normal watch, before, during and after the procedure. The Alpha Mio watch has a high accuracy, with a non-significant error of 0.1±0.3bpm and a precision of 3±1 bpm, which is comparable to the accuracy of an electrocardiogram (EGC)[48] The real-time heart rate data was transmitted to a Bluetooth Smart device. The electronics are integrated into the back plate of the wristband and include an accelerometer which enables the electro-optical cells to detect the pulsing volume of blood flow, also known as photoplethysmography.

Modified Dental Anxiety Scale – secondary outcome

The Modified Dental Anxiety Scale was used to estimate overall dental anxiety (situation-specific trait anxiety). It is a widely used anxiety scale with high reliability.[35,49-52] In this study, a Dutch version of the MDAS was used (Appendix C: Modified Dental Anxiety Scale). Patients have to indicate their emotional reaction to 5 items during a dental visit; when in the waiting room, in anticipation of drilling, scaling and local anesthetic injection. Responses are scored from 1 to 5, providing total scores ranging from 5 (not anxious at all) to 25 (extremely anxious). Dental anxiety scores of 19 or higher are considered as indicative of high dental trait anxiety.

Procedure

Presurgery procedure

All consecutive patients fulfilling the inclusion/exclusion criteria were asked by phone to participate when they were scheduled for surgery. When interested, patients received written and verbal information about the course and aims of the study and a copy of the informed consent form (Appendix A, B)).

When arrived at the department of Oral and Maxillofacial Surgery, verbal information regarding to what was expected of participants and what they might expect from this study was repeated in a consulting-room. After obtaining written informed consent, the participants were explained how to use the MDAS questionnaire and the method of acquiring heart rate measurements. Next, participants completed the MDAS questionnaire and demographic data such as age, gender, medical history and the use of medication were recorded. After that, heart rate was measured using the continuous heart rate monitor (Alpha MIO watch). A Bluetooth link was established with an iPad. Using the Bluetooth Low Energy (BLE) heart rate monitor application, the heart rate was monitored and recorded. From that moment on, heart rate was monitored continuously and stored every second. The recorded data consisted of a Unix Time Stamp, which comprises of the date and time of the measurement and the heart rate. From the moment the participant wore the watch, a stopwatch at the application of the Bluetooth smart device was started automatically. The application showed how much time had elapsed since heart rate measurement began. The exact elapsed time was noted on a form during different stages of the procedure in order to identify these moments for further data analysis. For example, when the participant returned to take a seat in the waiting-room, the exact elapsed time was registered on a form. The exact time of onset of the following events were registered:

1. Taking a seat in the waiting-room.
2. The moment the patient settles down in the dental chair.
3. Moment of application of local anesthesia.
4. Moment when the patient was being covered with surgical drapes.
5. Moment of time-out.
6. Moment of the first incision.

7. Moment of removal of bone using a drill (alveolotomy).
8. Moment of removal of the lower third molar.
9. Moment when suturing starts.
10. At the end of the procedure the moment of removal of the surgical drapes.

Additionally, other events were recorded such as when patients experienced and complained about pain during the procedure and the the moment of administration of additional local anesthetic (Appendix D).

After the patient was connected to the heart rate monitor, the patient returned to his or her seat in the waiting room. The patient had to remain in the waiting room for at least one minute, so their heart rate had the chance to stabilize. If necessarily, an orthopantomopgram was taken and once again the patient returned to the waiting room. An orthopantomogram was taken when no high-quality orthopantomogram was present in the electronic patient records or was not attached with the referrer form from the dentist. Subsequently, the patient was accompanied to the operating room and settled down in the dental chair. The patient received routine verbal information and reassurance by the operating surgeon immediately before, during and immediately after the procedure. The surgical procedure was standardized and the participating oral and maxillofacial surgeons were familiar with the aim of this study and standardized surgical procedures.

Surgical procedure

The surgical field was anesthetized by mucosal infiltration and by blocking the inferior alveolar nerve and lingual nerves. In case of removal of an additional upper third molar removal, the local anesthetic technique consisted of infiltration of the superior alveolar nerve and nervus palatinus major. In all cases local anesthesia consisted of 40mg articainehydrochloride with 0.01 mg epinephrine. Each carpule contained 1.7 ml injectable fluid and no more than 3 carpules were initially used for each patient. After local anesthesia, the surgical field was isolated using sterile drapes, leaving only the nose and mouth of the patient exposed. The time-out protocol followed, consisting of the verification of the patients' identity and the aim of the procedure. After checking the anesthetic state of the mucosa, the surgeon made an incision and created a mucoperiosteal flap. The need for bone removal with a drill could be established at this point. When sufficient surgical access was obtained, the need for sectioning of a molar could be determined. The third molar was then removed and a burr was be used to smooth any sharp or rough edges of bone, if necessary. Subsequently, the surgical wound was cleaned and subjected to curettage. The flap was repositioned, and the wound was finally sutured.

If the patient complained of pain or discomfort during the procedure, a limited volume of additional anesthetic solution was administered, and the procedure was delayed until patients' discomfort subsided.

Postsurgery procedure

After surgery, routine postoperative instructions, such as use of prescribed medications, were provided to the patient. Additionally all patients received a brochure which contained perioperative information and information about the postoperative course regarding third molar removal. Participants were told that after the surgical procedure they were expected in the consulting room again to remove the heart rate monitor and finish the contact properly.

Recording procedure

During the procedure the real time data obtained from the Alpha Mio Watch was transmitted to and recorded by a Bluetooth Smart device. Data was saved as a Training Center XML-file (TCX-file) and was converted into a comma-separated values file (CSV-file) by using a TCX-converter. Subsequently, the file could be opened by using Microsoft Excel. The file consisted of a Unix Time Stamp, the date and time of the measurement and the heart rate. From the moment the participant wore the watch, a stopwatch at the application of the Bluetooth smart device was initiated automatically. The exact time of important events during the procedure were noted on a form in order to identify them in the CSV-file.

Power analysis

A clinical significant change in heart rate was set at 10 bpm.[53] A power analysis with a power of 90% and a significance of 0.004, two-sided (with 11 tests as primary outcome) was used. A significance of 0.004 was used because of the 11 tests as primary outcome to counteract the problem of multiple comparisons. This was calculated by dividing p=0.05 by 11 (Bonferroni correction). Calculations of the sample size are based on paired samples t-tests, in which a non-parametric analysis was taken into account by adding 10% to the total sample size. Sample size calculations were performed by using Sample Power 2.0 (SPSS inc. Chicaco, IL, USA). A significant change in heart rate of 10 bpm, with a standard deviation of the paired difference of 10.7 (based on standard deviation of 12 bpm and a correlation between the paired observations of r=0.6), resulted in a sample size of 25. By adding 10% for the possibility of non-parametric testing, sample size resulted in 27.5. Another 10% was added for the attrition rate. This resulted in a total sample size needed of n=31.

Data analysis

Heart rate changes due to one event

We chose to process data in two different ways. One approach consisted of selecting 15 seconds of heart rate before and 15 seconds after the moment of the onset of an event. This approach was used in order to investigate the impact of an individual event on the heart rate of a participant. We estimated 15 seconds to be representative of the heart rate during a specific event.

Heart rates compared between different events

The second approach of data processing consisted of only selecting 15 seconds of heart rate after the onset of an event. This time-period directly after the onset of an event, was representative of the heart rate during that event. This approach was chosen to compare the various events and to determine if any differences in heart rate occurred between the events during the whole treatment process.

The various events were therefore defined by time-periods of 15 seconds. The table below gives an overview of the different time-periods and their respective meanings.

Table 1. Description of measurement time periods.

Period	**Description of measurement time points**
Period 1	Heart rate during waiting room
Period 2	Heart rate during dental chair
Period 3	Heart rate during local anesthesia
Period 4	Heart rate during surgical drapes
Period 5	Heart rate during time out
Period 6	Heart rate during incision
Period 7	Heart rate during alveolotomy
Period 8	Heart rate during removal of third molar
Period 9	Heart rate during suturing
Period 10	Heart rate during end of procedure
Period Pain	Heart rate during pain
Period additional anesthetics	Heart rate during the administration of additional anesthesia.

<u>*Statistical analysis*</u>

IBM SPSS Statistics 22 was used to store the database and analyze the data. The variables in the database included age, gender, MDAS score and descriptive values of the heart rate. The mean, standard deviation, median, minimum and maximum of each period were included in the database. These values were used for a descriptive analysis of the data and the median of a period was used for statistical analysis. Due to a limited number of measurements (15 heart rate measurements per period), the median of heart rate during a period was used in the statistical analysis rather than the mean.

According to the sample size calculations, we concluded a required sample size of 31 participants. However, an alveolotomy was not always carried out. Inclusion was therefore continued until 31 heart rate measurements of patients during alveolotomy were met. The data of the patients without the use of a drill were included in the analysis.

Distribution of the data

To determine the normal distribution of the data, the Shapiro-Wilk test, the Kolmogorov-Smirnov test and graphical interpretation of Normal Q-Q plots were used. If data did not appeared to be normally distributed, skewness and kurtosis were evaluated and square root, reflect and square root, logarithmic, reflect and logarithmic, inverse, or reflect and inverse transformation were applied in order to obtain a normal distribution. Subsequently, the normal distribution was evaluated again after transformation.

More detailed information about the used statistical tests and all Normal Q-Q Plots are displayed in appendix E for evaluation of the distribution of the data. If transformation was applied, the Normal Q-Q Plots of this transformed data is included as well. Additionally, the box-plots are included to determine any significant outliers in the data.

Normal distributed data

When data appeared to be normally distributed, the paired-samples t-test was used to determine whether the mean difference between the paired observations was statistically significantly different from zero. This test was used to compare continuous heart rate before and after the onset of an event during the procedure. The assumptions of the paired-samples t-test were evaluated in the database. The distribution of the differences between the two periods of heart rate measurements (before and after the onset of an event) should be approximately normally distributed in order to use the paired-samples t-test.

Non-normal distributed data
When assumptions of the paired-samples t-test were not met, the Wilcoxon signed rank test was used.

Analysis of changes in heart rate over time
Data of the heart rate during the events were not all normally distributed and multiple outliers were detected. Transformation was applied, but did not result in a normal distribution in all periods. Thus, the data did not comply with the assumptions of the repeated measures analysis of variance (ANOVA). For the analysis of changes in heart rate over time the Friedman's test was used. A post hoc test was used to determine where exactly the differences between periods lie. Pairwise comparisons were performed (IBM SPSS Statistics 22). We adjusted for multiple comparisons by using the Bonferroni adjustment. We tested ten hypotheses (ten periods) with a desired $\alpha = 0.05$. With the Bonferroni correction taken into account each individual hypothesis was tested at $p = 0.05/10 = 0.005$.

Differences between men and women
Differences in heart rate between men and women were evaluated by using the independent samples t-test for each event during the procedure if criteria for this test were met. If the data did not show a normal distribution, the Mann-Whitney U test was used.
Mean heart rate during the procedure was calculated by using period 1 to period 10. Differences in mean heart rate between men and women were evaluated by using the Mann-Whitney U test, due to a non-normal distribution and two detected outliers.

MDAS
Mean MDAS-score was described and to assess the differences in mean MDAS-score between males and females, the independent samples t-test was used. After square-root transforming the data, it showed a normal distribution.

The correlation between the MDAS and the heart rate was evaluated by using a scatterplot. Despite that the scatterplot revealed a non-linear correlation; the Spearman's rho was calculated.

Correction for multiple comparisons
All statistical tests were adjusted for multiple comparisons by using the Bonferroni adjustment, except for comparisons of the mean heart rate during the whole procedure (all 10 periods combined) and the MDAS-score. With the Bonferroni correction taken into account each individual hypothesis was tested at $\alpha = 0.005$.

RESULTS

The surgery was performed under local anesthesia and was performed by five different, experienced and licensed oral and maxillofacial surgeons.
A total of 84 participants were approached to participate in the study, of which 7 were excluded because they did not met the inclusion criteria. All patients, who were asked to participate in this study, were willing to take part in the study. Additionally, 3 participants were deleted from the database due to an alternative treatment or adjustment of the treatment plan during the anamnesis, examination or after evaluation of the orthopantomogram. Furthermore, one patient was excluded from the study because a vasovagal collapse during the procedure occurred. One anxious patient, who persisted to remove the sterile drapes from the face, was also excluded from the study. Another participant showed an extremely high heart rate (209 bpm), in which case surgery was temporarily stopped to assess stability of the patient. The participant claimed not to suffer from any physical complaints. However, intercollegiate consultation with a cardiologist took place. Although he concluded no intervention was needed, the general practitioner of the participant was informed of the situation in order to take action if he considered the latter necessary. Furthermore, data of 29 participants had to be excluded from analysis due to technical problems leading to recording difficulties, because heart rate measurements were not recorded during the whole procedure.
Thus, a total of 48 patients, aged from 18 to 40 years (mean age 25±6 years) were included for data analysis, including 20 men (mean age 25±7 years) and 28 women (mean age 24±5 years). Of the 48 patients 42% were male and 58% were female. 36 (75%) patients were having a mandibular third molar removal and 12 (25%) of participating patients had a combination of maxillary and mandibular third molar removal. Of the 48 participants 8 patients had evident pain complaints during treatment and 11 patients needed application of additional anesthetics. Of the 48 participants, 31 participants were subjected to alveolotomy.

Mean duration between time-periods (Table 1.)
In this study 15 seconds of heart rate before and after the onset of an event were used for analysis, which was representative for the heart rate change due to that event. During the treatment, mean duration between two successive events varied. Four of the patients showed an overlap between this time-period and consisted of an overlap of 4s, 5s and two patients showed an overlap of 2s. Two patients showed an overlap between the time-out procedure and the first incision of 2s and 3s. The mean duration between the successive events are shown in table 1. The mean duration between the time-periods represents the time between the time-periods from 15 seconds after the onset of the event and 15 seconds before the onset of the successive event.

Table 1. Mean duration between the successive time-periods

Event	Successive event	Time between two successive events (s)
Waiting room	Dental chair	418
Dental chair	Local anesthesia	153
Local anesthesia	Surgical drapes	110
Surgical drapes	Time-out procedure	142
Time-out procedure	First incision	49
First incision	Alveolotomy	65
Alveolotomy	Third molar removal	123
Third molar removal	Suturing	51
Suturing	End of procedure	83

Changes in heart rate before and after the onset of an event

All data, except data of the period ‘pain’ and the period ‘additional anesthetics’, was not normally distributed. Transformation was applied, but did not result in a normal distribution. As a result, the Wilcoxon-signed rank test was used for analysis. The data of the period ‘pain’ and the period ‘additional anesthesia’ showed a normal distribution and no outliers (of more than 1.5 box-lengths from the edge of the box in the boxplot) were detected, so the paired samples t-test was used.

Period 1: Heart rate during time in waiting-room
Of the 48 participants recruited to the study, the waiting in the waiting room elicited an increase in heart rate in 21 participants, whereas 4 participants saw no increase and 23 participants had a lower heart rate after sitting down in the waiting-room. There was *no significant increase in heart rate* when subjects were waiting in the waiting room (median=91.0 bpm) compared to the time before this event (median=89.0 bpm), z=-0.427, p=0.670 (Wilcoxon signed-rank test, non normal distribution, Appendix E).

Period 2: Heart rate during sitting down in dental chair
Of the 48 participants recruited to the study, the action of sitting down in the dental chair elicited an increase in heart rate in 37 participants compared to the time before sitting down in the dental chair, whereas 4 participants saw no increase and 7 participants had a lower heart rate after the event. There was a *statistically significant* median increase in heart rate (median=5.5 bpm) when subjects were sitting down in the dental chair (median=93.5 bpm) compared to the time before this event (median=88.0 bpm), z=4.382, p<0.0005 (Wilcoxon signed-rank test, non normal distribution, Appendix E).

Period 3: Application of local anesthesia
Participants showed a minimal higher heart rate before administering anesthetics (median=89.5 bpm) compared to their heart rate during the injection (median=88.5 bpm). The injection of anesthetic fluid elicited an increase in heart rate in 22 participants and a decrease in 23 participants, whereas 3 participants showed no difference. Differences in heart rate were *not statistically significant*, z=-0.650, p=0.516 (Wilcoxon signed-rank test, non normal distribution, Appendix E).

Period 4: Heart rate during applying surgical drapes
Of the 48 participants recruited to the study the applying surgical drapes elicited an increase in heart rate in 32 participants compared to the time before this event, whereas 3 participants saw no increase in heart rate and 13 participants had a lower heart rate after the event. This was *a statistically significant median increase* in heart rate (median=4.0 bpm) when subjects were covered with surgical drapes (median=100.5 bpm) compared to the time before this event (median=92.5 bpm), z = 3.548, p < .0005 (Wilcoxon signed-rank test, non normal distribution, Appendix E).

Period 5: Heart rate during time-out
Participants showed a lower heart rate before the time-out procedure (median=103.0 bpm) compared to their heart rate during the time-out procedure (median=104.5 bpm); with a median difference of 1.0 bpm. Of the 48 participants, the time-out elicited an increase in heart rate in 25 participants and a decrease in 18 participants, whereas 5 participants showed no difference. This was *not statistically significant*, z=1.149, p=0.250 (Wilcoxon signed-rank test, non normal distribution, Appendix E).

Period 6: Heart rate during incision

Of the 48 participants, the incision caused an increase in heart rate in 20 participants and a decrease in 24 participants, whereas 4 participants showed no difference (figure 6). The heart rate before and during incision was 105.5 bpm, with no significant difference. (z=-0.719, p=0.472 , Wilcoxon signed-rank test, non normal distribution, Appendix E).

Period 7: Heart rate during alveolotomy

31 participants were subjected to alveolotomy during the removal of the third molar. The alveolotomy caused an increase in heart rate in 18 participants and a decrease in 8 participants, whereas 5 participants showed no difference in heart rate. There appeared to be a lower heart rate before the alveolotomy procedure (median=101.0 bpm) compared to their heart rate during the alveolotomy (median=102.0 bpm); with a median difference of 1.0 bpm. This differences revealed a trend, however was *not statistically significant*, z= 1.896, p=0.058 (Wilcoxon signed-rank test, non normal distribution, Appendix E).

Period 8: Heart rate during removal of the third molar

Of the 48 participants, the removal of the third molar caused an increase in heart rate in 17 subjects and a decrease in 29 subjects, whereas 2 subjects showed no difference. There was a median heart rate before the removal of the third molar of 99.5 bpm and during the incision the median heart rate was 100.5 bpm, with a median difference of -2.0 bpm. Differences in heart rate were *not statistically significant*, z=-1.064, p=0.287 (Wilcoxon signed-rank test, non normal distribution, Appendix E).

Period 9: Heart rate during suturing

Before and during suturing, a same median heart rate of 95.0 bpm was found. Of the 48 participants, the suturing elicited an increase in heart rate in 16 participants and a decrease in 22 participants, whereas 10 participants showed no difference (figure 9). Differences in heart rate were *not statistically significant*, z=-0.851, p=0.427 (Wilcoxon signed-rank test, non normal distribution, Appendix E).

Period 10: Heart rate during end of procedure

The end of the procedure caused an increase in median heart rate in 26 subjects and a decrease in 17 subjects, whereas 5 subjects showed no difference. Although the same median heart rate of 90.5 bpm before the end of the procedure and a median heart rate of 90.5 bpm during the end of the procedure was found, the median difference between moments was 2.0 bpm. This difference revealed a trend*;* z=1.778, p=0.075.(Wilcoxon signed-rank test, non normal distribution, Appendix E).

Period pain

Participants had a slight higher heart rate before patients indicated their pain (mean 117 ± 15.2 standard deviation) compared to their heart rate after that moment (mean 116.5 ± 15.2 SD). A *statistically non-significant* decrease of -0.5 (95% CI, -2.499 to 1.499) bpm was found, t(7)=-0.592, p=0.573, d=0.209 (Paired samples t-test, normal distribution, Appendix E.)

Period additional anesthetics

Participants had a higher heart rate before patients administered additional anesthesia (mean bpm 104.6 ± 15.8 SD) compared to their heart rate during this event (mean 100.4 bpm ± 16.6 SD). A *statistically significant* decrease of -4.3 (95% CI, -6.697 to -1.849) bpm was found, p=0.003, t(10)=-3.928, , d=1.18 (Paired samples t-test, normal distribution, Appendix E.).

Figure 12a. Boxplot: Changes in median heart rates (in bpm) before and during the events.

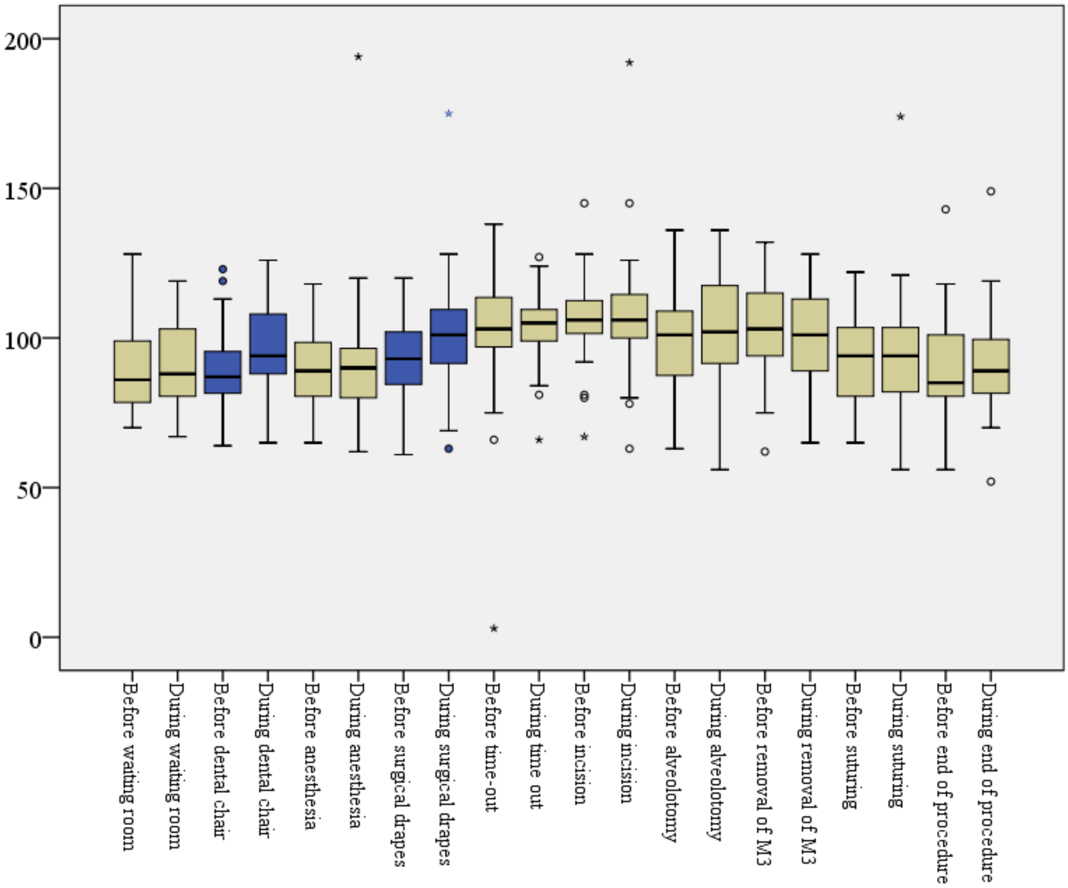

Figure 12a. Boxplot: Changes in median heart rate (in bpm) before and during the events. The blue bars represent the significant median changes in heart rate before and during that event. The sitting down in the dental chair elicited a significant increase in median heart rate. The appliance of surgical drapes also elicited a significant increase in median heart rate. (p<0.005). Circles and asterisks represent outlier value of more than 1,5 box-lengths from the edge of the box (circles) and of more than 3 box-lengths from the edge of the box (asterisks). The boxes represent the 1st quartile, the median in the middle and the 3rd quartile. The area between the 1st quartile and 3rd quartile is the interquartile range (IQR)

Figure 12b. Boxplot: Differences in median heart rate (in bpm) before and during the individual events.

