



# USER'S GUIDE

# **Kubios HRV Scientific**

& Kubios HRV Scientific Lite

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# **Abbrevations**

AC Heart rate acceleration capacity (AC) index ACQ AcgKnowledge data file format (Biopac Inc.)

ANS Autonomic nervous system
ApEn Approximate Entropy

AR Autoregressive (model or process)

ASCII Text file using the ASCII character set, which is the most common format for English-

language text files

CSV Comma separated value (file format)
DC Heart rate deceleration capacity (DC) index

DFA Detrended fluctuation analysis

ECG Electrocardiogram

EDF European data format (file format)

EE Energy expenditure FFT Fast Fourier transform

GDF General data format (file format)

GUI Graphical user interface

HF High frequency (refers to HRV frequency band, by default 0.15-0.4 Hz)

HR Heart rate

HRV Heart rate variability

IBI Inter-beat-interval (same as RR interval)

LF Low frequency (refers to HRV frequency band, by default 0.04-0.15 Hz)

MAT MATLAB data file format (Mathworks Inc.)

MSE Multiscale entropy

NNxx Number of successive RR interval pairs that differ more than xx msec

PDF Portable document format (file format)

pNNxx Relative number of successive RR interval pairs that differ more than xx msec

PNS Parasympathetic nervous system

PPG Photoplethysmogram (measurement of blood volume changes)

QRS QRS complex of electrocardiogram

RESP Respiration rate

RMSSD Root mean square of successive RR interval differences

RPA Recurrence plot analysis

RR Time interval between successive ECG R-waves (RR interval, same as IBI)

SampEn Sample entropy ShanEn Shannon entropy

SDANN Standard deviation of the averages of RR intervals in 5-min segments

SDNN Standard deviation of normal-to-normal RR intervals

SDNNI Mean of the standard deviations of RR intervals in 5-min segments

SI Stress index

SNS Sympathetic nervous system

SPSS Statistical analysis software package(IBM Corp.)
TINN Triangular interpolation of normal-to-normal intervals

TRIMP Training impulse

VLF Very low frequency (refers to HRV frequency band, by default 0-0.04 Hz)

## 1 About Kubios HRV Scientific

Kubios HRV Scientific is a gold-standard heart rate variability (HRV) analysis software designed for research and professional use. Kubios HRV software is used at roughly 1800 universities in 149 countries. With Kubios HRV, you can turn your ECG or HR monitor into a powerful tool to probe the cardiovascular system or to evaluate the effect of stress and recovery on heart health. The software is suitable for clinical and public health researchers, professionals working on human well-being, or sports enthusiasts; for anybody who want to perform detailed analyses on heart rate variability, e.g. to examine autonomic nervous system function.

Kubios HRV software has been developed within the past 20+ years. The development started in year 2000 as academic research work at the Department of Technical Physics, University of Eastern Finland, Kuopio, Finland. Kubios Ltd. was founded in Jan 2016 and has continued the development of Kubios HRV software ever since. Kubios HRV software version history is summarized below.

► Kubios HRV (ver. 1.1 | Win | 2002): First release of Kubios HRV software, incl. RR data support and standard HRV analysis. Software description published in [20]

Niskanen J-P, Tarvainen MP, Ranta-aho PO, and Karjalainen PA. Software for advanced HRV analysis. *Comp Meth Programs Biomed*, 76(1):73-81, 2004

- ➡ Kubios HRV (ver. 2.0 | Win & Linux | 2008): Extended RR data support and nonlinear HRV analysis parameters
- ► Kubios HRV (ver. 2.1 | Win & Linux | 2012): ECG data support and QRS detector. Software description published in [29] Tarvainen MP, Niskanen J-P, Lipponen JA, Ranta-aho PO, and Karjalainen PA. Kubios HRV Heart rate variability analysis software. Comp Meth Programs Biomed, 113(1):210-220, 2014
- ➤ Kubios HRV (ver. 2.2 | Win, Linux & Mac | 2014): ECG derived respiration and input data support extended
- ➡ Kubios HRV Premium & Standard (ver. 3.0 | Win, Linux & Mac | Jan 2017): First commercial version, release notes available online for this version and later
- ► Kubios HRV Premium & Standard (ver. 3.1 | Win, Linux & Mac | Mar 2018)
- ➤ Kubios HRV Premium & Standard (ver. 3.2 | Win, Linux & Mac | Jan 2019)
- ➤ Kubios HRV Premium & Standard (ver. 3.3 | Win, Linux & Mac | Jun 2019)
- ➤ Kubios HRV Premium & Standard (ver. 3.4 | Win, Linux & Mac | Jun 2020)
- ➤ Kubios HRV Premium & Standard (ver. 3.5 | Win, Linux & Mac | Jul 2021)
- ► Kubios HRV Scientific (ver. 4.0 | Win, & Mac | Sep 2022)
- ➤ Kubios HRV Scientific (ver. 4.1 | Win & Mac | Oct 2023)

Kubios HRV is a scientifically validated software and the most commonly used HRV analysis software in scientific research (used in roughly 5900 publications). The key publications describing certain functionalities of Kubios HRV software products and providing validation results for our HRV pre-processing and analysis algorithms can be found at <a href="https://www.kubios.com/publications">www.kubios.com/publications</a>.

More information about Kubios HRV Scientific is available online:

**Product page** (software features and screenshots)

Full version: www.kubios.com/hrv-scientific Lite version: www.kubios.com/hrv-scientific-lite

Download page: www.kubios.com/download (download latest installers)

Release notes: www.kubios.com/release-notes (recent changes, new features and bug fixes)

**EULA:** www.kubios.com/hrv-scientific-license (end user license agreement)

FAQ: www.kubios.com/support (frequently asked questions)

About HRV: www.kubios.com/about-hrv/ (learn about HRV and its analysis)

**Want to stay updated about Kubios HRV products?** Please sign up for Kubios Newsletter. You can also follow us on Facebook, LinkedIn, Twitter, Instagram and YouTube.

1.1 System requirements

#### 1.1 System requirements

Kubios HRV is developed using MATLAB®¹ and is compiled to a standalone application with the Matlab Compiler. Therefore, Kubios HRV installers include the MATLAB Runtime (MATLAB Runtime is free and you don't need to have a Matlab license to use Kubios HRV). The latest installers for Kubios HRV can be downloaded from <a href="https://www.kubios.com/download">https://www.kubios.com/download</a>. System requirements for running Kubios HRV software are similar to those of Matlab (see <a href="https://www.mathworks.com/support/sysreq/">https://www.mathworks.com/support/sysreq/</a>) and only 64-bit operating systems are supported. Please note that correct version of MATLAB Runtime (included in the installer or available at Kubios download page) must be installed in order to run Kubios HRV.

**Windows (10 or 11, 64-bit)** operating system with 8 GB of RAM, 3-5 GB of disk space, screen resolution of 1024×768 or higher, and the MATLAB Runtime installation.

**Mac OS X (Big Sur, Monterey, or Ventura)** operating system with 8 GB of RAM, 3-5 GB of disk space, screen resolution of  $1024 \times 768$  or higher, and the MATLAB Runtime installation

#### 1.2 Installation

In order to run Kubios HRV, you need to install Kubios HRV (including MATLAB Runtime) on your computer. Installers can be download from <a href="https://www.kubios.com/download">https://www.kubios.com/download</a>. The first time you launch Kubios HRV, you will be prompted to sign in to your Kubios account and then to activate the software with your License key. This will link the license key to your Kubios account. If you don't already have a Kubios account, you need to register a new account. The license key has been emailed to you when you purchased Kubios HRV. A short description of the installation process at different operating systems is given below. Activation will link the license with your Kubios account and you will be able to use the software on any computer where you have Kubios HRV installed.

**Windows:** Make sure that you have administrative privileges (you will need them to install Kubios HRV). Download and run the Kubios HRV installer for Windows. This will install both Kubios HRV and Matlab Runtime on your computer (Note: Matlab Runtime is downloaded during the installation, unless a correct version of Matlab Runtime is already installed). You can launch Kubios HRV by using the Desktop icon (if created) or by selecting it from the Start Menu. Please note that Kubios HRV also starts the MATLAB Runtime and may take some time especially with older computers. The first time you start Kubios HRV, you also need to sign in with your Kubios account and activate the software using your personal license key.

**Mac OS:** Download and run the Kubios HRV installer for macOS. This will install both Kubios HRV and Matlab Runtime (Note: Matlab Runtime is downloaded during the installation, unless a correct version of Matlab Runtime is already installed) into separate folders under your Applications. To start Kubios HRV, you need to click the Kubios HRV application under the KubiosHRV folder in your Applications. You can also find Kubios HRV application with Spotlight (click  $^{\mathbf{Q}}$  on the upper-right corner of the menu bar or press Command+Space). NOTE: If you have an earlier version of Kubios HRV software installed in your Mac, we recommend that you uninstall the older version first by moving the installed Kubios HRV and earlier version of Matlab Runtime to trash.

#### 1.3 Uninstallation

**Windows:** The software can be uninstalled through "Windows Settings > Apps > Installed apps". The uninstaller does not remove your Kubios HRV preference files. If you also want the delete these, you can find them from the following folder: C:\Users\<username>\AppData\Roaming\Kubios\Kubios\KubiosHRVScientific

Mac OS: Move the installed applications (MATLAB Runtime and Kubios HRV application) to trash.

<sup>&</sup>lt;sup>1</sup>MATLAB®, The Mathworks, Inc.

# 2 Importing measurement data to the software

## 2.1 Kubios HRV mobile app

Kubios HRV mobile app and its custom measurement mode enables an easy approach for making various kinds of HRV measurements. The app is available for Android and iOS, and supports HR sensors providing beat-to-beat RR data. For longer term recordings we recommend that you use the Android app (in Android, the custom measurement runs on a background service with uninterrupted operation). The app is powered by Polar SDK, and thus we recommend to use the app with Polar sensors:

- Polar H10: RR, ECG and ACC data available in online mode. For longer term recordings, offline measurement where RR data is stored in the sensor's memory can be used
- Polar Verity Sense: PPI, PPG and ACC data available in online mode (offline mode not available).

To use the custom measurement mode, you need to sign in to the app with your Kubios account (the same account that you are using with Kubios HRV scientific). Follow these steps to make new custom measurements:

- 1. Connect your sensor with the app (in case of connection problems, please see the mobile app section at <a href="https://www.kubios.com/support/">https://www.kubios.com/support/</a>)
- Choose data channels that you want to record. When measuring with Polar H10, you will also have the option to perform an offline measurement, where the RR data is stored in the sensor and downloaded from the sensor when you finish the measurement.
- 3. Provide subject information when necessary. Managing of measurement subjects is easy and subject information is linked to the measurements that you make with the app.
- 4. Perform the measurement and save it. Please note that you can insert event markers with text label during online measurements.
- 5. Download your measurements from Kubios Cloud into your computer using Kubios Cloud sync (see Appendix B).

For more information about measuring with Kubios HRV app, please see the video tutorial available at https://youtu.be/28qjeTBCM1M.

\*Note: The custom measurement mode is not available for the Lite version users.

# CUSTOM ECG (mV) (mV) 3 5.00 5.02 5.04 5.06 5.08 00:05:10 Discard Save

# 2.2 Other supported data formats and devices

Kubios HRV software products are designed to work with a range of HR monitors, ECG devices, and PPG monitors. A detailed list of supported devices is provided at <a href="https://www.kubios.com/supported-devices/">https://www.kubios.com/supported-devices/</a>. Please note that the provided list of supported devices is not a complete list, several other devices may also be able to export the RR/IBI, ECG or PPG data in a format supported by the software. File formats supported by Kubios HRV software are listed in Table 1. Please contact our support (support@kubios.com) if you want to verify the compatibility of your device. \*Note: Please note that the Lite version supports only RR/IBI data. For analyzing raw ECG or PPG data, the full version is required.

For accurate HRV analysis, we recommend either an ECG device or a good quality heart rate monitor. PPG devices can also be used for HRV analysis, but the accuracy is not comparable to ECG based recordings (see <a href="https://www.kubios.com/hrv-time-series/">www.kubios.com/hrv-time-series/</a>). Binary file formats, such as EDF/EDF+ or ISHNE Holter ECG formats are recommended for storing raw ECG (or PPG) data. When these binary files are read to the software, Kubios HRV

automatically tries to determine the ECG (or PPG) channel from the channel labels. If the channel cannot be determined (or more than one channels are identified as the appropriate data channel), the software prompts the user to select the appropriate channel.

When using heart rate monitors such as Polar, Suunto or Garmin, **make sure that your device can measure and store RR intervals in beat-to-beat**. Please note that HR monitors typically store the RR data only when you use an external HR sensor (chest strap) during the measurement. If HR is measured from the wrist using the PPG sensor integrated to the sport watch, only averaged HR data, but not the beat-to-beat RR data, is typically stored. Averaged HR (e.g. HR at 1-sec or 5-sec intervals) cannot be used for HRV analysis. RR data is supported for example in FIT format as well as in simple text file formats (RR intervals provided inside a text file in msecs).

In addition to simple text file formats, a custom text file option is also provided. Using this option you can import text files including header lines and/or several data columns. Once you have selected an input file, an interface for importing the file into Kubios HRV is opened. This interface and the options that you need to set according to your data file are shown in Fig. 1.

Finally, the software supports also MATLAB MAT files saved by Kubios HRV. When you are using Kubios HRV, you can save the analysis session into a MATLAB MAT file as described in Section 5.4. The MAT files include all the analysis results and settings as well as the raw data (ECG, PPG or RR data). Therefore, you are able to reopen the analysis session by opening the saved MAT file again in Kubios HRV.

**NOTE:** Please note that Kubios HRV Scientific supports also analysis of animal HRV data. When analyzing animal HRV data, you need to choose the species from software preferences (see Section 6), which adjusts certain analysis parameters and pre-processing algorithms so that they are suitable for analyzing the HRV data of that animal. For details, see Appendix D.

