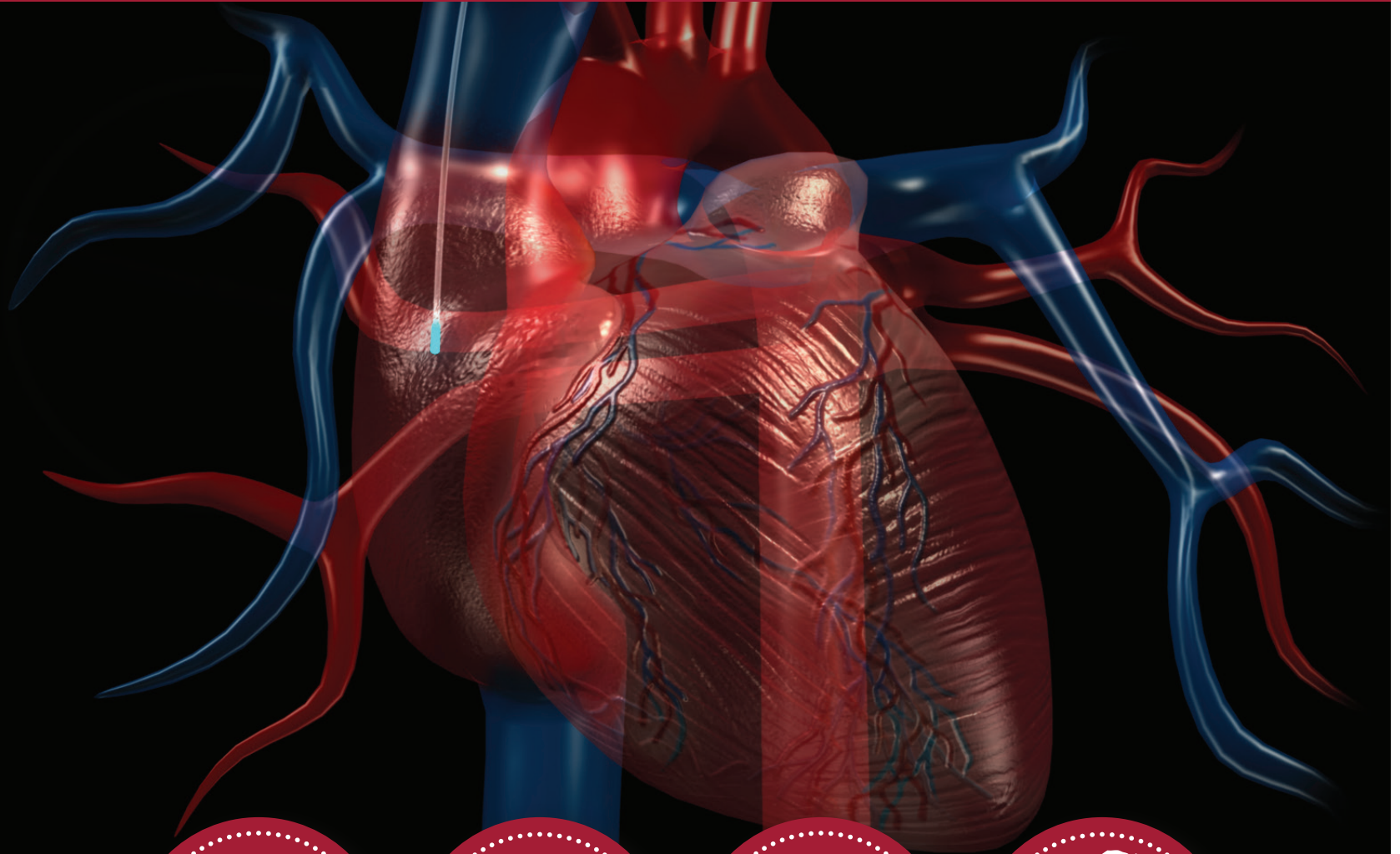


Advancing Research

A Review of Common Cardiovascular Biomarkers



A Review of Common Cardiovascular Biomarkers

Introduction

The cardiovascular system includes the heart and a complex network of vasculature which is influenced by cardiac output, humoral signaling, nervous system inputs, and other elements. As many factors play a role in the system's functionality, it is imperative for researchers to consider multiple cardiovascular biomarkers when trying to understand underlying molecular mechanisms and complete cardiovascular functionality. Despite advances in various in vitro and in silico experimental methods, in fields such as pharmacology, toxicology, and physiology it is acknowledged in vivo experimental models are required to understand the complete picture on how a new drug, medical device, or alternate therapy affects the body. This whitepaper provides an overview of common cardiovascular biomarkers using preclinical in vivo models including:

- Cardiac Contractility
- Heart Rate Variability
- Arrhythmia
- Systemic Blood Pressure
- Pulse Wave Velocity
- Baroreflex Sensitivity

Whole animal in vivo studies where subjects are unrestrained and unanesthetized provide unique insights into cardiovascular function. The biomarkers in this overview may be collected using technologies which enhance animal welfare and reduce signal artifact, ensuring the highest quality data.

Cardiac Contractility

Cardiac contractility is the force of cardiomyocyte contraction resulting from the interaction between actin and myosin at the cellular level. Contractility is a significant area of research due to its direct relationship to heart stroke volume (the amount of blood pushed out of the left ventricle during each heartbeat). Contractility is influenced by many factors, including the autonomic nervous system, afterload, preload, heart rate, or pharmacological drugs. As cardiac contractility plays a role in the body's cellular oxygenation, researchers continue to study

