Aus der Medizinischen Klinik und Poliklinik I
des Klinikums der Universität München
Direktor: Professor Dr. med. Steffen Massberg

# Influence of the autonomic nervous system on cardiac arrhythmogenesis

**Einfluss des autonomen Nervensystems auf die kardiale Arrhythmogenese**

Kumulative Habilitationsschrift
zur Erlangung der Lehrbefähigung
für das Fach Innere Medizin und Kardiologie

vorgelegt von
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## 1. Original publications summarized within this work

Tomsits P, Volz L, Xia R, Chivukula A, **Schüttler D*,** Clauß S*. Medetomidine/midazolam/fentanyl narcosis alters cardiac autonomic tone, leading to conduction disorders and arrhythmias in mice. *Lab Anim (NY). 2023 (accepted)* ***authors contributed equally.**

**Schüttler D,** Hamm W, Kellnar A, Brunner S, Stremmel C. Comparable Analysis of Acute Changes in Vascular Tone after Coffee versus Energy Drink Consumption. *Nutrients 2022, 14(9), 1888.*

**Schüttler D,** Rudi WS, Bauer A, Hamm W, Brunner S. Impact of energy drink versus coffee consumption on periodic repolarization dynamics: an interventional study. *Eur J Nutr. 2022 Mar 10.*

**Schüttler D,** Tomsits P, Bleyer C, Vlcek J, Pauly V, Hessen N, Sinner M, Merkus D, Hamers J, Kääb S, Clauss S. A practical guide to setting up pig models for cardiovascular catheterization, electrophysiological assessment and heart disease research. *Lab Anim (NY). 2022 Feb;51(2):46-67.*

**Schüttler D,** Krammer S, von Stuelpnagel L, Sams L, Bauer A, Hamm W, Brunner S. Estimation of anaerobic threshold by cardiac repolarization instability: a prospective validation study. *BMC Sports Sci Med Rehabil. 2021 Aug 6;13(1):85.*

**Schüttler D,** Schönermarck U, Wenner F, Toepfer M, Rizas KD, Bauer A, Brunner S, Hamm W. Large potassium shifts during dialysis enhance cardiac repolarization instability. *J Nephrol. 2020 Oct 15.*

**Schüttler D,** vonStülpnagel L, Rizas KD, Bauer A, Brunner S, Hamm W. Effect of Hyperventilation on Periodic Repolarization Dynamics. *Front. Physiol., 18 September 2020*.

Clauss S*, **Schüttler D*,** Bleyer C, Vlcek J, Shakarami M, Tomsits P, Schneider S, Maderspacher F, Chataut K, Trebo A, Wang C, Kleeberger J, Xia R, Baloch E, Hildebrand B, Massberg S, Wakili R, Kääb S. Characterization of a porcine model of atrial arrhythmogenicity in the context of ischaemic heart failure. *PLoS One. 2020 May 4;15(5):e0232374.* ***authors contributed equally.**

Hamm W, Kassem S, von Stülpnagel L, Maier F, Klemm M, **Schüttler D,** Grabher F, Weckbach LT, Huber BC, Bauer A, Rizas KD, Brunner S. Deceleration Capacity and Periodic Repolarization Dynamics As Predictors of Acute Mountain Sickness. *High Alt Med Biol. 2020 Nov 4*

Hamm W, Maier F, Kassem S, **Schüttler D,** Bauer A, Rizas KD, von Stülpnagel L, Brunner S. Deceleration Capacity of Heart Rate and Periodic Repolarization Dynamics during Normobaric Hypoxia. *Scand J Med Sci Sports. 2020 Apr 7.*

## 2. Introduction

The autonomic nervous system (ANS) modulates our cardiorespiratory system providing physiological adaptation in times of rest, stress, or exercise. Finely balanced and usually opposed sympathetic and parasympathetic afferent and efferent activities within the central nervous system (CNS), the heart, lungs and vessels accurately regulate our blood pressure, respiration, heart rate and cardiac contractility in response to environmental factors and the body's requirements. [1-3] This interaction between the cardiovascular system and the ANS occurs continually and imbalances in sympathetic and parasympathetic activities are tightly connected to the development of cardiovascular diseases (CVDs). [4] In addition, cardiovascular diseases lead to autonomic dysfunction vice versa. Many CVDs such as hypertension, acute myocardial infarction (AMI) or heart failure (HF) are characterized by pronounced alterations within the ANS. [5] An imbalance with increased sympathetic activity and concomitant vagal withdrawal is a typical feature of diseases such as ischemic heart failure (IHF). [6] This so called autonomic remodeling fortifies disease maintenance and progression. [7]

The implications of ANS dysfunction are huge: imbalances between the sympathetic and parasympathetic branches with overactivity in catecholaminergic nerves and suppressed vagal activities are known to facilitate the origin of malignant, potentially fatal ventricular arrhythmias. [5] Thus, dysfunctions of the ANS play a major role in the development of sudden cardiac death (SCD). [8] In addition, supraventricular arrhythmias such as atrial fibrillation (AF), which is the most common arrhythmia among the elderly and has a high socioeconomic burden due to its high prevalence and comorbidities such as hard failure and stroke, are also based on ANS remodeling processes.[9]
On histological and molecular level ANS dysfunction is often reflected by changes in innervation patterns. A dog model of IHF for example was able to detect an increased sympathetic nerve sprouting both in the infarcted LV area and remote of infarction. This nerve sprouting was partly mediated by nerval growth factor (NGF) and growth associated protein 43 (GAP43). [10]

Up to date most of the obtained data regarding autonomic remodeling is based on research performed in small animal models, especially rodents. To reliably study the pathophysiological alteration, which set base to arrhythmogenesis, valid translational models are needed which closely resemble human hemodynamics, anatomy, and electrophysiology. In two major reviews we were able to show that especially porcine models provide these characteristics in an excellent way and higher acceptance regarding ethical considerations (Fig. 1). [11,12]

To non-invasively assess the fine balance between sympathetic and parasympathetic influences on cardiac function, various ECG-based biomarkers have been developed over the past decades. Among those, parameters which are based on heart rate variability (HRV) have been investigated in detail to record ANS changes during physiological and pathological conditions. Besides these classical HRV-indices, particularly periodic repolarization dynamics

(PRD) has recently gained broad scientific interest: This parameter mirrors efferent sympathetic activity on the level of the ventricular myocardium. Its calculation is based on

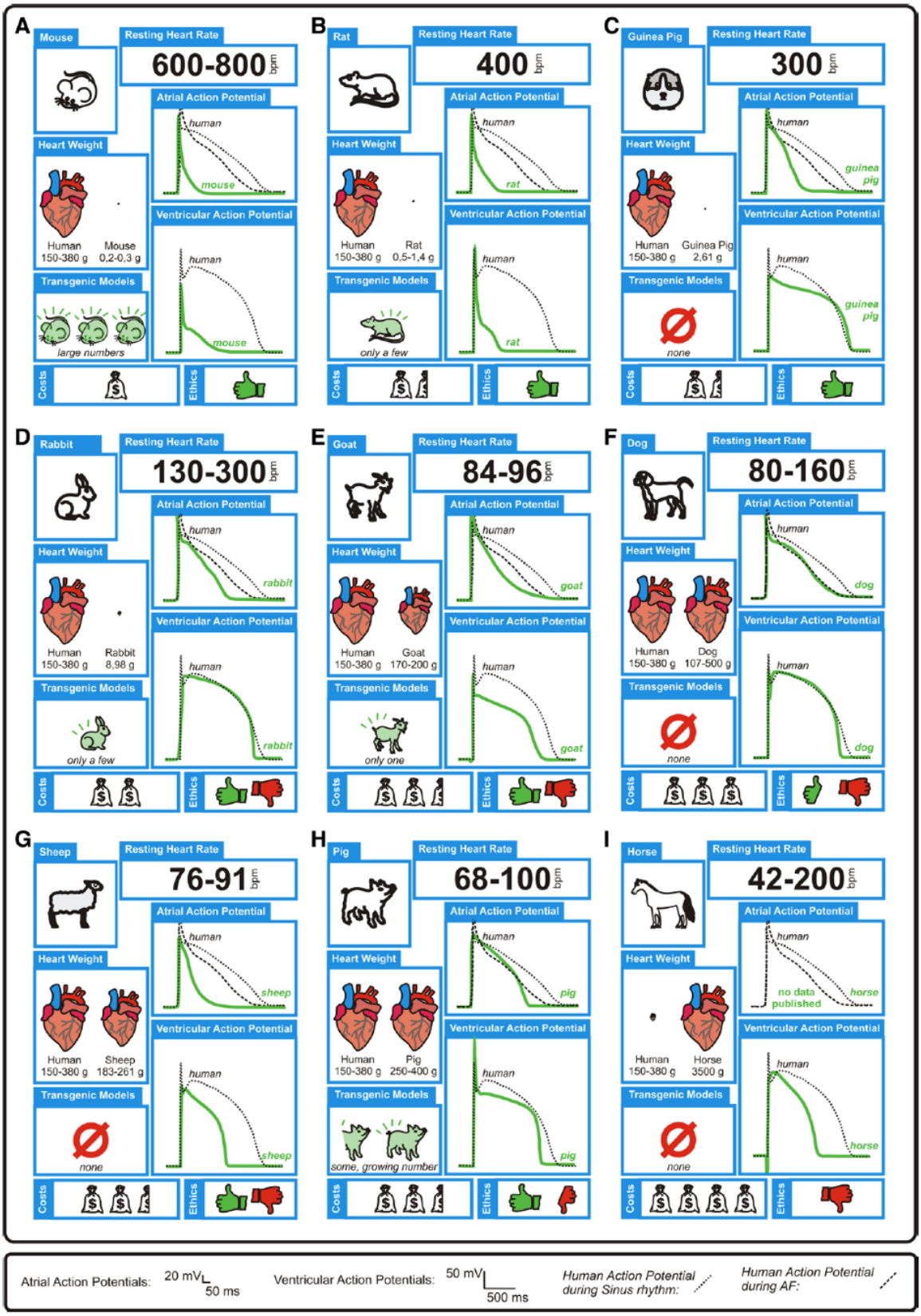



***Figure 1.*** *Overview of commonly used animal models in arrhythmia research regarding their atrial and ventricular action potentials (AP), heart weight, heart rate, costs, ethical acceptance, and availability of transgenic models. (Schüttler et al. Circ. Res. 2020)*

beat-to-beat variation of the T wave vector which is assessed via high resolution ECG (1000 Hz) recorded in Frank-lead configuration. [13] The spatiotemporal information of each T wave is integrated into a single vector T. [13] The instantaneous degree of repolarization instability can then be calculated by the angle dT° between two successive repolarization vectors. [13] When plotted over time, the dT°-signal shows its characteristic oscillations in the low-frequency range (ca. 0.1 Hz) (Fig. 2). [13] PRD is then calculated by wavelet analysis.