**Table 1:** Data formats supported in Kubios HRV software.

	File format	File extension					
1.	Kubios KDF files (RR data only)	(*.kdf)					
2.	Polar CSV, TXT and HRM files (Polar Electro Ltd.)	(*.txt,*.hrm)					
3.	Suunto FIT files (Suunto Ltd.)	(*.fit)					
	Suunto SDF, STE and XML files (older formats)	(*.sdf,*.ste,*.xml)					
4.	Garmin FIT files (Garmin Ltd.)	(*.fit)					
5.	RR interval text files	(*.txt,*.dat,*.csv)					
6.	Custom text data files (RR data only)	(*.txt,*.dat,*.csv)					
*Not	*Note: File formats below are not supported in the Lite version.						
7.	Kubios KDF files (RR, ECG and PPG data)	(*.kdf)					
8.	Direct export from Polar Flow (in FIT format)	(*.fit)					
9.	ECG and PPG data text files	(*.txt,*.dat,*.csv)					
10.	Custom text data files (RR, ECG and PPG data)	(*.txt,*.dat,*.csv)					
11.	Biopac ACQ3 files (Biopac Systems Inc.)	(*.acq)					
12.	Cardiology XML files	(*.xml)					
13.	European data format (EDF and EDF+) files	(*.edf)					
14.	General data format (GDF) files	(*.gdf)					
15.	ISHNE Holter ECG data format files	(*.ecg)					
16.	Physionet MIT files	(*.hea, *.dat)					
17.	Zephyr BioHarness ECG and RR data files	(*.csv)					
18.	Kubios HRV Matlab MAT files	(*.mat)					

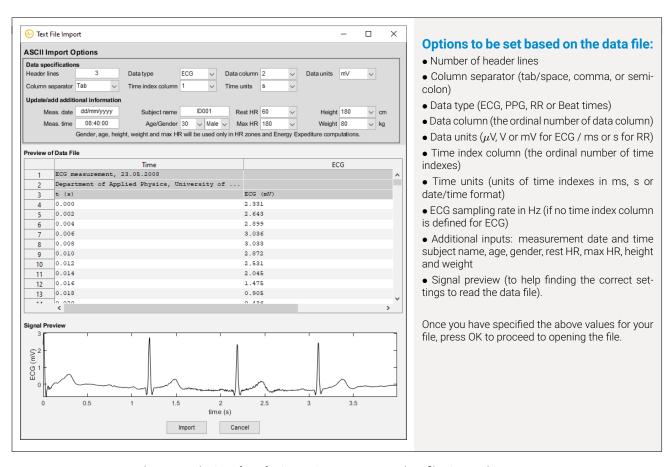


Figure 1: The interface for importing custom text data files into Kubios HRV.

# 3 Beat detection and pre-processing

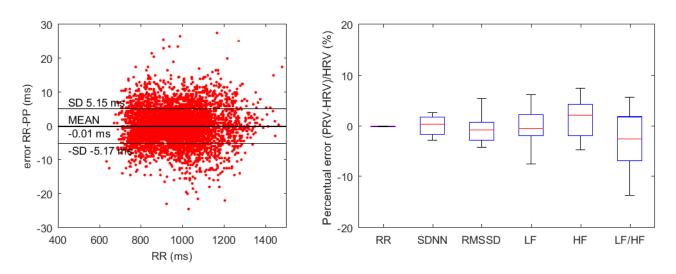
## 3.1 Beat detection algorithms

Kubios HRV Scientific has a built-in accurate QRS and pulse wave detectors for detecting heart beats from ECG and PPG data, respectively. \*Note: Beat detection is not available in the Lite version which supports only IBI data.

In case ECG data is imported into Kubios HRV Scientific, the R-wave time instants are automatically detected by applying the built-in QRS detection algorithm. This in-house developed detection algorithm is based on the Pan-Tompkins algorithm [23]. The detector consists of a preprocessing part followed by decision rules. The preprocessing part includes bandpass filtering of the ECG (to reduce power line noise, baseline wander and other noise components), squaring of the data samples (to highlight peaks) and moving average filtering (to smooth close-by peaks). The decision rules include amplitude threshold and comparison to expected value between adjacent R-waves. Both of these rules are adjusted adaptively every time a new R-wave is acceptably detected. Before R-wave time instant extraction, the R-wave is interpolated at 2000 Hz to improve the time resolution of the detection. This up-sampling will significantly improve the time resolution of R-wave detection when the sampling rate of the ECG is relatively low (200 Hz or higher recommended).

The pulse wave detector is based on matched filtering approach. Firstly maximum of 1st derivative representing the steepest part of the pulse wave is used for initial pulse location estimation. Secondly, template for the pulse wave (and matched filter) is constructed using the initial pulses. Decision of the final pulse wave locations are defined by comparing the filtered signal against varying threshold and comparing normalized error between the template and PPG signal. Allowed normalized error between template and pulse wave under inspection can be adjusted in software preferences. That is, the smaller the acceptance threshold percent is the more similar the pulse wave have to be with the template in order to be accepted.

The accuracy of the pulse wave detection algorithm is shown in Fig 2. The left panel showing the Bland-Altman plot illustrating the agreement between detected PP intervals and corresponding RR intervals during a resting measurement. The right panel shows error percentages of commonly used HRV parameters estimated from PP interval compared to RR interval time series. Used dataset contains 20 healthy volunteers with wide age scale (20 to 50 years). Error between the RR and PP time series is  $-0.01\pm5.16$  ms (mean  $\pm$  SD). This  $\pm5$  ms error in heart beat detection produces approximately  $\pm10\%$  maximum errors to the HRV parameters.



**Figure 2:** Accuracy of the PP interval vs. RR interval during resting measurement is presented on left panel. In right panel, errors between the PRV and HRV parameters are presented. Blue box indicates region between the 25-75 percentile and black lines are maximum and minimum value.

#### 3.2 Noise segments

Kubios HRV Scientific software supports noise detection, which automatically identifies noise segments based on the raw ECG data (if available) and from the interbeat interval data (RR or pulse-to-pulse intervals). The noise

detection level can be changed between Very low, Low, Medium and Strong (default Medium) and you can turn it off by switching it to None level. Automatic noise detection is extremely helpful for those analyzing long-term ambulatory ECG, PPG or RR recordings, where signal quality is not good throughout the recording. Please note that noise detection aims to identify noise segments which distort several consecutive beat detections, and thus, would affect on HRV analysis accuracy. Individual intermittent abnormal beat intervals (e.g. ectopic beats) are not typically marked as noise, since these can be reliably corrected by the automatic beat correction (which is by default performed after noise detection). All segments marked as noise will be excluded from HRV analysis. Please note that you can also manually edit the noise segment markings if necessary, see Fig. 3.

\*Note: Noise detection is not available in the Lite version.

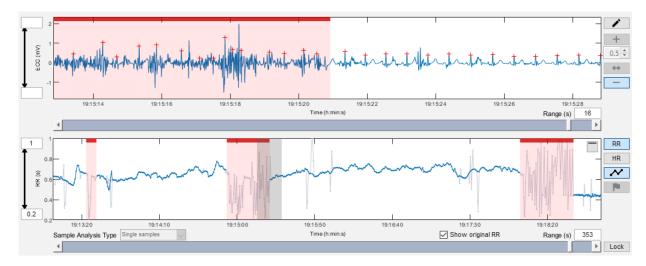


Figure 3: Illustration of noise segment markings in Kubios HRV Scientific software. Noise segments can be manually edited from ECG or RR data axes with mouse (right-click to remove an existing noise marking, CTRL + left mouse button (Win) or Command + left mouse button(macOS) to mark/unmark noise).

#### 3.3 **Beat correction algorithms**

Artefacts in the IBI time series can cause significant distortion to HRV analysis results, and thus, all artefacts should be either corrected or excluded from analysis as recommended also in [31]. Typical artefacts include missing, extra or misaligned beat detections as well as ectopic beats such as premature ventricular contractions (PVC) or other arrhythmias.

Kubios HRV includes two methods for correcting artefacts and ectopic beats present in the IBI data: 1) Threshold based correction, in which the artefacts and ectopic beats are simply corrected by comparing every RR interval value against a local average interval; 2) Automatic correction, in which artefacts are detected from a time series consisting of differences between successive RR intervals. The automatic correction is more accurate and the method has been validated in [14]. More information about the beat correction algorithms is available at www.kubios.com/hrv-preprocessing/.

In addition to these data driven correction methods, the beat detections can be corrected manually when raw ECG or PPG data is used for analysis (see Section 4.2).

\*Note: Automatic beat correction is not available in the Lite version.

#### 3.4 IBI time series trend removal

Another common feature that can alter the analysis significantly are the slow linear or more complex trends within the analyzed time series. Such slow nonstationarities are characteristic for HRV signals and should be considered before the analysis. The origins of nonstationarities in HRV are discussed, e.g., in [3]. Two kinds of methods have been used to get around the nonstationarity problem. In [33], it was suggested that HRV data should be systematically tested for nonstationarities and that only stationary segments should be analyzed. Representativeness of these segments in comparison with the whole HRV signal was, however, questioned in [9]. Other methods try to remove the slow nonstationary trends from the HRV signal before analysis. The detrending is usually based on first

3.4 IBI time series trend removal

order [15, 18] or higher order polynomial [26, 18] models. In addition, Kubios HRV software includes an advanced detrending method originally presented in [30]. This method is based on smoothness priors regularisation and is suitable also for more complex trends of the time series. The method works like a time-varying high-pass filter, smoothing the data according to the value of the smoothing parameter. The bigger the smoothing parameter is the lower is the cutoff frequency of the filter. We recommend using the smoothness priors method for removing IBI time series nonstationarities. The cutoff frequency should be below the low frequency band (< 0.04 Hz) not to remove any parts of the normal short term HRV.

#### 4 Kubios HRV user interface

In this section, the graphical user interface (GUI) of Kubios HRV analysis software is explained. Please note that functionality available in the GUI may depend on the version you are using. All the screen captures shown in this documentation are taken from the Kubios HRV Scientific (version 4.1.0).

The user interface window of Kubios HRV is shown in Fig. 4. The user interface is divided into three segments: 1) the RR interval series options segment on the top left corner, 2) the data browser segment on the top right corner, and 3) the results view segment on the bottom. These segments are described in Sections 4.1, 4.2 and 4.3, respectively.



Figure 4: The graphical user interface of Kubios HRV analysis software.

#### 4.1 RR interval series options

The RR interval series options shown in Fig. 5 include three functions: 1) *Noise detection*, 2) *Beat correction*, and 3) *Samples for analysis*. The purpose of noise detection is to mark time periods where data is too noisy to be analyzed. You can adjust the automatic noise detection level (the default level can be changed from preferences) and you can also manually edit noise segment markings. Time periods marked as noise are excluded from HRV analysis (see Section 3.2). The beat correction options can be used to correct artifacts from a corrupted RR interval series. The user can select between two methods: 1) Automatic correction and 2) Threshold correction. The automatic correction is an accurate algorithm for detecting artifacts (missed, extra and misaligned beat detections) as well as ectopic beats. The threshold correction simply compares every beat interval against a local mean RR, and identifies the beat as artifact if it exceeds the specified threshold. The threshold should be selected individually, because normal variability in RR intervals can be quite different between individuals, and therefore, a fixed threshold could over or under correct the RR data. For details on these correction algorithms, see Section 3.3.

When using any of the beat correction methods, Kubios HRV displays on the RR data axis the corrected RR interval time series in blue and original uncorrected data in light grey as shown in Fig. 6 A. In this case, the RR interval data

4.2 Data browser

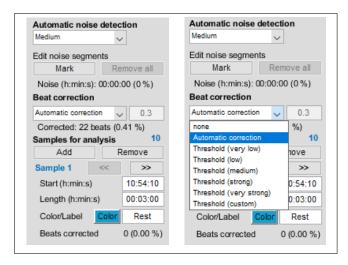


Figure 5: The RR interval series options segment of the user interface.

includes two clear artifacts: 1) a simulated ectopic beat at 10:13:40 (short interval followed by longer interval) and 2) a simulated missed beat detection at 10:15:30, which are both nicely corrected by using the automatic correction. The number of corrected artifacts in the whole recording is given under the Artifact correction controls and the number of artifacts corrected from the analysis sample is given under Samples for analysis controls. A piecewise cubic spline interpolation method is used in the corrections.

The importance of artifact correction is highlighted in Figs. 6 B-C. Please observe that having only two artifacts within the 5-min analysis segment has a significant effect on the time-domain HRV parameters, especially on SDNN, RMSSD and TINN. Thus, even single artifacts should always be either corrected or excluded from analysis. On the other hand, the number of corrected beats should not be too high (preferably <5%) not to cause significant distortion (suppressed variability) to analysis results. Finally, if ECG is measured, you should first try to directly correct the R-wave detections shown in the ECG data axis as described in Section 4.2.

Using the Samples for analysis options, you can add as many analysis samples that you want (the number of samples is not limited) and you can easily modify the positioning and length of these samples. You can edit the samples using the Add (adding a new sample), Remove (removing the active sample), Start (changing sample onset), Length (changing sample length), Sample Label (an illustrative name for the sample), and Sample Color (e.g. color-code different types of samples) controls. In addition, you can also edit the samples using mouse directly on the RR data axis as described in Section 4.2. Sample Label and Color are optional. By default, Kubios HRV computes analysis results for every selected sample, but you can also choose to merge all samples into one longer sample before analysis using the Sample analysis type control under the RR data axis.

NOTE: In Kubios HRV Scientific, you can automatically generate analysis samples by editing desired sample information within a structured CSV file ("Kubios\_Samples.csv"). The file can be generated from Tools menu according to active analysis samples, but you can also find a template file under the Sample Data folder. In the CSV file you can define Sample Label, Color, Start time and End time for as many samples as necessary. Analysis samples can be defined differently for every file in the folder or you can also define general sample definitions that will be used for every file in the folder. More information about editing the CSV file is provided on the file header. If the CSV file is placed in the same folder with the data files and sample definitions for a given data file are found, the analysis samples will be automatically imported into Kubios HRV. This feature will make analysis of larger data sets with known sample times much easier.

**\*Note:** Noise detection, automatic beat correction, and automatic analysis sample generation are not supported in the Lite version.

#### 4.2 Data browser

The data browser segment shown in Fig. 7 displays the measured ECG signal and the extracted RR interval series. If RR interval data is given as input (no ECG data), then only the RR data axis will be displayed in a bigger size. The ECG and RR interval data axes can be controlled by using Sliders (scrolling the data), Range edit boxes (to change data range shown on the axes), and Y-limit edit boxes (to change the y-axis scaling). The range of the ECG axis is displayed as a grey patch on the RR axis, which can be moved with left mouse button. ECG and RR axes can also

4.2 Data browser



**Figure 6:** RR interval artifact correction. A) The artifact corrected series is visualized on top of the raw RR interval series, summary of corrected beats within the recording is given on the right side of RR data axis. Time-domain analysis results B) before artifact correction and C) after the artifacts have been corrected.

be locked (Lock button) and scrolled together.