how intrinsic and extrinsic factors affect contractility, particularly when it comes to drug safety assessment. Current applications exploring cardiac contractility include:

- Heart Failure
- Cardiac Hypertrophy
- Cardiomyopathy
- Myocardial Infarction (MI)
- Hypertension
- Myocardial Ischemia
- Arrhythmias
- Drug Safety

Rodents, canines, and nonhuman primates remain the most common models used to evaluate cardiac contractility in whole animal studies. Left ventricular dP/dt_{max} is a common, robust, translatable, and sensitive indicator of changes in cardiac contractility. dP/dt_{max} measures the maximal change of left ventricular pressure (LVP) over time and presents as a wave form (see figure 1). LVP can be collected by implanting a pressure catheter into the left ventricle in both anesthetized and conscious animal models. Echocardiograms can also be used in conjunction with anesthetized models, but due to cost and expertise required, they remain a less accessible collection tool. Additional physiologic endpoints researchers typically collect with LVP include systemic blood pressure, electrocardiogram (ECG), temperature, and activity.

Heart Rate Variability

Heart rate variability (HRV) is the physiologic

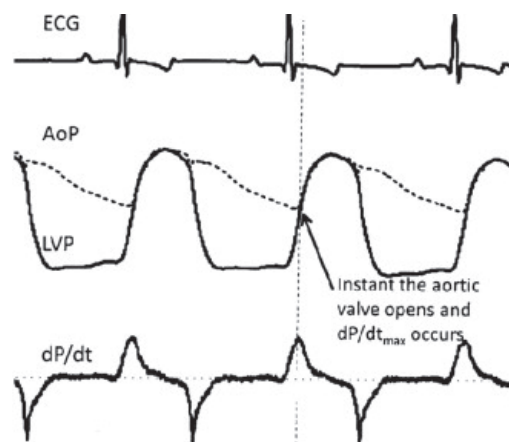


Figure 1: dP/dt indicates the change in pressure over time.¹

phenomenon of variation in the time interval between heartbeats and is measured by looking at variation in the beat-to-beat interval. Heart rate and blood pressure spontaneously fluctuate even while resting or during steady-state conditions. HRV allows observation of specific frequencies resulting from fluctuations and provides insight to autonomic function. HRV is one method used to help diagnose cardiovascular (myocardial infarction, congestive heart failure, coronary artery disease, hypertension) and non-cardiovascular diseases (stroke, diabetes, alcoholism, cancer, glaucoma, etc.). High HRV is an indication of healthy autonomic and cardiovascular response. Low HRV may indicate the sympathetic and parasympathetic nervous systems are not properly coordinating to provide an appropriate heart rate response.