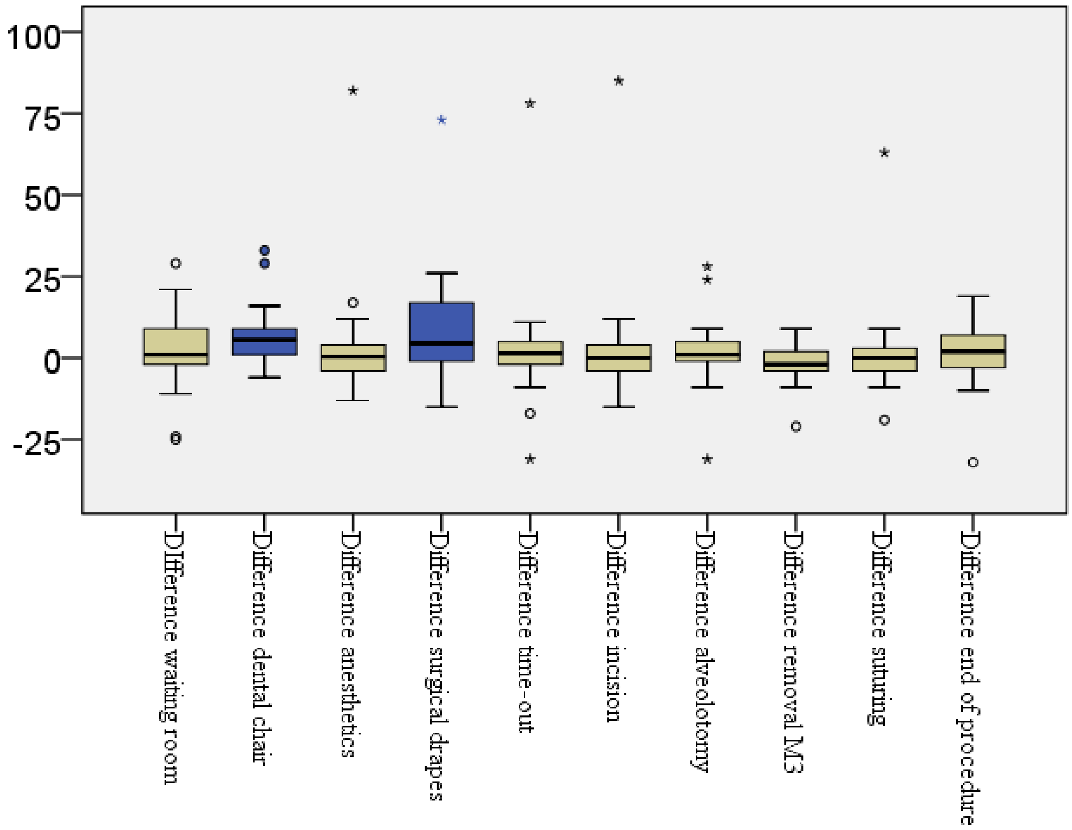

Figure 12b. Summary of differences in median heart rate (in bpm) before and during the individual events. For example, the difference in heart rate between median heart rate during the waiting room minus the median heart rate before the waiting room represent the 'difference waiting room' value. The blue bars represent the significant median changes in heart rate before and during that event. The sitting down in the dental chair elicited a significant median increase of 5,5 bpm. The appliance of surgical drapes elicited a significant median increase of 4,0 bpm ($p<0.005$).Circles and asterisks represent outlier values. The boxes represent the 1st quartile, the median in the middle and the 3rd quartile. The area between the 1st quartile and 3rd quartile is the interquartile range (IQR)

Heart rates compared between different events

Heart rate was statistically significant different at the time points during the whole procedure, $\chi 2$ (9) = 72.933, $p < .0005$ (Friedman's test, pairwise comparisons, Bonferroni adjustment, significancy set at p=0.005, Appendix E).

Post hoc analysis revealed statistically differences in:

- Heart rate from sitting down in the dental chair (median = 93.5 bpm) to the incision (median = 105.5 bpm, p<0.0005)
- Heart rate from applying the surgical drapes (median = 100.5 bpm) to the incision (median = 105.5 bpm, p=0.004)

- Heart rate from anesthesia (median=88.5) to time-out procedure (median=104.5 bpm, p< 0.0005), to applying surgical drapes (median=100.5, p=0.002), to incision (median= 105.5, p< 0.0005), to alveolotomy (median=102.0 bpm, p< 0.0005) and to the removal of the third molar (median= 100.5 bpm, p< 0.0005).

- Heart rate from the end of the procedure (median = 90.5 bpm) to the time-out procedure (median = 104.5 bpm, p<0.0005)to the incision (median = 105.5 bpm, p<0.0005) to alveolotomy (median = 102.0, p<0.0005), and to removal of the third molar (median = 100.5 bpm, p=0.001).

- Heart rate from the waiting-room (median = 91.0 bpm) to the time-out procedure (median = 104.5, p<0.0005), to the incision (median = 105.5 bpm, p<0.0005) and to the alveolotomy (median = 102.0 bpm, p=0.001).

- Heart rate from the suturing (median = 95.0 bpm) to the time-out procedure (median = 104.5 bpm) (p<0.0005), to the incision (median = 105.5 bpm), to the alveolotomy (median = 102.0 bpm, p=0.002).

Summarizing the lowest median heart rate values were recorded in the waiting room (91.0 bpm), during suturing (95.0 bpm) at the end of the procedure (90.5 bpm) and during anesthesia (88.5 bpm), whereas the highest values were obtained during the time-out procedure (104.5 bpm), incision (105.4 bpm) and the alveolotomy (102.0 bpm). The heart rate differences between the highest and lowest values were *statistically significant* (p<0.005).

Table 2 shows a total overview of data characteristics, including median, mean minimum and maximum heart rate and standard deviation. Figure 14 shows fluctuations of median heart rates during the treatment process. Visual interpretations of significant changes in median heart rate obtained by using the Friedman's test are depicted in Appendix F.

Table 2. Heart rate during the different events of the procedure. Values are heart rates (beats per minute)

	Waiting room	**Dental chair**	**Anesthesia**	**Surgical drapes**	**Time-out**
Median	91.0	93.5	88.5	100.5	104.5
Mean	92.9	96.1	91.9	101.4	103.3
SD	13.9	13.9	23.5	20.3	12.6
Minimum	67.0	65.0	62.0	63.0	66.0
Maximum	119.0	126.0	194.0	175.0	127.0

Table 2. Continued

	Incision	alveolotomy	Removal third molar	Suturing	End of procedure
Median	105.5	102.0	100.5	95.0	90.5
Mean	108.2	101.0	100.0	94.5	91.6
SD	22.5	20.9	17.8	22.0	18.1
Minimum	63.0	56.0	65.0	56	52.0
Maximum	193.0	136.0	128.0	174	149.0

Figure 14. Summary of median heart rate (in bpm) during the time point measurements.

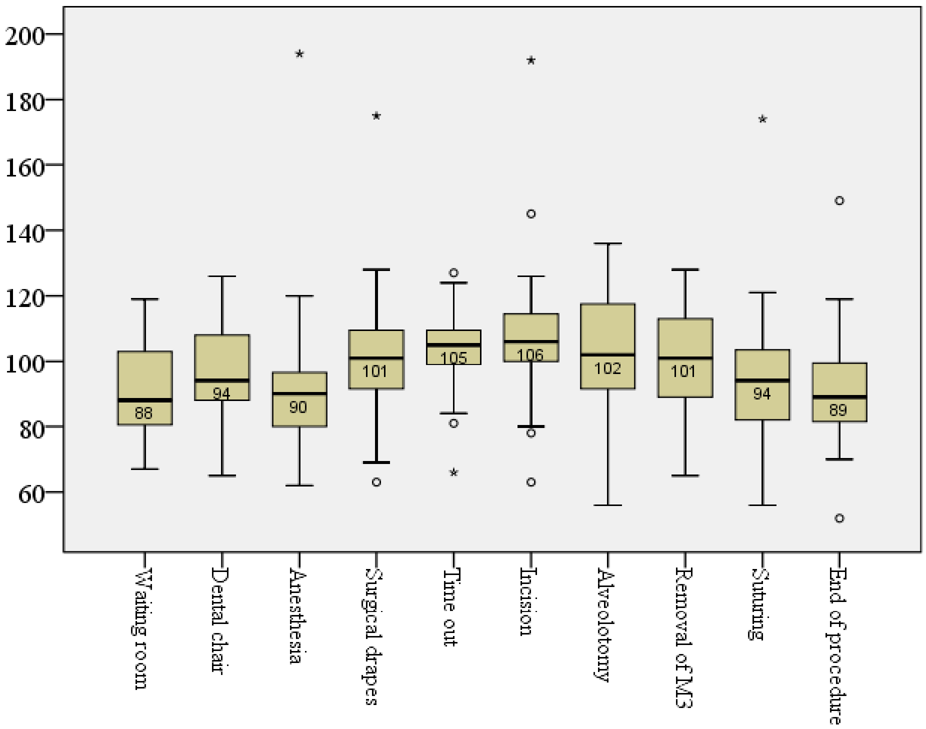

Figure 14. Summary of median heart rate (in bpm) during the time point measurements. Only the median heart rates of participants during the events are displayed. For example, only the median heart rate of participants after the onset of sitting down in the waiting room is included in the figure (not the heart rate before that moment). The numbers represent the median heart rates. Circles and asterisks represent outlier values. The boxes represent the 1st quartile, the median in the middle and the 3rd quartile. The area between the 1st quartile and 3rd quartile is the interquartile range (IQR).

Differences in heart rate between men and women

Mean heart rate during the total treatment time

Female heart was normally distributed, but male heart rate was not. (Appendix E). Heart rate for males (mean rank = 19.25) and females (mean rank = 28.25) was statistically different. Median heart rate for males (91.4 bpm) was *statistically significant lower* than median heart rate for females (94.9 bpm), U=385, z=2.196, p=0.028, Mann-Whitney U.

Mean heartrate per event

Evaluated per event, the heart rate of female was significantly higher afer giving local anesthesia, time-out, suturing and at the end of the procedure (period 3,5,9,10), however after Bonferroni correction this significancy did not remain (Fig 15.)

Figure 15. Summary of median heart rates between men and women.

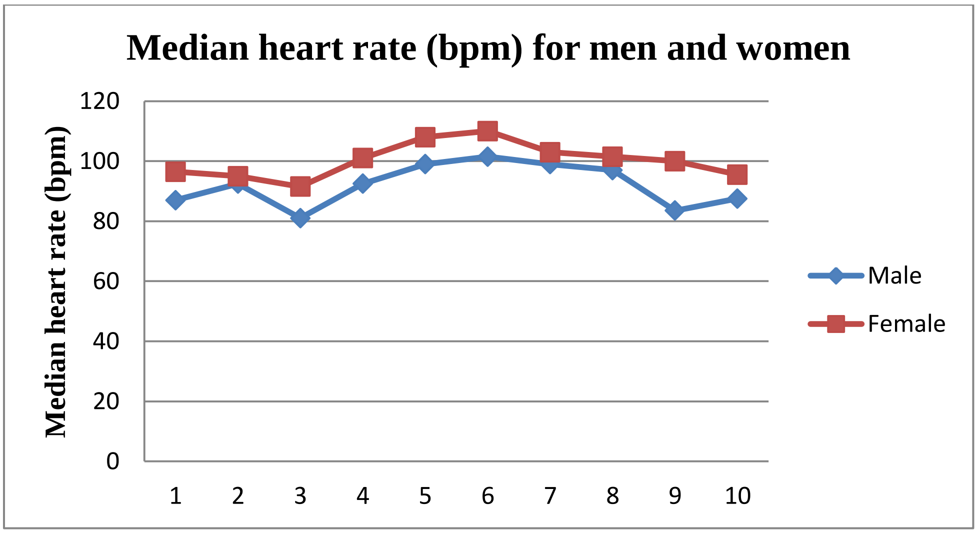

Figure 15. Summary of median heart rates (bpm) of men and women during the procedure. Numbers on the X-axis represent the different time periods: 1, waiting room; 2, dental chair; 3, local anesthesia; 4, surgical drapes; 5, time-out; 6, incision; 7, alveolotomy; 8, removal of the third molar; 9, suturing; 10, end of the procedure.

Modified Dental Anxiety Scale

The mean MDAS-score was 9 (Standard deviation ± 3.3), with a median of 8 and a minimum score of 5 and a maximum of 18. All scores of the MDAS consisted of low or moderate anxiety levels. The mean degree of dental anxiety before the surgical procedure was mild according to the MDAS score. None of the participants was extremely anxious according to the MDAS.[50,54]

Males had a lower mean MDAS-score (mean 8.1 ± 2.37 SD, n=20) as opposed to females (mean 10.3 ± 3.67 SD, n=28). Male mean MDAS score was 2.2 (95% CI, -4.084 to -0.316) lower than female mean MDAS-score. This was a *statistically significant* difference, t(46)= -2.331, p=0.024 (independent sample t-test, normal distribution, Appendix E).

Figure 16. Boxplot of MDAS

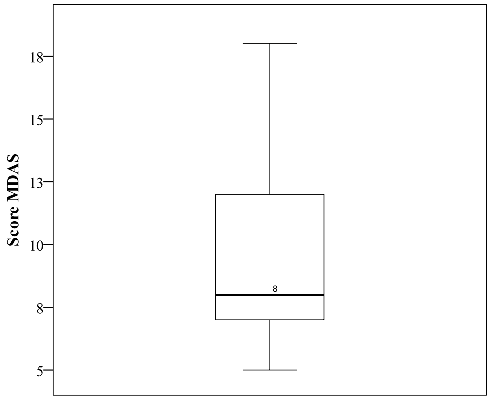

Figure 16. Boxplot of MDAS score. The number represents the median MDAS score.

Figure 17. Boxplot of MDAS Score for men and women

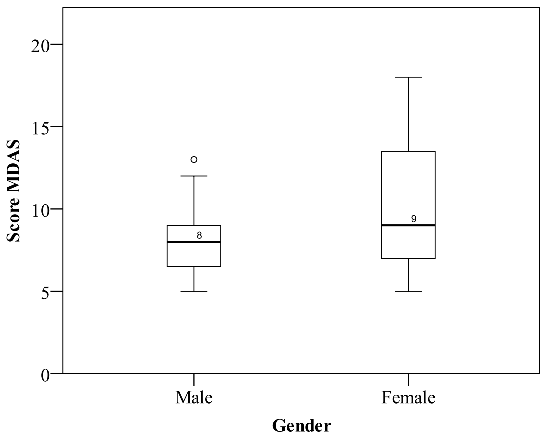

Figure 17. Boxplot of the mean MDAS score for men and women. Numbers represent the median MDAS score.

Correlation between MDAS and heart rate

No linear relationship was found between the preoperative MDAS-score and the heart rate during the time in the waiting room, as assessed by visual interpretation of the scatterplot below (figure 18a). Additionally, a Spearman's rank-order correlation was run to assess the relationship between the heart rate during the waiting room and the MDAS-score. There was a non-significant positive correlation between the heart rate and the MDAS, rs(46) = 0.140, p=0.343.

Also, no linear relationship was found between the preoperative MDAS-score and mean heart (of all 10 periods), as assessed by the visual interpretation of the scatterplot (figure 18b). The Spearman's rank-order correlation was used and it revealed a non-significant positive correlation between the mean heart rate and MDAS (rs(46) = 0.178, p=0.226).

Figure 18a. Scatterplot of heart rate during the waiting room and preoperative MDAS-score

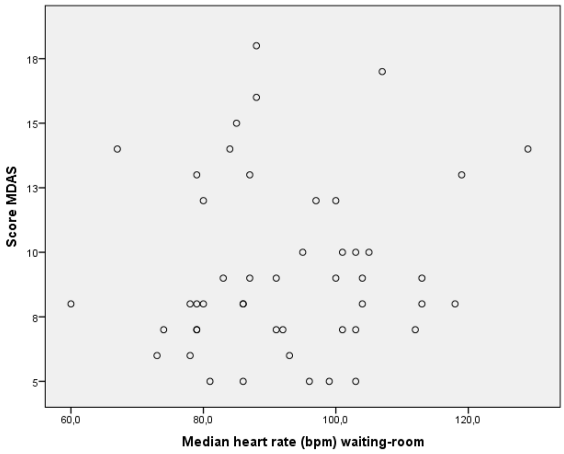

Figure 18a. Scatterplot of heart rate during the waiting room and preoperative MDAS-score. No linear relationship was found, based on visual interpretation of this scatterplot.

Figure 18b. Scatterplot of mean heart rate (of all 10 periods) and preoperative MDAS-score

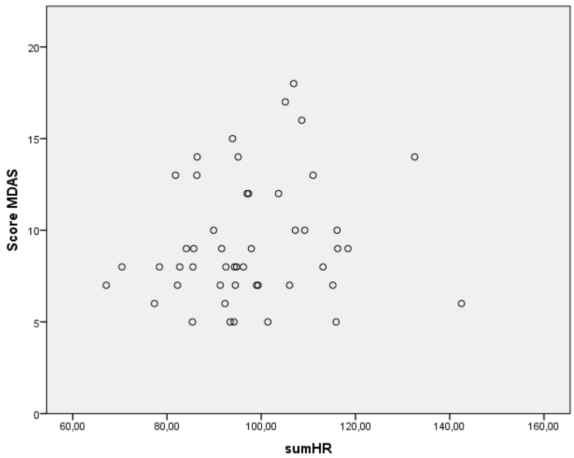

Figure 18b. Scatterplot of mean heart rate (of all 10 periods combined) and preoperative MDAS-score. No linear relationship was found, based on visual interpretation of this scatterplot.

Discussion

In the present study we found significant increases in heart rate during the action of sitting down in the dental chair and during the application of surgical drapes and a significant decrease in heart rate during the injection of anesthesia. A decrease in heart rate was measured during the administration of additional anesthesia ($p<0.005$). The lowest heart rate values were recorded in the waiting room (median 91.0 bpm), during suturing (95.0 bpm) at the end of the procedure (90.5 bpm) and during anesthesia (88.5 bpm), whereas the highest values were obtained during the time-out procedure (104.5 bpm), incision (105.4 bpm) and the alveolotomy (102.0 bpm), $p<0.005$).

These findings are partly in accordance with the study of Alemany-Martinez et al where the lowest heart rate values were recorded before the start of the surgical procedure and the highest values were obtained during incision and flap raising.[47] Also, Houston et al found a considerably increased heart rate of 12 bpm during the action of sitting down in the dental chair.[31] However, Meyer et al and Goldstein et al pointed out that the most marked changes in heart rate were observed just before application of local anesthesia and during the extraction.[28,46]

In our study, a trend of a higher heart rate before administering anesthetics (median 89.5 bpm) compared to the heart rate during the injection (median 88.5 bpm) was observable, but these findings did not reach statistical significance. Also, the application of additional anesthetic fluid elicited a significant decrease in heart rate. The latter could be explained by the pain reducing effect of the additional anesthesia. The literature on cardiovascular changes before and during the injection of anesthetic fluid is inconclusive. Significant increases were observed in mean heart rate (+2 to +18 bpm) immediately before the injection of local anesthetic.[28,29,46] During the actual injection, the heart rate decreased.[28] The study of Liao et al reported an elevated heart rate during the delivery of local anesthesia,[6] which is contrary to earlier studies that reported a decrease in heart rate.[55,56] Paramaesvaran et al found that 33 of the 35 patients who received local infiltrations had a decrease in heart rate during or after anesthesia.[55] Yokobayashi et al found that administration of local anesthesia lowered the heart rate, and local infiltration resulted in a more significant effect than block anesthesia.[56] Meyer et al suggested that the hemodynamic changes caused by emotional stress mask those due to exogenously active catecholamine and that an abrupt relative deficiency of epinephrine, known as epinephrine collapse, may occur after stress.[28]

Modified dental anxiety scale

The Modified Dental Anxiety Scale (MDAS) is a widely used anxiety scale with high reliability, developed in 1969 by Corah and was modified in 1995.[35,49-52]The Modified Dental Anxiety Scale is similar to Corah's Dental Anxiety Scale, but includes an extra question about a local anesthetic injection, as well as a simplified and a consistent answering scheme across all five questions. The purpose of the MDAS is to estimate overall dental anxiety (situation-specific trait anxiety). In this study, a Dutch version of the MDAS was used.

The mean MDAS-score in this study was 9 with and a minimum score of 5 and a maximum of 18. All scores of the MDAS consisted of low or moderate anxiety levels. The mean degree of dental anxiety before the surgical procedure was mild according to the MDAS score. None of the participants was extremely anxious according to the MDAS.[50,54] We found a positive correlation between the MDAS-score and the mean heart rate; however this correlation did not reach statistical significance. This finding is in accordance with the study of Martinez et al, where the most anxious patients had the highest heart rates during third molar removal, although the differences did not reach statistical significance either.[47]Another study, by Coldwell and colleagues, showed a positive relation between the DAS and physical symptoms before oral surgery. [57] In addition, the study of Liao et al showed that the dental axiety scale is a useful tool for estimating the impact of anxiety on the heart rate during the administration of local dental anesthesia. [6]

Differences between men and women

In this study, women revealed to have a higher mean heart rate compared to men ($p<0.005$). This finding is in line with the in general slightly faster sinus rate in women than compared to men. [58] Mean heart rate in women between 20-29 years is 69 bpm and between 30 to 39 years their mean heart rate is 70 bpm. Mean heart rate for men between 20-29 years is 64 bpm and 67 bpm between 30 and 39 years of age.[59]

The fact that women showed a significant higher mean heart rate and had a higher MDAS-score than men in this study, is in accordance with other studies assessing gender differences in anxiety provoking stimuli.[17,60-64] These findings are consistent with the current view on gender differences in anxiety disorders.[65, 66] This seems to apply to the dental setting as well, since in women the heart rate before dental treatment is higher than in men.[67] The anticipatory response to the dental setting shows a difference between men and women. Brand et al found that before dental treatment, the heart rate in women is higher than in men. However, the difference in heart rate appears to depend on the type of dental treatment. Immediately before a scheduled extraction, the difference is much greater (15 bpm) than before checkups or restorative procedures (7 bpm). The difference in heart rate is a long-term effect: even 24 hours before a scheduled checkup the mean heart rate of women is higher.[12] This is in agreement with psychological studies, which suggest that women display a stronger anticipatory response to stressful events in general.[68,69] Although we found a trend of a higher heart rate in women compared to men, no statistical difference between men and women during their time in the waiting room was found.