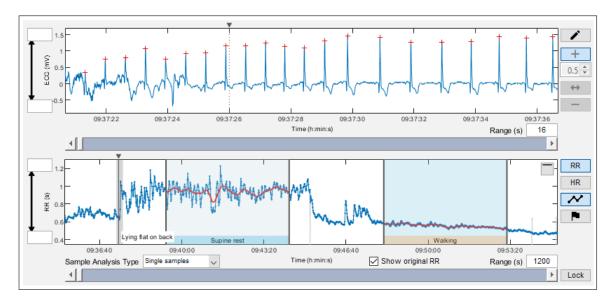


Figure 7: The data browser segment of the user interface.

The main functionalities of the data browser segment are described below.

**Correcting beat detections** – If ECG (or PPG) data is available, each detected beat is marked in the ECG (or PPG) axis with a "+" mark. Beat detection markers can be easily edited using the tools on the right-hand side

of the ECG (or PPG) axis. The button actions (from top to bottom) are as follows:

 Turn on/off the beat detection editing mode. When editing beat detections, HRV analysis results are not updated (auto-refresh results turned off) to guarantee smooth editing. When exiting the beat editing mode, any changes made in beat detection become effective and analysis results are updated accordingly.

- Add (shortcut "a") beat detection markers for missed beats.
- Adjust beat detection marker snapping window width (shortcut SHIFT+Scroll) when in Add mode.
- Move/shift (shortcut "s") existing beat detection markers for misaligned beat detections.
- Remove/delete (shortcut "d") beat detection markers for extra beat detections.

**Data markers** – Event markers stored in the recording (if any) are shown both at top of ECG (or PPG) and RR interval data axes as small black triangles. On mouse-over, the text of the marker is displayed. Analysis samples snap to event markers, to ease-up analyzing the data according to available markers. By clicking the "\mathbb{"}" button on the right, you can choose which markers are displayed.

**Extra data** – Extra data channels visualization is supported for certain data files (KDF, FIT and EDF formats). When you have opened such a file for analysis, you can choose to visualize on the ECG data axes any of the channels available in the data file. For example, if your EDF file includes (in addition to raw ECG data) acceleration data for X, Y and Z coordinates, you can choose to view the acceleration on the data axes to guide your HRV analysis.

**RR data plot options** – From the right side of the RR interval axis, you choose if the data axes shows RR interval or HR values, and if the data is plotted as a dotted line or normal line ("""). Also, from the button on top-right corner, you can choose to enlarge the RR data axis.

#### Mouse gestures/shortcuts

- Pan (scroll, SHIFT + scroll) and zoom (Win: CTRL + scroll, macOS: Command + scroll) data on RR and ECG data views by using mouse scroll wheel.
- Pan both RR and ECG data views by dragging with middle mouse button.
- Add a new analysis sample or noise segment (Win: CTRL + left mouse button + drag, macOS: Command + left mouse button + drag).
- Delete analysis sample or noise marking, or duplicate an analysis sample (right click to open menu).
- Move or resize analysis samples by dragging them with left mouse button from the middle of the sample (to move) or from the left or right edge (to resize).
- Center ECG data view to current cursor position in RR data view (Shift + left mouse click).

\*Note: Beat detections and extra data channels are not available in the Lite version which supports only IBI data.

#### 4.3 Results view

The results for the selected RR interval sample are displayed in the results view segment (see Fig. 4, which shows results for a maximal cardiopulmonary exercise test). The results are divided into

- Results overview
- 2. Time-domain HRV parameters
- 3. Frequency-domain HRV parameters
- 4. Nonlinear HRV analysis parameters
- 5. Time-varying analysis results
- 6. ECG waveform analysis parameters

The results of each category are displayed by pressing the corresponding button on the top of the results view segment. The results are by default updated automatically whenever analysis samples or analysis settings are changed. The processing time required increases as function of measurement length and number of analysis samples. In case of lack of usability, you can disable the automatic update by unchecking the "Auto-refresh" check box on top of the results view segment. This will prevent the software from updating the analysis results, until you press the "Refresh" button.

\*Note: Time-varying analysis and ECG waveform analysis are not available in Lite version.



#### 4.3.1 Results overview

The results overview section shown in Fig. 8 displays 1) a comparison between HRV parameters of selected analysis segment and normal resting values and 2) an overview of recording. The normal values for the HRV parameters (Mean RR, Mean HR, RMSSD, SD1 (%) and SD2 (%)) are obtained or derived from the quantitative systematic review by Nunan et al. 2010 [21]. The Stress index is the square root (to make the index normally distributed) of the Baevsky's stress index proposed in [1] and values of Baevsky's stress index between 7 and 12 are considered normal. The six HRV parameters divided into those reflecting parasympathetic nervous system (PNS) tone (Mean RR, RMSSD and SD1 (%)) and those reflecting sympathetic nervous system (SNS) tone (Mean HR, Stress index and SD2 (%)) are illustrated on top of the normal value distributions. These graphs give a quick view about the level of subject's HRV with respect to normal values. NOTE: the normal values are from rest measurements, thus exercise or stressful situations are expected to produce higher heart rate and lower HRV. PNS and SNS indexes are computed to provide an overall measures of these nervous system activities as compared to normal resting values (read more: www.kubios.com/hrv-ans-function).

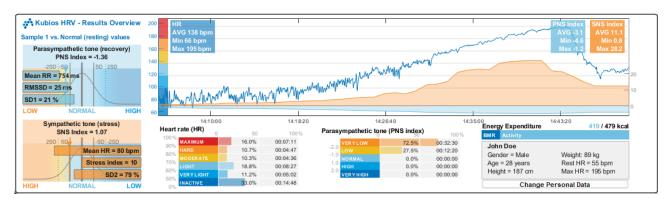


Figure 8: Results overview segment of Kubios HRV.

The overview of the recording includes an illustration of HR, PNS index and SNS index time trends; HR zones graph (time spent in each zone); PNS index zones (time spent in each zone); and energy expenditure (basal metabolic rate and activity related energy expenditure). These information are also provided in the time-varying report sheet (see Section 5.2), where also SNS index zones are illustrated. HR, PNS index and SNS index zones are defined as follows:

	HR zones		PNS zones		SNS zones
	(of HR <sub>max</sub> )				
MAXIMUM:	90-100%	VERY LOW:	< -2	VERY HIGH:	> 2
HARD:	80-90%	LOW:	-21	HIGH:	$1 \dots 2$
MODERATE:	70-80%	NORMAL:	$-1 \dots 1$	NORMAL:	$-1 \dots 1$
LIGHT:	60-70%	HIGH:	$1 \dots 2$	LOW:	-21
VERY LIGHT:	50-60%	VERY HIGH:	> 2	VERY LOW:	< -2
INACTIVE:	<50 %				

The total energy expenditure is divided into 1) Basal metabolic rate (BMR) and 2) Activity related energy expenditure (EE). Energy expenditure (EE) is computed using the Keytel's model without a measure of fitness ( $VO_{2,max}$ ) according to [13]. The BMR is estimated using the Mifflin-St Jeor equations [17], which have been found to be the most accurate. The energy expenditure computations are based on heart rate, body weight, height and age, which can be defined in Preferences. In Kubios HRV Scientific, these settings can also be changed by clicking the Change Personal Data button (to enable a quick way to update personal details for the current recording).

\*Note: In the Lite version, the time trends shown in the overview are computed using defaults settings since time-varying analysis is not enabled.

#### 4.3.2 Time-domain results view

The time-domain results view shown in Fig. 9, displays the time-domain HRV parameters in a table on the left. The two figure on the right show the RR interval histogram and HR deceleration capacity (DC) and acceleration capacity (AC) graphs. All HRV parameters are calculated from the detrended RR interval data (if detrending is applied), but mean RR, mean HR as well as min and max HR values are exceptions (marked with the \* symbol). In the edit boxes below the RR histogram, you can define fixed lower and upper limits for RR values. These limits

are saved in software preferences, so you only need to enter them once. These limits have effect on how the RR histogram is displayed, not only in the results view segment but also in the report figure described in Section 5.2. If you leave the edit boxes empty, the histogram is auto-scaled according to the minimum and maximum values of the data. \*Note: HR deceleration and acceleration capacities are not available in the Lite version.

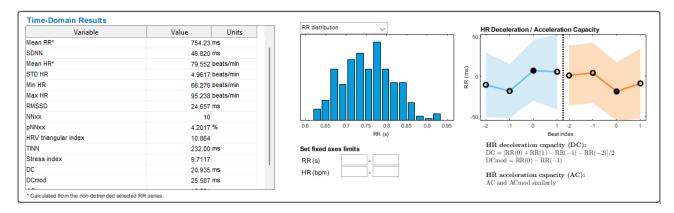


Figure 9: Time-domain HRV analysis results view of Kubios HRV software.

#### 4.3.3 Frequency-domain results view

The frequency-domain results view shown in Fig. 10, displays the results for both FFT and AR spectrum estimation methods. Both methods are applied to the detrended RR series. The spectra of the two methods are presented in the two axes (FFT spectrum on the left and AR spectrum on the right). Please note that you can choose in software preferences (see. Section 6) to use Lomb-Scargle periodogram instead of FFT based Welch's periodogram. The frequency axes of the spectra are fixed to range from 0 Hz to the upper limit of HF band plus 0.1 Hz. Thus, for the default frequency band settings the frequency axis range is 0–0.5 Hz. The results for both spectra are displayed in the table on the left. An estimate of respiration rate (RESP) is also computed from the ECG (if available) and RR data. Respiration rate is shown as a vertical line in both spectrum estimates and the numeric value is shown below the spectrum Y-limit options. \*Note: Lomb-Scargle periodogram and RESP are not available in the Lite version.

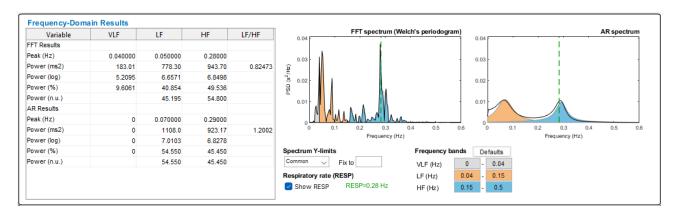


Figure 10: Frequency-domain HRV analysis results view of Kubios HRV software.

The frequency-domain results view includes the following settings. The power axes limits, can be adjusted with the options below the spectrum axes. The power axes can be selected to have either common (same limits for FFT/Lomb and AR spectra) or separate upper Y-limits. If common Y-limit is selected, it can also be entered manually into the edit box beside the selection button. The selected power axis options apply also for the report sheet. Below the spectrum Y-limits options, there is a checkbox, which can be used to show/hide the respiration rate (RESP). In addition, you can find settings for the very low frequency (VLF), low frequency (LF), and high frequency (HF) bands limits. The default values for the bands are VLF: 0-0.04 Hz, LF: 0.04-0.15 Hz, and HF: 0.15-0.4 Hz according to [31]. The default values for the bands can be restored by pressing the Defaults button. Adjustments to the frequency bands here apply only for the current session, if you want to change these settings permanently, you need to do it by editing software preferences (see Section 6).

#### 4.3.4 Nonlinear results view

The nonlinear results view shown in Fig. 11, displays all the calculated nonlinear variables in one table. The Poincaré plot and the DFA results are also presented graphically in the two axes. In the Poincaré plot (left hand axis), the successive RR intervals are plotted as blue dots and the SD1 and SD2 variables obtained from the ellipse fitting technique are presented. In the DFA plot (right hand axis), the detrended fluctuations F(n) are presented as a function of n in a log-log scale and the slopes for the short term and long term fluctuations  $\alpha_1$  and  $\alpha_2$ , respectively, are indicated. \*Note: Correlation dimension ( $D_2$ ), Recurrence Plot Analysis (RPA), and Multiscale entropy (MSE) are not available in the Lite version.

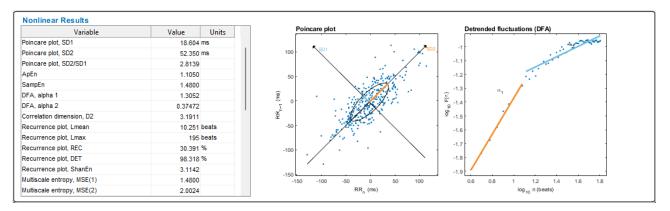


Figure 11: Nonlinear HRV analysis results view of Kubios HRV software.

#### 4.3.5 Time-varying results view

The time-varying results view shown in Fig. 12, displays the time-varying trend of the selected variables. Time-varying analysis is by default applied to the whole measurement, but you can also change Preferences to perform time-varying analysis for every analysis sample (see Section 6). You can either view two selected HRV variables or the time-varying spectrum. Plot style of selected variables can be changed between line plot and filled patch plot from the toggle buttons. You can also choose if you want a background grid and data point markers to be plotted (checkboxes below variable selections). When the time-varying spectrum is selected for view, a color bar indicating the power values is also shown on the right. The color map of the spectrum can be changed with the Color map dropdown button. The adjustable options for the time-varying analysis include the window width and grid interval for the moving window, which is used to calculate the results. \*Note: Time-varying analysis is not available in the Lite version.

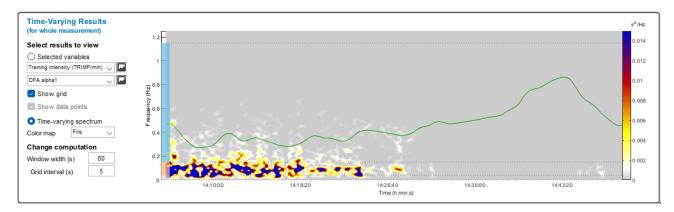


Figure 12: Time-varying HRV analysis results views of Kubios HRV software.