HRV can be affected by the following factors:

- Reflexes (baroreceptors, chemoreceptors, cardiopulmonary receptors)
- Respiration
- Renin-angiotensin System
- Physical or Mental Stress
- Exercise
- Cardiovascular and Non-Cardiovascular Disease States
- Age
- Drugs (beta-blockers, atropine, glycosides, anesthetics, etc.)

HRV analysis requires a series of successive heart beat intervals. HRV is typically derived from the R-R intervals of ECG signals (as shown in figure 2) or inter-beat-intervals from systolic to systolic peaks of blood pressure signals.

Analysis methods for HRV data exist in the time-domain and frequency-domain. Each method of analysis is very different but contains a wealth of

information. Note, the quality of analysis results is highly dependent on the quality of the original data and performance in detecting cardiac cycles.

False detections or missed detections can have a profound effect on the results.

Arrhythmia

Arrhythmia is an irregular rate or rhythm disturbance in the heart's conduction system. Examples of acute causes of arrhythmia include stress, anxiety, or ingestion of stimulants. Examples of chronic comorbidities of

arrhythmia may include disease, shock, infection, or structural changes of the heart. Many arrhythmias are benign and have no clinical significance. However, some have serious implications and may lead to cardiac arrest or sudden death. Researchers are particularly interested in monitoring arrhythmias, due to changes in the incident rate or arrhythmia type which may indicate disease progression or treatment effect.

Arrhythmias are identified by recording and reviewing an ECG signal and assessing the rate, regularity, and morphology of the heartbeat. As the heart beats, cellular membrane polarity changes in the electrical conduction system throughout the heart, leading to depolarization and repolarization of atrial and ventricular cardiac cells by causing them to contract and relax. Figure 3 shows the conduction system

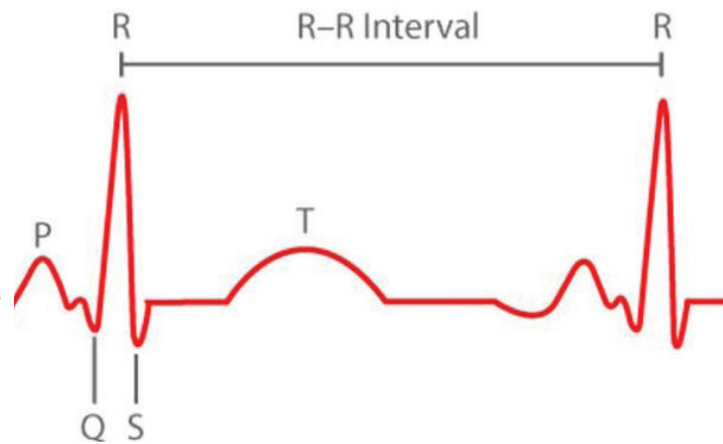


Figure 2: HRV may be calculated from the ECG's R-R Interval.²

The Cardiac Conduction System

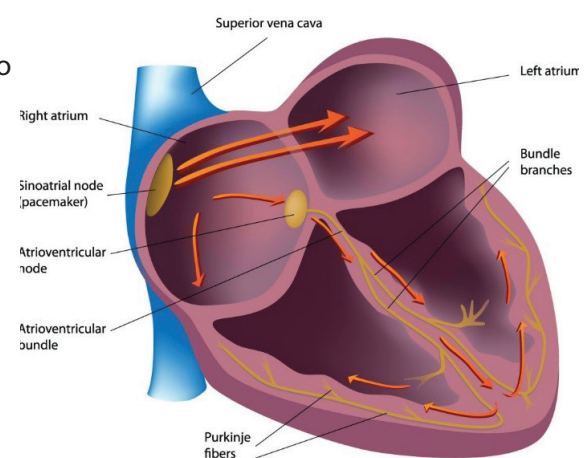


Figure 3: Conduction network of the human heart.³

of the heart. The cardiomyocyte chain reaction of depolarization ultimately facilitates the heart's ability to oxygenate blood and pump it throughout the arterial tree. This contraction (depolarization) and relaxation (repolarization) can be measured using electrodes placed in different combinations and configurations on the chest and limbs to produce a series of ECG complexes. An ECG complex is comprised of different waves which represent the electrical activity in specific regions of the heart.