Methods of assessing anxiety

Various physiological manifestations of anxiety can be observed to determine the presence of anxiety. For example, increased respiration, muscle tension, blood pressure, plasma epinephrine levels, electrical activity of the brain and palmar sweat index are physiological measures of fear. However, these physiological indices suffer from a number of methodological problems, including poor reliability and low practicality in naturalistic field testing. The amount of stress experienced is usually measured by the level of anxiety reported by the patient. However, the use of physiological measures to gauge anxiety in test situations would seem to have an advantage over self-report measures, because it is difficult to control voluntarily autonomic nervous system responses. In this study, to obtain a proper index of anxiety, a self-report index is combined with a physiological measure. Heart rate measurements are relatively easy to obtain and therefore probably have a low effect on anxiety. Also, the completion of a brief dental anxiety questionnaire before seeing the dentist has a non-significant effect on state anxiety.[70]

Heart rate

The normal heart rate has been considered historically to range from 60 to 100 bpm, with sinus tachycardia being defined as a sinus rhythm with a rate exceeding 100 bpm and bradycardia as a rhythm below 60 bpm. The pulse rate should be between 60 and 80 bpm when an adult is lying quietly in bed. The rhythm is regular except for a slight quickening in early inspiration and a slowing in expiration (sinus arrhythmia), but the heart rate increases or decreases no more than five percent during quiet respiration.[27] During inspiration,

parasympathetic tone falls and the heart rate quickens, and on expiration the heart rate falls. This variation is normal, particularly in children and young adults.[58] In summary, heart rate in the general population has a normal range of 60 to 100 bpm, however the interpersonal heart rate has a much smaller range in normal circumstances (± 5%).
In our study, by measuring interpersonal heart rate changes, the patient served as its own control. Therefore, a relative small group size was determined to detect clinical significant changes in heart rate during the surgical removal of a third molar.
Also, a relative small age group was taken to address variations in heart rate with age (slower with increasing age).[71-73]

Ideally, the heart rate should be measured at baseline at a non-anxiety provoking time and place. However, a visit to the dental surgery induces cardiovascular changes. Even if patients know that the visit entails only a blood pressure measurement, the blood pressure in the dental clinic is 5 mmHg higher than outside the dental office.[76] Anticipatory cardiovascular reactions are not limited to the dental setting. Comparable increases in blood pressure are observed in hospitals when patients visit physicians, an affect known as the 'white-coat-phenomenon.[77] A possibility of obtaining heart rate during a relaxed state of mind was to educate patients to measure their own heart rate while being at home. However, we expected this option to be highly admissible to errors.
Although no real baseline heart rates could be appropriately measured in this study, differences in heart rate during the treatment could be obtained. Additionally, baseline values were determined by Mason et al in general population.[59]

<u>*Limitations*</u>
In this study 15 seconds of heart rate before and after the onset of an event were used for analysis, which was representative for the heart rate change due to that event. This method has as disadvantage that it does not take into account a possible overlap between periods when two successive periods follow each other rapidly (i.e., within 15 seconds).
The mean duration between the time-periods represents the time between the time-periods from 15 seconds after the onset of the event and 15 seconds before the onset of the successive event. Although no mean overlap between the time-periods was found, six patients showed an overlap. As result of the overlap, the results obtained in our data could have been affected in two different ways. First, in case of a very stressful event and the successive event starts before the 15s after the onset of that event, the second event could be named as more stressful than it actually is. Second, if the successive period elicits a decrease in heart rate and this event shows overlap with the previous event. The previous event could be interpreted as less stressful than it actually is. However, despite of the occurrence of overlap between some of the successive periods, there was no mean overlap between the periods. Moreover, one of the aims of the procedure was to provide an efficient and patient-friendly surgical treatment.
The overlap could in theory be avoided by letting the patient wait at least 30s after the onset of an event before the next event takes place, but this method raises ethical concerns by counteracting with the aim of an efficient and patient-friendly treatment. Another approach of the avoidance of the overlap is to reduce the fixed time periods to less than 15 seconds. One major disadvantage is the smaller amount of heart rate measurements that could be obtained. This causes the possibility of a less accurate representation of the reality and the risk of drawing inappropriate conclusions. In summary, overlap between some of the successive time-periods occurred incidentally, and no average overlap was found; therefore the effect of the overlap was considered minimal.

Furthermore, data of 29 participants were excluded from analysis due to recording difficulties, because heart rate measurements were not recorded during the whole procedure. The technology of the Alpha Mio watch consist of an accelerometer which detects the pulsing volume of blood flow, also known as photoplethysmography. The use of an Alpha Mio watch has one disadvantage however, namely that it frequently rejects heart rate data. Valenti et al stated that this frequent data rejection was the result of low blood perfusion of the skin and therefore reduced the signal amplitude and therefore the signal-noise ratio.[48] The latter could have caused our data rejection of 29 participants because patients were lying quietly during treatment and therefore could have shown a low blood perfusion.

In this study we used the MDAS score to evaluate dental anxiety in general. Although this is a widely used anxiety scale with high reliability, many different other scales for measuring dental anxiety have been presented in the literature by researchers and clinicians.[78] Several anxiety indexes can be used in order to predict anxiety before oral surgery. In an investigation of dental patients, state and trait anxiety were reported to be related to discomfort and greater worry about dental visits.[79] A psychological questionnaire, ‘The State-Trait Anxiety Inventory’ (STAI) has been used to measure the anxiety of dental patients[43,80-82], because it assessed both state and trait anxiety simultaneously and quantitatively.[83] Fuentes et al found a significant association between dental anxiety and trait anxiety. However, their data indicates that subjects with high dental anxiety tend to present high trait anxiety, yet the opposite is not true such that high trait anxiety individuals seem not to present a predisposition to dental anxiety.[84] Furthermore, in a study from Newton et al MDAS was found to discriminate between the three groups of participants defined by self-reported anxiety level, and correlates highly with the Spielberger Trait Anxiety Inventory.[52]
By contrast, the Oral Surgery Confidence Questionnaire (OSCQ) is the only measure developed specially to predict the stress experienced during oral surgery. Kaakko et al hypothesized that the OSCQ would be more predictive of the anxiety experienced before oral surgery than would measures of dental anxiety or measures of general trait anxiety. However, their study showed that OSCQ scores and trait anxiety were not related to anxiety reported before oral surgery. They also stated that the use of multiple scales is the best way to accurately investigate dental anxiety within a study population; but it is complicated to conduct and evaluate the anxiety of patients by using multiple different scales.[43]
In summary, evidence for the best instrument to be used to estimate anxiety in oral and maxillofacial surgery remains inconclusive.

Another aspect of the impact of anxiety is the duration of the state of mind. Brand et al described the increase in heart rate as a long-term effect.[67] Even 24 hours before a scheduled checkup the mean heart rate of women is higher. Lopez-Jórnet et al evaluated anxiety by using the MDAS, STAI and DFS before, immediately after and one week after dental extraction.[85] However, in the latter study anxiety was only evaluated by anxiety questionnaires. Further evaluation of the long-term effects of anxiety seems useful to extend the knowledge of the procedural anxiety.

Future perspectives
One of the objectives of our study was to gather meaningful information concerning the course and intensity of anxiety during oral and maxillofacial surgery. This, in turn may help in finding anxiety reducing interventions to reduce patients stress and subsequent negative stress-related effects. Although all participants stated their anxiety level, according to the MDAS, as mild to moderate, their anxiety fluctuated during the procedure as measured by their real time heart rate. The action of sitting down in the dental chair and the application of

the surgical drapes induced a significant increase in heart rate. Especially the latter action could be relevant for clinical implications such as the usage of an alternative surgical drape. Special attention should be paid to the most anxiety provoking moments during the procedure such as the time-out procedure, the appliance of surgical drapes, the incision, the alveolotomy and the actual removal of the third molar. With this in mind, these findings can guide us in the search for anxiety reducing implications in clinical setting.

The intensity and course of anxiety was observed in this study during third molar surgery. This covers only one aspect of the surgical profession and very little is known about the anxiety during other oral and maxillofacial surgery procedures. However, the specific pattern and level of anxiety during third molar surgery found in this study most likely show similarities with anxiety during other oral procedures, such as apicoectomy and dental implant surgery. The key properties of third molar surgery, such as the usage of local anesthesia, full awareness of the patient, the out-patient setting and the relatively short duration of the procedure, are similar to other minor surgical procedures. Therefore, it must not escape our attention that our basic findings may be applicable to other surgical disciplines where minor surgical procedures are carried out under local anesthesia with full awareness of the patient. A few examples of minor surgical procedures include minor dermatologic surgeries, surgical excisions, biopsies, some of the cosmetic surgeries, refractive surgery and vasectomies. However, this is subject for future studies.

CONCLUSION

The intensity and course of anxiety has a specific pattern during the removal of a third molar. It seems that anxiety is most present before the third molar is removed, and declines afterwards.

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APPENDIX A T/M E

Appendix A: Description of the study

Informed Consent formulier bestemd voor deelnemende patiënten.

Mw. M. Hollander, Afdeling Mondziekten, Kaak- en Aangezichtschirurgie, Scheperziekenhuis, Emmen

Dit informed consent formulier bestaat uit 2 delen:

- **Deel 1: Informatiebrochure**
- **Deel 2: Toestemmingsverklaring (informed consent)**

Deel 1: Informatiebrochure

Introductie
Graag wil ik u uitnodigen om deel te nemen aan het onderzoek naar stress rondom een kaakchirurgische behandeling.
Voordat het onderzoek begint, is het belangrijk dat u op de hoogte bent van de procedure die in dit onderzoek wordt gevolgd. Lees daarom onderstaande tekst zorgvuldig door en aarzel niet om opheldering te vragen over deze tekst, mocht deze niet duidelijk zijn. De onderzoeker zal eventuele vragen graag beantwoorden.

Doel van het onderzoek
Kaakchirurgische ingrepen geven de patiënt vaak stress. Zij zien tegen de behandeling op en zijn wellicht bang voor pijn.
De wens is om inzicht te krijgen in de mate van emotionele stress en het beloop ervan gedurende het bezoek aan de kaakchirurg. Als bekend is op welke momenten de patiënt veel stress ervaart, zouden wellicht veranderingen plaats kunnen vinden om de stress te verminderen en daarmee de kwaliteit van zorg te verhogen.

Type onderzoek
Ongeacht of u wel of niet deelneemt aan dit onderzoek, uw behandeling zal precies hetzelfde zijn. Als u besluit deel te nemen aan het onderzoek zullen echter aanvullende metingen gedaan worden. Enkele minuten voorafgaand aan de behandeling wordt van u gevraagd een korte vragenlijst in te vullen om in te schatten in welke mate u gespannen bent. Daarnaast zult u voor, tijdens en kort na de behandeling een polshorloge dragen die uw hartfrequentie zal registreren. Na afloop van de behandeling zal u opnieuw gevraagd worden om een korte vragenlijst in te vullen.

Patiëntselectie
We nodigen alle patiënten uit om deel te nemen aan dit onderzoek, die de polikliniek kaakchirurgie bezoeken voor het verwijderen van een verstandskies en aan de criteria voldoen om te mogen deelnemen aan dit onderzoek. Voor deelname aan het onderzoek is het van belang dat u geen eerdere kaakchirurgische behandeling hebt ondergaan in de afgelopen 6 maanden, dat u tussen 18 en 40 jaar oud bent, dat u niet lijdt aan hart- en vaatziekten en geen medicijnen gebruikt die de hartfrequentie kan beïnvloeden.

Vrijwillige deelname
De keuze om deel te nemen aan dit onderzoek is geheel vrijwillig. Verder kunt u te allen tijde besluiten om tijdens het onderzoek te stoppen zonder dat dit voor u consequenties heeft, waarbij ook geen reden aangegeven hoeft te worden. Verder kunt u ook na afloop van het onderzoek, tot 24 uur daarna, alsnog besluiten dat de gegevens niet verder mee worden genomen in het onderzoek.
Als u besluit af te zien van deelname aan dit onderzoek, zal dit op geen enkele wijze gevolgen voor u hebben.

Gang van zaken
U hebt een geplande afspraak bij de kaakchirurg voor het verwijderen van een verstandskies. Een aantal minuten voordat de behandeling wordt gestart zult u een korte vragenlijst invullen. U krijgt een hartslagmeter om uw pols gedurende bezoek om uw hartfrequentie te registreren. Vervolgens zult u de behandeling ondergaan. Na afloop van de behandeling kunt u de hartslagmeter weer afdoen.

Duur
Het onderzoek vindt plaats in de reguliere behandeltijd voor het verwijderen van een verstandskies. Daarnaast zal de vragenlijsten enkele minuten in beslag nemen.

Risico
Er zijn geen risico's aan dit onderzoek verbonden.

Voordelen
Uw deelname zal u op korte termijn geen voordelen opleveren, maar u deelname helpt wel om inzicht geven in de mate van emotionele stress en het beloop ervan gedurende het bezoek aan de kaakchirurg.

Vertrouwelijke informatie
Met uw gegevens zal op vertrouwelijke wijze worden omgegaan. De anonimiteit van uw gegevens wordt gewaarborgd en zullen nooit aan derden zonder uw toestemming worden verstrekt.

Contact
Mocht u vragen hebben over dit onderzoek, vooraf of achteraf, dan kunt u zich wenden tot de onderzoeker mw. M. Hollander. Ook kunt u contact opnemen met Dr. J. Schortinghuis, kaakchirurg voor verdere informatie.

Met vriendelijke groet,

Marijke Hollander
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E-mail: m.hollander@sze.nl
Afdeling Mondziekten, Kaak- en Aangezichtschirurgie
Scheper ziekenhuis
Boermarkeweg 60
7824 AA Emmen

Appendix B: Informed Consent form

Deel 2: Toestemmingsverklaring

Dit formulier hoort bij de schriftelijke informatie die u hebt ontvangen over het onderzoek waar u aan kunt deelnemen. Met ondertekening van dit formulier verklaart u dat u de deelnemersinformatie heeft gelezen en begrepen. Verder geeft u met de ondertekening te kennen dat u akkoord gaat met de gang van zaken zoals deze staat beschreven in de informatiebrochure.

Deelnemer

"Ik verklaar hierbij op voor mij duidelijke wijze te zijn ingelicht over de aard en methode van het onderzoek, zoals uiteengezet in de bovenstaande informatie. Mijn vragen zijn naar tevredenheid beantwoord. Ik stem geheel vrijwillig in met deelname aan dit onderzoek. Ik behoud daarbij het recht deze instemming weer in te trekken zonder dat ik daarvoor een reden hoef op te geven en besef dat ik op elk moment mag stoppen met het experiment. Indien mijn onderzoeksresultaten gebruikt zullen worden in wetenschappelijke publicaties, dan wel op een andere manier openbaar worden gemaakt, zal dit volledig geanonimiseerd gebeuren. Mijn persoonsgegevens zullen niet door derden worden ingezien zonder mijn uitdrukkelijke toestemming."

Aldus getekend:

Datum:

……………………………...........	……………………………
Naam proefpersoon	*handtekening*

Onderzoeker

"*Ik heb toelichting verstrekt op het onderzoek. Ik verklaar mij bereid nog opkomende vragen over het onderzoek naar vermogen te beantwoorden.*"

Datum:

……………………………...........	……………………………
Naam onderzoeker	*handtekening*

Appendix C: Modified Dental Anxiety Scale

KUNT U ONS VERTELLEN HOE BANG U BENT, OF JUIST NIET, VOOR EEN BEZOEK AAN DE TANDARTS?

GRAAG AANGEVEN MET EEN 'X' IN HET TOEPASSELIJKE VAKJE

1. **Als u MORGEN naar uw tandarts zou gaan voor een BEHANDELING, hoe zou u zich dan voelen?**

Totaal Niet Bang ☐	Een Beetje Bang ☐	Nogal Bang ☐	Heel Bang ☐	Panisch Angst ☐

2. **Indien u in de WACHTKAMER zou zitten (wachtend op uw behandeling), hoe zou u zich dan voelen?**

Totaal Niet Bang ☐	Een Beetje Bang ☐	Nogal Bang ☐	Heel Bang ☐	Panisch Angst ☐

3. **Indien u op het punt zou staan om GEBOORD TE WORDEN, hoe zou u zich dan voelen?**

Totaal Niet Bang ☐	Een Beetje Bang ☐	Nogal Bang ☐	Heel Bang ☐	Panisch Angst ☐

4. **Indien u vlak voor TANDSTEEN VERWIJDERD EN POLIJSTEN zou staan, hoe zou u zich dan voelen?**

Totaal Niet Bang ☐	Een Beetje Bang ☐	Nogal Bang ☐	Heel Bang ☐	Panisch Angst ☐

5. **Indien u op het punt zou staan om een PLAATSELIJKE VERDOVING in uw tandvlees vlak boven een kies in uw bovenkaak te krijgen, hoe zou u zich dan voelen?**

Totaal Niet Bang ☐	Een Beetje Bang ☐	Nogal Bang ☐	Heel Bang ☐	Panisch Angst ☐

Appendix D: Heart rate measurements form and personal information

Naam:

Geboortedatum:

Geslacht:

Datum en tijd behandeling:

Nummer element:

Voorgeschiedenis:

Medicatie:

	s
1. Wachtkamer	
2. OK-stoel	
3. Anesthesie	
4. Afdekken	
5. Time Out	
6. Incisie	
7. Boren	
8. Kies 1 eruit	
9. Start hechten	
10. Einde (doek eraf)	
11. Rust	
Pijn	
Bijverdoven	
OPG	

Appendix E: Statistics, Normal Q-Q Plots and Boxplots.

More detailed information about the used statistical tests and all Normal Q-Q Plots are displayed in this appendix. If transformation was applied, the Normal Q-Q Plots of this transformed data is included also. Additionally, the box-plots are included to determine any significant outliers in the data.

Statistical tests, Normal Q-Q Plots and Boxplots of differences in heart rate before and during the onset of an event.

Period 1: Heart rate during time in waiting-room

The difference scores for the 'during-waiting-time' and 'before-waiting-time' data were not normally distributed, as assessed by Shapiro-Wilk's test (p=0.038) and the Kolmogorov-Smirnov test (p=0.002). Difference in heart rate scores appeared to be normally distributed, as assessed by visual inspection of Normal Q-Q Plots (figure 1a). However difference scores appeared to be normally distributed with a skewness of 0.300 (standard error = 0.343) and kurtosis of 1.518 (standard error = 0.674). We accepted a statistical significance level of 0.1, which equates to a z-score of ±2.58. The z-score for skewness (0.875) and kurtosis (2.252) are within this boundary.

Five outliers were detected that were more than 1.5 box-lengths from the edge of the box in a boxplot (figure 1b). Inspection of their values did not reveal them to be extreme and they were kept in the analysis. However, due to the outliers in the data and the questionable normal distribution, the Wilcoxon signed rank test was used. Of the 48 participants recruited to the study, the waiting in the waiting room elicited an increase in heart rate in 21 participants, whereas 4 participants saw no increase and 23 participants had a lower heart rate after sitting down in the waiting-room (figure 1c). The Wilcoxon signed-rank test determined that there was *no significant increase in heart rate* when subjects were waiting in the waiting room (median=91.0 bpm) compared to the time before this event (median=89.0 bpm), z=-0.427, p=0.670

Figure 1a. Normal Q-Q Plot of differences

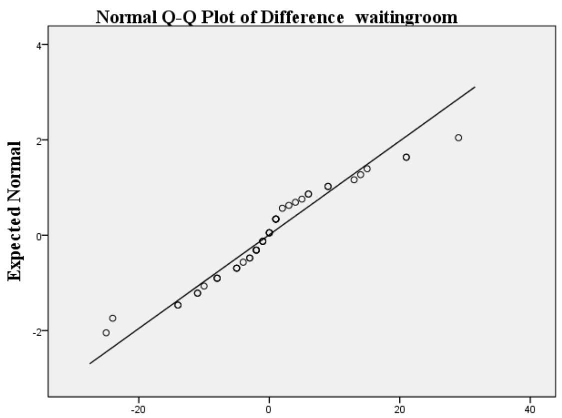


Figure 1a. Normal Q-Q Plot of the difference in values between heart rate of the participants before the waiting room and during the waiting room.

Figure 1b. Boxplot

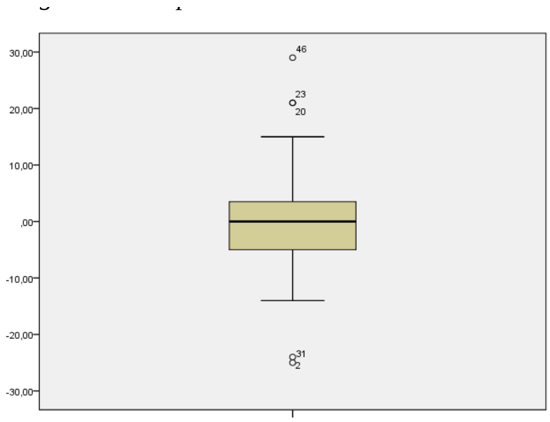


Figure 1b. Boxplot of the difference in values between heart rate of the participants before the waiting room and during the waiting room. Dots represent outliers of more than 1,5 box lengths from the edge of the box (numbers represent the identification number).

Figure 1c. Related Samples Wilcoxon Signed Rank Test

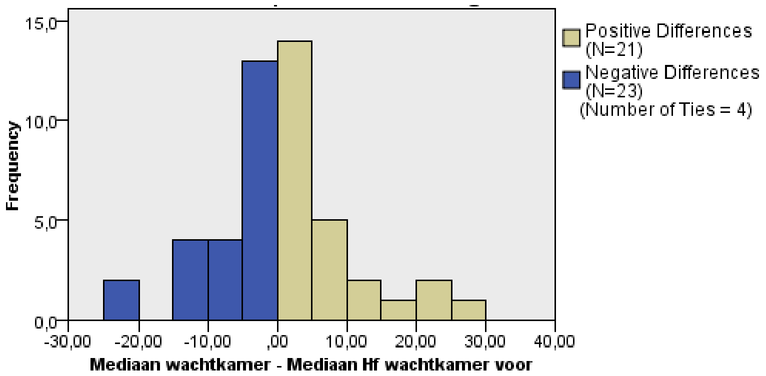


Figure 1c. Histogram of the related samples Wilcoxon Signed Rank test. Differences between the heart rate of participants before the waiting room and during the waiting room are displayed. 23 participants showed a negative difference (e.g., their heart rate decreased during the event) and 21 participants showed a positive difference (e.g., their heart rate increased during the event).

Period 2: Heart rate during sitting down in dental chair

The scores of the difference between sitting down in the dental chair and the time before that event were not normally distributed, as assessed by the Shapiro-Wilk's test ($p<0.0005$), the Kolmogorov-Smirnov test ($p=0.001$) and by visual inspection of the Normal Q-Q Plots (figure 2a). Difference scores were not-normally distributed with a skewness of 4.755 (standard error = 0.343) and kurtosis of 3.594 (standard error = 0.674). The z-score for skewness (4.755) and kurtosis (5.332), are outside boundaries (z-score of ±2.58) so the data is positively skewed and positively kurtosed. Additionally, histograms also showed a positively skewed data. Square root transformation, logarithmic transformation and inverse transformation did not result in a normal distribution of the data (figure 2b,2c,2d). Additionally, 2 outliers of more than 1.5 box lengths from the edge of the box and one outlier of more than 3 box lengths from the edge of the box were detected (figure 2e). Subsequently, the Wilcoxon signed rank test was carried out. Data are medians unless otherwise stated. Of the 48 participants recruited to the study, the action of sitting down in the dental chair elicited an increase in heart rate in 37 participants compared to the time before sitting down in the dental chair, whereas 4 participants saw no increase and 7 participants had a lower heart rate after the event (figure 2f). The Wilcoxon signed-rank test determined that there was a *statistically significant median increase in heart rate* (5.5 bpm) when subjects were sitting down in the dental chair (93.5 bpm) compared to the time before this event (88 bpm), $z=4.382$, $p<0.0005$.

Figure 2e. Boxplot

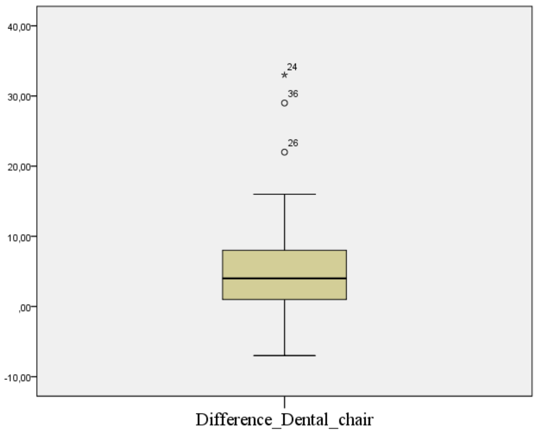


Figure 2e. Boxplot of the difference in values between heart rate of the participants before sitting down in the dental chair and during this event. Dots represent outliers of more than 1,5 box lengths from the edge of the box and asterisks represents outliers of more than 3 box lengths from the edge of the box (numbers represent the participants identification number).