4.4 Menus and toolbar buttons

#### 4.3.6 ECG waveform analysis

The ECG waveform analysis results view in presented in Fig. 13. Please note that ECG waveform analysis is intended only for research purposes, not for clinical use. Furthermore, this analysis module is available only if you have imported raw ECG data into Kubios HRV and if the ECG waveform analysis feature is turned on in software preferences. ECG waveform analysis is carried out for every analysis sample and the results of the currently selected sample are illustrated. ECG waveform analysis results include P-wave and QRS-complex duration, PQ and QT/QTc times, and P, Q, R, S and T wave amplitudes. Parameters are computed from an averaged ECG waveform. The criteria deciding which beats are chosen for calculation of the average waveform can be adjusted in software preferences (see. Section 6). If the ECG amplitude is not correctly scaled (not in millivolts), a scaling factor can be manually entered to fix this. ECG fiducial points (P onset and end, Q onset, R peak, S end, and T end) are automatically detected, but may need to be manually corrected especially if other than V2 lead is used for the analysis. \*Note: ECG waveform analysis is not available in the Lite version.

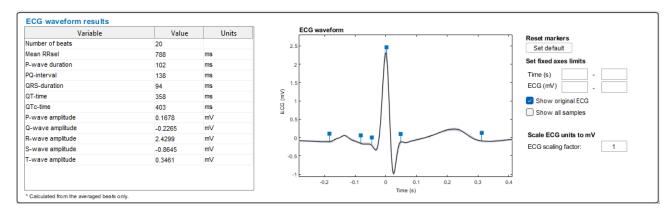


Figure 13: ECG waveform analysis results view of Kubios HRV software.

#### 4.4 Menus and toolbar buttons

The user menus and toolbar buttons are located on the upper left hand corner of the user interface. There are all together four user menus and eight toolbar buttons. The toolbar button icons and their actions are explained below

- Open new data file button is for opening a new data file for analysis. If the results of the current analysis have not been saved, user is prompted to do so.
- **Save results** button is for saving the analysis results. The results can be saved in ASCII, PDF, and MATLAB MAT file format (see Section 5 for details).
- Append results to "SPSS friendly" batch file button is for adding the current analysis session results into an existing (or creating a new) "SPSS friendly" batch file (see Section 5.5 for details).
- HRV report button opens the HRV analysis report (see Section 5.2 for details).
- **Training report** button opens the training data analysis report (see Section 5.7 for details).
- **ECG waveform report** button opens the ECG waveform analysis report (see Section 5.3 for details).
- **ANS function report** button opens the autonomic nervous system function report (see Section 5.8 for details).
- **Edit preferences** button opens a preferences window in which you can, e.g., change the default values for analysis options (see Section 6 for details).
- **About Kubios HRV** button opens the about dialog including information about the software version.
- **Zoom in/out** buttons can be used to zoom in or out on the ECG and RR data axes (please note that in other axes zooming is not enabled). You can also quickly zoom these axes using mouse (Win: CTRL + scroll, macOS: Command + scroll)
- **Show/hide markers** button toggles between showing and hiding event marker time points over the ECG and RR axes.

4.4 Menus and toolbar buttons

- **Show/hide marker lines** button toggles between showing and hiding event marker vertical dashed lines over the ECG and RR axes.
- Navigate markers buttons can be used to navigate to first, previous, next and last marker, respectively (if several markers).
- **Close file** button closes the current data file. If the results of the current analysis have not been saved, user is prompted to do so.

All the above actions are also available on the user menus. The **File menu** includes Open, Save Results, Save Results As, Append Results to "SPSS friendly" Batch File, Edit Preferences, Close, Kubios user account options (manage your account or sign out), and Quit commands. If you sign out, you need to sign in again with your Kubios credentials before you are able to use the software. The File menu also displays the last nine opened data files. The **Markers menu** includes Show All Markers, Hide All Markers, and Select Markers to Show options. The **ECG/PPG** printout option prints the ECG (or PPG) data in a standard millimeter paper style (an example of ECG printout is shown in Fig. 29). The **Tools menu** includes Kubios Cloud Sync, Polar Flow Export, and Create Analysis Sample Template (based on current samples) options. Kubios Cloud sync enables you to access the measurements that you have performed with Kubios HRV mobile app (see Appendix B for details). Polar Flow export provides an easy way to export all your Polar Flow measurements into your computer in FIT file format to be analyzed in Kubios HRV (see Appendix C for details). Finally, the **Help menu** includes links to Kubios HRV User's guide, Kubios home page, Kubios Support pages, Contact us by e-mail, License information, and the About Kubios HRV dialog. The license information opens a dialog showing the status of your license(s).

\*Note: In the Lite version, unsupported menu items and toolbar buttons are disabled.

# 5 Saving the results

The analysis results can be saved by selecting Save Results or Save Results As from the File menu or by pressing the save button on the toolbar. This will open a file save dialog, which enables you to save the results in three different formats.

- 1. HRV analysis results (and ECG waveform analysis results if enabled) can be stored in a CSV file (Section 5.1).
- 2. The reports of HRV analysis results (and ECG waveform analysis results if enabled) can be saved in PDF format (Sections 5.2 and 5.3).
- 3. The analysis session can be saved in a MATLAB MAT-file format (Section 5.4).

In addition, you have the following export/save options:

- 4. Append to "SPSS friendly" batch file. Using this option you can save HRV analysis results (and ECG waveform analysis results if enabled) into an existing batch file, which is ideal for saving group results (Section 5.5).
- 5. ECG/PPG printout. Choose ECG/PPG Printout report from user menu and click "Save". This saves the ECG (or PPG) waveform data in a standard millimeter paper layout in PDF format (Section 5.6).
- 6. Training report. Choose Training Report from toolbar or user menu and click "Save". This saves the training data report in PDF format and exports the numeric results of the analysis in a CSV file (Section 5.7).
- 7. ANS function report. Choose ANS Function Report from toolbar or user menu and click "Save". This saves the ANS function analysis report in PDF format and exports the numeric results of the analysis in a CSV file (Section 5.8).

#### 5.1 HRV analysis results - CSV export

When you save the HRV analysis results in a CSV format, the numeric results of HRV analysis (and ECG waveform analysis if enabled) will be written in a comma separated text file. A preview of the CSV export showing standard analysis results is presented in Fig. 23 and showing time-varying results in Fig. 24. The columns of the file are separated with comma or semicolon (can be adjusted in software preferences) so that the results could easily be imported to, e.g., spreadsheet programs such as the Microsoft Excel<sup>®</sup> for further inspection. The exported file includes the following information:

- 1. Software, user, and data file informations
- 2. Used analysis parameters
- 3. Samples selected for analysis
- 4. Results overview
- 5. Time-domain results
- 6. Frequency-domain results
- 7. Nonlinear results
- 8. ECG waveform analysis results
- 9. Time-varying results
- 10. Pre-processed RR interval data, spectrum estimates and averaged ECG waveforms for analysis samples.

\*Note: ECG waveform analysis and time-varying analysis are not available in the Lite version.

#### 5.2 HRV analysis results - PDF report

When you save the HRV analysis results in a PDF format, you will have a multipage report. Standard analysis results (time-domain, frequency-domain and nonlinear analysis results) for each analysis sample are displayed on separate pages (one page per analysis sample). An example report page for one analysis sample is displayed in Fig. 25. In addition, one page (or several pages if time-varying analysis has been set to be applied for analysis samples instead of whole recording in Preferences) including time-varying analysis results is produced. An example report page showing time-varying analysis results for an exercise test data is displayed in Fig. 26. \*Note: Time-varying analysis results are not available in the reports generated from the Lite version.

# 5.3 ECG waveform analysis results - PDF report

When you choose to save the HRV analysis results in a PDF format, a PDF report for the ECG waveform analysis results will also be exported (if ECG waveform analysis has been enabled). ECG waveform analysis results and the corresponding averaged ECG waveforms are shown for each analysis sample separately (results for three analysis per page). An example report page for three analysis samples is displayed in Fig. 27. \*Note: ECG waveform analysis is not available in the Lite version.

# 5.4 Analysis session - Matlab MAT-file export

The entire analysis session (including analysis parameters and analysis results) can be saved in a MATLAB MAT-file format. Also the raw data (ECG, PPG or RR data) is stored in the MAT-file. \*Note: MAT-file export is not available in the Lite version.

MAT-file saving option has two purposes:

- 1. The main purpose of the MAT-file is that by opening the MAT-file in Kubios HRV, you can return to the previously performed analyses session as it was (all settings and analysis samples are presented as they were) when the analysis was originally performed. Thus, the MAT file makes it easy for you to change something in the analysis (e.g. add a new analysis sample or change some settings) and re-analyze the data. Thus, we recommend that you save the analysis results always in MAT format as a backup of the analysis you have performed.
- 2. In addition, the MAT files are useful for anyone working with MATLAB (further analysis or processing can be performed easily by loading the MAT-files into MATLAB).

The MAT files include a single structured array variable named Res. The Res variable includes the numeric results as well as the RR interval data and all the analysis options. The Res structure includes four fields which are shortly described as follows

f\_name: File name of the analyzed data filef\_path: Full path for the analyzed data file

HRV: Used analysis options, RR interval data, and all analysis results

ECG\_waves: ECG waveform analysis data and results

CNT: Basic information of the data file

ExtraData: Additional data channels loaded to the data axes during analysis.

The HRV field is the most essential one of these fields. The HRV field includes six fields the contents of which are shortly described as follows

Param: The analysis options used in the calculation of the results

Data: The RR interval data

Summary: Results overview including PNS and SNS indexes

Statistics: Time-domain analysis results
Frequency: Frequency-domain analysis results

NonLinear: Nonlinear analysis results
TimeVar: Time-varying analysis results

The variable names of the different fields are more or less self-descriptive and are not documented here.

# 5.5 HRV analysis results - "SPSS friendly" batch file

The "SPSS friendly" batch file export option is ideal for saving group results. For example, if you need to analyze HRV data of several subjects and want to be able to have the group results easily available for statistical testing e.g. in MS Excel or SPSS. Alternatively, you can use the batch file saving option for saving HRV results of repeated personal recordings, e.g. to monitor training effect or daily stress levels. Kubios uses Comma Separated Values (CSV) file format for the batch file, which can be easily imported into many spreadsheet and statistical software packages (MS Excel, SPSS). \*Note: "SPSS friendly" export is not available in the Lite version.

The Append to "SPSS friendly" batch file functions as follows:



5.6 ECG/PPG printout 23

1. When saving the analysis results of the first subject (i.e. when you want to initialize a new CSV file), select the destination and file name for the new CSV file from the file dialog. In this case, Kubios HRV will initialize the CSV file by writing the column labels and add the analysis results into the first row below the column labels

2. When saving the analysis results of other subjects, simply select the previously saved CSV file. In this case, Kubios HRV will add a new row at the end of the CSV file and writes the results of the current analysis session onto the last row

The structure of the "SPSS friendly" batch file is presented in Fig. 28. Every row of the batch file consists of the file name string and used analysis parameters values ([1x18] array); followed by the following information for every analysis sample: sample info consisting of sample onset/offset time and beat correction rate ([1x2] array), HRV analysis results ([1x86] array), and ECG waveform analysis results ([1x12] array). For more details on the different HRV analysis variables please see Table 2.

# 5.6 ECG/PPG printout

By choosing the ECG/PPG printout from the Reports user menu, you can save the ECG (or PPG) waveform data in a standard millimeter paper layout. The print speed of the printout can be adjusted (default:  $25 \text{ mm/sec} \rightarrow 76 \text{ seconds}$  of ECG per page). The report can be generated for the entire recording or for a selected analysis sample. Please note that report generation may take a considerable time for longer-term recordings (or long analysis samples). An example of ECG printout is shown in Fig. 29. \*Note: ECG/PPG printout is not available in the Lite version.

# 5.7 Training data analysis export

The training data analysis provides detailed performance analytics and heart rate recovery for different types of training and exercise sessions, as well as physical workload assessments. Training data analytics consists of heart rate (HR), respiratory rate (RESP), training intensity (TRIMP), ventilatory threshold (VT) assessment, oxygen uptake (VO2) estimate, and heart rate recovery metrics. More information about the training data analysis is available at: kubios.com/about-sport-and-exercise-analysis/. \*Note: Training data analysis is not available in the Lite version.

To run training data analytics on your data file, choose the Training Report from the toolbar button or Reports user menu. You can choose to either have a preview of the report or save the training data analysis results (the report will be saved in PDF format and the numeric results in CSV format). Training data analysis can be carried out for the entire recordings (the recording is an exercise recording) or for a selected analysis sample (if you have performed a longer term recording, which includes an exercise period). Please note that the recovery period, from which heart rate recovery metrics are computed, is automatically detected within the training data analysis. However, you may also manually mark the desired recovery period, i.e. mark the desired recovery period as an analysis sample and then use this sample as the recovery period when you generate the training report. An example of training report is shown in Fig. 30.

# 5.8 ANS function analysis export

The autonomic nervous system (ANS) function analysis provides detailed analytics for the Valsalva maneuver, deep breathing, and head-up tilt tests, which are the three standard tests for assessing ANS function. The output of the analysis includes the standard ANS function parameters including the Valsalva ratio, HR response to deep breathing, and the 30:15 ratio. In addition, several HRV parameters are reported for the deep breathing and head-up tilt challenges. More information about the ANS function analysis is available at: kubios.com/about-autonomic-nervous-system-function-analysis/. \*Note: ANS function analysis is not available in the Lite version.

If you data file includes at least one of the supported tests (Valsalva / Deep breathing / Head-up tilt), you can run the ANS function analytics by choosing the ANS Function Report from the toolbar button or Reports user menu. You can choose to either have a preview of the report or save the ANS function analysis results (the report will be saved in PDF format and the numeric results in CSV format). In order to run the report, you need identify the tests with the following analysis samples:

**Deep breathing:** Mark the deep breathing period (1 min period with controlled deep breathing at 6 breaths/min) as one analysis sample (sample label e.g. "Deep breathing" or "DB").

**Head-up tilt:** Mark the period when the subject was resting in supine position as one sample (sample label e.g. "Supine") and the period when the subject was in upright position as one sample (sample label e.g. "Standing" or "Upright"). Supine and upright position are recommended to last 5 mins. Do not include the tilting phase in these samples (the two samples should be separated by at least 30 seconds).

An example of ANS function report is shown in Fig. 31.

# 6 Kubios HRV preferences

Kubios HRV includes several settings related to how the ECG (or PPG) and RR interval data is processed and analyzed. The default values for these settings are designed to be suitable for short-term HRV recordings, but you may sometime want to redefine some of the settings. Some of these settings can be adjusted in the user interface to apply for the current analysis session, but in order to make permanent changes into these settings you need to edit them at software preferences. Preferences can be edited by selecting Edit Preferences from the File menu or by pressing the corresponding toolbar button. The preferences are divided into the following categories:

- User information
- · Input data & pre-processing
- Analysis options
  - Time/frequency-domain
  - Nonlinear
  - Time-varying
  - ECG waveform
  - Report settings
- · Select species.