Systemic Blood Pressure

Blood pressure is a surrogate endpoint in the clinic, meaning it is "a laboratory measurement or physical sign that is used in therapeutic trials as a substitute for a clinically meaningful end point that is a direct measure of how a patient feels, functions, or survives and is expected to predict the effect of the therapy".^{4,5} As blood pressure is a strong factor in many cardiovascular diseases, regulating it can improve the patient's overall well-being. Figure 4 shows a diagram of an aortic pressure waveform. Blood pressure is also a highly translatable endpoint from animal models to humans and is used in a wide variety of applications including the desire to understand mechanisms behind various cardiovascular diseases, stress responses, metabolic disorders, sleep apnea, drug safety evaluation, and more.

In 2005, the American Heart Association published a paper on recommendations for measuring blood pressure in both humans and animal models. These recommendations recognize telemetry as the most

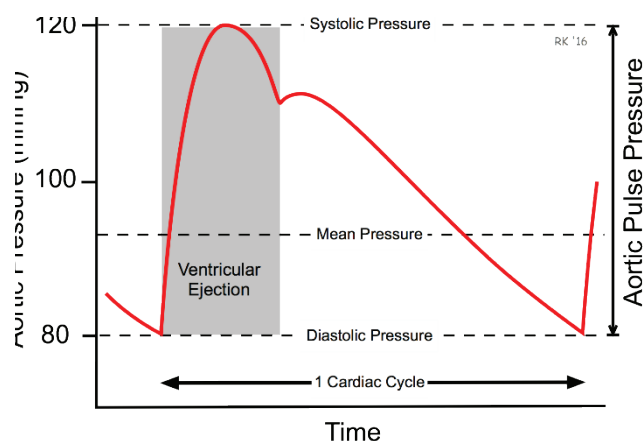


Figure 4: Diagram of aortic pressure waveform.⁶

reliable method for measurement in animal models over indirect methods of measurement, such as a tail cuff solution.⁷ In addition, the ICH S7a guidelines for safety pharmacology studies stress a preference for unrestrained, conscious methods, such as telemetry, for in vivo studies.⁸

Pulse Wave Velocity

Pulse Wave Velocity (PWV) is a measure of the rate at which pressure waves move down a blood vessel, and increased speed correlates with arterial stiffness. It has been established as a highly reliable indicator for cardiovascular morbidity and mortality in a variety of adult populations including older adults, patients with end-stage renal disease, diabetes, and hypertension.

During systole of the heart, contraction of the left ventricle and ejection of blood into the ascending aorta acutely dilates the aortic wall and generates a pressure wave which moves along the arterial tree. The velocity of this movement gives a measurement of arterial compliance. With age, or changes in the arterial wall, these vessels become stiffer and the speed at which pressure waves move through the system increases.

In addition, there are reflected pressure waves which move back towards the heart at the end of the systolic period. When pressure waves move faster through the arteries, the reflected waves will also move quicker. As higher systolic pressure is needed to overcome the afterload, the cardiovascular system must work harder.