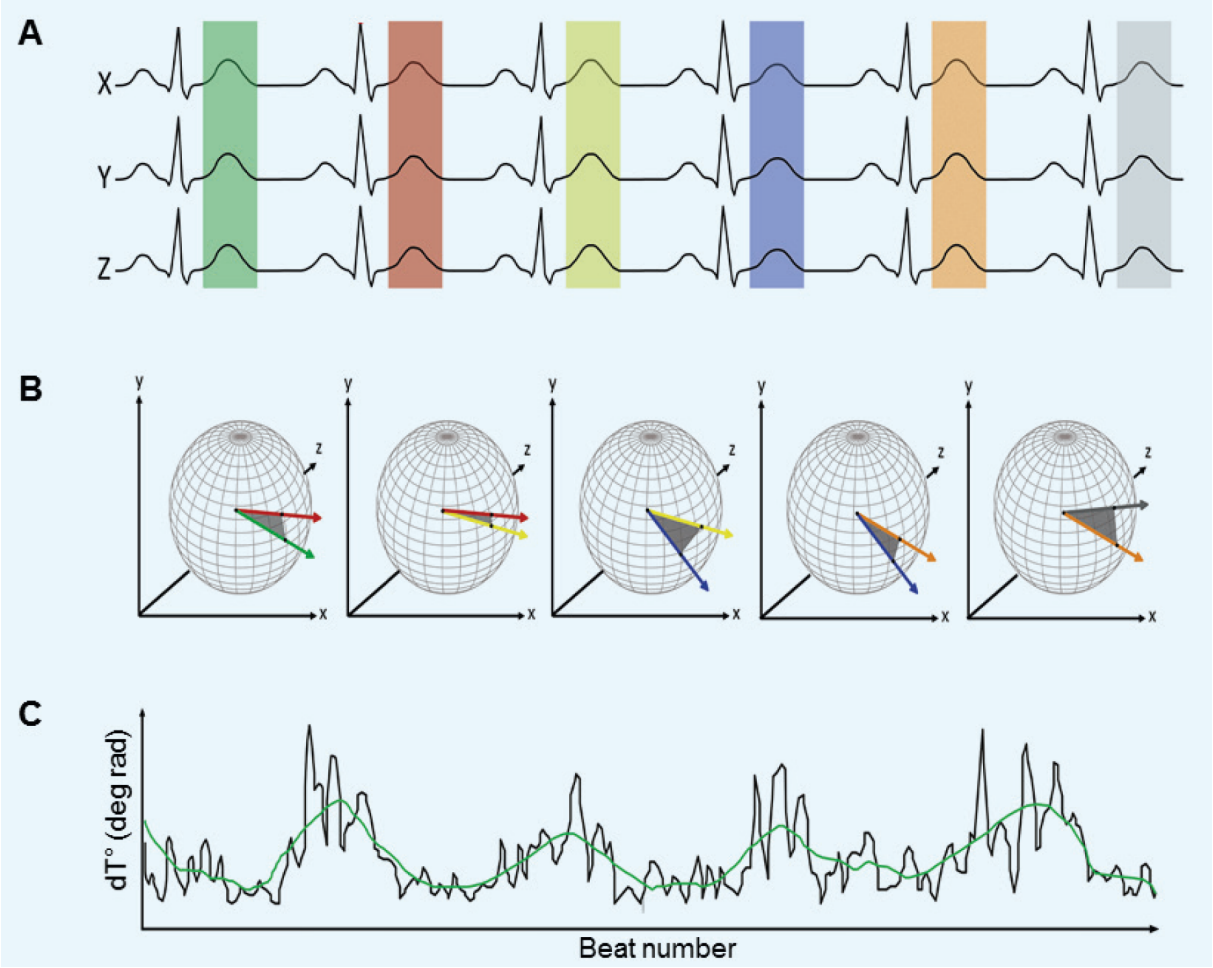



***Figure 2**. Illustration of PRD assessment: ECG is recorded in Frank-lead (X,Y,Z) configuration (A). Visualization of successive T-wave vectors in virtual spheres (B). The angle dT° of two successive T-wave vectors of each heartbeat is illustrated in the y-axis. When plotted over time dT° reveals a periodic pattern in the low frequency range (C). PRD is quantified by wavelet analysis. (Schüttler et al. Dtsch Z Sportmed. 2020)*

Large clinical trials were able to demonstrate that PRD levels exceeding a cut-off of 5.75 deg$^2$, predict the occurrence of malignant arrhythmias as well as of subclinical arrhythmias following myocardial infarction. [14,15] Additionally, PRD seems to work as a predictive tool in patients with non-ischemic cardiomyopathy (CMP) identifying patients which show profit of ICD implantation. [16]

**However, little is known how these ECG-based biomarkers behave under certain physiological and environmental changes. Thus, with our research projects we aimed at further investigating the influence of environmental, physiological, and pathological**

**conditions on autonomic nervous function and the ECG-based biomarker PRD. Additionally, we intended to develop a porcine model of IHF with a proarrhythmic phenotype allowing to reliably investigate arrhythmia-related remodeling processes especially on autonomic level.**

# 3. Results and discussion

## 3.1 Alterations of ANS biomarkers in response to environmental, physiological and pathological conditions

### 3.1.1 Autonomic changes in response to hyperventilation

**Schüttler D,** vonStülpnagel L, Rizas KD, Bauer A, Brunner S, Hamm W. Effect of Hyperventilation on Periodic Repolarization Dynamics. *Front. Physiol., 18 September 2020*.

Respiration and heart rates as well as heart function are closely connected and under control of the autonomic nervous system: Our heart rates slow during expiration while a relative tachycardia evolves during inspiration due to inhibitory effects on the parasympathetic branch, known as respiratory sinus arrhythmia. [17] Impaired cardiorespiratory coupling is connected to cardiovascular diseases. [18] Hyperventilation was demonstrated to activate the sympathetic nervous system resulting in physiological changes of the cardiovascular system such as increased heart rate and blood pressure. [19]Additionally, hyperventilation can affect the repolarization phase of the cardiac cycle by inducing repolarization abnormalities including ST

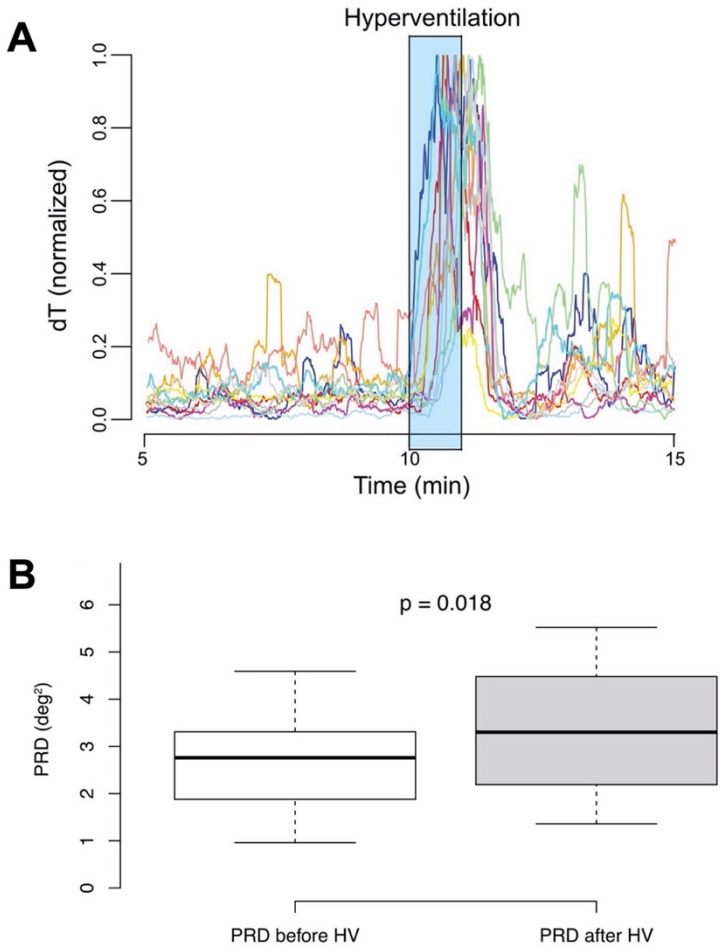



***Figure 3.*** *Normalized dT° signal of study participants showing a characteristic pattern with a marked delay after start of hyperventilation (A). PRD levels before and after hyperventilation (HV) (B). (Schüttler et al Front. Phys. 2020)*

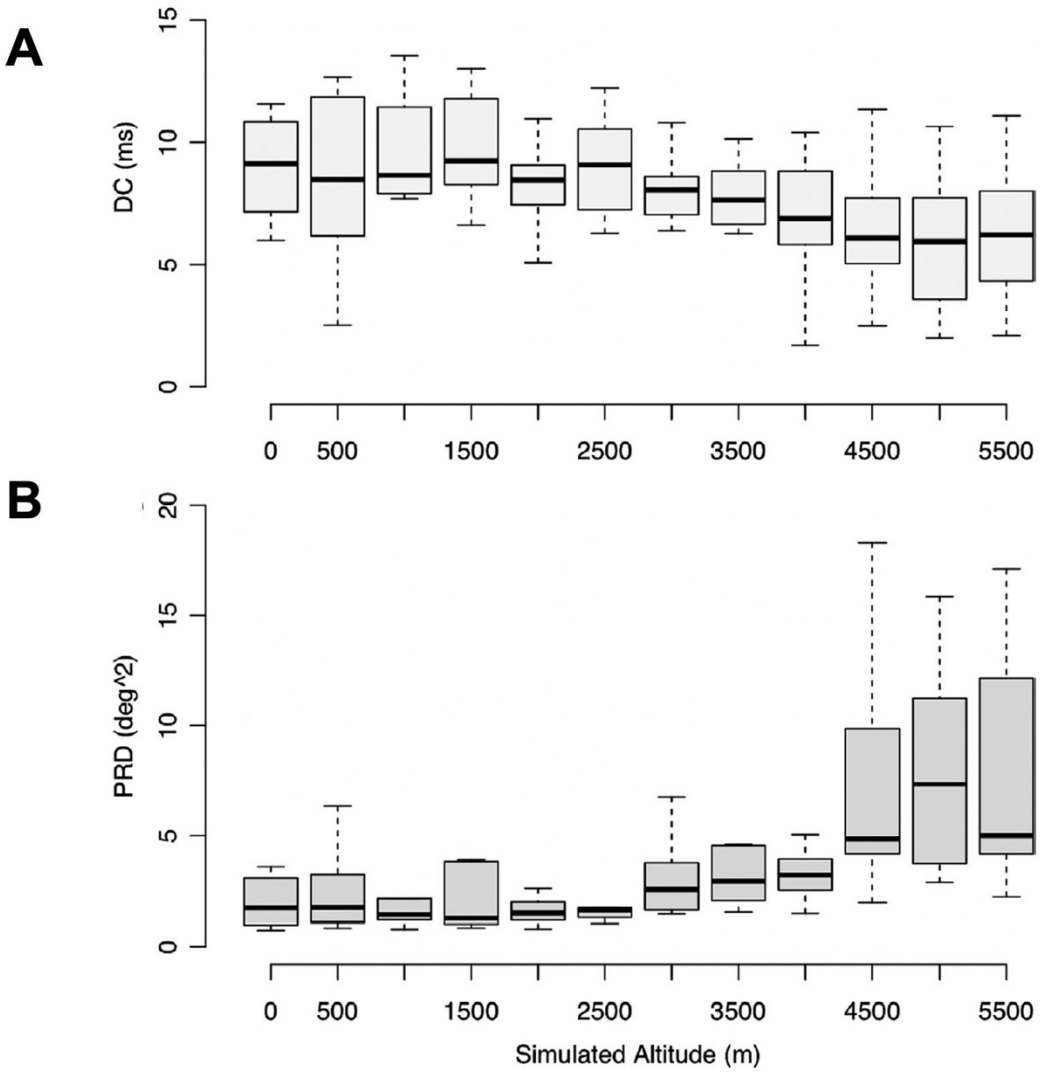
depression and T wave inversion. [20] However little is known about the behavior of dT° and PRD under hyperventilation. We included 11 healthy volunteers in our study and performed a standardized hyperventilation maneuver: [21] We were able to detect a characteristic dT° pattern in response to hyperventilation (Fig. 3). We could demonstrate that normalized dT° values were significantly higher during hyperventilation then compared to baseline (0.063 (IQR 0.032) vs. 0.376 (IQR 0.093), $p < 0.001$] and recovery [0.082 (IQR 0.029) vs. 0.376 (IQR 0.093), $p < 0.001$]. During recovery, dT° showed higher levels compared to baseline ($p = 0.019$). When calculating PRD, we found significantly increased PRD values after hyperventilation compared to baseline [3.30 (IQR 2.29) $deg^2$ vs. 2.76 (IQR 1.43) $deg^2$, $p = 0.018$] (Fig.3). Linear regression analysis revealed that increases in PRD level were independent of heart rate ($p = 0.63$). Hyperventilation thus seems to represent a non-invasive method to induce efferent sympathetic activity on the ventricular myocardium and indicates that these changes can be assessed by measuring PRD and its underlying dT° signal.