Any modifications to the preferences can be saved by pressing the OK button. Please note that some changes may take effect only after restarting Kubios HRV. You can reset default preferences by pressing Reset preferences button. In addition to above described analysis options, there are various other editable options which have mainly influence on the usability of the software. Such options are e.g. the Range and Y-limit values of the data axis and various visualization options. The values of these options are preserved in memory and any changes made to them will be applied in the future sessions.

All the preferences and editable options available in Kubios HRV software are saved in user specific folders<sup>2</sup>.

Windows 10 or 11:

 $\label{lem:hamme} \label{lem:hamme} HRV\ Scientific: C:\Users\cientific aming\Kubios\Kubios\HRV\ Scientific aming\cientific aming\cientific$ 

Mac OSX:

HRV Scientific: ~/Library/Preferences/Kubios/KubiosHRVScientific

where <username> is the name of your user profile. The folder will include a file named KubiosHRVprefs.mat, which includes all the preferences for the analysis options, user information and user interface usability. The file is created when Kubios HRV is started for the first time and it will be updated whenever the preference values are edited/updated.

\*Note: In the Lite version, preference settings related to unsupported features are disabled.

**User information** settings are shown in Fig. 14 (NOTE: These are local user settings and they can be different to your Kubios account information). You can set up your personal contact information (Name, Department, and Organization). This information will be included in the bottom left corner of the reports that you generate with the software and in the beginning of the CSV export including the analysis results (to identify who has carried out the analyses). In addition, preferences for gender, date of birth, height, weight, rest HR, max HR, and VO2max can be adjusted. These preference values will be used for HR zones, training impulse (TRIMP), energy expenditure (EE) and oxygen uptake (VO2) computations, unless you have inputted data file specific subject information in Personal Data section (see 4.3.1). When you analyze measurements made using the Kubios HRV mobile app, personal preferences available on the measurement are used in computations.

**Input data & pre-processing** settings are shown in Fig. 15. The default input data type is All Supported Files, but you can change this to any of the file formats mentioned in Section 2.2 if you want to filter the files within your data folders. The automatic signal quality detection level, default artifact correction method, and acceptance threshold can be modified (see Sections 3.2 and 3.3). By default the acceptance threshold is set to 5%, meaning that analysis samples having over 5% beat correction will trigger a warning of low quality data. Under Signal type

<sup>&</sup>lt;sup>2</sup>Note that the AppData folder in Windows is hidden by default and is not visible in the File Explorer if the "Show hidden files and folders" is not selected from the "Folder Options" section of the File Explorer.

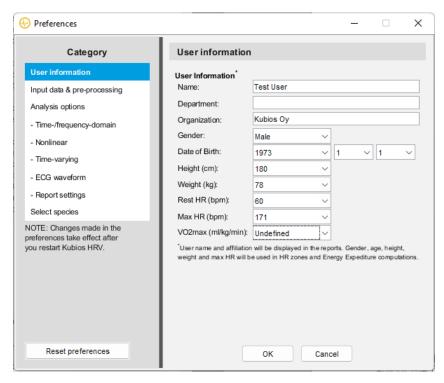


Figure 14: Set up preferences window of the software – User information settings.

you can specify if you are importing ECG or PPG data for your HRV analysis (i.e. if QRS detector or pulse wave detector should be applied for the data). When ECG is selected as signal type, you can modify QRS detection settings. You can force Kubios to look for the R-waves either from positive of negative amplitudes, or let Kubios to decide (R-wave polarity=Automatic). Also, you can manually fix the prior guess for the average RR interval (used by the QRS detector as initial value), or let Kubios try to estimate it automatically. By default the QRS detection settings are set to automatic and there is no reason to change them unless you are experiencing problems in R-wave detection. If PPG is selected as signal type, you can modify the pulse acceptance threshold (visible when PPG is selected as signal type), which adjusts the sensitivity of the pulse detector algorithm. In addition, the interpolation rate (by default a 4 Hz cubic spline interpolation is applied to form equidistantly sampled time series from the IBI data) and detrending method (by default smoothness priors method is used to remove very low frequency trend components) can be adjusted here. The default detrending settings will remove most of the very low frequency components (frequencies below 0.04 Hz) from the RR interval series prior to analysis. By doing so the HRV analysis results are not affected by the slow baseline changes in heart rate and are more sensitive to the short-term HRV regulated by the autonomic nervous system. We recommend removing the trend using the Smoothness priors method which was proposed in [30].

**Analysis options** general settings are shown in Fig. 16. You can select if both 1) Standard analysis (all time-domain, frequency-domain and nonlinear analysis for selected analysis samples) and 2) Time-varying analysis are performed. Unchecking the unnecessary analysis type will speed up the computations. Analysis samples settings include an option to identify the file where you have defined specifications (including number of samples and their positions) for the analysis samples that you want to apply for the data files you analyze. Alternatively, you can set the number of analysis samples and the default sample length here. Also, the minimum analysis sample length can be defined between 10, 30 and 60 seconds. In case of several samples, you can choose the analysis type between Single samples (in this case, Kubios will perform analysis for every sample separately) and Merge samples (the samples are merged into one longer sample for which analysis is then performed). The Update mode can be changed between Automatic (analysis results are refreshed automatically) and Manual (you need to refresh results manually, which is recommended when analyzing long-term recordings). Finally, you can also choose the Representation of time between Recording start time (if you want to see the actual clock time in the UI and reports) and 00:00:00 (if you want the time to start from zero).

**Analysis options: Time-/frequency-domain** analysis settings are shown in Fig. 17. For time-domain methods, you can adjust the width of the moving average window which is used to extract instantaneous HR values (default: 5 beats), and the threshold used in the computation of NNxx and pNNxx parameters (default: 50 ms  $\rightarrow$  NN50 and pNN50). The very low frequency (VLF), low frequency (LF), and high frequency (HF) bands of HRV frequency-domain analysis can be adjusted. The default values for these frequency bands are VLF: 0–0.04 Hz, LF: 0.04–0.15

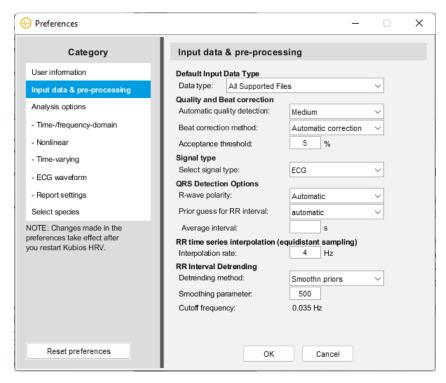


Figure 15: Software preferences – Input data & pre-processing settings.

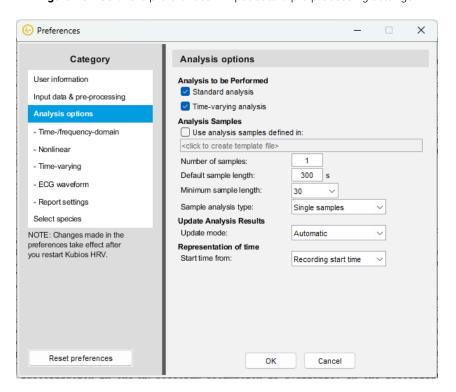


Figure 16: Software preferences – Analysis options settings.

Hz, and HF: 0.15–0.4 Hz according to [31]. The rest of the settings relate to spectrum estimation methods and rarely need to be adjusted. The resolution of the spectrum estimate may be increased (via Fourier interpolation) by increasing the points in frequency-domain setting (default is equal to the window with). The spectrum for the selected RR interval sample is calculated both with Welch's periodogram method (FFT spectrum) and with an autoregressive model (AR spectrum). In the Welch's periodogram, the used window width (default: 300 s) and window overlap (default: 50%) can be adjusted by editing the corresponding fields. The default values produce three overlapping windows for a 10-min (600 sec) analysis sample. As an alternative to FFT spectrum, you can select to use the Lomb-Scargle periodogram, which does not assume equidistant sampling and has been recom-

mended for HRV spectral analysis in some studies. The default smoothing window for this spectrum estimate is 0.02 Hz. Regarding the AR spectrum, the order of the used AR model can be adjusted (default: 16) and the use of spectral factorization in the AR spectrum estimation is available. In the factorization the AR spectrum is divided into separate components and the power estimates of each component are used for the band powers. Spectral factorisation has been shown to provide some advantage especially when e.g. the HF component is partially overlapping with the LF band [28].

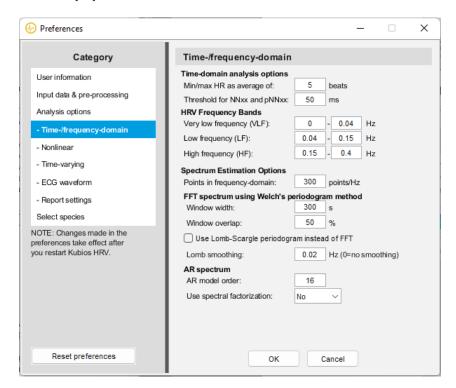


Figure 17: Software preferences – Analysis options: time-/frequency-domain methods.

Analysis options: Nonlinear analysis settings are shown in Fig. 18. You can choose if detrended RR data is also used when computing the nonlinear parameters (default: TRUE). The embedding dimension m (default: 2 beats) and the tolerance value r (default:  $0.2\times {\rm SD}$ , i.e. adjusted in relation to standard deviation of RR data) used in approximate entropy (ApEn) and sample entropy (SampEn) computations can be modified. The limits of the short-term (N1) and long-term (N2) fluctuations assessed in the Detrended fluctuation analysis (DFA) can be modified (default: N1=4-12 and N2=13-64 beats). Finally, the embedding dimension (default: 10 beats) used in the computations of correlation dimension ( $D_2$ ) and recurrence plot analysis (RPA), and the threshold level (default:  $\sqrt{10}\times {\rm SD}$ ) used in RPA, can be modified.

Analysis options: Time-varying analysis settings are shown in Fig. 19. You can choose to apply time-varying analysis on the whole measurement (default) or for each analysis sample. You can adjust the width of the moving window used for HRV analysis (default: 300 s), width of the moving window used for energy expenditure (EE) and TRIMP (default: 10 s), and grid interval which defines how much the window is moved at every step (default: 60 sec). For example, if you want to performe time-varying analysis at 10-min non-overlapping windows for the whole duration of your recording, you need to select "Whole recording" as the analysis mode and define the window width and grid interval both to 600 seconds. Effective data threshold setting adjusts how noise segments affect time-varying analysis results, by default results are not computed unless the effective RR data length (length of data excluding noise) is at least 50% of the analysis window width. For the time-varying spectrum estimation there are two options: 1) the well known spectrogram (default) and 2) a Kalman smoother spectrum estimate proposed in [28].

**Analysis options: ECG waveform** analysis settings are shown in Fig. 20. You can choose if you want to apply ECG waveform analysis (default: FALSE) for your data files. You can set a fixed scaling factor (if your ECG data is not correctly imported in mV units). Notch filter to remove powerline noise can be selected when necessary (default: None) and the cut-off frequency of the high-pass filter used for removing ECG baseline wander can be adjusted (default: 1 Hz). The ECG waveform analysis is carried out based on an average ECG waveform extracted from the analysis window. Averaged beats can be chosen based being close to the mean RR, minimum RR or maximum RR interval within the window (default: mean RR). In addition, number of beats chosen for averaging (default: 30

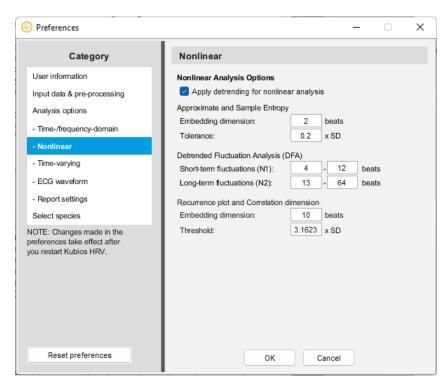


Figure 18: Software preferences – Analysis options: nonlinear methods.

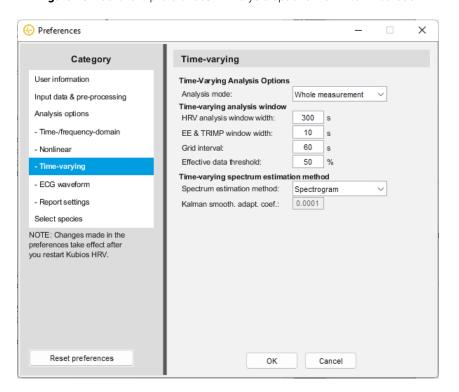


Figure 19: Software preferences – Analysis options: time-varying methods.

beats) and rejection rate for divergent beats (default: 33%) can be selected.

**Analysis options: Report settings** are shown in Fig. 21. You can select the default results save directory (Results folder or Data folder) and your preferred paper size used in PDF reports (default: A4). Regarding HRV report, you can select to include in the report the analysis sample results and/or time-varying results, select if FFT/Lomb or the AR spectrum estimate is displayed in the report (default: FFT/Lomb), and select four optional HRV parameters to be displayed on the time-varying analysis report page. For the training data report you can select if the HR zones are computed with respect to HR max or HR reserve (default: HR max), if ventilatory thresholds are estimated using Kubios VT estimation algorithm or DFA alpha 1 (default: Kubios VT algorithm), and you can edit the DFA alpha 1

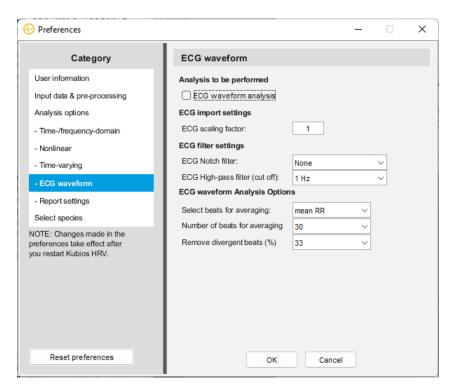


Figure 20: Software preferences – Analysis options: time-varying methods.

cutoff points (default HRV thresholds (HRVT): HRVT1=0.75 and HRVT2=0.50). Relating to the CSV exports, you can select the used column separator (default: comma ",") and decimal separator (default: dot "."). Finally, you can also change the color theme if you are not satisfied with the default theme.