Figure 2a. Normal Q-Q Plot of differences

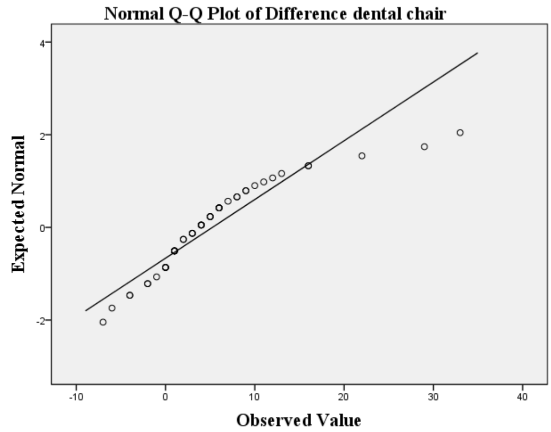


Figure 2b. Normal Q-Q Plot of differences (square root)

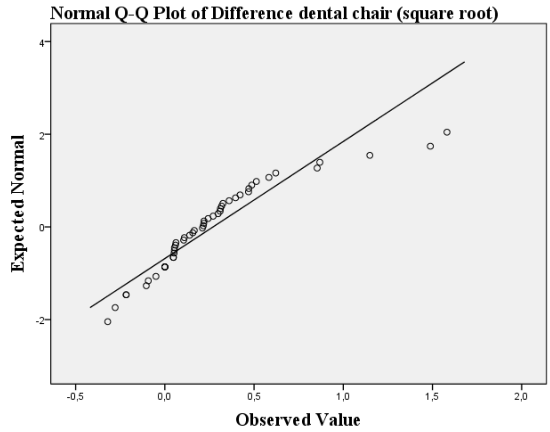


Figure 2c. Normal Q-Q Plot of differences (log10)

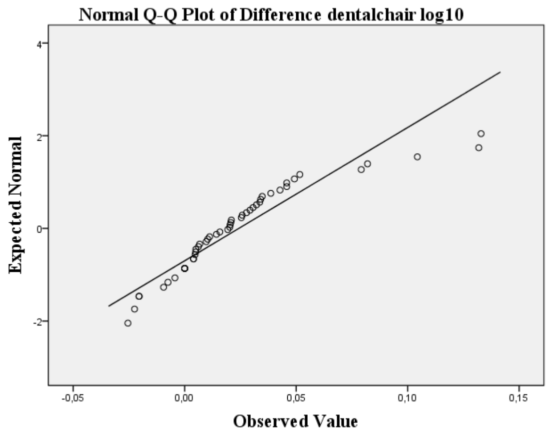


Figure 2d. Normal Q-Q Plot of differences (inverse)

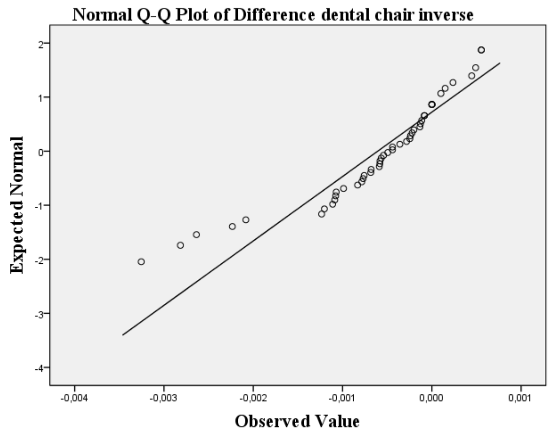


Figure 2a,b,c,d. Normal Q-Q Plot of the difference in values between heart rate of the participants before sitting down in the dental chair and during this event (Figure 2a) and after square root (Figure 2b), logarithmic (Figure 2c) and inverse (figure 2d) transformation.

Figure 2f. Related Samples Wilcoxon Signed Rank Test

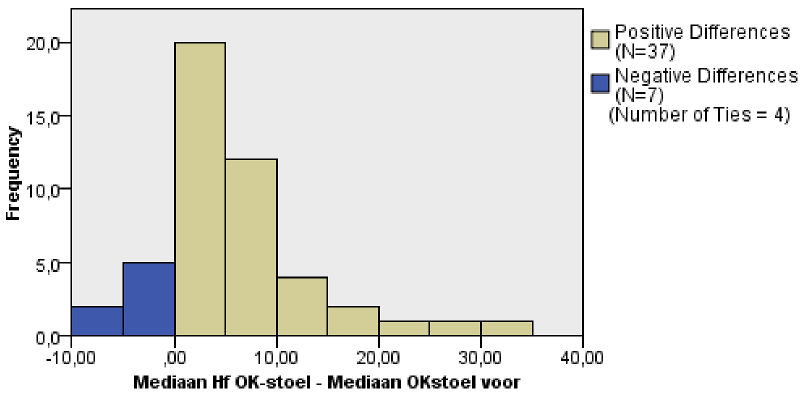


Figure 2f. Histogram of the related samples Wilcoxon Signed Rank test. Differences between the heart rate of participants before sitting down in the dental chair and during this event are displayed. 7 participants showed a negative difference (e.g. their heart rate decreased during the event) and 37 participants showed a positive difference (e.g. their heart rate increased during the event).

Period 3: Heart rate during application of local anesthesia

The difference scores for the heart rate during application of anesthetics and before this event were not normally distributed, as assessed by Shapiro-Wilk's test ($p<0.0005$), the Kolmogorov-Smirnov test ($p<0.0005$) and by inspection of the Normal Q-Q Plots (figure 3a). Difference scores revealed a skewness of 4.799 (standard error = 0.343) and a kurtosis of 28.841 (standard error = 0.674), the z-score for skewness (13.991) and kurtosis (42.791) are outside accepted boundaries (z-score of ±2.58). Two outliers were detected. One outlier of more than 1.5 box-lengths from the edge of a box in the boxplot, but by inspection the value did not reveal them to be extreme and it was kept in the analysis. However, the second outlier was a data point more than 3 box-lengths away and was interpreted as an extreme outlier (figure 3b). The outlier was kept in analysis. In view of the circumstances, we chose to use the Wilcoxon signed-rank test instead of the paired samples t-test. Data are medians unless otherwise stated. Participants showed a higher heart rate before administering anesthetics (89.5 bpm) compared to their heart rate during the injection (88.5 bpm); with no median difference. Of the 48 participants, the injection of anesthetic fluid elicited an increase in heart rate in 22 participants and a decrease in 23 participants, whereas 3 participants showed no difference (figure 3c). Differences in heart rate were *not statistically significant*, $z=-0.650$, $p=0.516$.

Figure 3a. Normal Q-Q Plot of differences

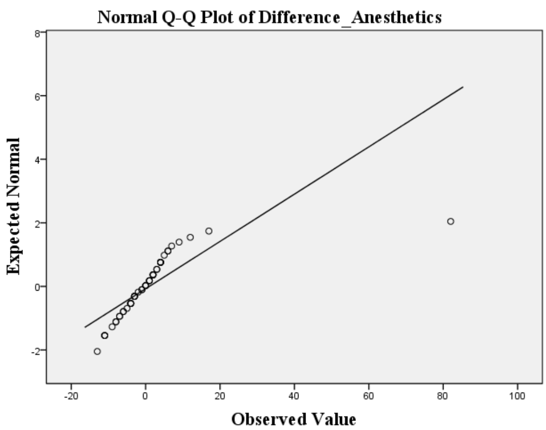


Figure 3a. Normal Q-Q Plot of the difference in values between heart rate of the participants before the local anesthesia and during this event.

Figure 3b. Boxplot

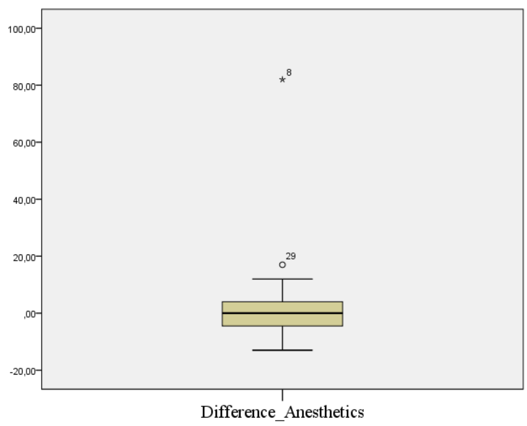


Figure 3b. Boxplot of the difference in values between heart rate of the participants before the local anesthesia and during this event. Dots represent outliers of more than 1,5 box lengths from the edge of the box and asterisks represents outliers of more than 3 box lengths from the edge of the box (numbers represent the participants identification number).

Figure 3c. Related Samples Wilcoxon Signed Rank Test

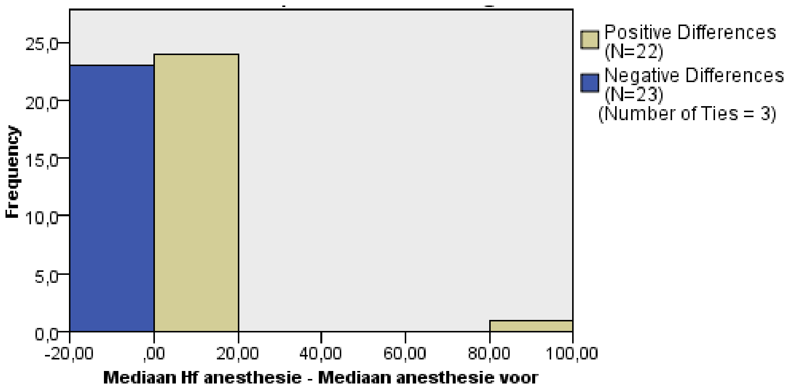


Figure 3c. Histogram of the related samples Wilcoxon Signed Rank test. Differences between the heart rate of participants before local anesthesia and during this event are displayed. 23 participants showed a negative difference (e.g. their heart rate decreased during the event) and 22 participants showed a positive difference (e.g. their heart rate increased during the event).

<u>Period 4: Heart rate during applying surgical drapes</u>

The scores of the difference between applying the surgical drapes and the time before that event were not normally distributed, as assessed by the Shapiro-Wilk's test (p=0.000), the Kolmogorov-Smirnov test (p<0.0005) and by visual inspection of the Normal Q-Q Plots (figure 4a). Difference scores were not-normally distributed with a skewness of 2.323 (standard error = 0.343) and kurtosis of 7.550 (standard error = 0.674). The z-score for skewness (6.773) and kurtosis (11.202), are outside boundaries (z-score of ±2.58) so the data has a non-normal distribution with a positive skewness and positive kurtosis. Two outliers were detected that were more than 3 box-lengths from the edge of the box in a boxplot and one outlier of more than 1.5 box-lengths from the edge (figure 4b). The outliers were kept in analysis and the Wilcoxon-signed rank test was used. Data are medians unless otherwise stated. Of the 48 participants recruited to the study the action of applying surgical drapes elicited an increase in heart rate in 32 participants compared to the time before this event, whereas 3 participants saw no increase in heart rate and 13 participants had a lower heart rate after the event (figure 4c). The Wilcoxon-signed rank test determined that there was a statistically significant median increase in heart rate (4.0 bpm) when subjects were covered with surgical drapes (100.5 bpm) compared to the time before this event (92.5 bpm), z = 3.548, p < .0005.

Figure 4a. Normal Q-Q Plot of differences

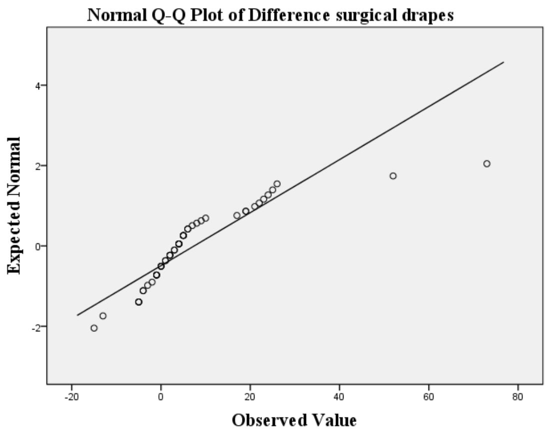


Figure 4a. Normal Q-Q Plot of the difference in values between heart rate of the participants before the application of surgical drapes and during this event.

Figure 4b. Boxplot

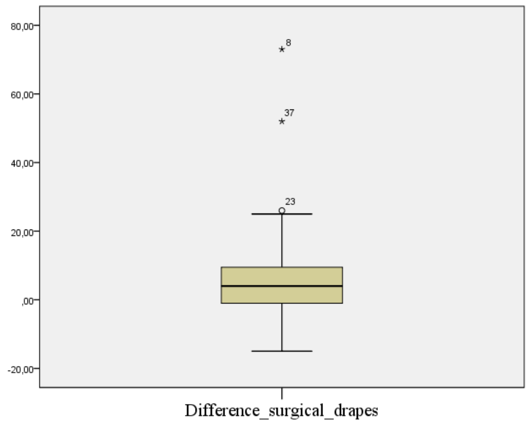


Figure 4b. Boxplot of the difference in values between heart rate of the participants before the application of surgical drapes and during this event. Dots represent outliers of more than 1,5 box lengths from the edge of the box and asterisks represents outliers of more than 3 box lengths from the edge of the box (numbers represent the participants identification number).

Figure 4c. Related Samples Wilcoxon Signed Rank Test

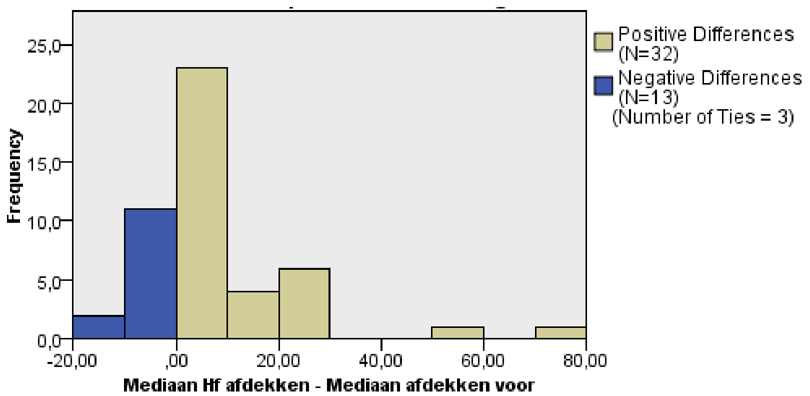


Figure 4c. Histogram of the related samples Wilcoxon Signed Rank test. Differences between the heart rate of participants before applying surgical drapes and during this event are displayed. 13 participants showed a negative difference (e.g. their heart rate decreased during the event) and 32 participants showed a positive difference (e.g. their heart rate increased during the event).

Period 5: Heart rate during time-out

The scores of the difference between the time-out and the time before that event were not normally distributed, as assessed by the Shapiro-Wilk's test ($p<0.0005$), the Kolmogorov-Smirnov test ($p<0.0005$) and by visual inspection of the Normal Q-Q Plots (figure 5a). Difference scores were not normal distributed with a skewness of 3.733 (standard error = 0.343) and kurtosis of 23.660 (standard error = 0.674). The z-score for skewness (10.833) and kurtosis (35.104) are outside boundaries (z-score of ±2.58). So the data has a non-normal distribution with a positive skewness and positive kurtosis. Two outliers were detected that were more than 3 box-lengths from the edge of the box in a boxplot and one outlier of more than 1.5 box-lengths from the edge (figure 5b). The outliers were kept in analysis and the Wilcoxon-signed rank test was used.

Data are medians unless otherwise stated. Participants showed a lower heart rate before the time-out procedure (103.0 bpm) compared to their heart rate during the time-out procedure (104.5 bpm); with a median difference of 1.0 bpm. Of the 48 participants, the time-out elicited an increase in heart rate in 25 participants and a decrease in 18 participants, whereas 5 participants showed no difference (figure 5c). Differences in heart rate were *not statistically significant*, $z=1.149$, $p=0.250$.

Figure 5a. Normal Q-Q Plot of differences

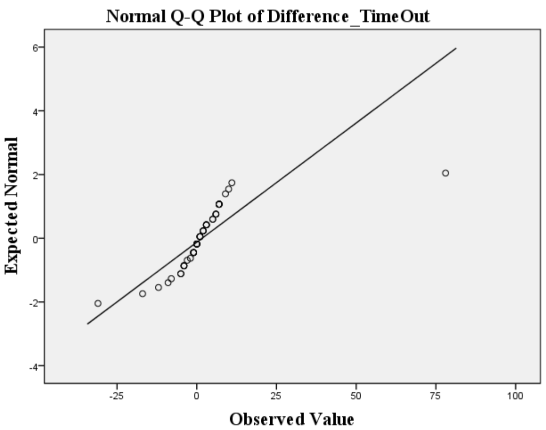


Figure 5a. Normal Q-Q Plot of the difference in values between heart rate of the participants before the time-out and during this event.

Figure 5b. Boxplot

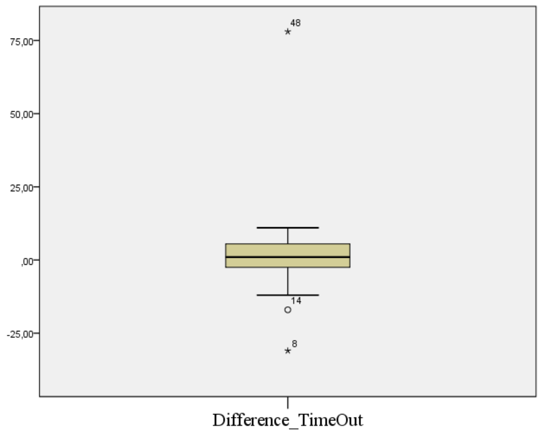


Figure 5b. Boxplot of the difference in values between heart rate of the participants before the Time-Out and during this event. Dots represent outliers of more than 1,5 box lengths from the edge of the box and asterisks represents outliers of more than 3 box lengths from the edge of the box (numbers represent the participants identification number).

Figure 5c. Related Samples Wilcoxon Signed Rank Test

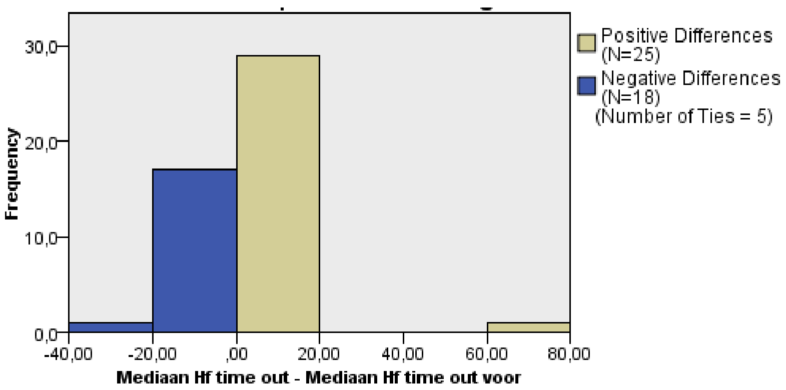


Figure 5c. Histogram of the related samples Wilcoxon Signed Rank test. Differences between the heart rate of participants before the Time-Out and during this event are displayed. 18 participants showed a negative difference (e.g. their heart rate decreased during the event) and 25 participants showed a positive difference (e.g. their heart rate increased during the event).

<u>*Period 6: Heart rate during incision*</u>

The scores of the difference between the incision and the time before that event were not normally distributed, as assessed by the Shapiro-Wilk's test (p=0.000), the Kolmogorov-Smirnov test (p<0.0005) and by visual inspection of the Normal Q-Q Plots (figure 6a). Data showed a skewness of 4.875 (standard error = 0.343) and a kurtosis of 30.004 (standard error=0.674), with the corresponding z-scores of 14.213 (skewness) and 44.516 (kurtosis), which also confirms a non-normal distribution and also shows a positive skewness and kurtosis.

Two outliers were detected at 1.5 box-lengths from the edge of a boxplot and one outlier was detected at more than 3 box-lengths from the edge (figure 6b). We kept the outliers in analysis and continued with the Wilcoxon-signed rank test. Data are medians unless otherwise stated. Of the 48 participants, the incision caused an increase in heart rate in 20 participants and a decrease in 24 participants, whereas 4 participants showed no difference (figure 6c). Subjects showed a heart rate before incision of 105.5 bpm and during the incision the heart rate remained 105.5 bpm; however data showed a median difference of -0.5 bpm. Thus, the heart rate was 0.5 bpm lower during the incision compared to the time before that moment. Differences in heart rate were *not statistically significant*, z=-0.719, p=0.472.

Figure 6a. Normal Q-Q Plot of differences

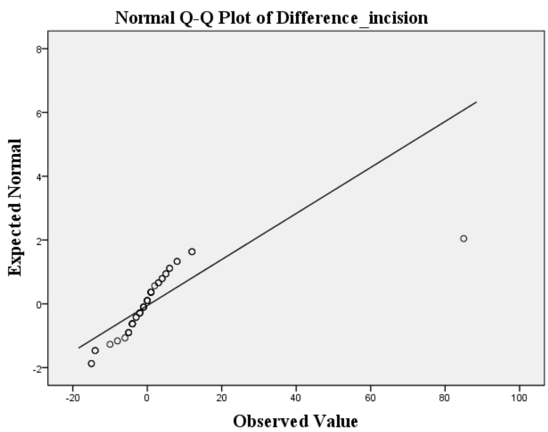


Figure 6a. Normal Q-Q Plot of the difference in values between heart rate of the participants before the incision and during this event.

Figure 6b. Boxplot

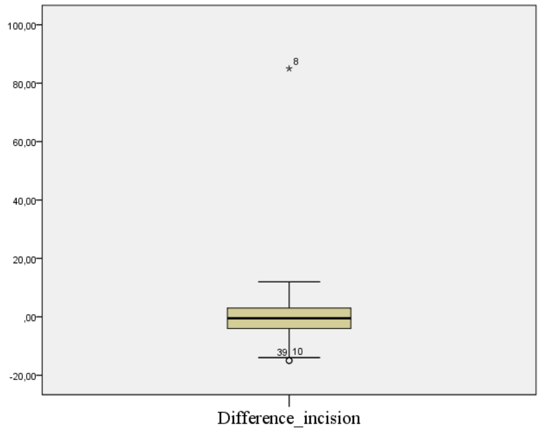


Figure 6b. Boxplot of the difference in values between heart rate of the participants before the incision and during this event. Dots represent outliers of more than 1,5 box lengths from the edge of the box and asterisks represents outliers of more than 3 box lengths from the edge of the box (numbers represent the participants identification number).

Figure 6c. Related Samples Wilcoxon Signed Rank Test

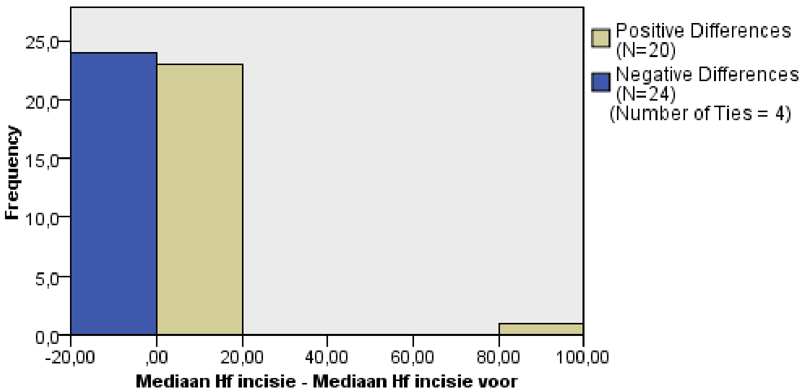


Figure 6c. Histogram of the related samples Wilcoxon Signed Rank test. Differences between the heart rate of participants before the incision and during this event are displayed. 24 participants showed a negative difference (e.g. their heart rate decreased during the event) and 20 participants showed a positive difference (e.g. their heart rate increased during the event).

Period 7: Heart rate during alveolotomy

The difference scores for the heart rate during alveolotomy were not normally distributed, as assessed by the Shapiro-Wilk's test (P=0.000), the Kolmogorov-Smirnov test (p=0.002) and the visual inspection of the Normal Q-Q-plot (figure 7a). Additionally, data were not normally distributed as assessed by the skewness of -0.385 (standard error=0.421) and kurtosis of 6.163 (standard error=0.821). The z-score of -0.914 (skewness) is within the set boundary of ±2.58, however the z-score of 7.507 (kurtosis) lies outside this boundary. Furthermore, 1 outlier of more than 1.5 box-lengths and 3 outliers of more than 3 box-lengths from the edge of a boxplot were detected (figure 7b). Outliers were kept in analysis and the Wilcoxon-signed rank test was carried out. Data are medians unless otherwise stated. Of the 31 participants who underwent alveolotomy, the alveolotomy caused an increase in heart rate in 18 participants and a decrease in 8 participants, whereas 5 participants showed no difference in heart rate (figure 7c). Participants showed a lower heart rate before the alveolotomy procedure (101.0 bpm) compared to their heart rate during the alveolotomy (102.0 bpm); with a median difference of 1.0 bpm. Differences in heart rate revealed a trend, however *not statistically significant*, z= 1.896, p=0.058.