### 3.1.2 Autonomic changes under high altitude (HA) exposure

Hamm W, Maier F, Kassem S, **Schüttler D,** Bauer A, Rizas KD, von Stülpnagel L, Brunner S. Deceleration Capacity of Heart Rate and Periodic Repolarization Dynamics during Normobaric Hypoxia. *Scand J Med Sci Sports. 2020 Apr 7.*

Hamm W, Kassem S, von Stülpnagel L, Maier F, Klemm M, **Schüttler D,** Grabher F, Weckbach LT, Huber BC, Bauer A, Rizas KD, Brunner S. Deceleration Capacity and Periodic Repolarization Dynamics As Predictors of Acute Mountain Sickness. *High Alt Med Biol. 2020 Nov 4*

The autonomic nervous system plays an important role under exposure to high altitude mediating adaptive responses. [2] A withdrawal of vagal activity and an increase of sympathetic activity are commonly found during acute exposure to HA.[22] To non-invasively assess vagal and sympathetic tone by ECG biomarkers deceleration capacity (DC), PRD and standard HRV parameters during a gradual exposure to normobaric hypoxia (FiO2 from 21.0% to 11.0%, corresponding to 0 to 5500 m simulated altitude) we recorded high resolution ECG in an altitude chamber in 10 healthy individuals. [23] DC is a marker based HRV and reflects the vagal branch of the cardiac ANS by integrating deceleration-related oscillations of heart rate. [24] In our study, DC significantly decreased from 8.88 ± 2.03 to 6.23 ± 2.57 ms (p=0.029) and PRD significantly increased from 1.89 ± 1.06 to 7.88 ± 5.29 $deg^2$ (p=0.002) (Fig.4 A+B). Changes became particularly obvious at a simulated altitude of above 4.500 m. Supporting our findings, HRV parameters reflecting the parasympathetic branch such as SDNN, RMSSD decreased while frequency-domain derived parameter high frequency decreased and the low frequency/high frequency ratio increased suggesting a shift towards sympathetic excitation (Fig. 4C).




C

| | Baseline | Maximal simulated altitude | *P*-value |
|---|---|---|---|
| SDNN, ms | 83.69 ± 27.68 | 61.80 ± 39.95 | .02 |
| RMSSD, ms | 37.08 ± 7.92 | 17.38 ± 11.45 | <.01 |
| LF, $ms^2$ | 1144.3 ± 564.1 | 895.6 ± 916.1 | .38 |
| HF, $ms^2$ | 391.5 ± 151.4 | 135.9 ± 138.7 | <.01 |
| LF/HF ratio | 3.19 ± 1.82 | 10.60 ± 6.91 | <.01 |

***Figure 4.*** *DC* ***(A)*** *and PRD* ***(B)*** *levels of study volunteers represented in 500 m increments of simulated altitude (normobaric hypoxia) in a HA chamber). P for trend 0.001 (DC) and 0.002 (PRD), respectively. Changes of standard HRV parameters between baseline and maximal simulated altitude of 5500m* ***(C)****. (HF, high frequency; HRV, heart rate variability; LF, low frequency; RMSSD, root mean square of successive differences; SDNN, standard deviation of NN intervals). (Hamm et al. Scand J Med Sci Sports. 2020)*

A complication under acute HA exposure is the occurrence of acute mountain sickness (AMS) which consists of nonspecific symptoms such as dizziness, headache and nausea in unacclimatized mountaineers. [25] As the ANS plays a vital role in acclimatization and mediates physiological changes in a hypobaric hypoxic environment, we aimed to investigate the behavior of ECG-based biomarkers DC and PRD with respect to the occurrence of AMS-related symptoms. In contrast to our previous study in an environmental chamber, we conducted this study under "real" environmental conditions at the Environmental Research Station "Schneefernerhaus" (2650 m) at the Zugspitze, Germany under moderate altitude conditions. [26] In this larger cohort we investigated DC and PRD using high resolution ECG in a resting supine position in 23 individuals 24 hours after arrival at the research station. ECG data were compared to values obtained at baseline recorded in Munich (521 m). Symptoms of AMS were assessed using the Lake Louise score for AMS. [27] Values of 3 or more were considered as significant AMS. PRD increased after 24 hours at HA from 1.50 ± 1.01 deg$^2$ to 3.51 ± 4.46 deg$^2$ (p=0.03) and DC declined from 11.48 ± 2.91 to 9.94 ± 2.78 ms (p=0.001). Volunteers with both an increase in PRD and a decrease in DC had high LLS scores (Fig. 5). A combined finding of a PRD increase and a DC decrease had a sensitivity of 100% and a specifity of 76.5% to diagnose AMS (LLS≥3).

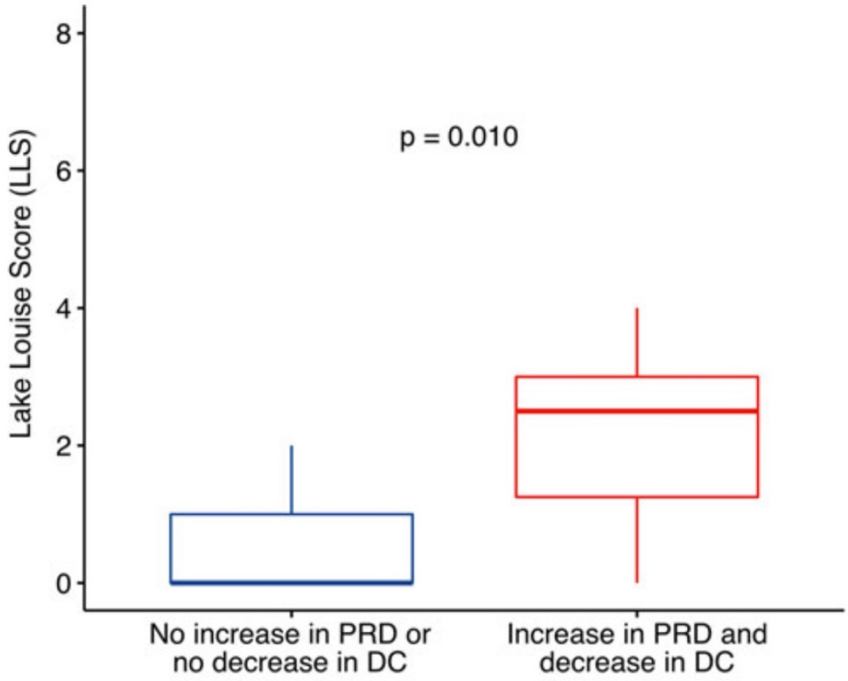



***Figure 5**. Lake Louise Score in study volunteers with no increase in PRD/no decrease in DC compared to participants with increase in PRD/decline in DC. (Hamm et. al High Alt Med Biol. 2020)*

Our data thus provides evidence that ANS function is altered both under moderate altitude exposure at 2650 m and under HA simulation at up to 5500 m. It underlines the key role of ANS function in guiding physiological changes in response to environmental changes. These changes in ANS balance can be assessed by measuring ECG-based biomarkers DC and PRD. DC and PRD measurements are useful to identify individuals suffering from AMS.

### 3.1.3 Autonomic changes during exercise

**Schüttler D,** Krammer S, von Stuelpnagel L, Sams L, Bauer A, Hamm W, Brunner S. Estimation of anaerobic threshold by cardiac repolarization instability: a prospective validation study. *BMC Sports Sci Med Rehabil. 2021 Aug 6;13(1):85.*

During exercise, the ANS exerts influences on our cardiorespiratory system by regulating heart rate, contractility, respiratory rate, and blood pressure to fulfil the increased demand on oxygen and cardiac output.

During increasing workloads our cardiorespiratory system passes different thresholds as lactate and $CO_2$ increases, buffer systems become insufficient and free $H^+$ ions are released. [28] These thresholds have been assessed so far by measuring lactate or by identifying ventilatory thresholds in cardiopulmonary exercise tests (CPET). When using lactate, the thresholds are called lactate threshold (LTs), when using CPETs ventilatory thresholds (VTs). [28] Of note, LTs and VTs show close correlation but are not identical and LT2/VT2 can be assessed to identify the region of the anaerobic threshold (AT), which is the highest sustained workload without blood lactate substantially elevating.[29,30] Modern training concepts especially in endurance athletes are based on these thresholds and certain amounts of training below, in-between, and above thresholds were shown to improve endurance capacity and overall performance.[31,32]

Recently, various study groups implemented the evaluation of autonomic biomarkers to control training intensities and to detect training-induced states of fatigue.[13] Heart rate variability (HRV)-derived parameters reflecting ANS activity showed an association with LTs.[13] Exercise affects repolarization phase of the heart noticeably. [13,33] Our study group recently showed that AT can be assessed by a characteristic pattern of repolarization instability in healthy young individuals. [34]

Within the present study validated these findings in a cohort of 65 professional and amateur team sports athletes who underwent a graded cycle ergometer test until maximal exhaustion. [35] We recorded high-resolution data from Frank's orthogonal lead ECG and analyzed its dT° signal during exercise. During exercise this dT° signal shows a characteristic three-phasic pattern and the anaerobic threshold which can be determined by the dT° signal ($AT_{dT°}$) is defined as the point of maximal discordance between dT° signal and heart rate (Fig.6).