PWV can be collected using two pressure catheters placed a known distance from one another, referred to as the Pulse Wave Distance. The time it takes the pressure wave to go from the upstream catheter

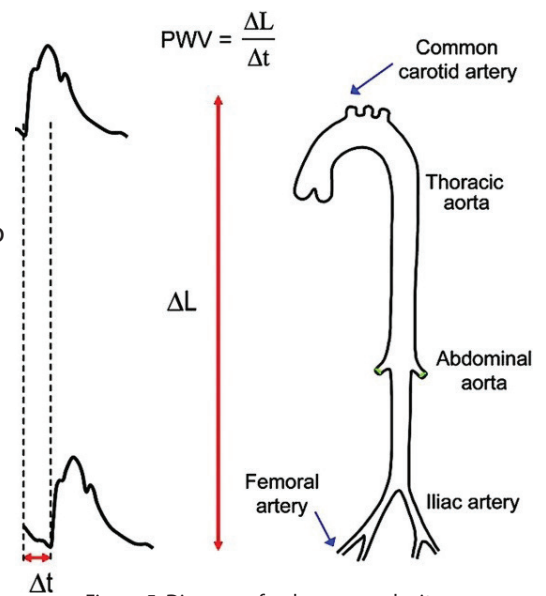


Figure 5: Diagram of pulse wave velocity measurement between carotid and femoral.⁹

to the downstream catheter provides the Pulse Transit Time (PTT). PWV can then be calculated by dividing distance by transit time, providing a measure of cardiovascular health. Figure 5 shows a diagram of pulse wave velocity measurement.

Baroreflex Sensitivity

Baroreflex is the fastest mechanism to regulate acute blood pressure changes by controlling heart rate, contractility, and peripheral resistance. Baroreceptors are mechanoreceptors located in the carotid sinus and aortic arch (as shown in figure 6). Their function is to sense pressure changes by responding to variations in tension of the arterial wall. The baroreflex mechanism is a fast response to changes in blood pressure. The baroreflex or baroreceptor sensitivity (BRS) index quantifies how much control the baroreflex has on the heart rate. BRS can be valuable in assessing the development and progression of cardiovascular diseases.

Reduced BRS can indicate:

- Neurological Disorders
- Hypertension
- Coronary Artery Disease
- Myocardial Infarction (MI)
- Heart Failure
- End-organ Damage
- Progression of Underlying Disease

BRS requires beat-to-beat information from both blood pressure and RR interval. Systolic blood pressure is typically derived from systemic arterial pressure, whereas the RR interval is derived from ECG. The spectral analysis method to assess baroreceptor sensitivity outputs the gain and phase of the transfer function. Gain corresponds to the effectiveness with which the baroreflex is able to maintain constant conditions. Phase is the time lag between systolic blood

pressure and RR.

Trusted Research Partner

Data Sciences International (DSI) has provided solutions to cardiovascular researchers for over 30 years and its PhysioTel™ blood pressure telemetry has been cited in over 5,000 publications. The PhysioTel telemetry platform is designed to monitor physiologic signals in conscious, freely moving animals ranging in size from mouse to primate. DSI offers the widest range of physiologic signal monitoring options, providing the flexibility needed to study cardiovascular diseases

in combination with comorbidities. The ability to measure various pressures (arterial, left ventricular, ocular, bladder, and intra-cranial), ECG, temperature, and activity is key in cardiovascular studies. For researchers looking to incorporate metabolic endpoints, DSI offers a solution to collect second-to-second measurements of blood glucose. Those interested in incorporating neuroscience endpoints have access to biopotentials including EEG, EMG, and EOG as well as sympathetic nerve activity which can be collected simultaneously with cardiovascular endpoints. Studies requiring respiratory endpoints can utilize telemetry or combine it with the Buxco® line of respiratory and inhalation solutions. In addition to the signal types listed here, DSI offers numerous derived parameters which can

be calculated from the signal waveforms. Examples of derived parameters include systolic, diastolic, and mean pressures.

Ponemah™ is a complete physiologic data acquisition and analysis software platform used by physiologists, pharmacologists, and toxicologists to confidently collect, accurately analyze, and quickly summarize cardiovascular study data. Ponemah's powerful tools simplify the analysis process and allow researchers to quickly analyze data to derive the biomarkers mentioned in this paper and many more. In addition, the Data Insights module automates recognition of patterns, such as arrhythmias, saving researchers valuable time by reducing the need to