Figure 7a. Normal Q-Q Plot of differences

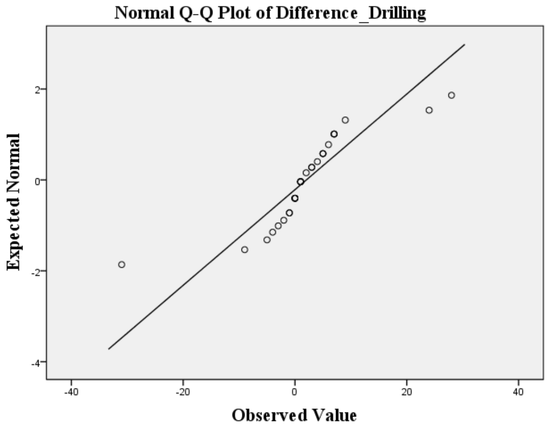


Figure 7a. Normal Q-Q Plot of the difference in values between heart rate of the participants before the alveolotomy and during this event.

Figure 7b. Boxplot

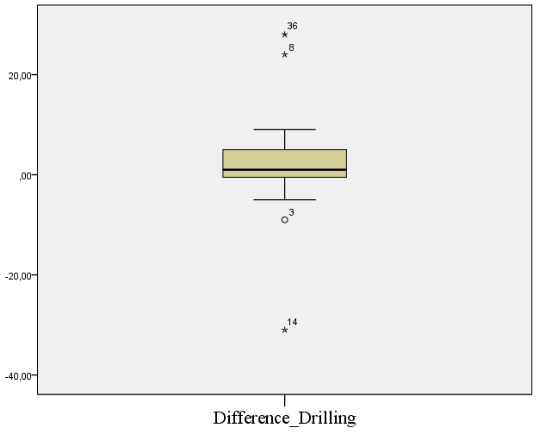


Figure 7b. Boxplot of the difference in values between heart rate of the participants before the alveolotomy and during this event. Dots represent outliers of more than 1,5 box lengths from the edge of the box and asterisks represents outliers of more than 3 box lengths from the edge of the box (numbers represent the participants identification number).

Figure 7c. Related Samples Wilcoxon Signed Rank Test

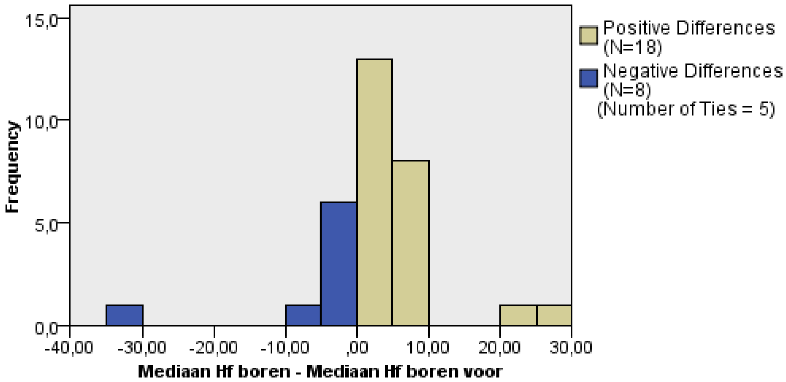


Figure 7c. Histogram of the related samples Wilcoxon Signed Rank test. Differences between the heart rate of participants before the alveolotomy and during this event are displayed. 8 participants showed a negative difference (e.g. their heart rate decreased during the event) and 18 participants showed a positive difference (e.g. their heart rate increased during the event).

Period 8: Heart rate during removal of the third molar

The difference scores for the heart rate during the removal of the third molar and the time before that moment were not normally distributed, as assessed by Shapiro-Wilk's test (p=0.005) and the Kolmogorov-Smirnov test (p=0.022). However, the visual inspection of the Normal Q-Q plots showed a normal distribution (figure 8a). However difference scores were normally distributed with a skewness of -0.852 (standard error = 0.343) and kurtosis of 3.185 (standard error = 0.674). We accepted a statistical significance level of 0.1, which equates to a z-score of ±2.58. The z-score for skewness (-2.484) lies within this boundary, but kurtosis (4.726) does not. One outlier was detected of more than 3 box-lengths from the edge of the box in a boxplot (figure 8b). Inspection of their values did not reveal them to be extreme and it was kept in the analysis.

A Wilcoxon-signed rank test followed. Of the 48 participants, the removal of the third molar caused an increase in heart rate in 17 subjects and a decrease in 29 subjects, whereas 2 subjects showed no difference (figure 8c). Subjects showed a heart rate before the removal of the third molar of 99.5 bpm and during the incision the heart rate remained 100.5 bpm, however data showed a median difference of -2.0 bpm. Thus, the heart rate was 2.0 bpm lower during the removal compared to the time before that moment. Differences in heart rate were *not statistically significant*, z=-1.064, p=0.287.

Figure 8a. Normal Q-Q Plot of differences

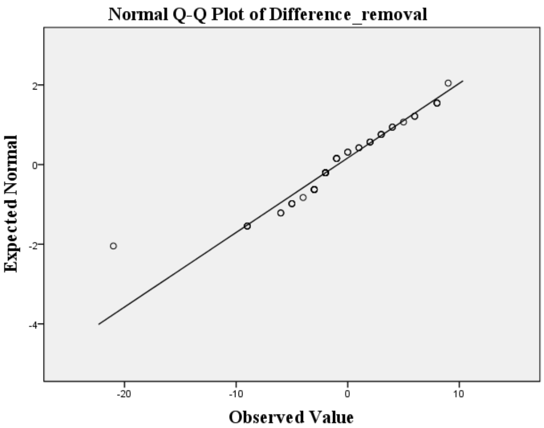


Figure 8a. Normal Q-Q Plot of the difference in values between heart rate of the participants before the removal of the third molar and during this event.

Figure 8b. Boxplot

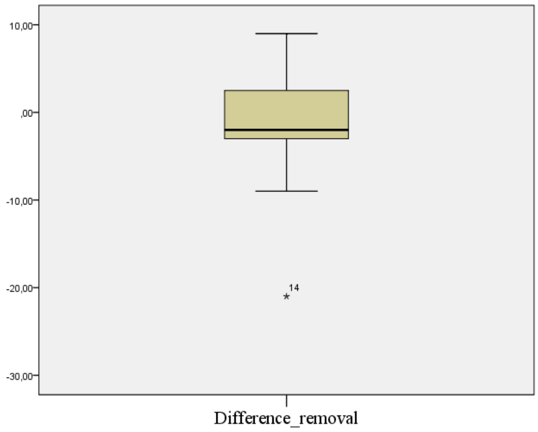


Figure 8b. Boxplot of the difference in values between heart rate of the participants before the removal of the third molar and during this event. Dots represent outliers of more than 1,5 box lengths from the edge of the box and asterisks represents outliers of more than 3 box lengths from the edge of the box (numbers represent the participants identification number).

Figure 8c. Related Samples Wilcoxon Signed Rank Test

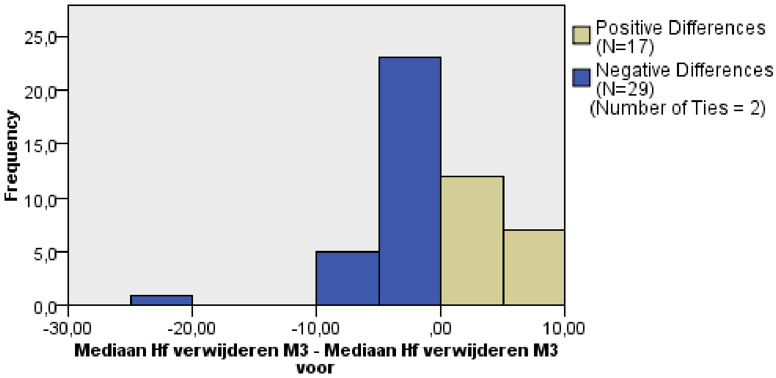


Figure 8c. Histogram of the related samples Wilcoxon Signed Rank test. Differences between the heart rate of participants before the removal of the third molar and during this event are displayed. 29 participants showed a negative difference (e.g. their heart rate decreased during the event) and 17 participants showed a positive difference (e.g. their heart rate increased during the event).

Period 9: Heart rate during suturing

The difference scores for the 'during-waiting-time' and 'before-waiting-time' data were not normally distributed, as assessed by Shapiro-Wilk's test ($p<0.0005$), the Kolmogorov-Smirnov test ($p<0.0005$) and the Normal Q-Q Plots (figure 9a). Difference scores were normally distributed with a skewness of 4.287 (standard error = 0.343) and kurtosis of 25.830 (standard error = 0.674). We accepted a statistical significance level of 0.1, which equates to a z-score of ±2.58. The z-score for skewness (12.499) and kurtosis (38.323) are outside this boundary, so the data is has a non-normal distribution and a positive skewness and kurtosis. One outlier was detected of more than 1.5 box-lengths, and one outlier of more than 3 box-lengths from the edge of the box in a boxplot (figure 9b). They were kept in the analysis. Due to outliers and a non-normal distribution we used the Wilcoxon-signed rank test to analyze the data. Dare medians unless otherwise stated. Participants showed a lower heart rate before the suturing of 95.0 bpm compared to their heart rate during the suturing, which was also 95.0 bpm. Thus, there was no median difference in heart rate found. Of the 48 participants, the suturing elicited an increase in heart rate in 16 participants and a decrease in 22 participants, whereas 10 participants showed no difference (figure 9c). Differences in heart rate were *not statistically significant*, $z=-0.851$, $p=0.427$.

Figure 9a. Normal Q-Q Plot of differences

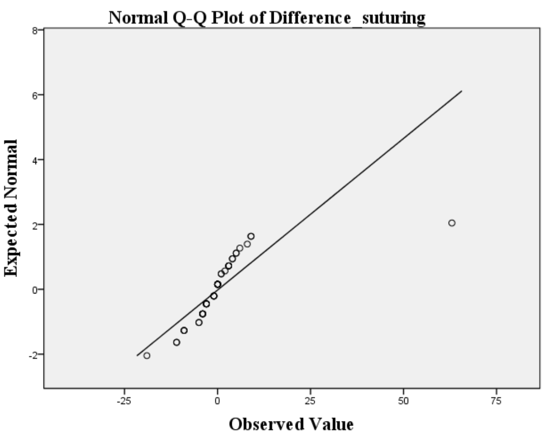


Figure 9a. Normal Q-Q Plot of the difference in values between heart rate of the participants before the suturing and during this event.

Figure 9b. Boxplot

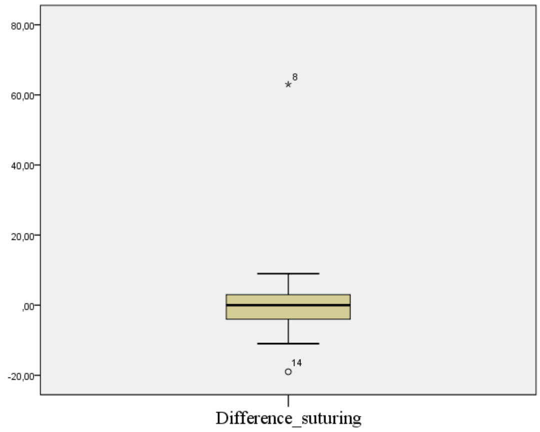


Figure 9b. Boxplot of the difference in values between heart rate of the participants before the suturing and during this event. Dots represent outliers of more than 1,5 box lengths from the edge of the box and asterisks represents outliers of more than 3 box lengths from the edge of the box (numbers represent the participants identification number).

Figure 9c. Related Samples Wilcoxon Signed Rank Test

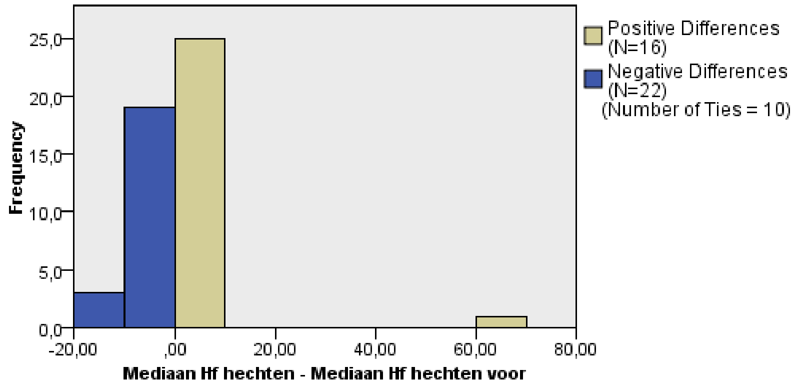


Figure 9c. Histogram of the related samples Wilcoxon Signed Rank test. Differences between the heart rate of participants before the suturing and during this event are displayed. 22 participants showed a negative difference (e.g. their heart rate decreased during the event) and 16 participants showed a positive difference (e.g. their heart rate increased during the event).

Period 10: Heart rate during end of procedure

The difference scores of the heart rate during the end of the procedure and the time before that event, were not normally distributed as assessed by Shapiro-Wilk's test (p=0.001) and by visual interpretation of the Normal Q-Q-Plot (figure 10a), but showed a normal distribution at the Kolmogorov-Smirnov test (p=0.073). Data was distributed with a skewness of -1.158 (standard error=0.343) and kurtosis of 5.707 (standard error=0.674). We accepted a statistical significance level of 0.1, which equates to a z-score of ±2.58. The z-score for skewness (-3.376) and kurtosis (8.467) are outside this boundary, so the data has a non- normal distribution and a negative skewness and positive kurtosis.

One outlier was detected of more than 3-box lengths from the edge of the box in a boxplot (figure 10b). Inspection of their values did not reveal them to be extreme, however due to the outlier and a non-normal distribution we used the Wilcoxon-signed rank test to analyze the data. Of the 48 participants, the end of the procedure caused an increase in median heart rate in 26 subjects and a decrease in 17 subjects, whereas 5 subjects showed no difference (figure 10c). Subjects showed a median heart rate of 90.5 bpm before the end of the procedure and a median heart rate of 90.5 bpm during the end of the procedure as well, however the median difference between moments was stated at 2.0 bpm. Differences in heart rate revealed a trend, however *not statistically significant*, z=1.778, p=0.075.

Figure 10a. Normal Q-Q Plot of differences

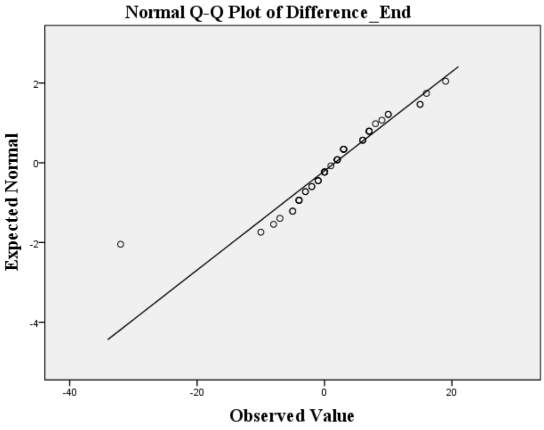


Figure 10a. Normal Q-Q Plot of the difference in values between heart rate of the participants before the end of the procedure and during this event.

Figure 10b. Boxplot

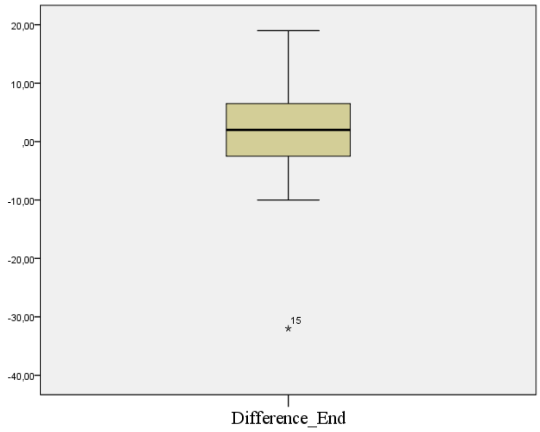


Figure 10b. Boxplot of the difference in values between heart rate of the participants before the end of the procedure and during this event. Dots represent outliers of more than 1,5 box lengths from the edge of the box and asterisks represents outliers of more than 3 box lengths from the edge of the box (numbers represent the participants identification number).

Figure 10c. Related Samples Wilcoxon Signed Rank Test

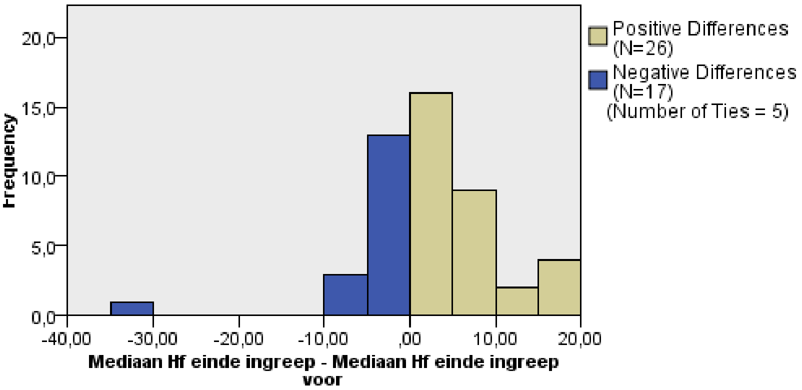


Figure 10c. Histogram of the related samples Wilcoxon Signed Rank test. Differences between the heart rate of participants before end of the procedure and during this event are displayed. 17 participants showed a negative difference (e.g. their heart rate decreased during the event) and 26 participants showed a positive difference (e.g. their heart rate increased during the event).

Period pain

The difference scores of the occurrence of pain and the time before this event were normal distributed, as assessed by Shapiro-Wilk's test (p=0.720), the Kolmogorov-Smirnov test (p=0.200) and the visual interpretation of the Q-Q Plot (figure 11a). There were no outliers in the data, as assessed by inspection of a boxplot for values greater than 1.5 box-lengths from the edge of the box (figure 11b). Paired samples t-test was used to analyze the data. Data are mean ± standard deviation, unless otherwise stated. Participants had a slight higher heart rate before patients indicated their pain (117 ± 15.2) compared to their heart rate during pain (116.5 ± 15.2). A *statistically non-significant* decrease of -0.5 (95% CI, -2.499 to 1.499) bpm was found, t(7)=-0.592, p=0.573, d=0.209.

Figure 11a. Normal Q-Q Plot of differences

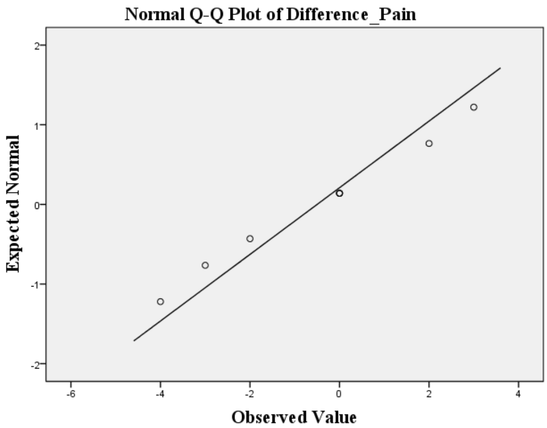


Figure 11a. Normal Q-Q Plot of the difference in values between heart rate of the participants before pain and during this event.

Figure 11b. Boxplot

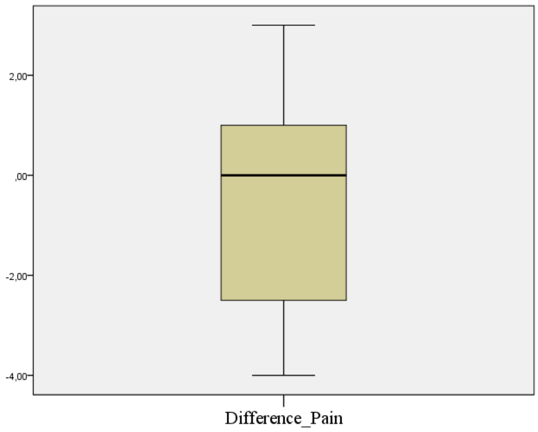


Figure 11b. Boxplot of the difference in values between heart rate of the participants before pain and during this event.

Period additional anesthetics

The difference scores of the application of additional anesthesia and the time before this event were normally distributed, as assessed by the Shapiro-Wilk's test (p=0.780), the Kolmogorov-Smirnov test (p=0.200) and the visual interpretation of the Q-Q Plot (figure 12a). There were no outliers detected (figure 12b). Paired samples t-test was used to analyze the data. Data are mean ± standard deviation, unless otherwise stated. Participants had a higher heart rate before patients administered additional anesthesia (104.6 ± 15.8) compared to their heart rate during this event (100.4 ± 16.6). A *statistically significant* decrease of -4.3 (95% CI, -6.697 to -1.849) bpm was found, t(10)=-3.928, p=0.003, d=1.18.

Figure 12a. Normal Q-Q Plot of differences

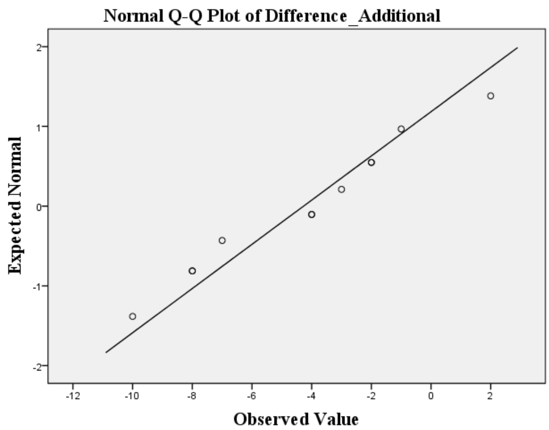


Figure 12a. Normal Q-Q Plot of the difference in values between heart rate of the participants before the additional anesthetics and during this event.

Figure 12b. Boxplot

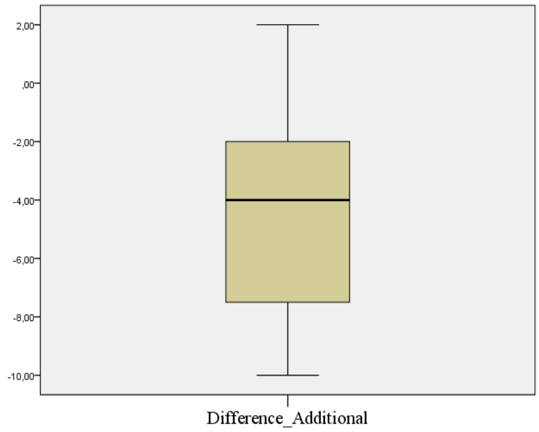


Figure 12b. Boxplot of the difference in values between heart rate of the participants before the additional anesthetics and during this event.

Statistical tests, Normal Q-Q Plots and Boxplots of changes in heart rate between different events.

To assess the changes in heart rate over time between the different events during the whole procedure, we initially used the repeated measures ANOVA. First, we determined if the assumptions of this statistical test were met.
Normal distribution was evaluated by using the Shapiro-Wilk's test (S-W), the Kolmogorov-Smirnov test (K-S) and by visual interpretation of the Normal Q-Q Plots (figure 13a – 13j)
Heart rate was normally distributed during time in the waiting room (S-W test p=0.113, K-S test p=0.052), sitting down in the dental chair (p=0.695, K-S test p=0.200), time-out (p=0.095, K-S test p=0.098), alveolotomy (p=0.382, K-S test p=0.200), the removal of the third molar (p=0.390, K-S test p=0.200) and at the end of the procedure (p=0.062, K-S test p=0.200). However, the data of the heart rate during anesthesia (S-W test p<0.0005, K-S test p=0.002), surgical drapes (S-W test p=0.005, K-S test p=0.056), incision (S-W test p=0.001, K-S test p=0.053) and suturing were not normally distributed, also assessed by normal Q-Q Plots. These non-normal distributed data points all showed a positive skewness and positive kurtosis. By square root and logarithmic transformation data showed no normal distribution. However, by inverse transformation, the data of anesthesia (p=0.734, K-S test p=0.200), surgical drapes (p=0.143, K-S test p=0.112) and suturing (p=0.258, K-S test p=0.013) were normally distributed, as assessed by Shapiro-Wilk's test, Kolmogorov-Smirnov test and Q-Q plots (figure 14a - d). However, normal distribution of data of incision could not be reached with inverse transformation (p=0.028, K-S test p=0.013). There were outliers detected in the data, as assessed by inspection of a boxplot. There were 5 extreme data point of more than 3 box-lengths away from the edge of their box during anesthesia, surgical drapes, time-out, incision and suturing. Additionally, there were 8 outliers detected of more than 1.5 box-lengths away from the edge of their box (figure 15). By identifying these outliers, we established that the outlier is neither a data entry error nor measurement error. We concluded that the outliers are most likely a genuinely unusual data points.