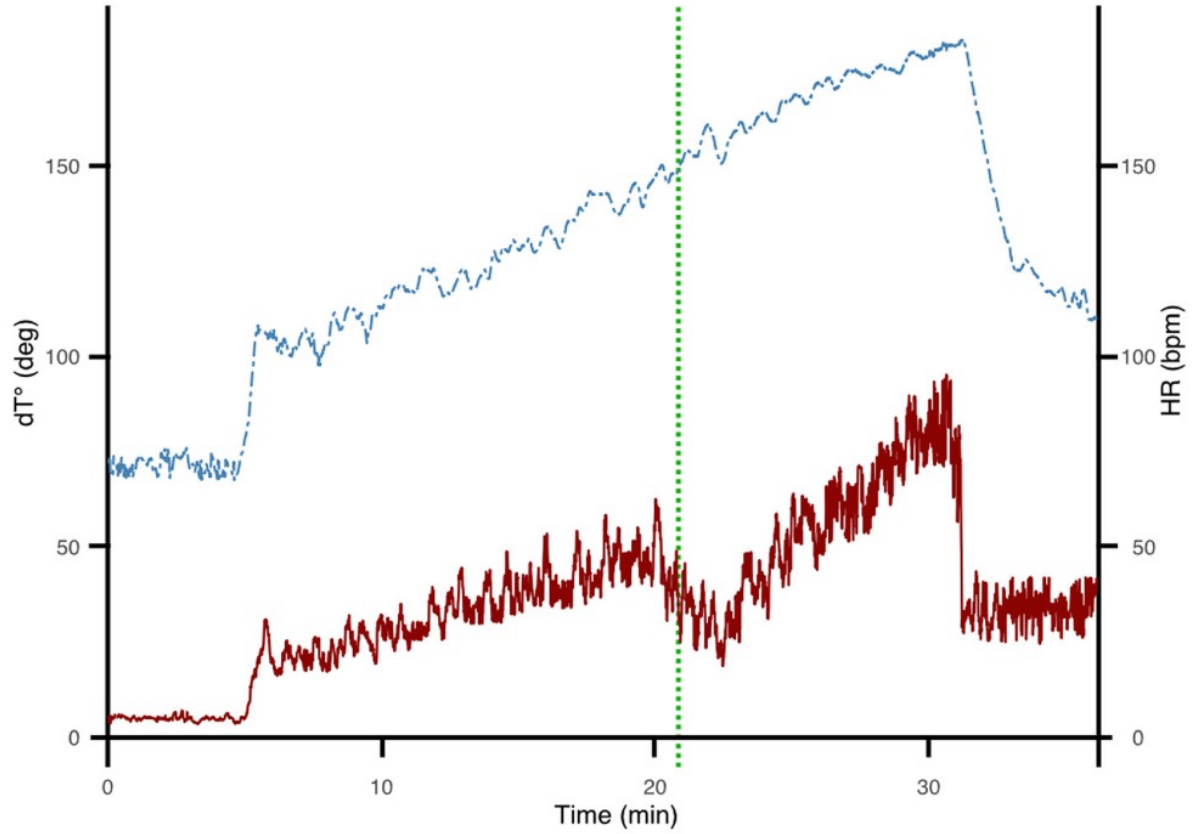



***Figure 6.*** *Exemplary dT° signal (red) and corresponding heart rate signal (blue) during graded exercise test showing the moment of maximal discordance between these signals defined as $AT_{dT°}$ (dotted green line). (Schüttler et al BMC Sports Sci Med Rehabil. 2021)*

Capillary blood lactate concentrations (in mmol/l) were measured) before exercise, during exercise at the end of each incremental step and after exercise. Lactate thresholds were determined according to the methods by Mader (fixed threshold at 4 mmol/l) and Dickhuth (lactate concentration 1.5 mmol/l above the lactate equivalent (defined as the lowest value of the lactate performance ratio marking the onset of the lactate increase during exercise)). Athletes showed anaerobic threshold as assessed by dT° via our high-resolution ECGs ($AT_{dT°}$) at 187.6 ± 44.4 W, $LT_{Dickhuth}$ at 181.1 ± 45.6 W and $LT_{Mader}$ at 184.3 ± 52.4 W. $AT_{dT°}$ correlated highly significantly with $LT_{Dickhuth}$ ($r = 0.96$, $p < 0.001$) and $LT_{Mader}$ ($r = 0.98$, $p < 0.001$) both in the entire cohort of athletes but also in the subgroups of professional and amateur athletes ($p < 0.001$ for all). (Fig. 7) Intra class correlation for these three methods was excellent with intraclass correlation coefficients of 0.95 (power output) and 0.93 (heart rate). Intergroup comparison of LTs and $AT_{dT°}$ revealed no significant differences for power outputs and heart rates at thresholds using Kruskal-Wallis tests ($p=0.73$ and $p=0.91$). Taken altogether, anaerobic threshold can be reliably and noninvasively assessed measuring repolarization instability via high-resolution ECG and correlates very well with established methods of LT assessment in both amateur and professional athletes.

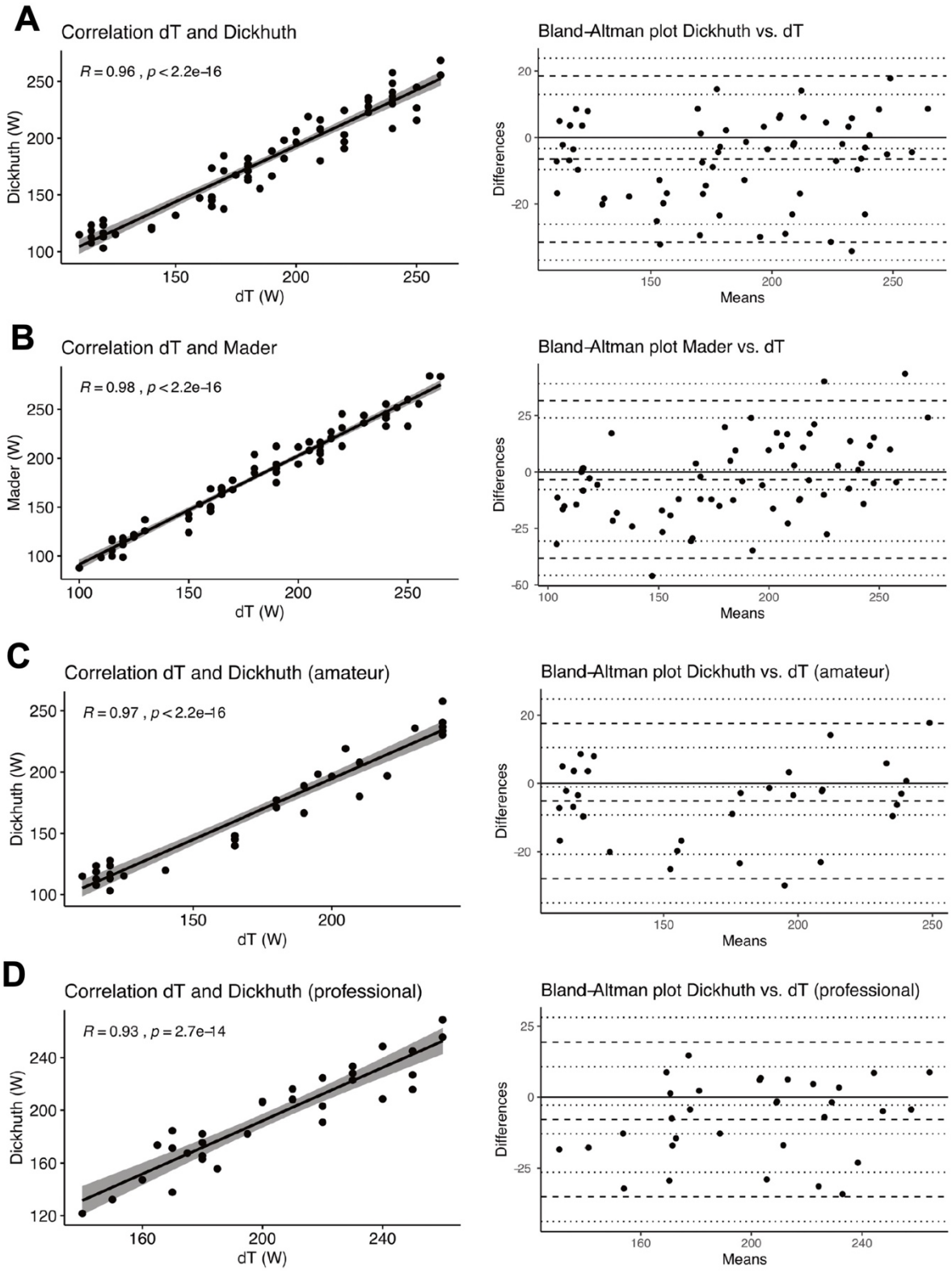



***Figure 7.*** *Pearson correlation coefficient test for power output between $AT_{dT°}$ and $LT_{Dickhuth}$ or $LT_{Mader}$ for all athletes (A+B) and between between $AT_{dT°}$ and $LT_{Dickhuth}$ for amateur athletes (C) and professional athletes (D). Bland-Altman plots indicate concordance between $AT_{dT°}$ and LTs. (Schüttler et al BMC Sports Sci Med Rehabil. 2021)*

### 3.1.4 Autonomic changes under caffeine exposure

**Schüttler D,** Hamm W, Kellnar A, Brunner S, Stremmel C. Comparable Analysis of Acute Changes in Vascular Tone after Coffee versus Energy Drink Consumption. *Nutrients 2022, 14(9), 1888.*

**Schüttler D,** Rudi WS, Bauer A, Hamm W, Brunner S. Impact of energy drink versus coffee consumption on periodic repolarization dynamics: an interventional study. *Eur J Nutr. 2022 Mar 10.*

Caffeinated beverages are consumed daily throughout the world but both adverse and beneficial side effects have been reported. While coffee consumption was found to be inversely associated with total and cause-specific mortality [36], other studies have raised the question if caffeine consumption is related to the occurrence of cancer, arrhythmias and other cardiovascular diseases.[37,38] A possible explanation for adverse cardiovascular effects of caffeine consumption might be the development of ANS dysfunction.[39]
In our interventional cross-over study we investigated the effect of efferent cardiac sympathetic activity on the ventricular myocardium by calculating PRD after consumption of 240 mg caffeine each by means of commercial energy drinks or coffee. [40] Consumption of energy drinks increased PRD levels significantly (3.64 vs. 5.85 $deg^2$; $p < 0.001$) 45 minutes after intake compared to baseline levels, whereas coffee consumption did not alter PRD levels (3.47 vs 3.16 $deg^2$; $p = 0.63$) (Fig. 8).

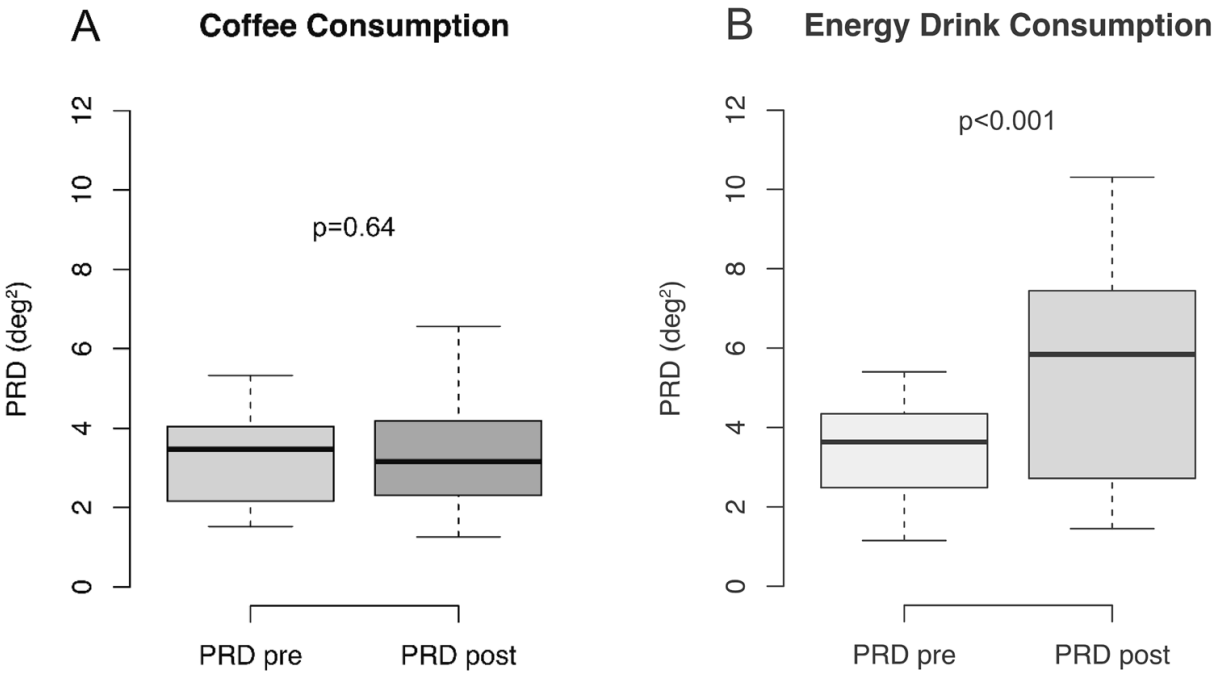



***Figure 8.*** *Box plots showing PRD values before and 45 minutes after consumption of 240 mg caffeine by means of coffee (A) and energy drinks (B). (Schüttler et al. Eur J Nutr 2022)*

Heart rates were not altered by caffeine consumption and Spearman analysis showed no significant correlation between PRD changes and heart rate changes (R = 0.34, p = 0.31 for

coffee, R = 0.31, p = 0.24 for energy drink). In addition, consumption of tap water (750 ml) did not alter PRD levels (2.94 deg2 vs. 2.77 deg$^2$, p = 0.79) which excludes that observed effects on ANS biomarker were mediated by volume effects. In conclusion, our results shows that energy drinks lead to enhanced sympathetic activity on the level of the ventricular myocardium but not coffee consumption. This sympathetic activity-mediated repolarization instability thus seems to be independent from caffeine and the observed ANS alterations might consequently be triggered by other ingredients or a combination of those. Enhanced repolarization instability after energy drink consumption might facilitate the occurrence of malignant ventricular arrhythmias and could therefore be a potential reason for observed sudden cardiac deaths SCDs after excessive intake.