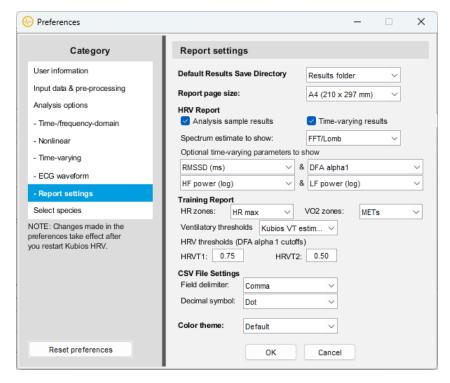


Figure 21: Software preferences – Report settings.

**Select species** settings are shown in Fig. 22. These settings should be changed only if you are analyzing non-human HRV data. Currently supported species include rat, mouse and pig (default: human). Once you select one of these species, you can edit the rest HR and max HR values specific for the species. Furthermore, you can also edit normal values for Mean RR, RMSSD, Poincaré plot SD1 and SD2, and stress index, which will be used

for PNS and SNS index computations. These settings affect the functioning of beat detection (QRS or pulse wave detection), signal quality detection, and beat correction algorithms. Thus, small animal data can also be processed and analyzed with Kubios HRV optimally.

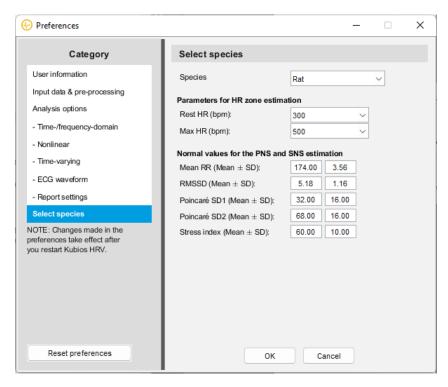


Figure 22: Software preferences – Species selection.

# A Appendix: Kubios HRV analysis parameters

Kubios HRV provides a detailed analyses of HRV (over 40 HRV analysis parameters), training data analysis, and ECG waveform analysis. Summary of the parameters calculated by Kubios HRV software is given in Table 2.

 Table 2:
 HRV parameters calculated by Kubios HRV software

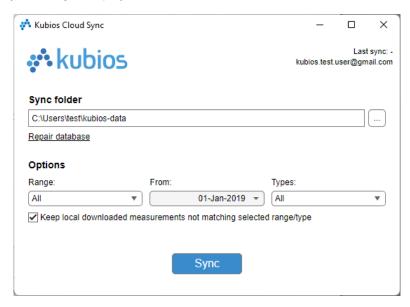
Parameter	Units	Description	References
Overview <sup>1</sup>	- Onno		
PNS index	-	Parasympathetic nervous system activity compared to normal resting values	
SNS index	-	Sympathetic nervous system activity compared to normal resting values	
Time-Domain			
Mean RR	[ms]	The mean of RR intervals	
STD RR (SDNN)	[ms]	Standard deviation of RR intervals	
Mean HR	[1/min]	The mean heart rate	
STD HR	[1/min]	Standard deviation of instantaneous heart rate values	
Min & Max HR	[1/min]	Minimum and maximum HR computed using $N$ beat moving average (default value:	N = 5)
RMSSD	[ms]	Square root of the mean squared differences between successive RR intervals	1. 0)
NNxx	[beats]	Number of successive RR interval pairs that differ more than xx ms (default value: xx	- 50)
pNNxx	[%]	NNxx divided by the total number of RR intervals	.— 00)
HRV triangular index	[,0]	The integral of the RR interval histogram divided by the height of the histogram	[31]
TINN	[ms]	Baseline width of the RR interval histogram	[31]
Stress index	[1110]	Square root of Baevsky's stress index	[1]
DC, AC*	[ms]	HR deceleration capacity (DC) and acceleration capacity (AC)	[2, 22]
SDANN	[ms]	Standard deviation of the averages of RR intervals in 5-min segments	[2, 22]
SDNNI	[ms]		[31]
	[1115]	Mean of the standard deviations of RR intervals in 5-min segments	[31]
Frequency-Domain			
Spectrum		Welch's (or Lomb-Scargle*) periodogram and AR spectrum estimates	
Peak frequency	[Hz]	VLF, LF, and HF band peak frequencies	
Absolute power	[ms <sup>2</sup> ]	Absolute powers of VLF, LF, and HF bands	
Absolute power	[log]	Natural logarithm transformed values of absolute powers of VLF, LF, and HF bands	
Relative power	[%]	Relative powers of VLF, LF, and HF bands	
		$VLF[\%] = VLF[ms^2]/total power[ms^2] \times 100\%$	
		LF [%] = LF [ms <sup>2</sup> ]/total power [ms <sup>2</sup> ] $\times$ 100%	
		$HF[\%] = HF[ms^2]/total power[ms^2] \times 100\%$	
Normalized power	[n.u.]	Powers of LF and HF bands in normalised units	
		$LF [n.u.] = LF [ms^2]/(total power [ms^2] - VLF [ms^2])$	
		HF [n.u.] = HF [ms <sup>2</sup> ]/(total power [ms <sup>2</sup> ] – VLF [ms <sup>2</sup> ])	
LF/HF	_	Ratio between LF and HF band powers	
RESP*	[Hz]	Respiration rate (derived from ECG and RR data)	
Nonlinear	را اکا	Nespiration rate (derived from 200 and NN data)	
SD1	[ms]	In Poincaré plot, the standard deviation perpendicular to the line-of-identity	[4, 5]
SD2	[ms]	In Poincaré plot, the standard deviation along the line-of-identity	[4, 0]
SD2/SD1	[IIIS]	Ratio between SD2 and SD1	
ApEn	_	Approximate entropy	[27, 8]
SampEn	_	Sample entropy	[27]
DFA, $\alpha_1$		In detrended fluctuation analysis, short term fluctuation slope	[24, 25]
	-	In detrended fluctuation analysis, snort term fluctuation slope	[24, 23]
DFA, $\alpha_2$	-	Correlation dimension	[10, 11]
$D_2^*$ RPA*:	_		. , .
	D	Recurrence plot analysis:	[32, 7, 34]
Lmean	[beats]	Mean line length	
Lmax	[beats]	Maximum line length	
REC	[%]	Recurrence rate	
DET	[%]	Determinism	
ShanEn	-	Shannon entropy	
MSE*	-	Multiscale entropy for scale factor values $ au=1,2,\ldots,20$	[6]
		ters shown above, except $D_2$ , RPA and MSE, plus)	
Energy expenditure	[kcal/min]	Activity related energy expenditure (EE) estimated using Keytel's model	[13]
	[TRIMP/min]	Training impulse (TRIMP) according to Banister's exponential model	[19]
Oxygen uptake	[l/min]	Oxygen uptake (VO2) according to personal VO2max estimate	[12]
Training Data Analysis*	,2		
HR	[bpm]	Instantaneous HR and HR zones (zones are based on HR max or HR reserve)	
	[breaths/min]	Instantaneous RESP and RESP zones	
	[TRIMP/min]	Instantaneous TRIMP, TRIMP zones and cumulative TRIMP (training load)	
VT1 & VT2	[bpm]	Instantaneous ventilatory threshold (VT) estimate, VT zones, and HR at VT1 and VT2	
DFA- $lpha_1$	-	Instantaneous DFA- $\alpha_1$ and VT zones estimated from it (HRV thresholds)	
V02	[mg/kg/min]	Instantaneous VO2 estimate and VO2 zones (0-3, 3-6, 6-9, and >9 MET)	
HRR	[bpm]	Heart rate recovery (HRR) at 60s, 120s and 300s increments as well as fast 30s HRR	(T30)
ANS Function Analysis*	.,3		
Valsalva ratio	_	Longest RR interval after maneuver divided with the shortest RR interval during mane	euver
HR response	[bpm]	Heart rate response to deep breathing	
E-I	[ms]	Difference between RR interval maximum and minimun during deep breathing	
E/I	-	Ratio between RR interval maximum and minimun during deep breathing	
30:15	-	Ratio between RR interval maximum ( 30th beat) and minimum ( 10th beat) during he	ead-un tilt
ECG Waveform Analysis	*	the state of the s	o - o - o - o - o - o - o - o - o
Time intervals	[ms]	P-wave duration, PQ-interval, QRS duration, QT-time, and heart rate corrected QT-time	(OTc)
Wave amplitudes	[mV]	P, Q, R, S and T-wave amplitudes	(4.0)
		version: 1 About PNS and SNS indexes, see kubios com/hrv-ans-function/: 2 About tra	

\*Parameter is not available in the Lite version; <sup>1</sup>About PNS and SNS indexes, see kubios.com/hrv-ans-function/; <sup>2</sup>About training analytics, see kubios.com/about-sport-and-exercise-analysis/; <sup>3</sup>About ANS function analytics, see kubios.com/about-autonomic-nervous-system-function-analysis/.



# **B** Appendix: Kubios Cloud sync

When you use Kubios HRV mobile app to measure HRV, your recordings are saved in Kubios Cloud (see our privacy policy). You can download your recordings to your PC using the Kubios Cloud Sync available in the Kubios HRV software. The Cloud Sync dialog is displayed below.



The first time you use the Cloud Sync, please select a folder where the app measurements should be downloaded (database containing your app measurements will be established at this folder). The sync options include:

- Range: All, Last 1 month, Last 3 months, Last 6 months, Last year, or Custom (defines the range which measurements are synched)
- From: Choose an arbitrary start date for the sync (Custom range sync)
- Types: All, Readiness, or Custom (defines which measurement types you want to sync)
- Keep local downloaded measurements not matching selected range/type (if you uncheck this, local downloaded measurements outside the selected measurement range and type will be deleted)

The measurements will be stored in the specified sync folder under folder named according to your Kubios user account ID number (unique UUID). The measurements are stored in Kubios Data Format (KDF) and the files are named according to

```
YYYY-MM-DDThhmmss (<measurement type>) - xxxxxx.kdf
OR
YYYY-MM-DDThhmmss - <measurement subject name> - xxxxxx.kdf
```

where YYYY-MM-DDThhmmss is the date and time when the measurement was started, measurement type is readiness or custom, measurement subject name is displayed if the custom measurement has been linked to a specific subject, and xxxxxx are the first six characters of the measurement UUID.

NOTE: Sometimes the local measurement database may be corrupted, in which case you may need to run the "Repair database".

NOTE: If you want to clean the local database, you can simply delete the entire sync folder (just make sure you haven't stored anything else than Cloud Sync generated files on the folder). Deleting the local database will not remove your files from Kubios Cloud.

# C Appendix: Polar Flow export

The Polar Flow export option provides an easy way to export your Polar Flow measurements into your computer hard drive in FIT file format, which can then be analyzed in Kubios HRV software. The exported FIT file will also include the manual and automatic (e.g. distance based) lap markers made during the exercise.

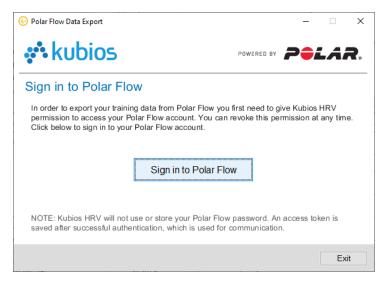
**NOTE: RR data is not available when measuring with the Polar Flow or Beat App. \*Note:** Polar Flow export is not available in the Lite version, but you can still manually export your RR data from Polar Flow in CSV format.

In order to start exporting your Polar Flow measurements, click Polar Flow Export in Tools menu and follow the given instructions. The steps needed to link your Polar Flow account with Kubios HRV are shortly described below. Please note that only those training sessions that are added to Polar Flow after linking with Kubios HRV are accessible through the Polar Flow API.

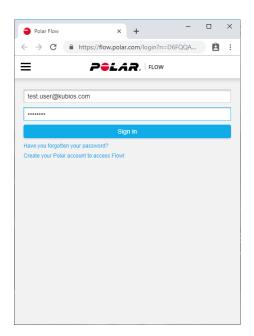
#### Please remember that:

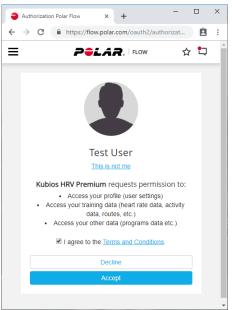
- 1. ONLY MEASUREMENTS MADE AFTER LINKING YOUR POLAR FLOW ACCOUNT ARE ACCESSIBLE
- 2. RR data is available only when a chest strap (H6, H7 or H10) has been used
- 3. Each measurement can be exported only once (take backup copies of exported data files).

When you click the Polar Flow Export option for the first time, a window instructing you to sign in to your Polar Flow account pops up



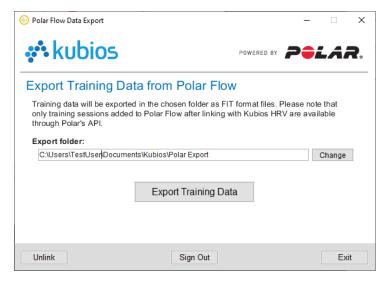
Click "Sign in to Polar Flow" to continue.



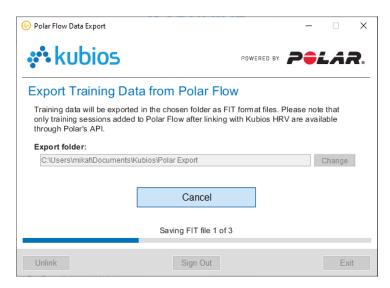


Next, you need to Sign In to your Polar Flow account and give Kubios HRV permission to access your Polar Flow account data. To continue, confirm that you agree to the Terms and Conditions and click Accept. You should then receive a notice that your Polar Flow account is successfully linked with Kubios HRV.

Once you have linked your Polar Flow account with Kubios HRV, you can export your training data by clicking the Polar Flow Export from Tools menu.



You can define the export folder, where your training data from Polar Flow will be exported. Every training measurement that you have uploaded into Polar Flow after linking your account with Kubios HRV or after the previous successful export, will be exported into the selected folder in FIT file format when clicking Export Training Data button.



If you do not have new measurements uploaded to Polar Flow, you will receive a notification "No new training data available in Polar Flow". Please note, that not all the FIT files exported from Polar Flow necessarily include RR data and can not thus be opened in Kubios HRV. RR data is available only when you have used a chest strap (Polar H6, H7 or H10) to record heart rate. RR data is not available when you have measured heart rate from your wrist (wrist-based HR) or by using the optical Polar OH1 armband.