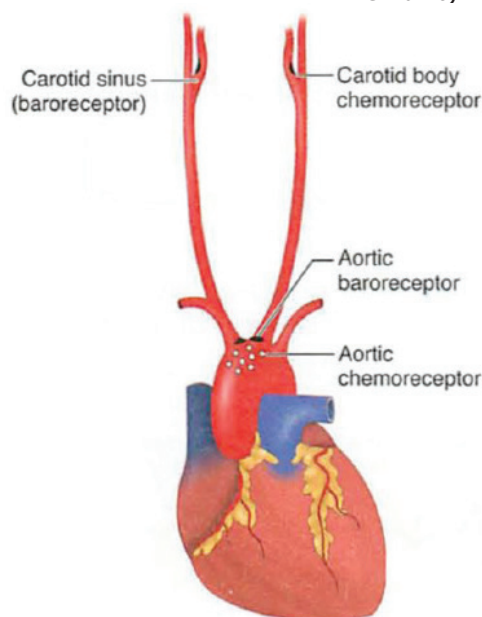


Figure 6: Diagram showing location of the carotid baroreceptors.¹⁰

manually read waveforms.

DSI assists researchers in streamlining studies by helping them make better informed decisions. The scientific services team can assist with improving success with implanting telemetry, executing preclinical in vivo studies, summarizing data for confidence in results, and meeting GLP validation requirements. DSI has a comprehensive services team to support researchers throughout their entire experience. Several training options are available including surgical implantation of telemetry, software use, and configuration of the system. In addition, researchers can take advantage of services such as having animals pre-implanted at DSI, installation of the system, analysis and reporting of data, and contracting the full study to DSI's services team.

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⁴Desai M, Stockbridge N, Temple R. (2006). "Blood Pressure as an Example of a Biomarker That Functions as a Surrogate". *American Association of Pharmaceutical Scientists Journal*. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2751433/pdf/12248_2008_Article_81146.pdf

⁵Temple R. (1998). "Are surrogate markers adequate to assess cardiovascular disease drugs?" *JAMA*. 282; 790-795. <https://jamanetwork.com/journals/jama/article-abstract/191287>

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⁹Ranjith R, Binu TG, George V, Madhu KG, Devika P, Subair K, Jayaram V, Mohanan KS, Radhakrishnan VV. (2014). "Aortic pulse wave velocity and its relationship with complexity of coronary artery disease based on SYNTAX score". *Heart Asia*. 6(1). <https://heartasia.bmj.com/content/6/1/109>

¹⁰Quizlet. (2019). "PBL: Acute control of blood pressure". <https://quizlet.com/164814823/pbl-acute-control-of-blood-pressure-flash-cards/>

Appendix: Publications Using DSI Technology

The following is an abbreviated list of peer-reviewed publications citing the use of DSI solutions in the above-mentioned applications.

Cardiac Contractility

Boulay E, Pugsley MK, Jacquemet V, Vinet A, Accardi MV, Soloviev M, Troncy E, Doyle JM, Pierson JB, Authier S. (2016). "Cardiac contractility: Correction strategies applied to telemetry data from a HESI-sponsored consortium." *Journal of Pharmacological and Toxicological Methods*. 87; 38-47. <https://doi.org/10.1016/j.vascn.2017.04.009>

Guth BD, Chiang AY, Doyle J, Engwall MJ, Guillon JM, Hoffmann P, Koerner J, Mittelstadt S, Ottinger S, Pierson JB, Pugsley MK, Rossman EI, Walisser J, Sarazan RD. (2015). "The evaluation of drug-induced changes in cardiac inotropy in dogs: Results from a HESI-sponsored consortium". *Journal of Pharmacological and Toxicological Methods*. 75; 70–90. <https://doi.org/10.1016/j.vascn.2015.02.002>

Chiang AY, Guth BD, Pugsley MK, Foley CM, Doyle JM, Engwall MJ, Koerner JE, Parish ST, Sarazan RD. (2018). "The evaluation of endpoint variability and implications for study statistical power and sample size in conscious instrumented dogs". *Journal of Pharmacological and Toxicological Methods*. 92; 43-51. <https://doi.org/10.1016/j.vascn.2018.02.009>