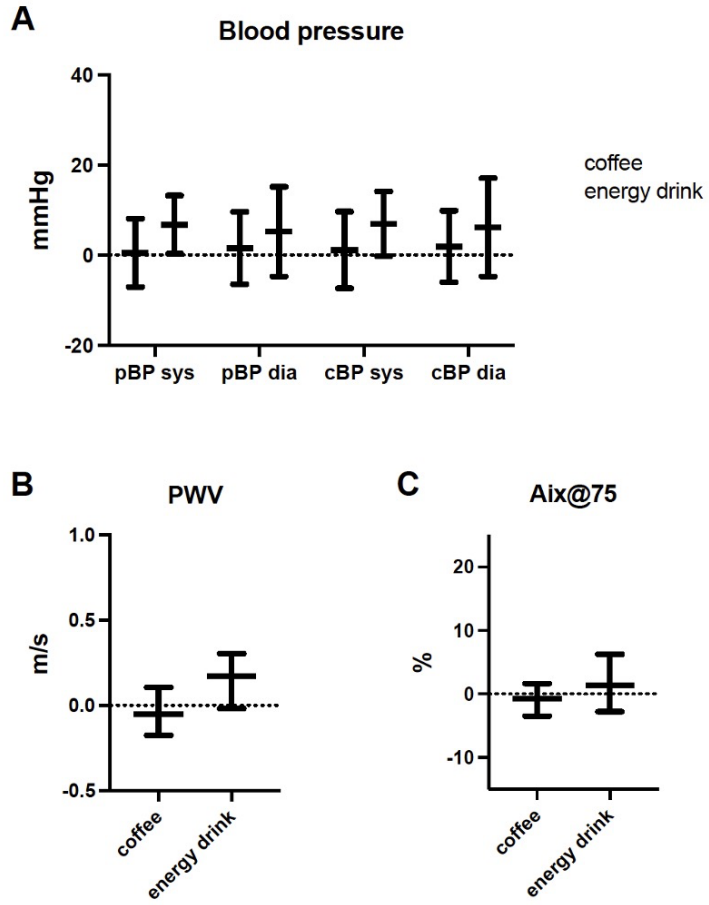



***Figure 9.*** *Hemodynamic parameters after caffeine consumption. Effect of energy drink and coffee consumption on systolic (sys) and diastolic (dia) peripheral (pBP) as well as central blood pressure (cBP) (A), pulse wave velocity (PWV) (B) and augmentation index (Aix) (C). (Schüttler et al. Nutrients 2022)*

In addition, in our cohort of 23 study participants we performed pulse wave analysis (PWA) after coffee and energy drink consumption. [41] PWA is a non-invasive, commonly used diagnostic tool which records multiple parameters of arterial stiffness. Pulse wave velocity (PWV), a parameter which can be assessed by PWA is associated with increased cardiovascular morbidity and mortality and correlates with end organ damage. [42] While blood pressure remained stable after coffee consumption, energy drinks led to an increase in peripheral (+6.35 mmHg) as well as central systolic blood pressure (+7.00 mmHg). Pulse wave velocity did not change after coffee intake but increased by 0.17 m/s after energy drink consumption, Augmentation index (Aix) remained stable under both conditions. (Fig. 9) Statistical significance was lost after correction for multiple testing. This data further strengthens the hypothesis that energy drinks mediate a negative impact on cardiovascular properties.

### 3.1.4 Autonomic changes during dialysis

**Schüttler D,** Schönermarck U, Wenner F, Toepfer M, Rizas KD, Bauer A, Brunner S, Hamm W. Large potassium shifts during dialysis enhance cardiac repolarization instability. *J Nephrol. 2020 Oct 15.*

Hemodialysis (HD) patients suffer from an estimated 14-fold increased mortality due to sudden cardiac death (SCD) compared to patients with normal kidney function, mostly caused by malignant cardiac arrhythmias. [43] Especially large potassium shifts and the use of dialysates with low potassium concentrations have been associated with increased mortality and occurrence of arrhythmias. [44,45] However, the exact mechanisms how arrhythmias evolve during dialysis are still insufficiently explored. We thus conducted a study to non-invasively assess repolarization instability during dialysis wondering if autonomic function is altered during dialysis. [46] Biomarker PRD was calculated out of ECG recordings from the first 30 min and last 30 min of dialysis session. There was a distinct correlation between the intradialytic potassium shift and an increase in PRD levels (Spearman correlation coefficient R = 0.62, p = 0.006). Patients who showed a potassium shift of above 1 mmol/l had significantly increased PRD values at the end of their dialysis session when compared to patients with potassium shifts below 1.0 mmol/l [delta PRD 2.82 (IQR 2.13) vs. −2.08 (IQR 3.60), p = 0.006]. Spearman analysis showed no significant correlation between PRD changes and overall fluid removal during dialysis (R = − 0.23, p = 0.36).

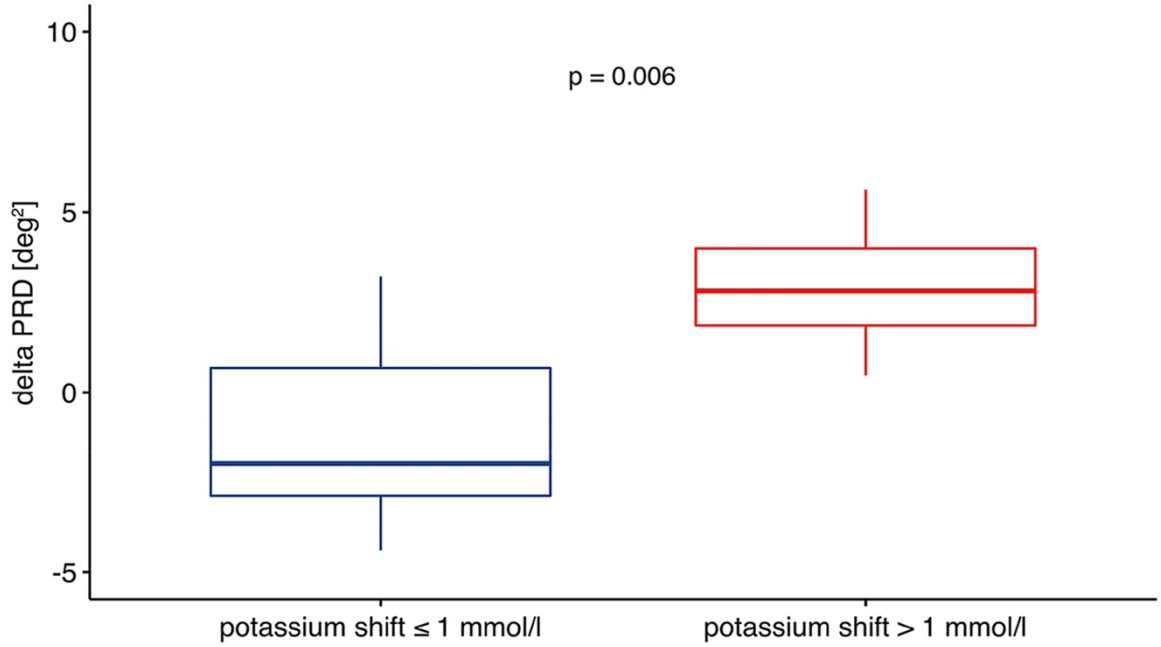



***Figure 10.*** *PRD changes in response to amount of potassium shift during hemodialysis. (Schüttler et al. J Nephrol. 2020)*

Our data suggests a possible physiological link between changes of potassium shift and the vulnerability of the heart to sympathetic activity-associated repolarization instability. As increases in efferent sympathetic activity are hypothesized to facilitate the occurrence of

arrhythmias, our observations could, at least in part, explain the increases in malignant arrhythmias described in literature. In addition, PRD might be a promising tool for risk stratification to arrhythmias in patients undergoing HD.

### 3.2 Establishing animal models to investigate autonomic function

As elucidated above most of the data investigating autonomic remodeling and its impact on arrhythmogenesis is based on research performed in rodent models. However, it is necessary to test the obtained findings in valid translational models which are usually larger animal models such as pigs or dogs.

All animals clearly display advantages and disadvantages, and it is crucial to select a model which is most suitable to address the aim of the study. [12] Especially autonomic function is highly vulnerable and can be easily influenced by intrinsic and extrinsic factors such as narcosis regimes which potentially disturb research results as recently shown by us:

#### 3.2.1 Autonomic function is altered by narcosis regimens in mice

Tomsits P, Volz L, Xia R,Chivukula A, **Schüttler D*,** Clauß S*. Medetomidine/midazolam/fentanyl narcosis alters cardiac autonomic tone, leading to conduction disorders and arrhythmias in mice. *Lab Anim (NY). 2023 (accepted)* **IF 9.7, *authors contributed equally.**

Mice are widely used to study arrhythmia-related mechanisms including autonomic dysfunction.[47] Mouse models exert an essential role as they can conveniently be genetically modified, have a short generation time and can be housed and bred in most institutions at reasonable costs. [12] To assess electrophysiological properties and induce arrhythmias, mice have to be sedated. Different narcosis regimens such as medetomidine/midazolam/fentanyl (MMF) and isoflurane/fentanyl (IF) are commonly used. However, there is a lack of knowledge if used anesthesia confounds electrophysiological properties, arrhythmogenesis and autonomic function.

Therefore, we investigated 29 C57BL/6N mice with either telemetry (without narcosis) or ECG/invasive electrophysiology (EP) study with MMF or IF narcosis. We assessed basic ECG parameters, occurrence of spontaneous arrhythmias and conduction disorders, heart rate variability (HRV) parameters, sinus node and AV node function, refractory periods as well as atrial and ventricular susceptibility to arrhythmias.