You can Unlink Kubios HRV from your Polar Flow account whenever you want. However, you should do this only if you do not anymore need to access your training data in Kubios HRV. If you want to temporarily disable the access to your Polar Flow account from Kubios HRV, you can Sign Out from your account instead. In this case, you are required to sign in again the next time you want to export data from Polar Flow, but all your latest training data are still accessible through the Polar Flow link.

## D Appendix: Analyzing animal HRV data

Kubios HRV Scientific supports also analysis of animal HRV data. When analyzing animal HRV data, you need to choose the species from software preferences (see Section 6). According to the selected species, adjustments summarized in Table 3 and described below are made. \*Note: Analysis of animal HRV data is not supported in the Lite version.

Regarding input data formats and pre-processing, in addition to supported file formats described in Section 2.2, Notocord (.dat) and visual sonic (.raw.physio) ECG files are specifically supported for importing animal ECG data. In addition, the preprocessing filters of the QRS detector are optimized for each species individually, and the detection logic is adjusted based on the normal heart rate of the selected species. Automatic noise detection is available for dog and rat data. The noise detection logic is adjusted according to the normal HRV of the species. Within the automatic beat correction algorithm, the limits of allowed heart rate range are changed according to selected species. The default value of the RR interpolation rate, which is used to make RR interval time series equidistantly sampled for the spectrum estimation, will be changed according to the normal heart rate of the species. The cutoff frequency of the detrending is also adjusted.

Regarding analysis settings, the default values for frequency band limits of VLF, LF and HF components are changed for each species and the default value of pNNxx threshold is changed according to normal heart rate of the selected species. Normal values of HRV-parameters used in PNS and SNS indexes' estimation are changed according to selected species (these are shown in software preferences and can be edited therein).

In ECG waveform analysis, the algorithm detecting ECG wave onset and offset times is adjusted based on the normal heart rate of the selected species. For murine ECG analysis, additional QRSp wave boundary marker is added, which can be used to mark end of the J-wave [16]. Two-time intervals for QRS complex duration will be calculated, QRS-duration (from Q to S) and QRSp-duration (from Q to QRSp). ECG-waveform report millimeter-paper timescale (mm/sec) will be adjusted according to normal heart rate of the selected species.

NOTE: The automatic noise detection algorithm is only available for Human, Dog and Rat data. Respiratory rate estimation is not available for animal data. In addition, estimates specifically defined for human data such as energy expenditure, oxygen uptake, and ventilatory thresholds are not available for animal data. Thus, the training data report is not available for animal data.

**Settings** Human Mouse Rat Pig Dog Pre-processing RR interpolation rate 20 Hz 20 Hz 4 Hz 4 Hz 4 Hz 0.035 Hz 0.035 Hz 0.035 Hz RR detrending cutoff frequency 0.11 Hz 0.11 Hz (smoothing parameter) (2000)(500)(2000)(500)(500)**Analysis parameters** Default resting HR 60 bpm 600 bpm 300 bpm 40 bpm 40 bpm Default maximum HR 175 bpm 800 bpm 500 bpm 240 bpm 270 bpm pNNxx threshold 50 ms 50 ms 6 ms 6 ms 50 ms VLF range 0 - 0.04 Hz $0 - 0.15 \, Hz$ 0 - 0.19 Hz $0 - 0.04 \, \text{Hz}$ 0 - 0.04 HzLF range  $0.04 - 0.15 \, Hz$ 0.15 - 1.5 Hz 0.19 - 0.75 Hz  $0.04 - 0.15 \, Hz$  $0.04 - 0.15 \, Hz$ 

1.5 - 5.0 Hz

0.75 - 2.5 Hz

0.15 - 0.4 Hz

0.15 - 1 Hz

0.15 - 0.4 Hz

**Table 3:** Species specific settings.

HF range

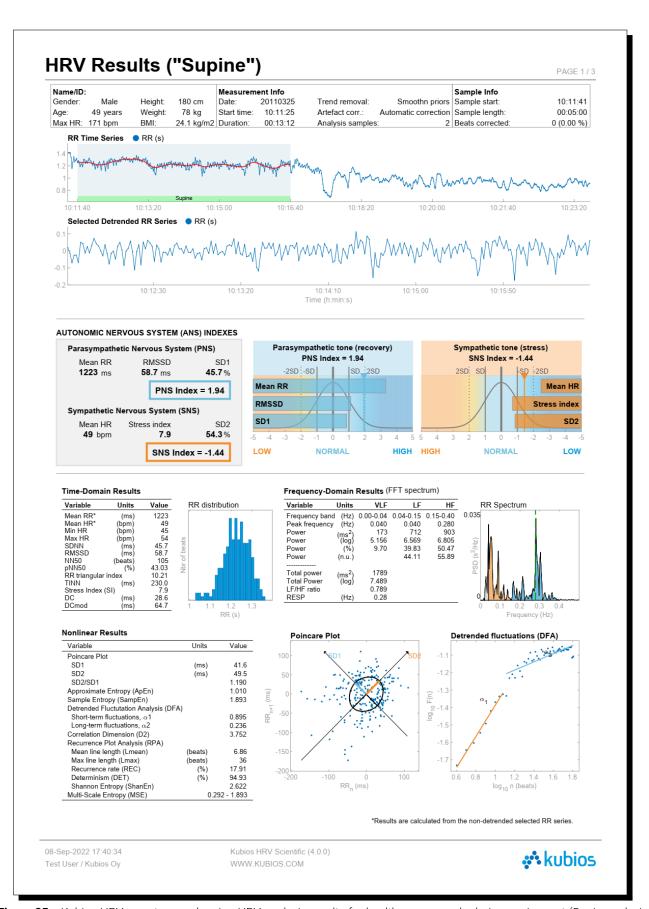
# **E** Appendix: Kubios HRV figures

0	A BB interval Secondar Selected for A	B	С	D	Е	F	G	Н		J
	RR Interval Samples Selected for A		/=1							- 1/-1
1		Rest(1)	Warm-up(2)	40W(3)	80W(4)	120W(5)	160W(6)	200W(7)	240W(8)	Peak(9)
2	Sample limits (hh:mm:ss):		10:59:56-11:02:	11:04:09-11:07:	11:07:10-11:10:	11:10:11-11:13:	11:13:12-11:16:	11:16:13-11:19:	11:19:14-11:22:	11:25:14-11:28
3	Sample Analysis Type: Single sam									
4	Beat correction: Automatic correc									
5	Beats total:	231	290	310	338	379	429	477	522	572
5	Beats corrected:	0	2	4	2	0	0	2	0	2
7	Beats corrected (%):	0	0.69	1.29	0.592	0	0	0.419	0	0.35
8	Effective data length (s):	180	180	180	180	180	180	180	180	180
9	Effective data length (%):	100	100	100	100	100	100	100	100	100
0										
1										
_	RESULTS FOR SINGLE SAMPLES									
	STANDARD RESULTS									
4		Rest(1)		Warm-up(2)		40W(3)		80W(4)		120W(5)
	Results Overview									
6	PNS index:	-0.8		-1.8674		-2.3531		-2.9086		-3.51
7	SNS index:	0.6073		2.3119		3.384		5.9657		8.7605
3	Stress index:	8.0995		10.5646		14.1578		25.2804		33.777
9										
_	Time-Domain Results									
1	Statistical parameters									
2	Mean RR (ms):	778.0303		620.5406		579.744		531.5622		474.6821
3	SDNN (ms):	58.8781		51.521		38.5571		20.952		9.9782
4	Mean HR (beats/min):	77.1178		96.6899		103.494		112.8748		126.4004
5	SD HR (beats/min):	5.9695		8.0154		6.5231		4.3521		2.5703
6	Min HR (beats/min):	64.8368		73.0105		78.7712		99.2392		106.5719
7	Max HR (beats/min):	92.5926		110.9262		119.7366		123.3046		139.8601
8	RMSSD (ms):	39.4455		28.6342		19.3617		10.1272		4.3621
9	NNxx (beats):	45		21		10		1		(
0	pNNxx (%):	19.5652		7.2664		3.2362		0.2967		(
1	SDANN (ms):	NaN		NaN		NaN		NaN		NaN
2	SDNN index (ms):	NaN		NaN		NaN		NaN		NaN
3	Geometric parameters									
4	RR tri index:	14.4375		9.666667		8.857143		5.451613		2.746377
5	TINN (ms):	289		234		192		94		65
6	DC (ms):	46.2283		37.9963		28.8461		12.6265		3.8611
7	DCmod (ms):	42.8331		33.1038		21.1257		9.9574		3.5325
8	AC (ms):	-46.0219		-31.7911		-24.4101		-10.2447		-4.7206
9	ACmod (ms):	-42.7762		-27.9293		-18.7914		-8.875		-4.0763
0	Action (ilis).	-42.7702		-21.3233		-10.7314		-6.673		-4.0702
$\rightarrow$	Frequency-Domain Results	FFT spectrum	AR spectrum	FFT spectrum	AR spectrum	CCTt	AD	CCT	AD	FFT
		rri spectrum	AK Spectrum	rri spectrum	AK Spectrum	FFT spectrum	AR spectrum	FFT spectrum	AR spectrum	FFT spectrum
	Peak frequencies		_		_		_			
3	VLF (Hz):	0.033333	0	0.026667	0	0.04	0	0.03	0	0.033333
4	LF (Hz):	0.093333	0.1	0.086667	0.093333	0.093333	0.093333	0.093333	0.093333	0.086667
5	HF (Hz):	0.15	0.2	0.173333	0.26	0.253333	0.276667	0.16	0.286667	0.196667
	Absolute powers									
7	VLF (ms^2):	255.2539	0	47.3955	327.9902	33.3018	249.1382	59.609	0	13.178
8	LF (ms^2):	2323.1262	2899.4314	2234.5719	2410.7425	429.3865	1288.3253	451.9943	451.7297	83.054
9	HF (ms^2):	946.442	617.2272	646.3698	190.2333	165.0663	66.6487	76.6464	47.8106	18.4172
0	VLF (log):	5.5423	0	3.8585	5.793	3.5056	5.518	4.0878	0	2.5786
1	LF (log):	7.7507	7.9723	7.7118	7.7877	6.0624	7.1611	6.1137	6.1131	4.419
2	HF (log):	6.8527	6.4252	6.4714	5.2483	5.1063	4.1994	4.3392	3.8672	2.913
3	Relative powers									
4	VLF (%):	7.2416	0	1.6185	11.1982	5.3049	15.5312	10.1333	0	11.494
5	LF (%):	65.9076	82.4485	76.3085	82.3069	68.4001	80.3139	76.8371	90.4291	72.440
6	HF (%):	26.8508	17.5515	22.0729	6.4949	26.2946	4.1549	13.0296	9.5709	16.063
7	Normalized powers									
8	LF (n.u.):	71.053	82.4485	77.5639	92.6861	72.2319	95.0812	85.5012	90.4291	
9	HF (n.u.):	28.947	17.5515	22.436	7.3139	27.7676	4.9188	14.4988	9.5709	~
_	Total power (ms^2):	3524.8221	3516.6586	2928.34	2928.9659	627.7575	1604.1123	588.2501	499.5403	114.651
	exercise test hrv	(+)				: [4]				

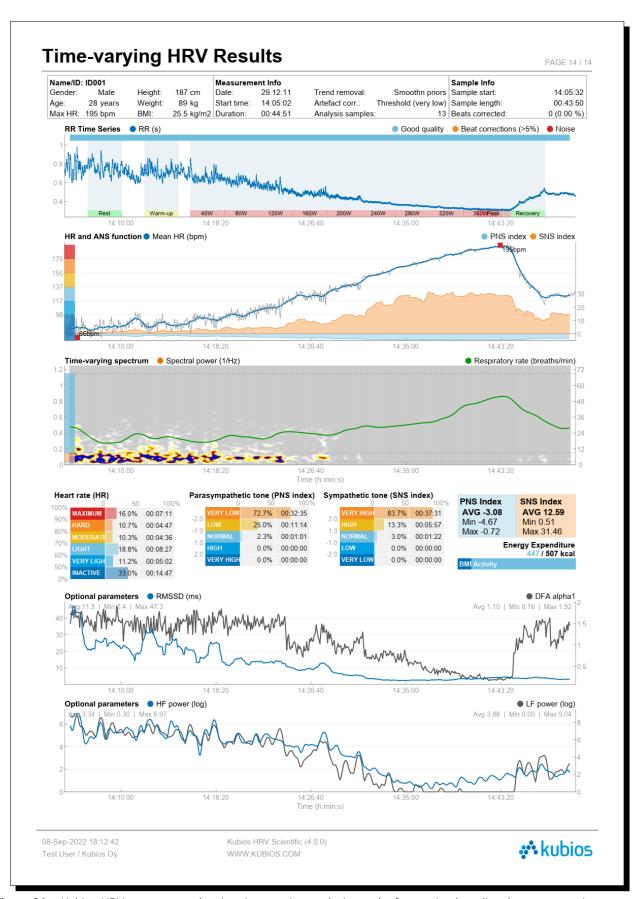
**Figure 23:** Kubios HRV results for exercise test saved as a CSV formatted text file, showing time-domain, frequency-domain and nonlinear HRV analysis results for different exercise protocol time periods (Rest, Warm-up, 40W load, 80W load, and 120W load).