Due to a non-normal distribution of the heart rate during incision and due to multiple outliers we continued our analysis by using the Friedman's test.

Figure 13a. Normal Q-Q Plot: waiting room

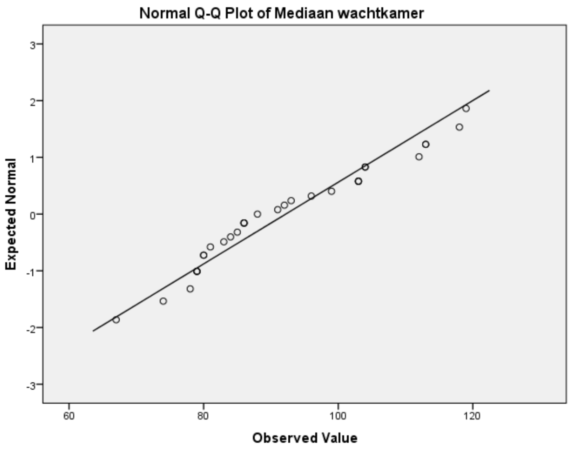


Figure 13a. Normal Q-Q Plot of median heart rate of patients during their time in the waiting room

Figure 13b. Normal Q-Q Plot: dental chair

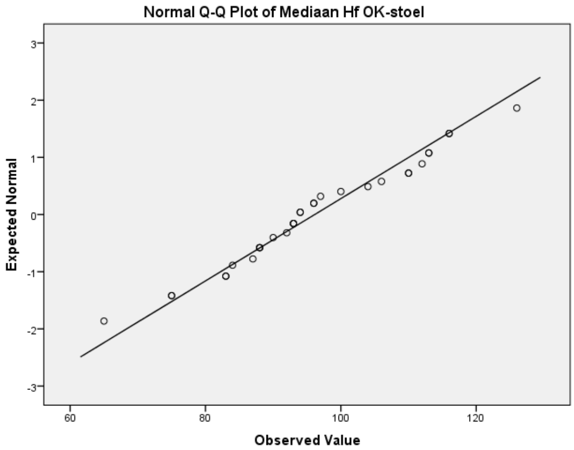


Figure 13b. Normal Q-Q Plot of median heart rate of patients during sitting down in the dental chair

Figure 13c. Normal Q-Q Plot: anesthesia

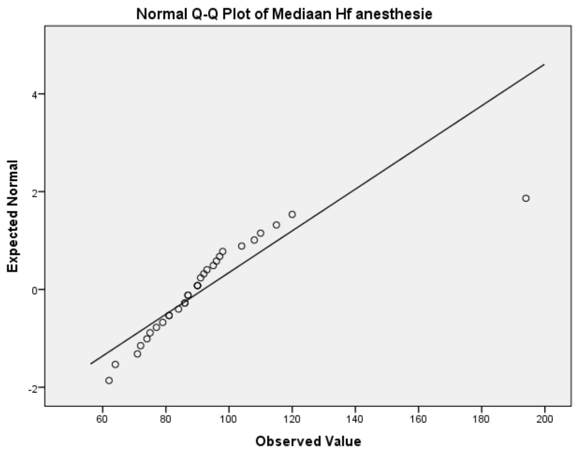


Figure 13c. Normal Q-Q Plot of median heart rates of patients during the application of local anesthesia.

Figure 13d. Normal Q-Q Plot: surgical drapes

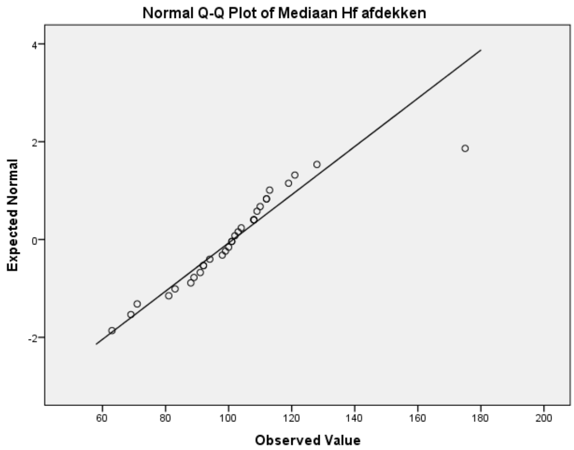


Figure 13d. Normal Q-Q Plot of median heart rates of patients during the application of surgical drapes

Figure 13e. Normal Q-Q Plot: time-out

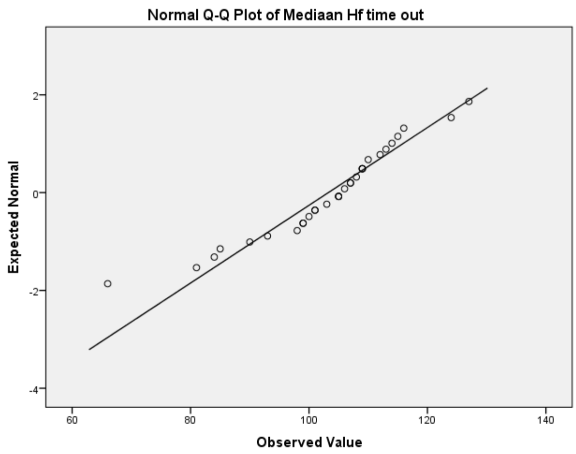


Figure 13e. Normal Q-Q Plot of median heart rates of patients during the time-out procedure.

Figure 13f. Normal Q-Q Plot: incision

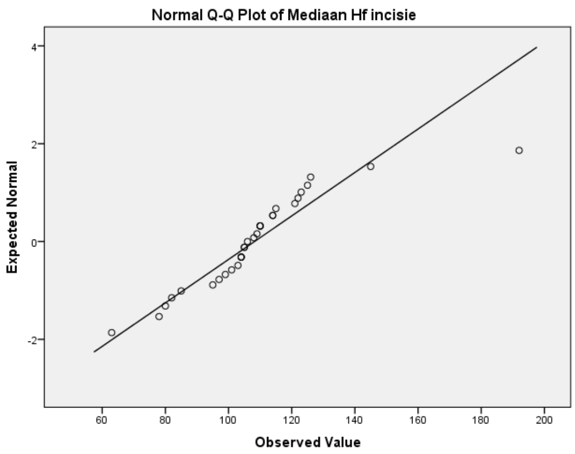


Figure 13f. Normal Q-Q Plot of median heart rates of patients during the first incision.

Figure 13g. Normal Q-Q Plot: alveolotomy

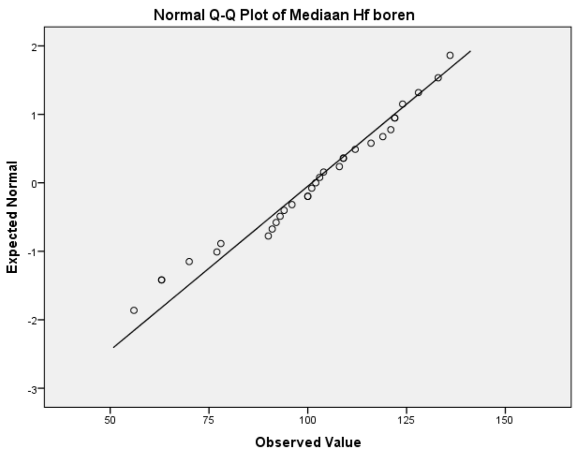


Figure 13g. Normal Q-Q Plot of median heart rates of patients during the alveolotomy.

Figure 13h. Normal Q-Q Plot: removal of M3

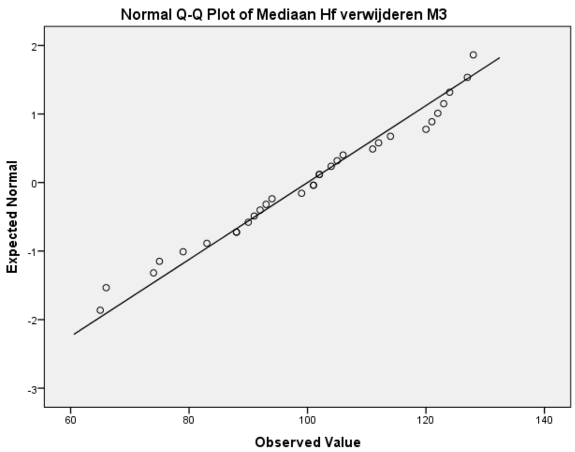


Figure 13h. Normal Q-Q Plot of median heart rates of patients during the removal of the third molar.

Figure 13i. Normal Q-Q Plot: suturing

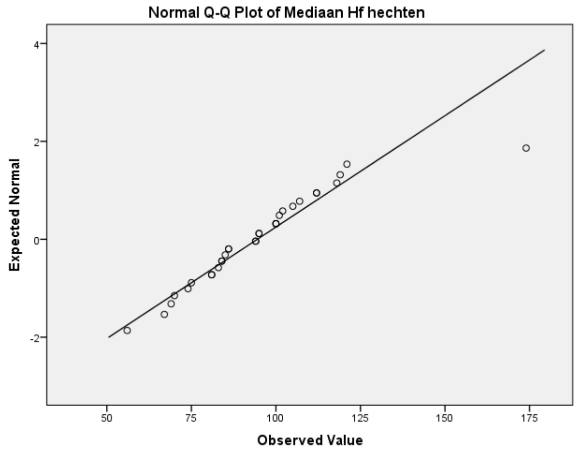


Figure 13i. Normal Q-Q Plot of median heart rates of patients during the suturing.

Figure 13j. Normal Q-Q Plot: end of procedure

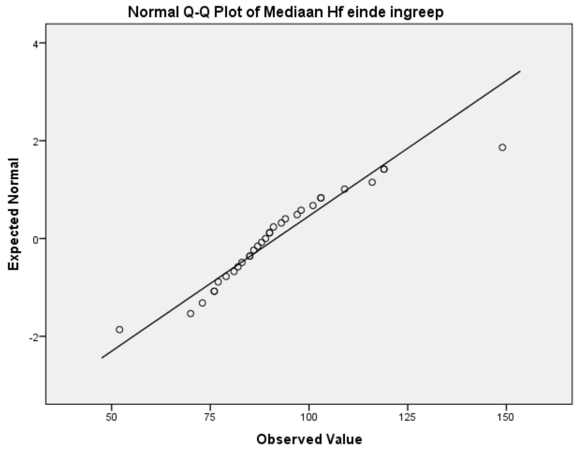


Figure 13j. Normal Q-Q Plot of median heart rates of patients during the end of the procedure.

Figure 14a. Normal Q-Q Plot: anesthesia (inverse transformation.)

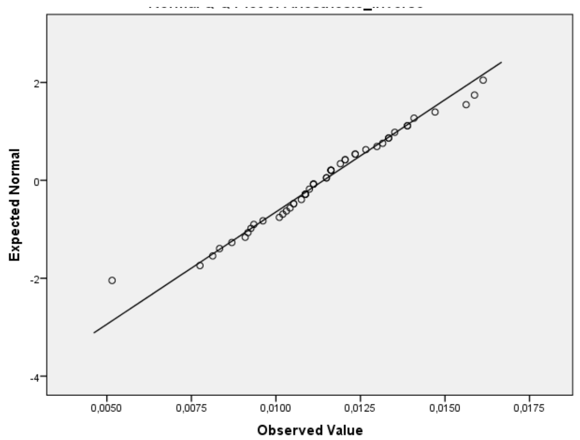


Figure 14a. Normal Q-Q Plot of median heart rates of patients during the anesthesia after inverse transformation.

Figure 14b. Normal Q-Q Plot: surgical drapes (inverse transformation)

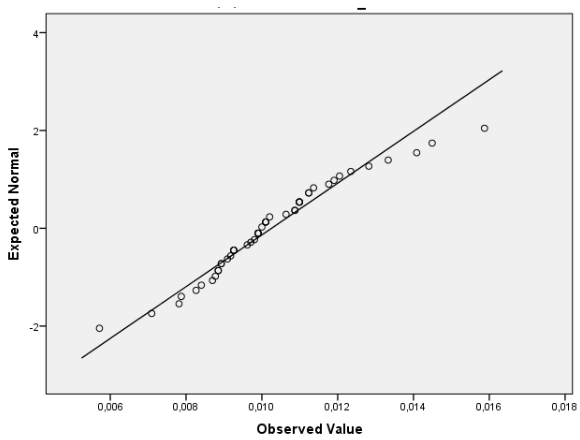


Figure 14b. Normal Q-Q Plot of median heart rates of patients during the application of surgical drapes after inverse transformation.

Figure 14c. Normal Q-Q Plot: suturing (inverse transformation)

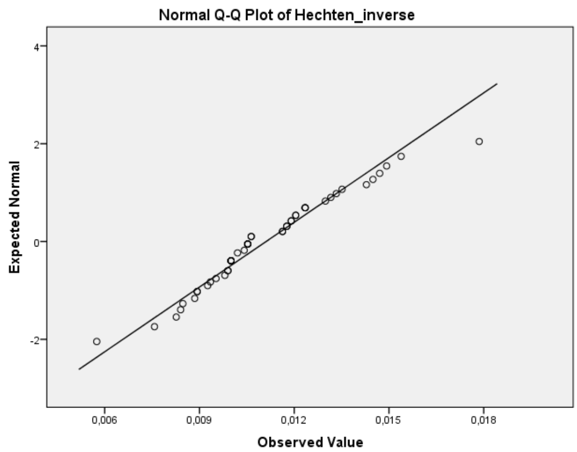


Figure 14c. Normal Q-Q Plot of median heart rates of patients during the suturing after inverse transformation.

Figure 14d. Normal Q-Q Plot: incision (inverse transformation)

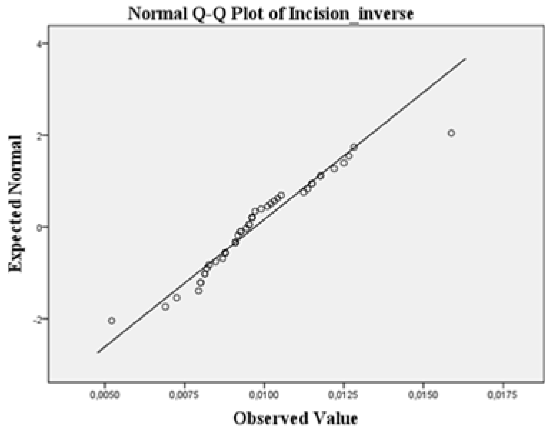


Figure 14d. Normal Q-Q Plot of median heart rates of patients during the incision after inverse transformation.

Figure 15. Boxplot of the median heart rates of the participants during the different periods.

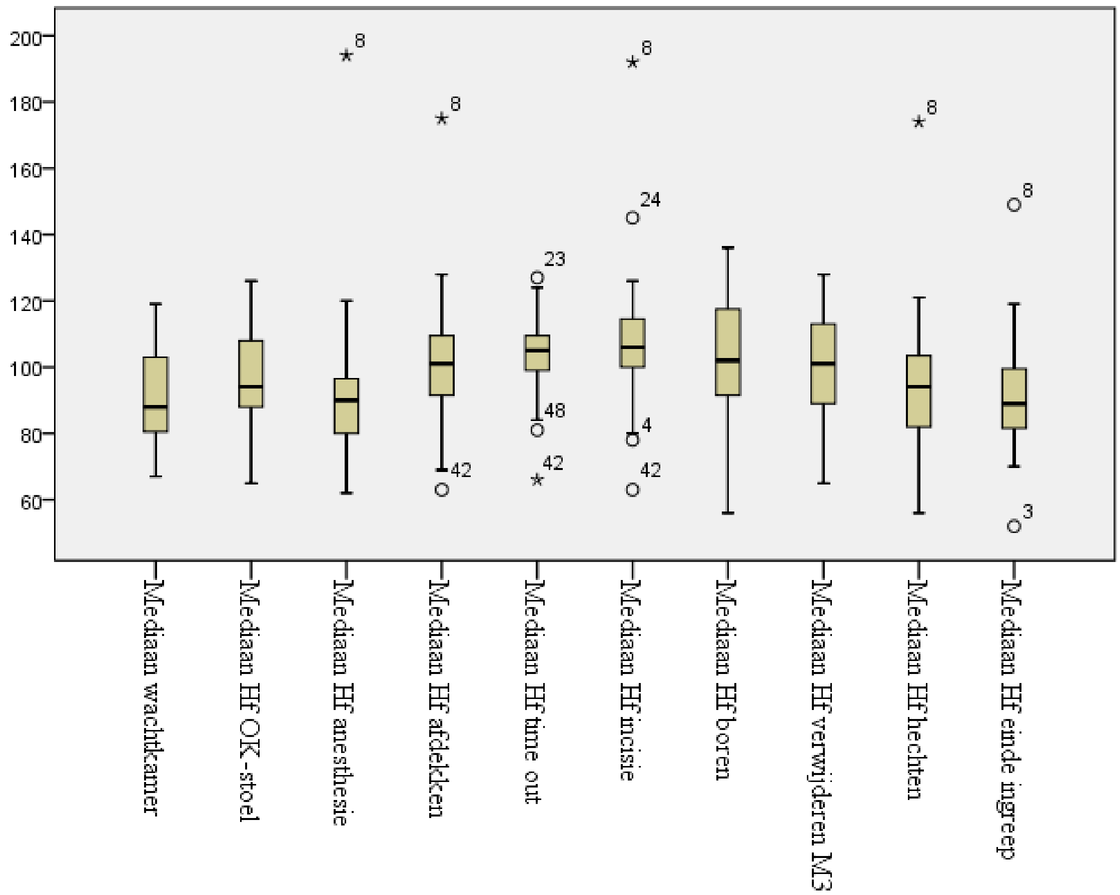


Figure 15. Boxplot of the median heart rates of the participants during the different periods. Dots represent outliers of more than 1,5 box lengths from the edge of the box and asterisks represents outliers of more than 3 box lengths from the edge of the box (numbers represent the participants identification number).

Mean heart rate during the total treatment time

Normal distribution for females, but not for males, as assessed by the Shapiro Wilk's test ($p=0.931$ for females, $p=0.010$ for males), the Kolmogorov-Smirnov test ($p=0.200$ for females, $p=0.123$ for males) and Q-Q Plot (figure 16a,b). One outlier was detected (figure 16c) A Mann-Whitney U test was run to determine if there were differences in mean heart rate between males and females. Heart rate for males (mean rank = 19.25) and females (mean rank = 28.25) was statistically different. Median heart rate for males (91.4 bpm) was *statistically significant lower* than median heart rate for females (94.9 bpm), $U=385$, $z=2.196$, $p=0.028$.

Figure 16a. Normal Q-Q Plot: mean heart rate (female)

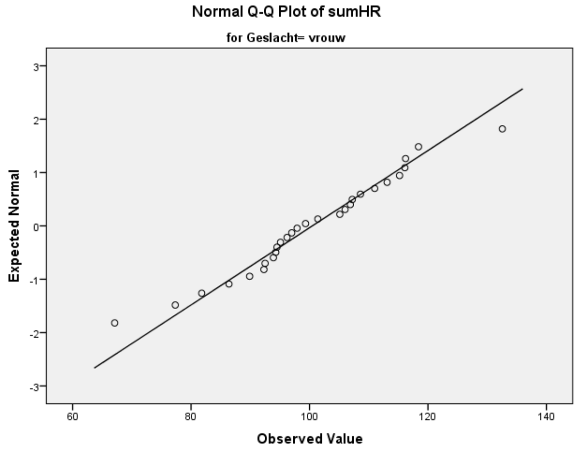


Figure 16a. Normal Q-Q Plot of mean heart rate during the total treatment time (female)

Figure 16a. Normal Q-Q Plot: mean heart rate (male)

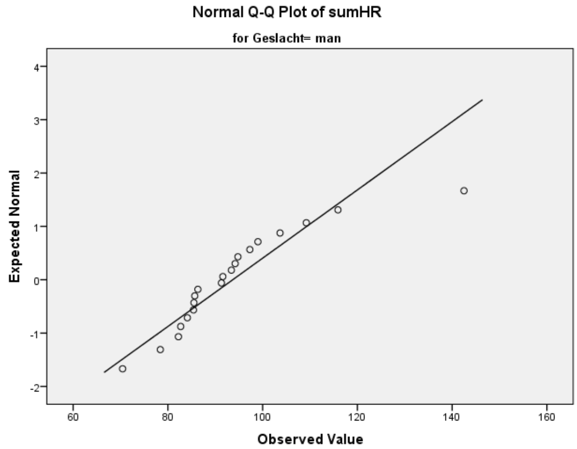


Figure 16b. Normal Q-Q Plot of mean heart rate during the total treatment time (male)

Figure 16c. Boxplot of mean heart rate (male and female)

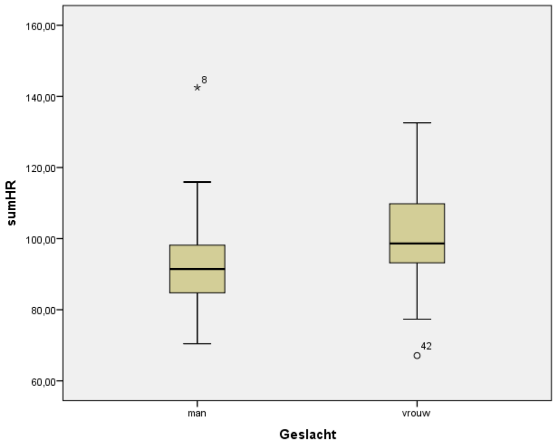


Figure 16c. Boxplot of mean heart rate during the whole treatment time. Dots represent outliers of more than 1,5 box lengths from the edge of the box and asterisks represents outliers of more than 3 box lengths from the edge of the box (numbers represent the participants identification number).

Period 1: Waiting room

Normal distribution, as assessed by the Shapiro-Wilk’s test (p=0.232 for females, p=0.823 for males), the Kolmogorov-Smirnov test (p=0.200 for females, p=0.200 for males) and the Normal Q-Q Plots (figure 17a,b). No outliers were detected (figure 17c). Data are mean ± standard deviation, unless otherwise stated. There were 20 male and 28 female participants. Mean female heart rate (95.5 ± 15.7) was higher than mean male heart rate (88.1 ± 11.1) during time in the waiting room. There was homogeneity of variances, as assessed by Levene's test for equality of variances (p = .051). Male mean heart rate was 7.49 (95% CI, -15.74 to 0.77) lower than female mean heart rate. However a trend was observed, these differences were *not statistically significant*, t(46) = -1.825, p = 0.074.

Figure 17a. Normal Q-Q Plot: Heart rate during waiting room (female)

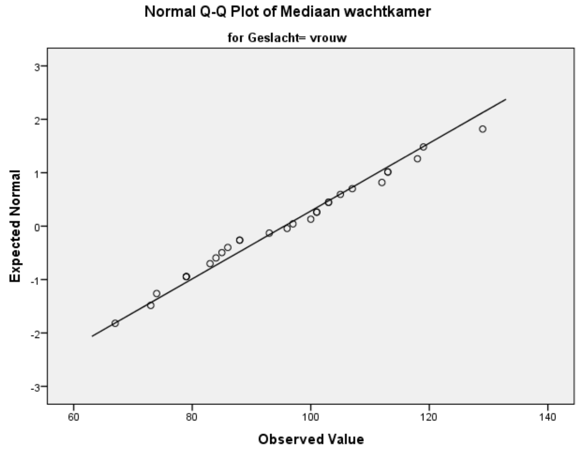


Figure 17a. Normal Q-Q Plot of heart rate during the waiting room (female)

Figure 17b. Normal Q-Q Plot: Heart rate during waiting room (male)

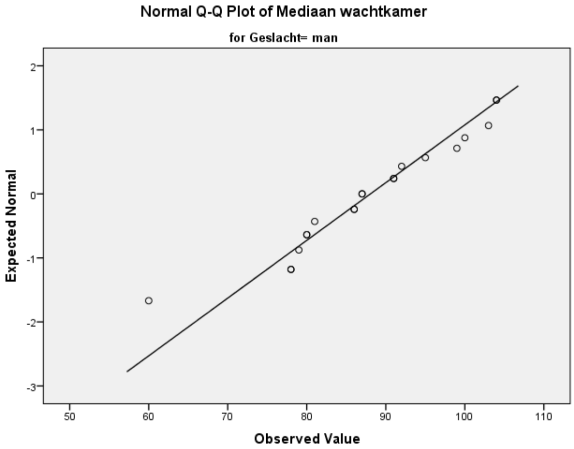


Figure 17b. Normal Q-Q Plot of heart rate during the waiting room (male)

Figure 17c. Boxplot of heart rate during waiting room (male and female)

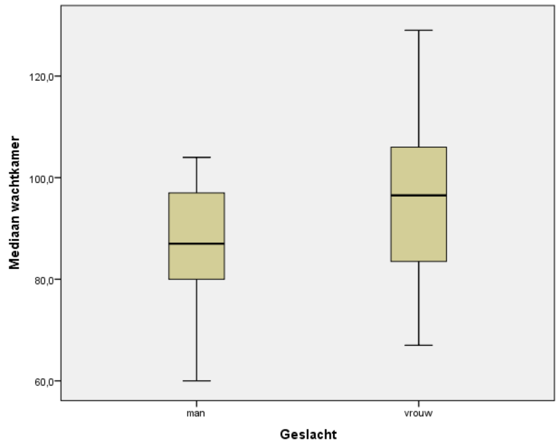


Figure 17c. Boxplot of heart rate during the waiting room.