We detected broad effects of MMF narcosis on electrophysiological properties. We recorded spontaneous arrhythmias (Fig. 11) and HRV biomarkers were significantly altered suggesting pronounced alteration of autonomic cardiac function under MMF narcosis. Especially SDRR, RMSSD and pRR50 were markedly increased (Fig. 12) which reflects a shift towards

predominant vagal tone. [48] In addition, frequency domains were altered under MMF with increases in the HF spectrum also indicating an increased vagal activity. This imbalance towards a parasympathetic predominance might be mediated by medetomidine which is known to inhibit the central release of norepinephrine. [49]

In contrast to MMF, IF narcosis only mildly affected heart rate without significant influences on HRV and arrhythmias. Therefore, we suggest using this regimen for studying cardiac arrhythmias and autonomic function in mice.

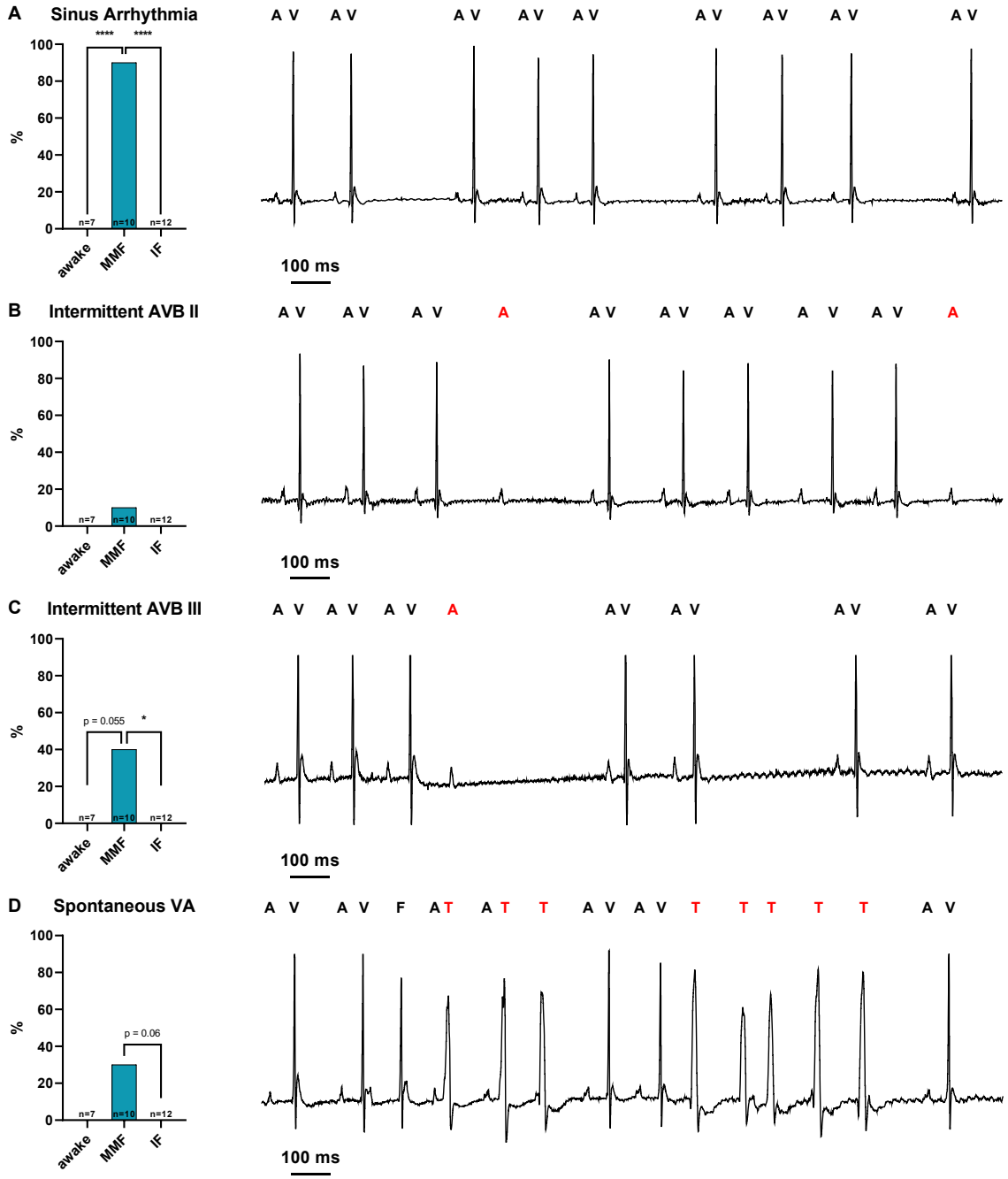



***Figure 11.*** *Quantification of spontaneously occuring arrhythmias (A: sinus arrhythmias, B: intermittent AVB II°, C: intermittent AVB III° D: spontaneous ventricular activity. A=atrial activity V= ventricular activity. *=p<0.05, **** p<0.0001 (Tomsits et al. Lab Animal 2023, accepted)*

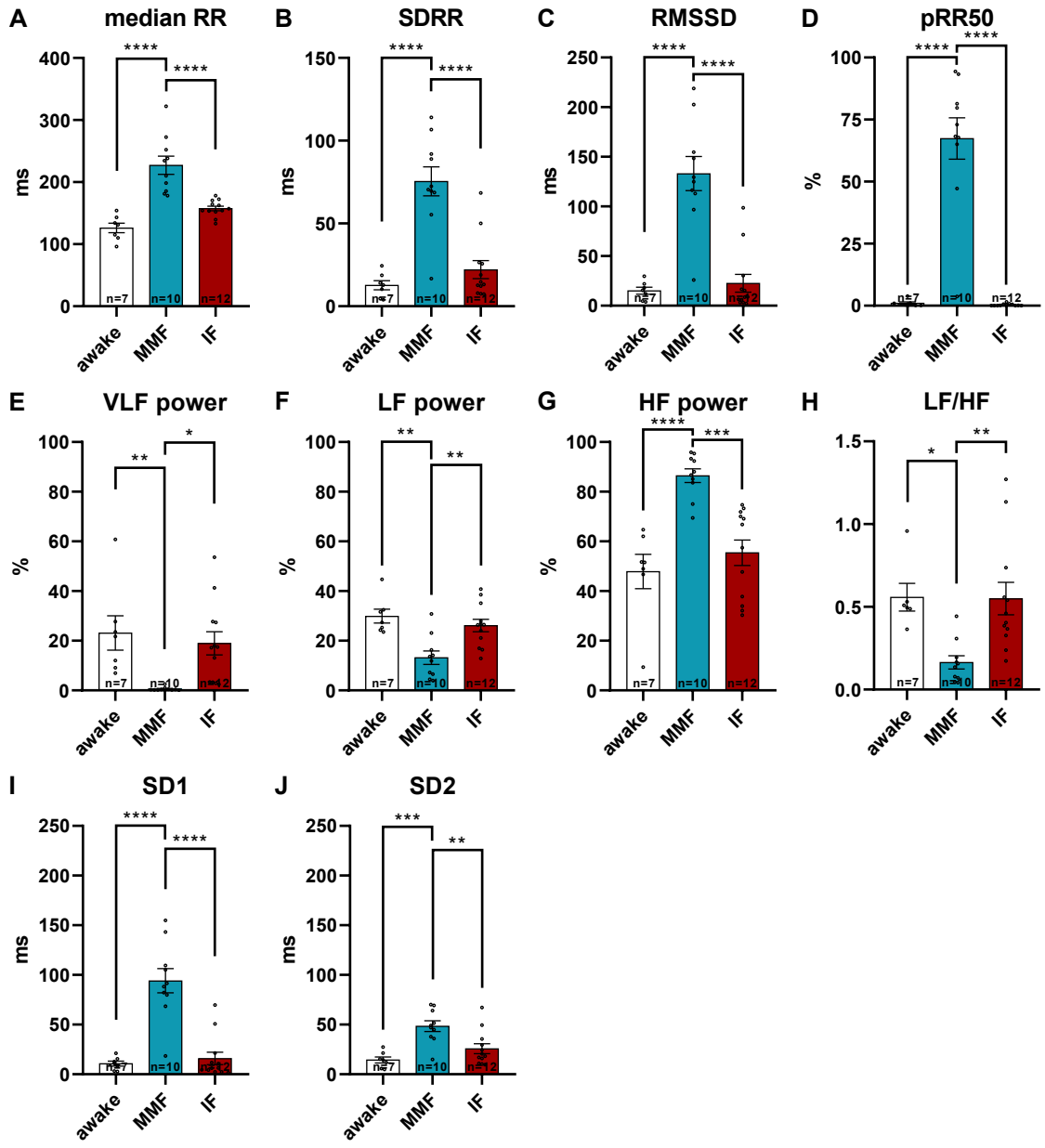



***Figure 12.*** *HRV parameters in awake mice or under MMF or IF narcosis**.** Median RR interval (A), standard deviation of all RR intervals (SDRR) (B), Square root of the mean of the sum of the squares of differences between adjacent RR intervals (RMSSD) (C), percentage difference between adjacent RR intervals that are greater than 50 ms (pRR50) (D). percentage of the very low frequency (VLF) oscillation (0.00-0.15 Hz) (E), percentage of the low frequency (LF) oscillation (0.15-1.50 Hz) (F), percentage of the high frequency (HF) oscillation (1.5-5.0 Hz) (G), LF/HF ratio (H)**,** Poincaré standard deviation 1 (SD1) (I), Poincaré standard deviation 2 (J). n=7 for awake. values presented as mean ± SEM.*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. (Tomsits et al. Lab Animal 2023, accepted)*

Compared to rodents, there is still a lack of valid large animal models to study autonomic remodeling processes in the context of arrhythmogenesis. However, in a step wise translational approach it is essential to test initial findings, which were obtained e. g. in murine models, in larger pre-clinical animal models before performing clinical tests in humans. Therefore, our aim was to establish a porcine model to study remodeling processes including autonomic assessment which facilitate the occurrence of arrhythmias. We chose pigs as this

species is ethically accepted, shows similar anatomic, electrophysiologic and hemodynamic properties like humans and as more and more genetically modified pigs become available: [11,12]

### 3.2.2 A porcine model to assess autonomic function and electrophysiological properties

**Schüttler D,** Tomsits P, Bleyer C, Vlcek J, Pauly V, Hessen N, Sinner M, Merkus D, Hamers J, Kääb S, Clauss S. A practical guide to setting up pig models for cardiovascular catheterization, electrophysiological assessment and heart disease research. *Lab Anim (NY). 2022 Feb;51(2):46-67.*

Handling large animals in an experimental set-up is not easy and some pitfalls are easily made which may cause loss of data, loss of tissue harvest and even unwanted death of the animal. To avoid complications, we developed a practical and detailed guide to set up porcine models for cardiovascular catheterization and research. Based on our long expertise in experimentally handling porcine models we provided detailed information about intubation (Fig. 13) - one extremely critical step as hypersalivation, laryngospasms and bad view at laryngeal opening