Pugsley MK, Guth B, Chiang AY, Doyle JM, Engwall M, Guillon JM, Hoffmann PK, Koerner JE, Mittelstadt SW, Pierson JB, Rossman EI, Sarazan DR, Parish ST. (2017). "An evaluation of the utility of LVdP/dt40, QA interval, LVdP/dtmin and Tau as indicators of drug-induced changes in contractility and lusitropy in dogs". *Journal of Pharmacological and Toxicological Methods*. 85; 1–21. <https://www.ncbi.nlm.nih.gov/pubmed/28065821>

Accardi MV, Troncy E, Abtout S, Ascah A, Maghezzi S, Authier S. (2016). "Rat cardiovascular telemetry: Marginal distribution applied to positive control drugs". *Journal of Pharmacological and Toxicological Methods*. 81; 120-127. <https://doi.org/10.1016/j.vascn.2016.03.005>

Heart Rate Variability

Cho JH, Zhang R, Aynaszyan S, Holm K, Goldhaber JI, Marbán E, Cingolani E. (2018). "Ventricular Arrhythmias Underlie Sudden Death in Rats With Heart Failure and Preserved Ejection Fraction". *Circulation: Arrhythmia and Electrophysiology*. 11(8). <https://doi.org/10.1161/CIRCEP.118.006452>

Fox BM, Becker BK, Loria AS, Hyndman KA, Jin C, Clark H, Johns R, Yanagisawa M, Pollock DM, Pollock JS. (2018). "Acute Pressor Response to Psychosocial Stress Is Dependent on Endothelium-Derived Endothelin-1". *Journal of the American Heart Association*. 7(4). <https://doi.org/10.1161/JAHA.117.007863>

Möller C, Maarten van Dijk R, Wolf F, Keck M, Schönhoff K, Bierling V, Potschka H. (2019). "Impact of repeated kindled seizures on heart rate rhythms, heart rate variability, and locomotor activity in rats". *Epilepsy & Behavior*. 92; 36-44. <https://doi.org/10.1016/j.yebeh.2018.11.034>

Arrhythmia

Cordes JS, Heyen JR, Volberg ML, Poy N, Kreuser S, Shoieb AM, Steidl-Nichols J. (2016). "Validation and utility of the PhysioTel Digital M11 telemetry implant for cardiovascular data evaluation in cynomolgus monkeys and Beagle dogs". *Journal of Pharmacological and Toxicological Methods*. 70; 72-79. <https://doi.org/10.1016/j.vascn.2016.01.006>

Liang D, Wu Y, Zhou L, Chen Y, Liu H, Xie D, Huang J, Zhang Y, Liu Y, Zhu W, Li J, Chen YH. (2019). "LRP5 controls cardiac QT interval by modulating the metabolic homeostasis of L-type calcium channel". *International Journal of Cardiology*. 275; 120-128. <https://doi.org/10.1016/j.ijcard.2018.06.029>

Mehendale C, Doyle JM, Kolin SM, Kroehle JP. (2016). "Unlock the information in your data: Software to find, classify, and report on data patterns and arrhythmias". *Journal of Pharmacological and Toxicological Methods*. 81; 99-106. <https://doi.org/10.1016/j.vascn.2016.05.007>

Systemic Blood Pressure

Collins T, Gray K, Bista M, Skinner M, Hardy C, Wang H, Mettetal JT, Harmer AR. (2018). "Quantifying the relationship between inhibition of VEGF receptor 2, drug-induced blood pressure elevation and hypertension". *British Journal of Pharmacology*. 175; 618-630. <https://bpspubs.onlinelibrary.wiley.com/doi/pdf/10.1111/bph.14103>

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Pulse Wave Velocity

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Baroreflex Sensitivity

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Yamamoto K, Eubank W, Franzke M, Mifflin S. (2012). "Resetting of the sympathetic baroreflex is associated with the onset of hypertension during chronic intermittent hypoxia". *Autonomic neuroscience : basic & clinical*. 173(1-2); 22-7. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3529839/>