Period 2: Dental chair

Normal distribution, as assessed by the Shapiro-Wilk's test (p=0.944 for males, p=0.156 for females), the Kolmogorov-Smirnov test (p=0.150 for females, p=0.200 for males) and the Normal Q-Q Plots (figure 18a,b). No outliers were detected (figure 18c). Mean female heart rate (98.0 ± 16.0) was higher than mean male heart rate (94.4 ± 14.7) during sitting down in the dental chair. There was homogeneity of variances, as assessed by Levene's test for equality of variances (p=0.575).

Male mean heart rate was 3.6 (95% CI, -12.73 to 5.53) lower than female heart rate. However, these differences were *not statistically significant*, t(46) = -0.794, p=0.431.

Figure 18a. Normal Q-Q Plot: Heart rate during dental chair (female)

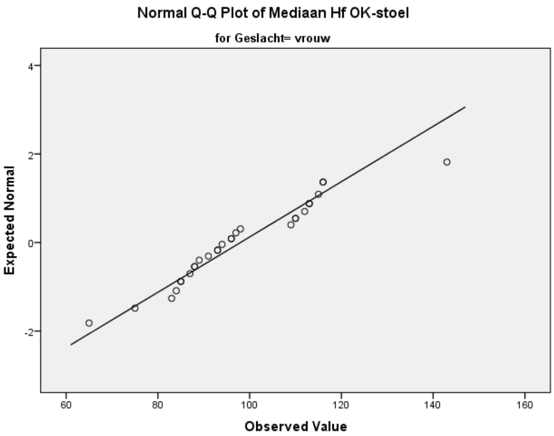


Figure 18a. Normal Q-Q Plot of heart rate during the dental chair (female)

Figure 18a. Normal Q-Q Plot: Heart rate during dental chair (male)

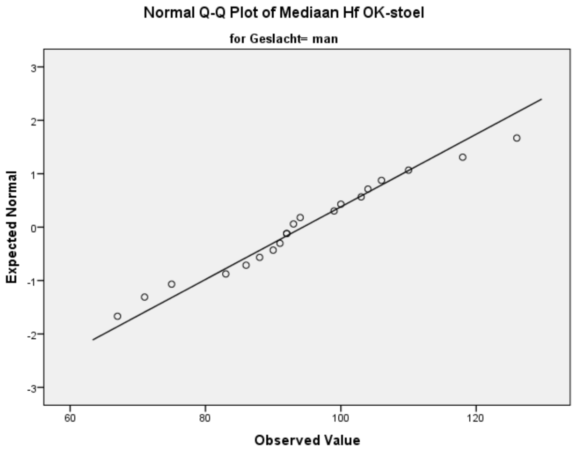


Figure 18b. Normal Q-Q Plot of heart rate during the dental chair (male)

Figure 18c. Boxplot of heart rate during dental chair (male and female)

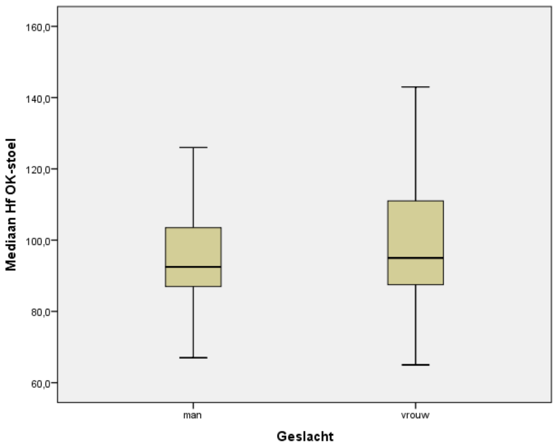


Figure 18c. Boxplot of heart rate during the dental chair (male and female)

Period 3: Anesthesia

Normal distribution for females, but not for males, as assessed by the Shapiro Wilk's test ($p=0.584$ for females, $p=0.000$ for males), the Kolmogorov-Smirnov test ($p=0.200$ for females, $p=0.000$ for males) and the Normal Q-Q Plots (figure 19a,b). One outlier was detected of more than 3 box-lengths from the edge of the box. A Mann-Whitney U test was run to determine if there were differences in heart rate between males and females. Median heart rate for males (81.0 bpm) was lower than median heart rate for females (91.5 bpm). However a trend could be observed, heart rate for males (mean rank = 18.75) and females (mean rank = 28.61) were *not statistically significantly* different, $U = 395$, $z = 2.406$, $p = 0.016$.

Figure 19a. Normal Q-Q Plot: Heart rate during anesthesia (female)

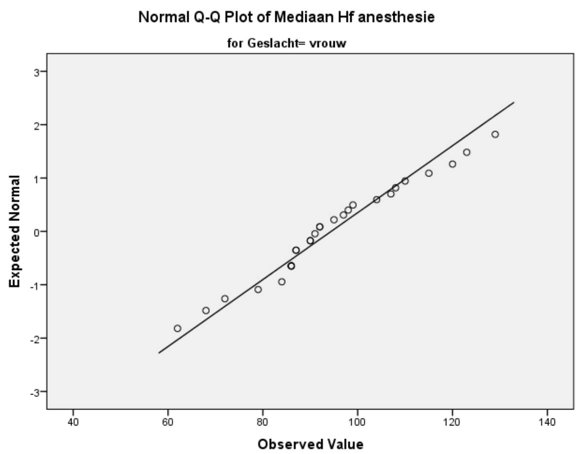


Figure 19a. Normal Q-Q Plot of heart rate during the anesthesia (female)

Figure 19b. Normal Q-Q Plot: Heart rate during anesthesia (male)

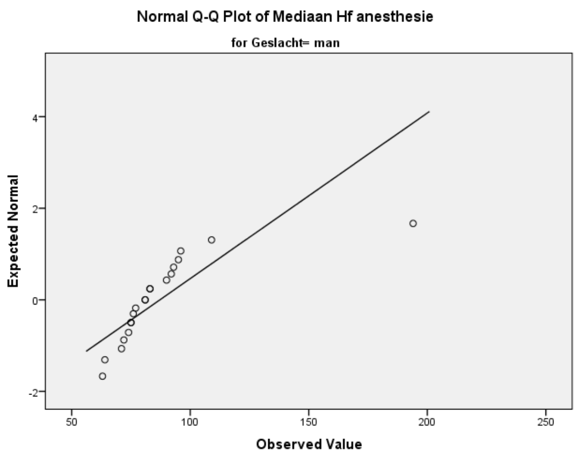


Figure 19b. Normal Q-Q Plot of heart rate during the anesthesia (male)

Figure 19c. Boxplot of heart rate during anesthesia (male and female)

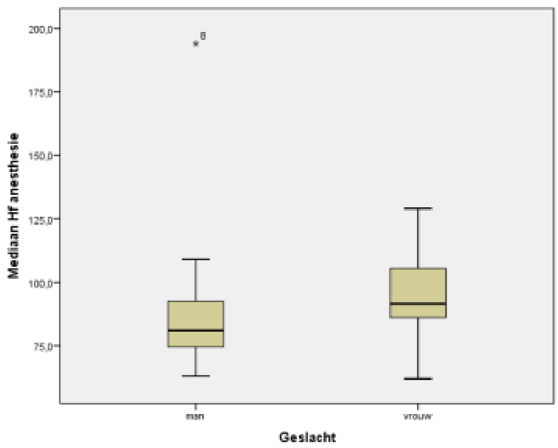


Figure 19c. Boxplot of heart rate during the anesthesia. The asterisk represent an outlier of more than 3 box-lengths from the edge of the box. The number represents the identification number.

Period 4: Surgical drapes

Normal distribution for females, but not for males as assessed by the Shapiro-Wilk's test ($p=0.232$ for females, $p=0.001$ for males), the Kolmogorov-Smirnov test ($p=0.200$ for females, $p=0.115$ for males) and Normal Q-Q plots (figure 20a,b). One extreme (male) outlier was detected of more than 3 box-lengths from the edge of the box (figure 20c). A Mann-Whitney U test was run to determine if there were differences in heart rate between males and females. Median heart rate for males (92.5 bpm) was lower than median heart rate for females (101.0 bpm). Heart rate for males (mean rank = 20.92) and females (mean rank = 27.05) were *not statistically significantly* different, $U = 351$, $z = 1.497$, $p = 0.135$.

Figure 20a. Normal Q-Q Plot: Heart rate during surgical drapes (female)

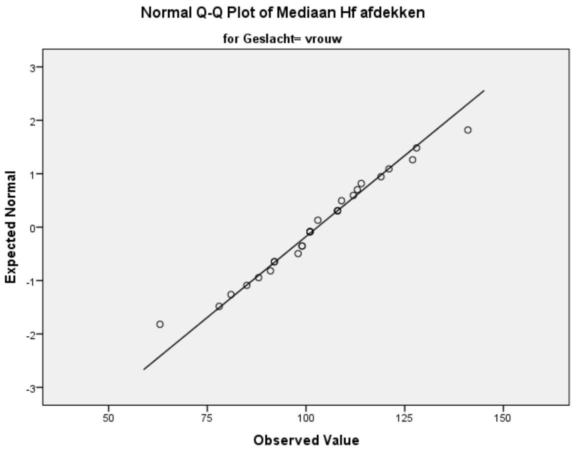


Figure 20a. Normal Q-Q Plot of heart rate during the surgical drapes (female)

Figure 20b. Normal Q-Q Plot: Heart rate during surgical drapes (male)

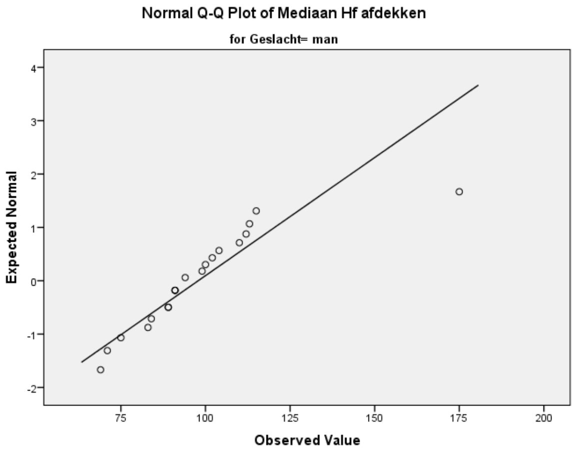


Figure 20b. Normal Q-Q Plot of heart rate during the surgical drapes (male)

Figure 20c. Boxplot of heart rate during surgical drapes (male and female)

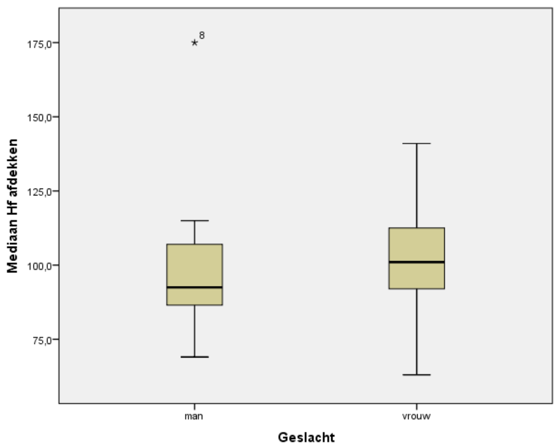


Figure 20c. Boxplot of heart rate during the surgical drapes. The asterisk represent an outlier of more than 3 box-lengths from the edge of the box. The number represents the identification number.

Period 5: Time-Out

Normal distribution, as assessed by the Shapiro-Wilk's test (p=0.326 for females, p=0.131 for males), the Kolmogorov-Smirnov test (p=0.200 for females, p=0.200 for males) and the Normal Q-Q Plots (figure 21a,b). Two outliers of more than 1.5 box-lengths from the edge of the boxplot were detected (figure 21c). A Mann-Whitney U test was run to determine if there were differences in heart rate between males and females. Distributions of the heart rate for males and females were not similar, as assessed by visual inspection. Median heart rate for males (99.0 bpm) was lower than median heart rate for females (108.0 bpm). However a trend could be observed, heart rate for males (mean rank = 18.90) and females (mean rank = 28.50) were *not statistically significantly* different, U = 392, z = 2.344, p = 0.019.

Figure 21a. Normal Q-Q Plot: Heart rate during time out (female)

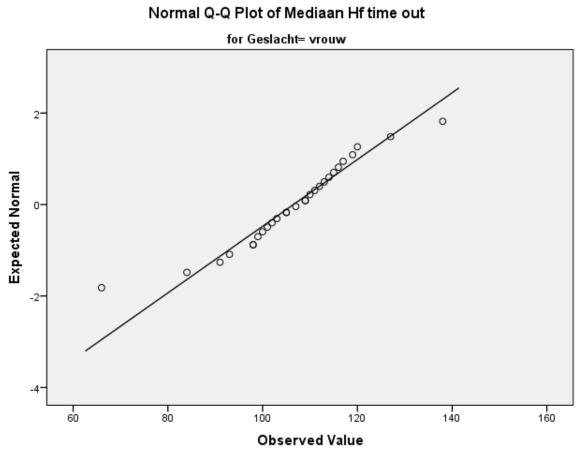


Figure 21a. Normal Q-Q Plot of heart rate during the time out (female)

Figure 21b. Normal Q-Q Plot: Heart rate during time out (male)

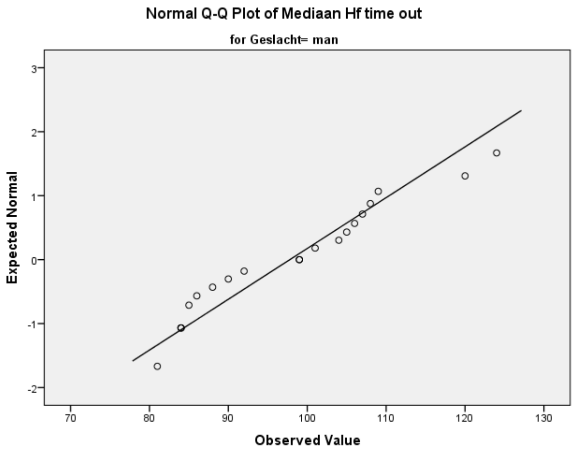


Figure 21b. Normal Q-Q Plot of heart rate during the time out (male)

Figure 21c. Boxplot of heart rate during time out (male and female)

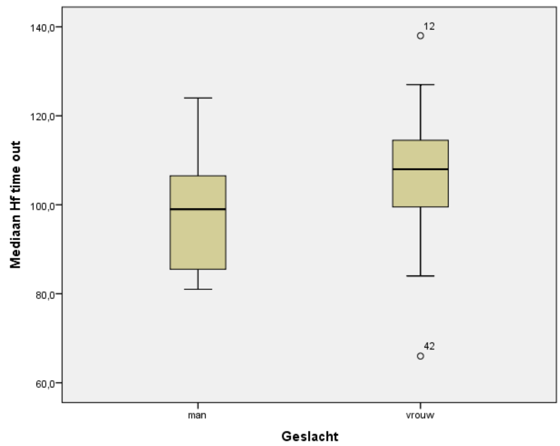


Figure 21c. Boxplot of heart rate during the time out. The circles represent an outlier of more than 1,5 box-lengths from the edge of the box. The number represents the identification number.

Period 6: Incision

Normal distribution for females, but not for males, as assessed by the Shapiro Wilk's test (p=0.110 for females, p=0.000 for males) and the Kolmogorov-Smirnov test (p=0.182 for females, p=0.001 for males) and the Normal Q-Q plots (figure 22a,b). One extreme (male) outlier was detected of more than 3 box-lengths from the edge of the box and 2 outliers of more than 1.5 box-lengths (figure 22c). A Mann-Whitney U test was run to determine if there were differences in heart rate between males and females. Distributions of the heart rate for males and females were not similar, as assessed by visual inspection. Median heart rate for males (101.5 bpm) was lower than median heart rate for females (110.0 bpm). However a trend could be observed, heart rate for males (mean rank = 19.85) and females (mean rank = 27.82) were *not statistically significantly* different, U = 373, z = 1.947, p = 0.052.

Figure 22a. Normal Q-Q Plot: Heart rate during incision (female)

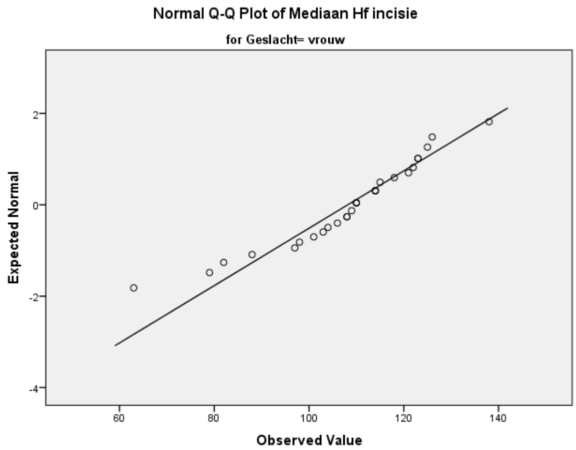


Figure 22a. Normal Q-Q Plot of heart rate during the incision (female)

Figure 22b. Normal Q-Q Plot: Heart rate during incision (male)

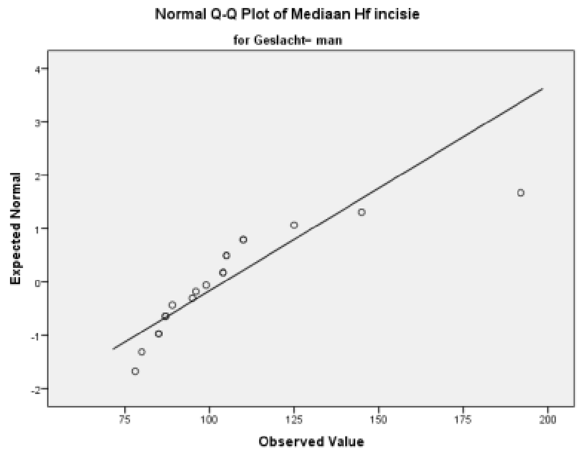


Figure 22b. Normal Q-Q Plot of heart rate during the incision (male)

Figure 22c. Boxplot of heart rate during the incision (male and female)

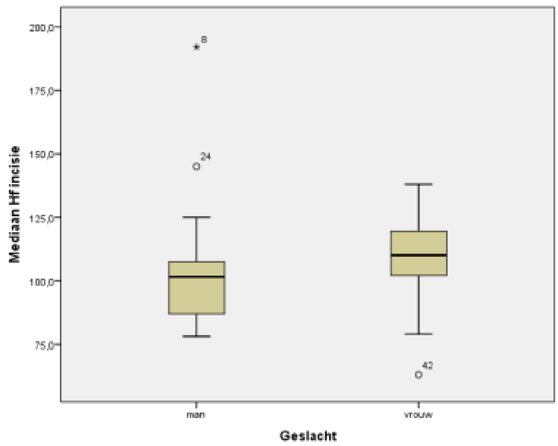


Figure 22c. Boxplot of heart rate during the incision. The circles represent an outlier of more than 1,5 box-lengths from the edge of the box, the asterisk represent an outlier of more than 3 box-lengths from the edge of the box . The number represents the identification number.

Period 7: Alveolotomy

Normal distribution, as assessed by the Shapiro-Wilk's test (p=0.091 for females, p=0.834 for males), the Kolmogorov-Smirnov test (p=0.200 for females, p=0.200 for males) and the Normal Q-Q plots (figure 23a,b). No outliers were detected (figure 23c). Mean female heart rate (102.2 ± 20.2) was higher than mean male heart rate (99.3 ± 22.6) during alveolotomy. There was homogeneity of variances, as assessed by Levene's test for equality of variances (p=0.637). Male mean heart rate was 2.9 (95% CI, -18.88 to 13.06) lower than female heart rate. However, these differences were *not statistically significant*, t(29) = -0.372, p=0.712.

Figure 23a. Normal Q-Q Plot: Heart rate during alveolotomy (female)

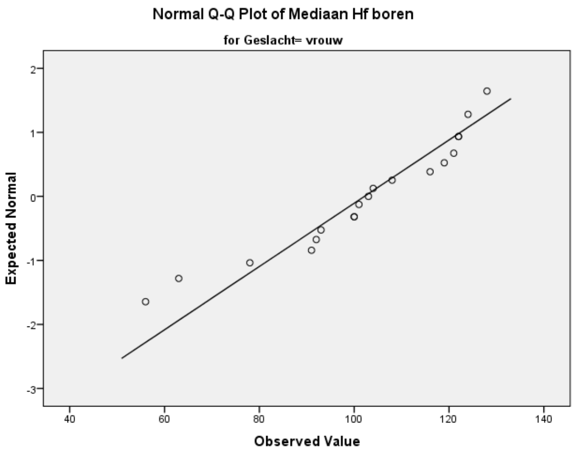


Figure 23a. Normal Q-Q Plot of heart rate during the alveolotomy (female)

Figure 23b. Normal Q-Q Plot: Heart rate during alveolotomy (male)

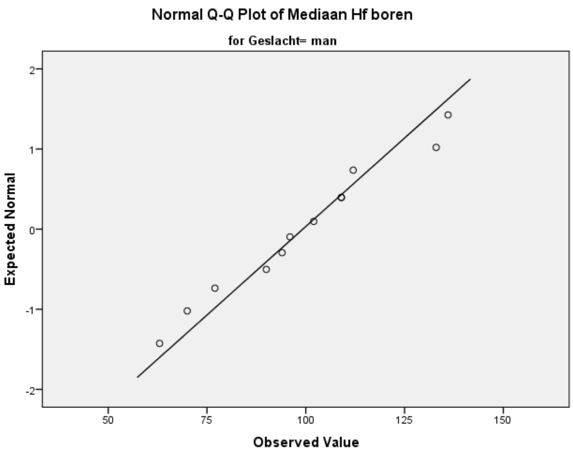


Figure 23b. Normal Q-Q Plot of heart rate during the alveolotomy (male)

Figure 23c. Boxplot of heart rate during the alveolotomy (male and female)

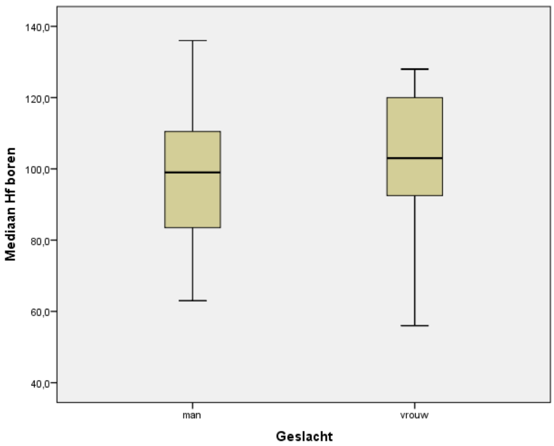


Figure 23c. Boxplot of heart rate during the alveolotomy.