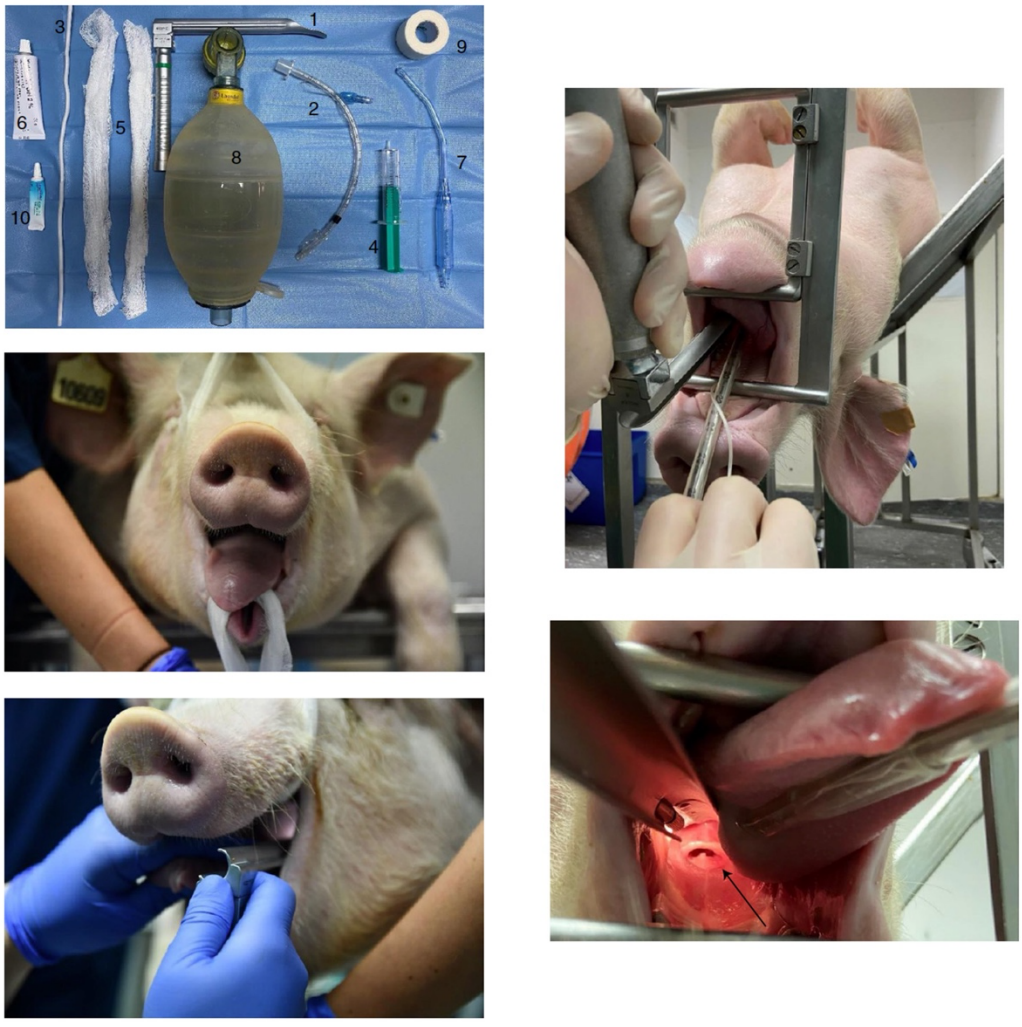



***Figure 13.*** *Overview of equipment for intubation and intubation techniques in ventral (left) and dorsal (right) recumbency. (Schüttler et al. Lab Animal 2022)*

can cause severe complications such as perforation, hypoxia or death. [50,51] Our guide provides a broad safety net for even long-lasting and potentially complicated intubation procedures as we experienced best results using ventral recumbency (dorsal recumbency is also feasible, though) and only mild sedation with pigs still spontaneously breathing. We explained possible anesthetic drugs elucidating their advantages and side effects, demonstrated possible access sites for sheath insertion, guided the reader step-by-step through cardiac catheterization, basic EPS, cardiac output (CO) and pressure recordings, pressure–volume (PV)-loop measurements and coronary angiography. We also describe a method for the induction of acute myocardial infarction, a common disease model in cardiovascular and arrhythmia research. [52] This model leads to ischemic heart failure and allows studying related remodeling processes.

Following our suggested protocol allows a detailed electrophysiological and hemodynamic assessment of pigs as well as a non-invasive assessment of autonomic biomarkers via accompanying ECG recordings. In addition, as experimental procedures can be safely conducted, sufficient organ harvest is usually possible which allows to further study the scientific issue.

We used our protocol for a porcine model of IHF to investigate the underlying remodeling processes which lead to occurrence of arrhythmias:

### 3.2.3 Induction of ischemic heart failure leads to a proarrhythmogenic substrate in a porcine model

Clauss S*, **Schüttler D*,** Bleyer C, Vlcek J, Shakarami M, Tomsits P, Schneider S, Maderspacher F, Chataut K, Trebo A, Wang C, Kleeberger J, Xia R, Baloch E, Hildebrand B, Massberg S, Wakili R, Kääb S. Characterization of a porcine model of atrial arrhythmogenicity in the context of ischaemic heart failure. *PLoS One. 2020 May 4;15(5):e0232374.* ***authors contributed equally.**

Acute myocardial infarction can cause heart failure. Occurring remodeling processes in response to ischemia facilitate the origin of both atrial and ventricular arrhythmias.[53] About 6 and 21% develop AF after myocardial infarction and this is associated with poor prognosis.[54,55] However, the exact mechanisms underlying arrhythmogenesis after AMI are still incompletely understood. To investigate these and to develop novel treatment strategies, clinically relevant, close-to-human large animal models are urgently needed.

We thus developed a porcine model of IHF allowing further investigation of mechanisms. Altogether 43 pigs were initially investigated, but as some pigs died due to ventricular fibrillation during AMI, 16 IHF pigs and 15 control pigs could be included in the final analyses. AMI was induced by balloon occlusion of the LAD distal of the diagonal branch for 90 minutes. Right heart and left heart catheterization were performed to measure hemodynamics and atrial EPS were performed to induce atrial arrhythmias via burst pacing. Ejection fractions were assessed by angiography and were significantly reduced after AMI showing a distinct

phenotype of IHF (Fig. 14). This was accompanied by increases in left ventricular end diastolic pressure, pulmonary capillary wedge pressure and right atrial pressure. In pigs with IHF we observed a significantly increased atrial arrhythmogenicity: A significantly higher number of IHF pigs we prone to develop atrial arrhythmic episodes including AF after stimulation compared to control pigs (3/15 vs. 10/16 pigs, p = 0.029) with longer average and total duration of episodes (Fig. 15).

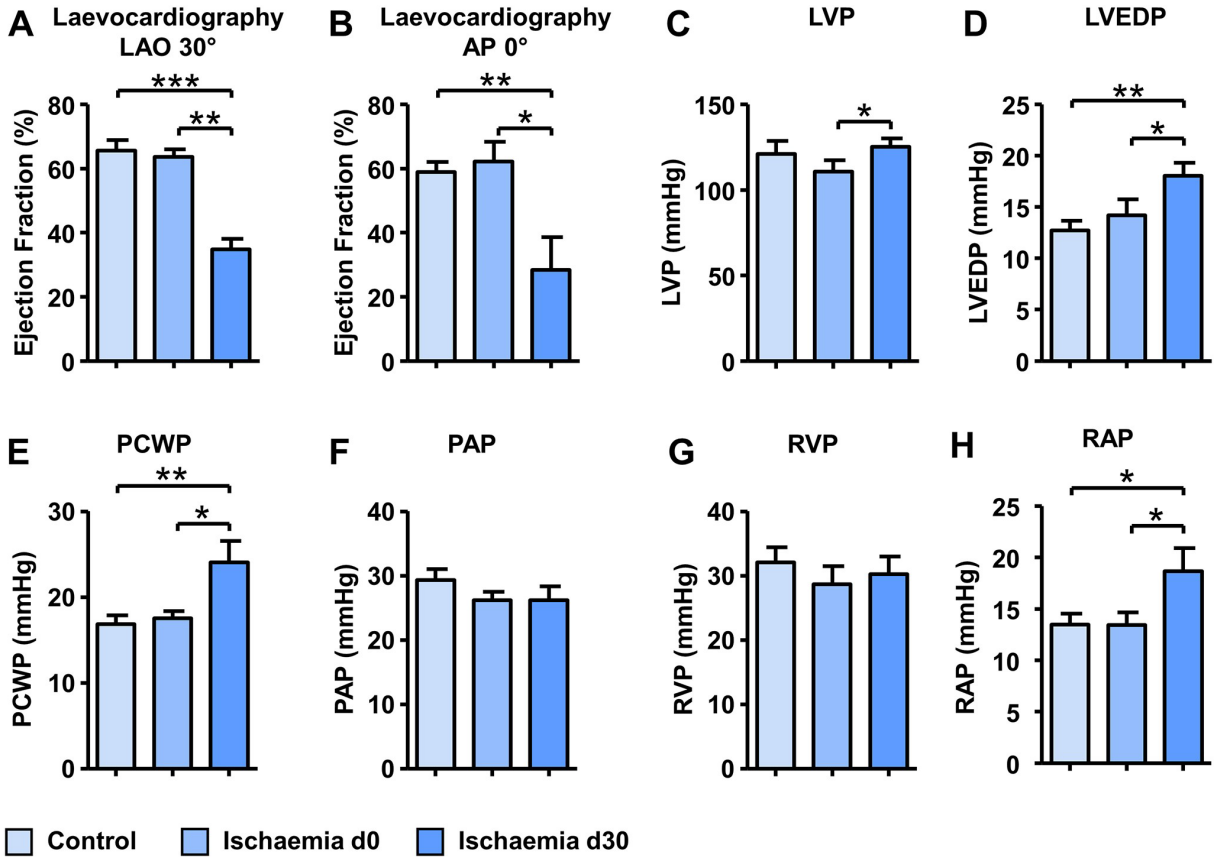



***Figure 14.*** *Hemodynamic measurements of left ventricular ejection fraction by levocardiography at two angulations (left anterior oblique (LAO) 30˚ (A) and anterior-posterior (AP) 0˚ (B)), left ventricular pressure (LVP) (C), left ventricular end-diastolic pressure(LVEDP) (D), pulmonary capillary wedge pressure (PCWP) (E), pulmonary artery pressure (PAP) (F), right ventricular pressure (RVP) (G) and right atrial pressure (RAP) (H) as obtained via right and left heart catheterization in control pigs and ischemic pigs at day 0 and day 30. * p<0.05, ** p<0.01, ***p<0.001. (Clauss*, Schüttler* et al. PlosOne 2020)*

Both atrial and left ventricular tissue samples remote of infarction showed increased amounts of fibrosis in IHF pigs 30 days after myocardial infarction compared to controls (6.3% vs. 12.8%, p = 0.008; 2.1% vs. 4.6%, p = 0.042; respectively) which could be a potentially underlying mechanism facilitation atrial arrhythmogenicity.

Taken together, selective AMI in this porcine model leads to IHF resembling the human situation after myocardial infarction. It causes elevated left ventricular end diastolic pressures, wedge pressures and atrial pressures inducing an atrial remodeling which sets basis for an arrhythmogenic substrate. Therefore, this close-to-human large model is highly suitable to

study pathomechanisms underlying atrial and potentially ventricular arrhythmogenesis after AMI such as electrical, structural and autonomic remodeling.

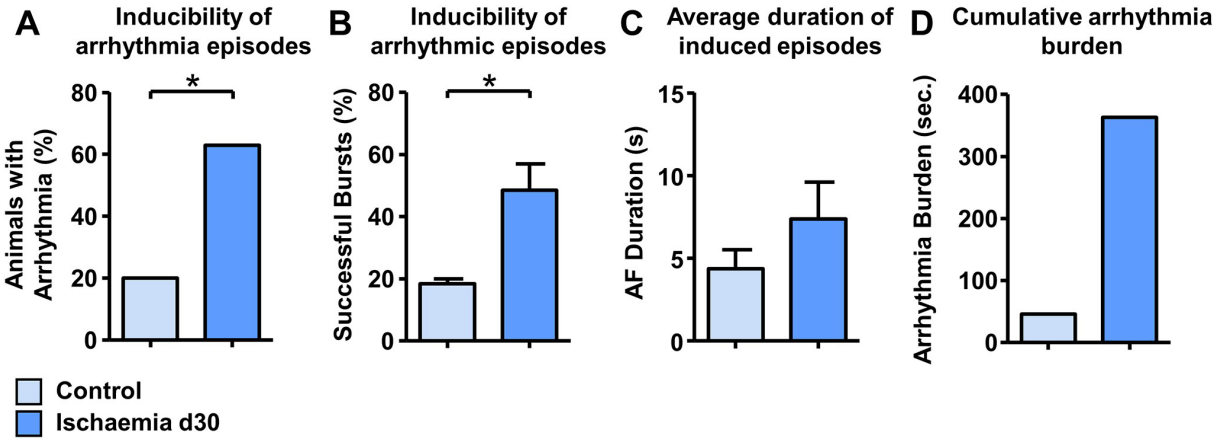



***Figure 15.*** *Electrophysiology studies demonstrating the inducibility of atrial arrhythmia episodes per animal (A) ,the percentage of successful bursts (B), the average duration of induced episodes (C), and the cumulative arrhythmia burden (D) in control and ischaemic pigs at day 30.* p<0.05 (Clauss*, Schüttler* et al. PlosOne 2020)*

### 3.3 Summary and future perspectives

The ANS permanently exerts its influence on cardiovascular properties providing the adaptive responses to intrinsic and extrinsic factors. However, visualization of the influence remains difficult. To mirror the influence of autonomic cardiac inputs different ECG based biomarkers were thus developed. These are mostly based on heart rate alterations and T wave changes as these phases are highly affected by the ANS. Especially parameters of heart rate variability have been investigated over the years. Recently novel biomarkers “periodic repolarization dynamics (PRD)”, which shows efferent sympathetic activity on the level of ventricular myocardium, and “deceleration capacity (DC)”, which mirrors changes in vagal tone, were postulated. However, little is known how these parameters behave under certain physiological and environmental changes.

Within our research work we consequently investigated the effect of intrinsic and extrinsic factors on ECG-based autonomic biomarkers. We were able to show that hyperventilation leads to characteristic changes in repolarization instability and elevates PRD levels. Gradual exercise triggers a characteristic three phasic dT° signal which can be used to non-invasively determine anaerobic threshold $AT_{dT°}$, highly correlates with standard lactate threshold concepts, and has thus potential to be used for threshold-based training concepts. Additionally, we were able to demonstrate that PRD and DC are changed under altitude exposure and that PRD elevations with concomitant DC decreases are highly predictive for development of acute mountain sickness. PRD levels are also elevated after consumption of high amount of energy drinks which might provide a link to the occurrence of arrhythmias after excessive intake as enhanced sympathetic activity is connected to arrhythmogenesis. In

addition, we also detected significantly higher PRD levels in response to high potassium shifts in dialysis patients which could in part explain occurrence of arrhythmias in this cohort.

As there is a lack of valid animals to study autonomic remodeling in the context of arrhythmogenesis, we developed a porcine model of ischemic heart failure which demonstrates an atrial proarrhythmic phenotype which is based on profibrotic remodeling of the atria. Current preliminary analyses show that this model is also suitable to investigate autonomic remodeling: Staining for autonomic (TH+) and sprouting (GAP43+) nerve fibers in IHF pigs shows upregulation in both ventricles and atria. This is accompanied by an upregulation of sympathetic markers in western blot and PCR analyses. Furthermore, autonomic biomarkers are altered in IHF pigs as PRD values are increased after AMI when compared to control pigs. These findings will be further investigated in future experiments to further identify mechanisms of autonomic remodeling and find potential therapeutic targets to counteract this unfavorable remodeling.

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## 5. Abbreviations

| | |
|---|---|
| AF | atrial fibrillation |
| Aix | augmentation index |
| AMI | acute myocardial infarction |
| AMS | acute mountain sickness |
| ANS | autonomic nervous system |
| AP | action potential |
| AP | anterior-posterior |
| AT | anaerobic threshold |
| AV | atrioventricular |
| AVB | atrioventricular block |
| CMP | cardiomyopathy |
| CNS | central nervous system |
| CPET | cardiopulmonary exercise test |
| CVD | cardiovascular disease |
| DC | deceleration capacity |
| ECG | electrocardiogram |
| EP | electrophysiology |
| EPS | electrophysiology study |
| GAP43 | growth associated protein 43 |
| HA | high altitude |
| HD | hemodialysis |
| HF | heart failure |
| HF | high frequency |
| HRV | heart rate variability |
| ICD | implantable cardioverter defibrillator |
| IF | isoflurane/fentanyl |
| IHF | ischemic heart failure |
| LAO | left anterior oblique |
| LF | low frequency |
| LLS | Lake Louise Score |
| LT | lactate threshold |

| | |
|---|---|
| LVEDP | left ventricular end diastolic pressure |
| LVP | left ventricular pressure |
| MMF | medetomidine/midazolam/fentanyl |
| NGF | nerval growth factor |
| PAP | pulmonary artery pressure |
| PCWP | pulmonary capillary wedge pressure |
| PRD | periodic repolarization dynamics |
| pRR50 | percentage difference between adjacent RR intervals that are greater than 50 ms |
| PWA | pulse wave analysis |
| PWV | pulse wave velocity |
| RAP | right atrial pressure |
| RMSSD | root mean square of successive differences |
| RVP | right ventricular pressure |
| SCD | sudden cardiac death |
| SD1 | Poincaré standard deviation 1 |
| SD2 | Poincaré standard deviation 2 |
| SDNN | standard deviation of NN intervals |
| SDRR | standard deviation of all RR intervals |
| VLF | very low frequency |
| VT | ventilatory threshold |

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## 7. Publication list

### I. Original publications as first or senior author

Tomsits P, Volz L, Xia R, Chivukula A, **Schüttler D*,** Clauß S*. Medetomidine/midazolam/fentanyl narcosis alters cardiac autonomic tone, leading to conduction disorders and arrhythmias in mice. *Lab Anim (NY). 2023 (accepted)* **IF 9.7, *authors contributed equally.**

**Schüttler D,** Hamm W, Kellnar A, Brunner S, Stremmel C. Comparable Analysis of Acute Changes in Vascular Tone after Coffee versus Energy Drink Consumption. *Nutrients 2022, 14(9), 1888.* **IF 6.7**

**Schüttler D**, Hamm W, Krammer S, Steffen J, Deuster E, Lauseker M, Egger F, Meyer T, Brunner S. Staying on the ball during COVID-19 pandemic: impact on training modalities in football players**.** *J Sports Med Phys Fitness. 2022 May;62(5):595-601.* **IF 1.7**

**Schüttler D,** Rudi WS, Bauer A, Hamm W, Brunner S. Impact of energy drink versus coffee consumption on periodic repolarization dynamics: an interventional study. *Eur J Nutr. 2022 Mar 10.* **IF 4.9**

**Schüttler D,** Tomsits P, Bleyer C, Vlcek J, Pauly V, Hessen N, Sinner M, Merkus D, Hamers J, Kääb S, Clauss S. A practical guide to setting up pig models for cardiovascular catheterization, electrophysiological assessment and heart disease research. *Lab Anim (NY). 2022 Feb;51(2):46-67.* **IF 9.7**

**Schüttler D,** Krammer S, von Stuelpnagel L, Sams L, Bauer A, Hamm W, Brunner S. Estimation of anaerobic threshold by cardiac repolarization instability: a prospective validation study. *BMC Sports Sci Med Rehabil. 2021 Aug 6;13(1):85.* **IF 2.4**

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## II. Original publications as co-author

Shoykhet M., Dervishi O., Menauer P., Hiermaier M., Moztarzadeh S., Osterloh C., Ludwig R.J., Williams T., Gerull B., Kaab S., Clauss S., **Schüttler D.**, Waschke J., Yeruva S. EGFR inihibition leads to enhanced desmosome assembly and cardiomyocyte cohesion via ROCK activation. *JCI Insight. 2023 Feb 16;e163763.* **IF 9.5**

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## III. Case Reports:

**Schüttler D,** Mourouzis K, Auernhammer CJ, Rizas KD. Development of severe intrapulmonary shunting in a patient with carcinoid heart disease after closure of a persistent foramen ovale: a case report. *Eur Heart J Case Rep. 2021 Dec 4;5(12):ytab494.* **IF n/a**

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## IV. Reviews

Xia R, Tomsits P, Loy S, Zhang Z, Pauly V, **Schüttler D***, Clauss S*. Cardiac Macrophages and Their Effects on Arrhythmogenesis. *Front Physiol. 2022 Jun 22;13:900094.* **IF 4.8, *authors contributed equally.**

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## V. Book chapters

**n/a**

## VI. Other publications

**Schüttler D**, Weckbach LT, Brunner S. Physical Rehabilitation in Patients with Heart Failure. *N Engl J Med. 2021 Sep 30;385(14):1339-1340.* **IF 176.1**

## 8. Teaching activities

| | | |
|---|---|---|
| Wintersemester 16/17 | 7M1292 | Bedside Teaching |
| Sommersemester 17 | 7M1292 | Bedside Teaching |
| | 7M1384 | Interdisziplinäres Tutorial Modul 23 |
| Wintersemester 17/18 | 7M1292 | Bedside Teaching |
| | 7M1384 | PBL-Tutorials |
| Sommersemster 18 | 7M1292 | Bedside Teaching |
| | 7M1384 | PBL-Tutorials |
| Wintersemester 18/19 | 7M1292 | Bedside Teaching |
| | 7M1384 | PBL-Tutorials |
| Sommersemster 19 | 7M1293 | Seminar kardiovaskuläres System |
| | 7M1292 | Bedside Teaching |
| | 7M1298 | Kardiologische Funktion |
| Wintersemester 19/20 | 7M1292 | Bedside Teaching |
| | 7M1384 | PBL-Tutorials |
| Sommersemester 20 | 7M1384 | PBL-Tutorials |
| | 7M1292 | Bedside Teaching |
| Wintersemester 20/21 | 7M1298 | Kardiologische Funktion |
| | 7M1292 | Bedside Teaching |
| | 7M1384 | PBL-Tutorials |
| Sommersemester 21 | 7M1298 | Kardiologische Funktion |
| | 7M1293 | Seminar kardiovaskuläres System |
| | 7M1292 | Bedside Teaching |
| Wintersemester 21/22 | 7M1293 | Seminar kardiovaskuläres System |
| | 7M1292 | Bedside Teaching |
| | 7M1298 | Kardiologische Funktion |
| Sommersemester 22 | 7M1292 | Bedside Teaching |
| | 7M1293 | Seminar kardiovaskuläres System |

## 9. Eidesstattliche Erklärung

Hiermit erkläre ich, Dominik Christian Schüttler, dass die schriftliche Habilitationsleistung selbständig verfasst wurde und die Herkunft des verwendeten oder zitierten Materials ordnungsgemäß kenntlich gemacht wurde. Zudem erkläre ich, dass außer dem derzeitigen kein weiteres Habilitationsgesuch im gleichen oder einem anderen Fach an der LMU München oder einer anderen Hochschule eingereicht wurde. Mir ist bisher kein akademischer Grad entzogen worden oder ein Verfahren gegen mich anhängig ist, welches die Entziehung eines akademischen Grades zur Folge haben könnte.

München, 05.03.2023 Dr. med. Dominik Schüttler