Period 8: Removal of third molar

Normal distribution, as assessed by the Shapiro-Wilk's test (p=0.939 for females, p=0.658 for males), the Kolmogorov-Smirnov test (p=0.200 for females, p=0.200 for males) and Normal Q-Q plots (figure 24a,b). No outliers were detected (figure 24c). Mean female heart rate (102.3 ± 17.6) was higher than mean male heart rate (96.2 ± 18.0) during the removal of the third molar. There was homogeneity of variances, as assessed by Levene's test for equality of variances (p=0.823). Male mean heart rate was 6.1 (95% CI, -16.59 to 4.35) lower than female heart rate. However, these differences were *not statistically significant*, t(46) = -1.177, p=0.245.

Figure 24a. Normal Q-Q Plot: Heart rate during third molar removal (female)

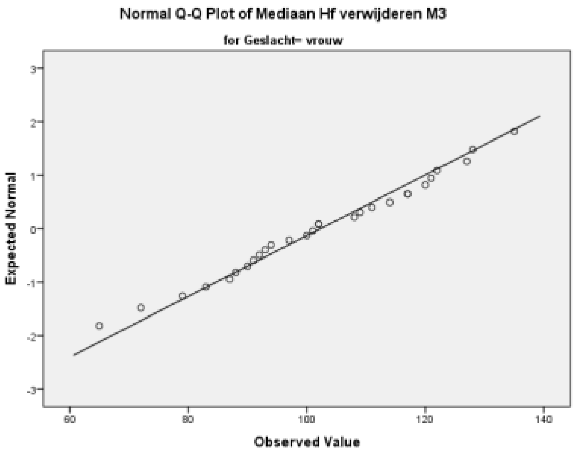


Figure 24a. Normal Q-Q Plot: Heart rate during third molar removal (female)

Figure 24b. Normal Q-Q Plot: Heart rate during third molar removal (male)

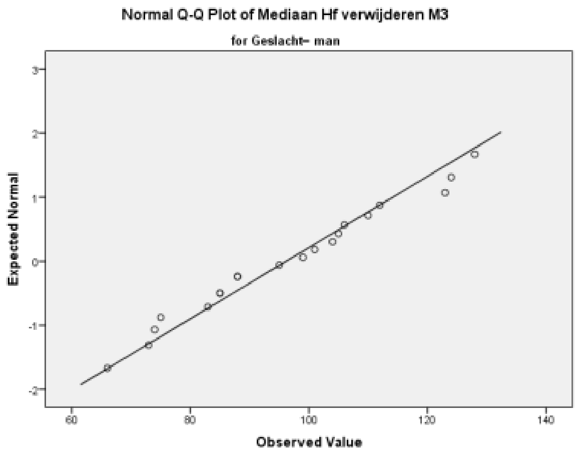


Figure 24b. Normal Q-Q Plot: Heart rate during third molar removal (male)

Figure 24c. Boxplot of heart rate during the third molar removal (male and female)

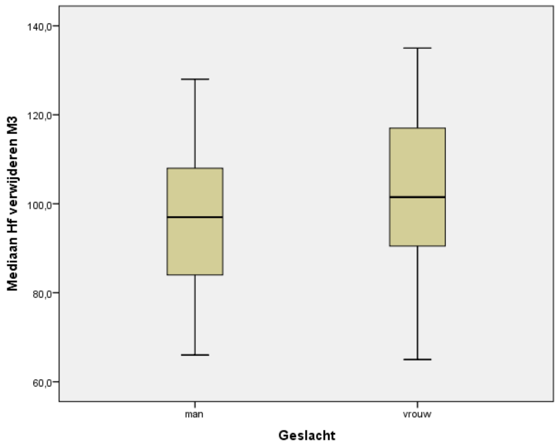


Figure 24c. Boxplot of heart rate during the third molar removal.

Period 9: Suturing

Normal distribution for females (p=0.931), but not for males (p=0.001), as assessed by the Shapiro Wilk's test, the Kolmogorov-Smirnov test (p=0.200 for females, p=0.052 for males) and the Normal Q-Q plots (figure 25a,b). One extreme (male) outlier was detected of more than 3 box-lengths from the edge of the box (figure 25c). A Mann-Whitney U test was run to determine if there were differences in heart rate between males and females. Distributions of the heart rate for males and females were not similar, as assessed by visual inspection. Median heart rate for males (83.5 bpm) was lower than median heart rate for females (100.0 bpm). However a trend could be observed, heart rate for males (mean rank = 18.72) and females (mean rank = 28.62) were *not statistically significantly* different, U = 395.5, z = 2.418, p = 0.016.

Figure 25a. Normal Q-Q Plot: Heart rate during suturing (female)

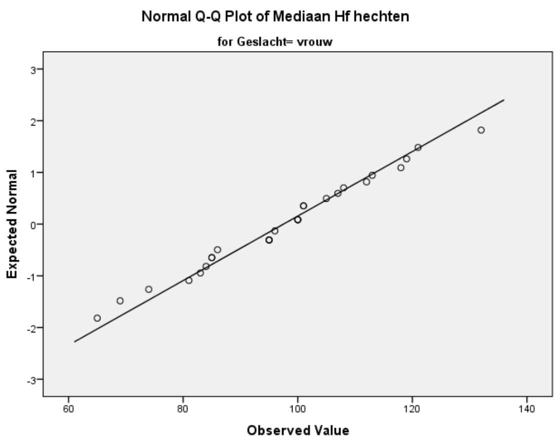


Figure 25a. Normal Q-Q Plot: Heart rate during suturing (female)

Figure 25b. Normal Q-Q Plot: Heart rate during suturing (male)

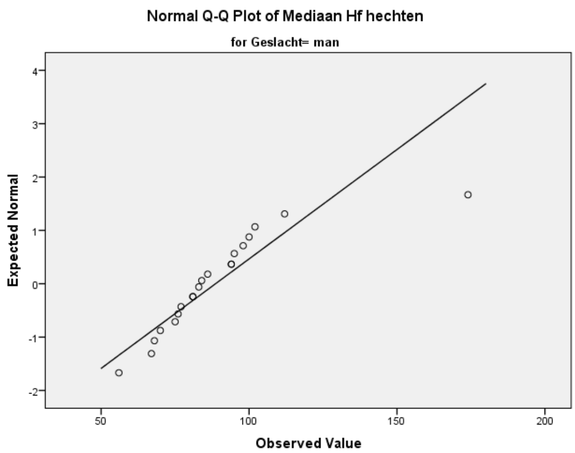


Figure 25b. Normal Q-Q Plot: Heart rate during suturing (male)

Figure 25c. Boxplot of heart rate during the suturing (male and female)

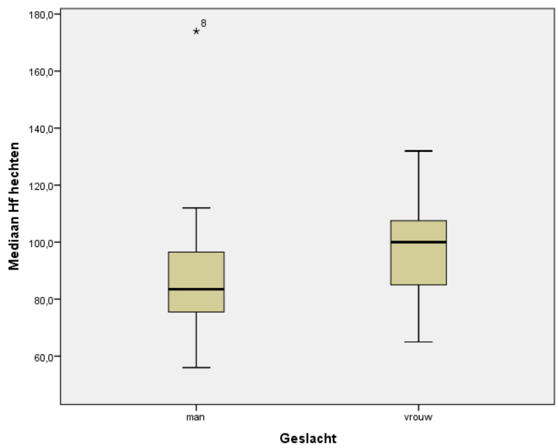


Figure 25c. Boxplot of heart rate during the suuturing. The asterisk represent an outlier of more than 3 box-lengths from the edge of the box. The number represents the identification number.

Period 10: End of procedure

Normal distribution for females, but not for males, as assessed by the Shapiro Wilk's test (p=0.754 for females, p=0.026 for males), the Kolmogorov-Smirnov test (p=0.200 for females, p=0.043 for males) and the Normal Q-Q plots (figure 26a,b). One male outlier was detected of more than 1.5 box-lengths from the edge of the box (figure 26c). A Mann-Whitney U test was run to determine if there were differences in heart rate between males and females. Distributions of the heart rate for males and females were not similar, as assessed by visual inspection. Median heart rate for males (87.5 bpm) was lower than median heart rate for females (95.5 bpm). However a trend could be observed, heart rate for males (mean rank = 19.58) and females (mean rank = 28.02) were *not statistically significantly* different, U = 378.5, z = 2.061, p = 0.039.

Figure 26a. Normal Q-Q Plot: Heart rate during end of procedure (female)

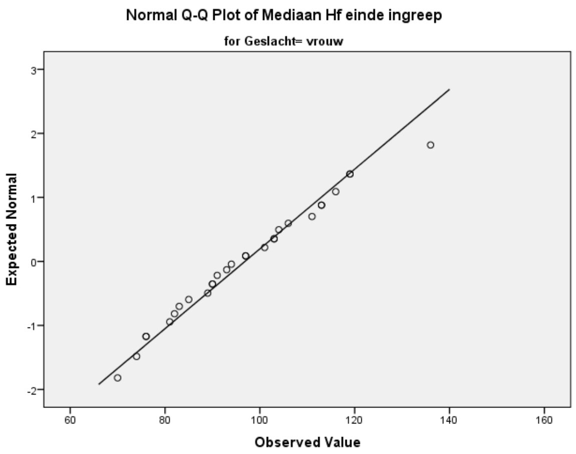


Figure 26a. Normal Q-Q Plot: Heart rate during end of procedure (female)

Figure 26b. Normal Q-Q Plot: Heart rate during end of procedure (male)

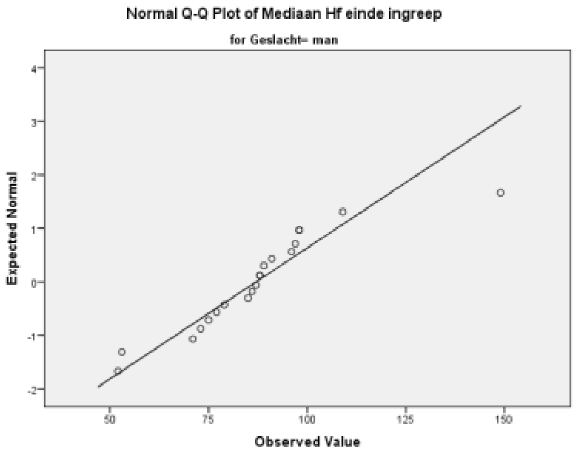


Figure 26b. Normal Q-Q Plot: Heart rate during end of procedure (male)

Figure 26c. Boxplot of heart rate during the end of the procedure (male and female)

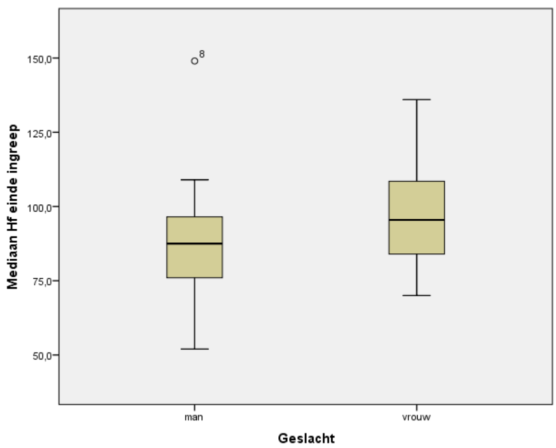


Figure 26c. Boxplot of heart rate during the end of procedure. The asterisk represent an outlier of more than 3 box-lengths from the edge of the box. The number represents the identification number.

Statistical tests, Normal Q-Q Plots and Boxplots of the Modified Dental Anxiety Scale

To assess the differences in mean MDAS-score between males and females, the independent samples t-test was used. The MDAS-score was normally distributed for males as assessed by the Shapiro-Wilk's test (p=0.098), the Kolmogorov-Smirnov test (p=0.200) and the visual interpretation of the Q-Q plot. However, the data was not normally distributed for females, with a p-value of 0.034 for the Shapiro-Wilk's test, the Kolmogorov-Smirnov test (p=0.037) and by interpretation of the Q-Q plot (figure 27a,b). Difference scores were normally distributed for females with a skewness of 0.610 (standard error = 0.441) and kurtosis of -0.787 (standard error = 0.858). We accepted a statistical significance level of 0.1, which equates to a z-score of ±2.58. The z-score for skewness (1.383) and kurtosis (-0.917) are within this boundary, so the data is has a normal distribution and a positive skewness and negative kurtosis. Subsequently, data showed a normal distribution for males and females by using a square root transformation as assessed by the Shapiro-Wilk's test (males p=0.159 and females p=0.110), the Kolmogorov-Smirnov test (males p=0.200 and females p=0.123) and by inspection of Normal Q-Q plots (figure 27c,d). Additionally, one outlier of more than 1.5 box-lengths from the edge of a boxplot was detected. However, after square root transformation no outliers were detected (figure 28a,b).
Data are mean ± standard deviation, unless otherwise stated. There were 20 males and 28 females. There was homogeneity of variances for MDAS scores for males and females, as assessed by Levene's test for equality of variances (p = 0.055). Males had a lower mean MDAS-score (8.1 ± 2.37) as opposed to females (10.3 ± 3.67). Male mean MDAS score was 2.2 (95% CI, -4.084 to -0.316) lower than female mean MDAS-score. There was a *statistically significant* difference in mean MDAS-score between males and females, t(46)= -2.331, p=0.024.

Figure 27a. Normal Q-Q Plot: MDAS-score (male)

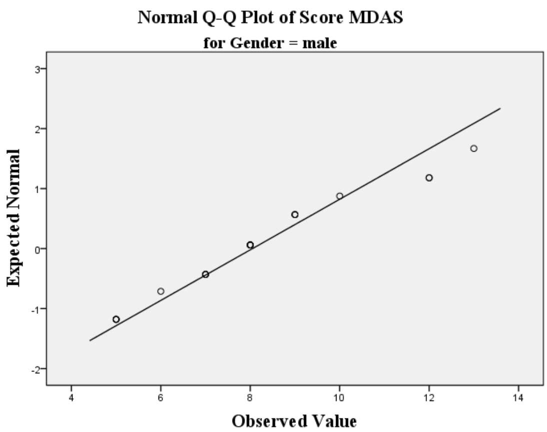


Figure 27a. Normal Q-Q Plot of MDAS-score of male participants.

Figure 27b. Normal Q-Q Plot: MDAS-score (female)

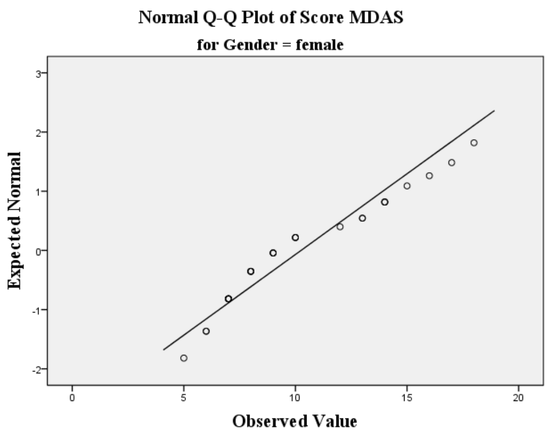


Figure 27b. Normal Q-Q Plot of MDAS-score of female participants.

Statistical tests, Normal Q-Q Plots and Boxplots of the Modified Dental Anxiety Scale

Figure 27c. Normal Q-Q Plot: MDAS-score for males (square root transformation)

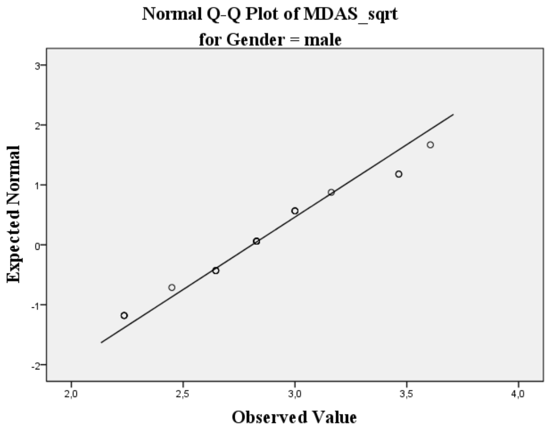


Figure 27c. Normal Q-Q Plot of MDAS-score of male participants after square root transformation.

Figure 27d. Normal Q-Q Plot: MDAS-score for females (square root transformation)

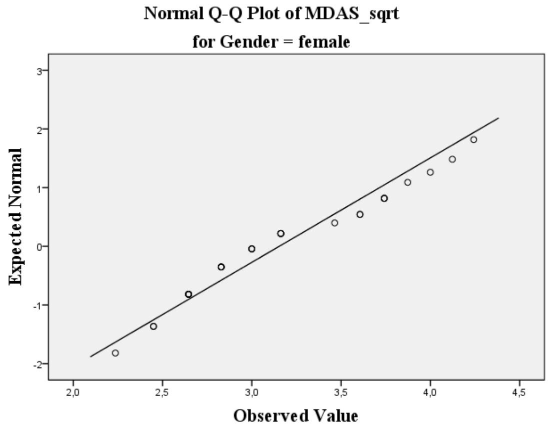


Figure 27d. Normal Q-Q Plot of MDAS-score of female participants after square root transformation.

Figure 28a. Boxplot MDAS-score

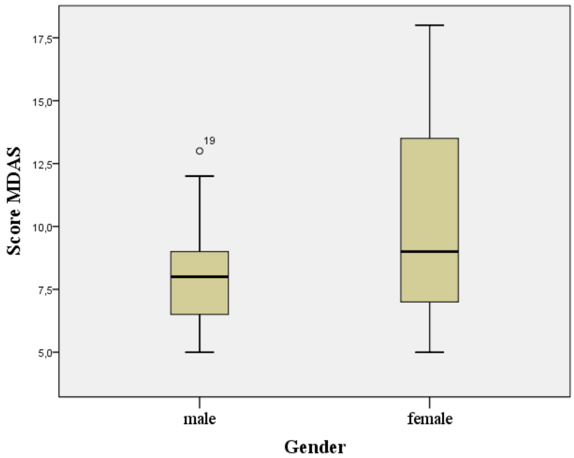


Figure 28a. Boxplot of the median heart rates of the participants during the different periods. Dots represent outliers of more than 1,5 box lengths from the edge of the box (numbers represent the participants identification number).

Figure 28b. Boxplot MDAS-score (square root)

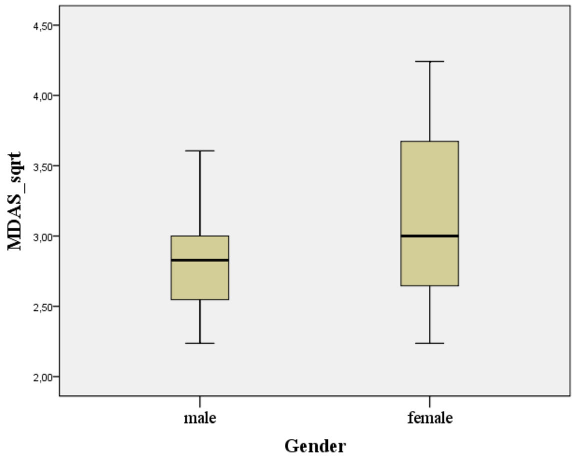
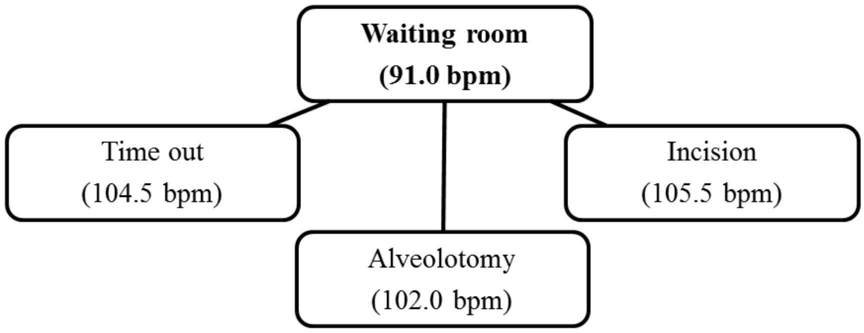


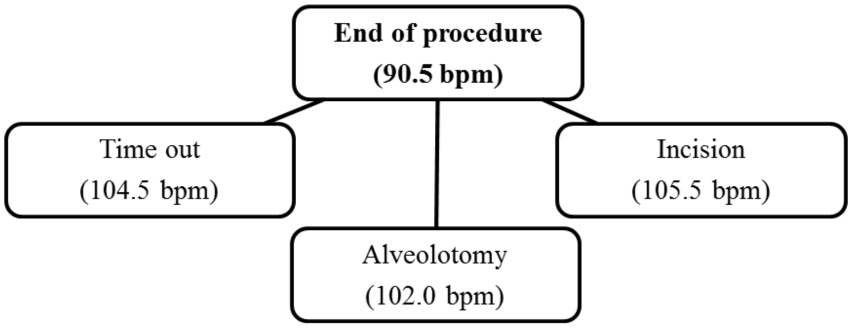
Figure 28b. Boxplot MDAS-score (after square root transformation)

Appendix F: Visual interpretation of outcomes of the Friedman's test

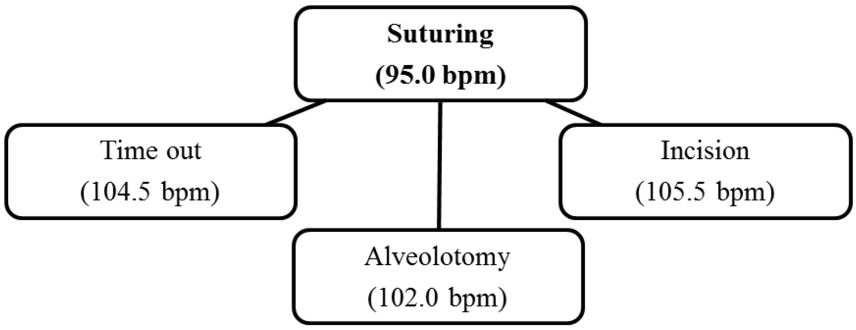
This appendix displays significant changes in median heart rate as assessed by the Friedman's test. The boxes show the time-point measurements with the median heart rate. The lines represent a significant change in median heart rate.



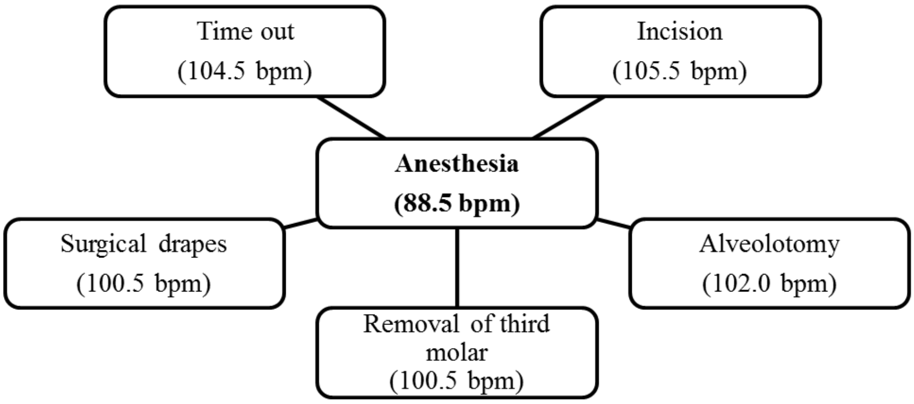
The median heart rate during the waiting room showed significant differences with the time-out procedure, the incision and the alveolotomy (P<0.005).



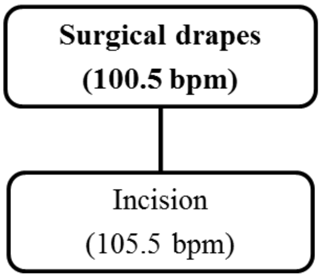
The median heart rate during the end of the procedure showed significant differences with the time-out procedure, the incision and the alveolotomy (P<0.005).



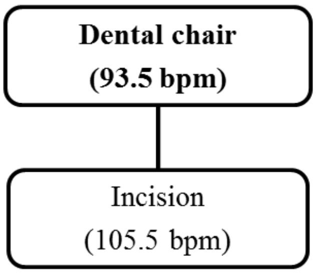
The median heart rate during the suturing showed significant differences with the time-out procedure, the incision and the alveolotomy (P<0.005).



The median heart rate during the anesthesia showed significant differences with the time-out procedure, the incision, the alveolotomy, the removal of the third molar and the application of surgical drapes (P<0.005).



The median heart rate during the application of surgical drapes showed significant differences with the incision (P<0.005).



The median heart rate during sitting down in the dental chair showed significant differences with the incision (P<0.005).