Α	В	С	D	E	F	G	Н		J	K	L	М
TIR #	IE MADVING DECIN	TC										
HIM	IE-VARYING RESUL	.18										
	WHOLE DATA											
							Overview					
	Time		1			Effective data le	PNS index	SNS index	Stress index	EE activity	EE activity	Intensity
	(hh:mm:ss)	(count)	(count)	(%)	(sec)	(%)				(kcal/min)	(kcal)	(TRIMP/min)
	10:54:19	184					-0.5403	0.3922	8.1832	2.6598	0.4433	0.1495
	10:54:29	186					-0.5999	0.4109	8.1212		0.7199	0.0998
	10:54:39	186				100	-0.6008	0.431	8.1237	2.3559	1.1125	0.1336
	10:54:49	185	0	0	150	100	-0.5823	0.4789	8.5857	2.2999	1.4959	0.1307
	10:54:59	186	0			100	-0.5423	0.4937	8.5679	1.595	1.7617	0.0969
	10:55:09	189	0	0	150	100	-0.6248	0.5473	8.516	1.6963	2.0444	0.1015
	10:55:19	190	0	0	150	100	-0.6917	0.5525	8.2368	1.5867	2.3089	0.0965
	10:55:29	191	0	0	150	100	-0.7387	0.5846	8.2231	1.7143	2.5946	0.1023
	10:55:39	191	0	0	150	100	-0.7428	0.6289	8.5534	2.9237	3.0819	0.1639
	10:55:49	192	0	0	150	100	-0.7746	0.6077	8.2225	2.447	3.4897	0.1383
	10:55:59	193	0	0	150	100	-0.7569	0.5354	7.6033	1.6997	3.773	0.1017
	10:56:09	195	0	0	150	100	-0.8039	0.626	7.8372	2.545	4.1971	0.1434
	10:56:19	196	0	0	150	100	-0.8316	0.7156	8.2385	3.201	4.7306	0.1798
	10:56:29	198	0	0	150	100	-0.8575	0.7928	8.4936	2.5276	5.1519	0.1425
	10:56:39	198				100	-0.9264	0.8074	8.5231	2.4329	5.5574	0.1375
	10:56:49	197	0			100	-0.9399	0.7967	8.4148		5.9462	0.1324
	10:56:59	197				100	-0.9089	0.6794	7.8465		6.3804	0.1466
	10:57:09	196				100	-0.9301	0.7546	8.3936		6.9107	0.1787
	10:57:19	197	0			100	-0.9609	0.7287	8.1172		7.5799	0.2303
	10:57:29	198				100	-1.0327	0.7735	8.231	2.5115	7.9985	0.1416
	10:57:39	197	0			100	-0.9645	0.6562	7.5966		8.4952	0.1671
	10:57:49	197	0			100	-0.9869	0.7073	7.9198		8.832	0.1168
	10:57:59	200				100	-1.0652	0.7451	7.6388		9.1304	0.1059
	10:58:09	205			150	100	-1.1912	0.945	8.1151		9.4638	0.1159
	10:58:19	210				100	-1.2933	1.0922	8.3333	1.8863	9.7782	0.1104
	10:58:29	213			150	100	-1.4049	1.3307	9.3416		10.1621	0.1309
	10:58:39	214			150	100	-1.4541	1.4205	9.674		10.6461	0.1629
	10:58:49	217	5		150	100	-1.5491	1.4648	9.3589	2.3576	11.0391	0.1023
	10:58:59	219			150	100	-1.6086	1.6464	10.0509	2.3370	11.5041	0.1566
	10:59:09	223			150	100	-1.6396	1.6779	9.8102	5.1427	12.3612	0.1300
		225				100						
-	10:59:19				150		-1.5874	1.6251	9.0598		13.4827	0.4494
	10:59:29	228			150	100	-1.6648	1.7235	9.0767	6.5404	14.5727	0.4313
	10:59:39	232			150	100	-1.7038	1.8044	9.0541	6.0142	15.5751	0.3832
	10:59:49	235			150	100	-1.7845	2.0343	9.8741	4.9726	16.4038	0.298
	10:59:59	237	5		150	100	-1.8109	2.1058	9.9633	5.6719	17.3492	0.3538
	11:00:09	240			150	100	-1.9415	2.3504	10.8696		18.2729	0.343
	11:00:19	243				100	-1.9862	2.432	10.915		19.0052	0.2559
	11:00:29	243			150	100	-1.8752	2.4382	11.1015		19.6694	0.2283
	11:00:39	241			150	100	-1.8242	2.3102	10.6845		20.5042	0.3007
	11:00:49	239				99.3333	-1.7791	2.1345	9.9009		21.2991	0.2828
	11:00:59	239			150	100	-1.8044	2.1233	9.7891	5.7222	22.2528	0.358
	11:01:09	240			150	100	-1.8249	2.2863	10.5469		23.0502	0.2839
	11:01:19	241	0			100	-1.8363	2.2404	10.2034	5.6351	23.9894	0.3507
	11:01:29	241	0	0	150	100	-1.813	2.1887	9.9231	5.353	24.8816	0.3277
	11:01:39	242		0		100	-1.8428	2.3299	10.6263		25.7193	0.3021
	11:01:49	244					-1.9775	2.7174				
	11:01:59	244				100	-1.9693	2.7117	12.7303	4.9016	27.2895	0.2926
	11:02:09	244				100	-1.9919	2.7087	12.6988	6.2086	28.3242	
	11:02:19	243	0	0	150	100	-1.9661	2.5106	11.6267	6.6299	29.4292	0.4399
	11:02:29	242	0	0	150	100	-1.9145	2.4563	11.4806	5.9593	30.4224	0.3784
	11:02:39	240	0	0	150	100	-1.8635	2.2781	10.7499	5.3189	31.3089	0.3249
	11:02:49	239	0	0	150	100	-1.8515	2.216	10.5699	5.3338	32.1979	0.3261
	11:02:59	237	0	0	150	100	-1.8216	2.1129	10.1748	5.8682	33.1759	0.3 🗸
	11:03:09	239	0	0	150	100	-1.8667	2.2894	10.9436	4.9542	34.0016	
	11:03:19	239	2	0.8368			-1.8763	2.3629				

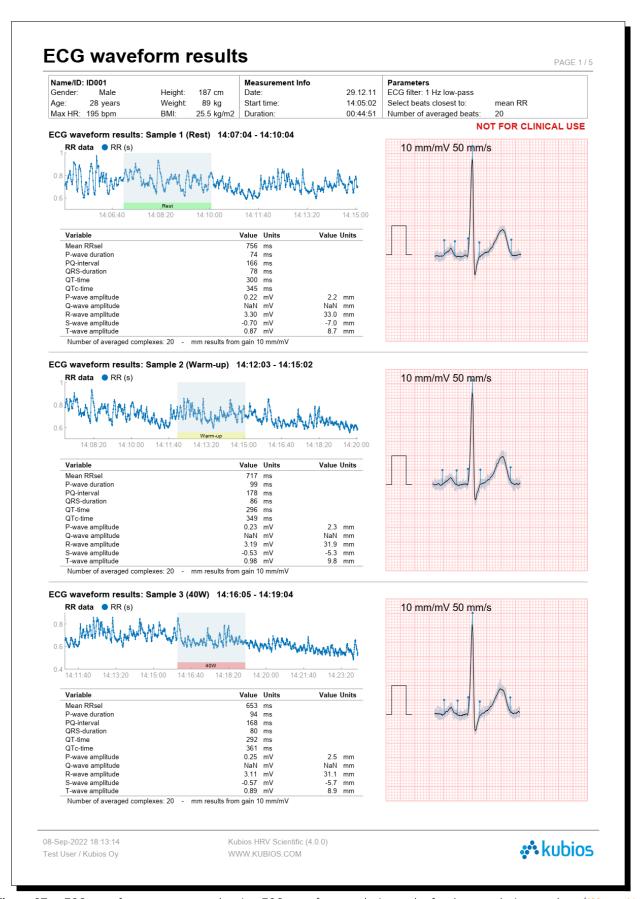
**Figure 24:** Kubios HRV time-varying analysis results saved as a CSV formatted text file. In this case, time-varying results were computed at 120-sec window with 10-sec grid interval. Note: only the first few columns of results are shown. (\*Note: Not available in the Lite version.)



**Figure 25:** Kubios HRV report page showing HRV analysis results for healthy young male during supine rest (5-min analysis sample).



**Figure 26:** Kubios HRV report page showing time-varying analysis results for maximal cardiopulmonary exercise test performed by healthy young male. (\*Note: Not available in the Lite version.)



**Figure 27:** ECG waveform report page showing ECG waveform analysis results for three analysis samples. (\*Note: Not available in the Lite version.)

-	•
Δ	u
_	M

		Sample 1 (S <sub>1</sub> )						
Files analyzed	Parameters	Info	HRV results	ECG results	Info	HRV results	ECG results	etc.
FileName	PRM#[name]	S1_[info]	S1_[name]	S1_[name]	S2_[info]	S2_[name]	S2_[name]	
subject_1.txt	[1x18] array	[1x2]	[1x86]	[1x12]	[1x2]	[1x86]	[1x12]	
subject_2.txt	[1x18] array	[1x2]	[1x86]	[1x12]	[1x2]	[1x86]	[1x12]	
:	:	:	:	:	:	:	:	
;		:	•	•	1			
	[4 40]	[ [4 6]	[4 00]	[4 40]	[4 0]	[4 00]	[4 40]	
subject_M.txt	[1x18] array	[1x2]	[1x86]	[1x12]	[1x2]	[1x86]	[1x12]	

B)

#### **Parameters**

Detrending: Detrending method used InterpRate: Interpolation rate of RR data MinMaxHR: Nbr of beats averaged for Min/Max HR NNxxThreshold: Threshold for NNxx and pNNxx in msec VLFband: VLF frequency band limits in Hz LFband: LF frequency band limits in Hz HFband: HF frequency band limits in Hz FreqPoints: Nbr of points in spectra (points/Hz) FFTorLomb: FFT (Welch) or Lomb periodogram used

#### Sample Info

Onset-Offset: Sample onset-offset times (hh:mm:ss)

#### **HRV** analysis variables

PNS index: Parasympathetic nervous system tone index SNS index: Sympathetic nervous system tone index Stress index: Square root of Baevsky's stress index Mean RR (ms): Mean of RR intervals SDNN (ms): Standard deviation of RR intervals Mean HR (bpm): Mean heart rate SD HR (bpm): Standard deviation of heart rate  ${\tt Min}\ {\tt HR}\ ({\tt bpm})$ : Minimum HR using N beat MA Max HR (bpm): Maximum HR using N beat MARMSSD (ms): RMS of successive RR interval differences NNxx (beats): Nbr or successive RRs > xx ms pNNxx (%): Percentage of successive RRs > xx ms HRV triangular index: RR histogram area/height TINN (ms): RR histogram baseline width DC (ms): HR deceleration capacity DCmod (ms): Modified HR deceleration capacity AC (ms): HR acceleration capacity ACmod (ms): Modified HR acceleration capacity SDANN (ms): SD of 5-min RR interval segment means SDNNI (ms): Mean of 5-min RR interval segment SDs VLFpeak\_FFT\* (Hz): VLF band peak frequency (FFT) LFpeak\_FFT (Hz): LF band peak frequency (FFT) HFpeak\_FFT (Hz): HF band peak frequency (FFT) VLFpow\_FFT (ms2): Absolute VLF power (FFT) LFpow\_FFT (ms2): Absolute LF power (FFT) HFpow\_FFT (ms2): Absolute HF power (FFT) VLFpow\_FFT (log): Log VLF power (FFT) LFpow\_FFT (log): Log LF power (FFT) HFpow\_FFT (log): Log HF power (FFT) VLFpow\_FFT (%): Relative VLF power (FFT) LFpow\_FFT (%): Relative LF power (FFT) HFpow\_FFT (%): Relative HF power (FFT) LFpow\_FFT (n.u.): Normalised LF power (FFT)

#### ECG waveform variables

Number of beats: Beats used for ECG waveform average Mean RRsel (ms): Mean of RR intervals for selected beats P-wave duration (ms): Duration of P-wave PQ-interval (ms): PQ-interval length QRS-duration (ms): QRS-complex duration QT-time (ms): QT-interval length

HFpow\_FFT (n.u.): Normalised HF power (FFT) TOTpow\_FFT (ms2): Total spectral power (FFT) LF\_HF\_ratio\_FFT: LF/HF power ratio (FFT)

WelchWindow: Window width (overlap) in Welch

LombWindow: Smoothing window width in Lomb periodogram

ARspectrum: Order of AR spectrum (factorisation) Entropy: Embedding dimension (tolerance) DFAshortterm: DFA, short-term fluctuations range DFAlongterm: DFA, long-term fluctuations range RecurrencePlot: RPA, embedding dimension (threshold)

NbrSamples: Number of analyzed samples ArtifactCorrection: RR artifact correction method

Beats corrected (%): Corrected beats within the sample

VLFpeak\_AR (Hz): VLF band peak frequency (AR spectrum) LFpeak\_AR (Hz): LF band peak frequency (AR spectrum) HFpeak\_AR (Hz): HF band peak frequency (AR spectrum) VLFpow\_AR (ms2): Absolute VLF power (AR spectrum) LFpow\_AR (ms2): Absolute LF power (AR spectrum) HFpow\_AR (ms2): Absolute HF power (AR spectrum) VLFpow\_AR (log): Log VLF power (AR spectrum) LFpow\_AR (log): Log LF power (AR spectrum) HFpow\_AR (log): Log HF power (AR spectrum) VLFpow\_AR (%): Relative VLF power (AR spectrum) LFpow\_AR (%): Relative LF power (AR spectrum) HFpow\_AR (%): Relative VLF power (AR spectrum) LFpow\_AR (n.u.): Normalised LF power (AR spectrum) HFpow\_AR (n.u.): Normalised HF power (AR spectrum) TOTpow\_AR (ms2): Total spectral power (AR spectrum) LF\_HF\_ratio\_AR: LF/HF power ratio (AR spectrum)

#### RESP (Hz): Respiration rate

SD1 (ms): Poincaré plot short term variability SD2 (ms): Poincaré plot long term variability SD2\_SD1\_ratio: SD2/SD1 ratio ApEn: Approximate entropy SampEn: Sample entropy D2: Correlation dimension DFA1: DFA, short term fluctuations slope DFA2: DFA, long term fluctuations slope RP Lmean (beats): RPA, mean line length

RP\_Lmax (beats): RPA, maximum line length RP\_REC (%): RPA, recurrence rate RP\_DET (%): RPA, determinism RP\_ShanEn: RPA, Shannon entropy

MSE\_1 ...MSE\_20: Multiscale entropy for scales  $\tau=1,\ldots,20$ 

QTc-time (ms): Corrected QT-interval length P-wave amplitude (mV): Amplitude of P-wave Q-wave amplitude (mV): Amplitude of Q-wave R-wave amplitude (mV). Amplitude of R-wave S-wave amplitude (mV): Amplitude of S-wave T-wave amplitude (mV): Amplitude of T-wave

 $^*$  If Lomb-Scargle periodogram is used instead of Welch's periodogram, FFT ightarrow Lomb

Figure 28: Structure of the "SPSS friendly" batch file: A) overview of the file structure and B) short description of the fields. (\*Note: Not available in the Lite version.)

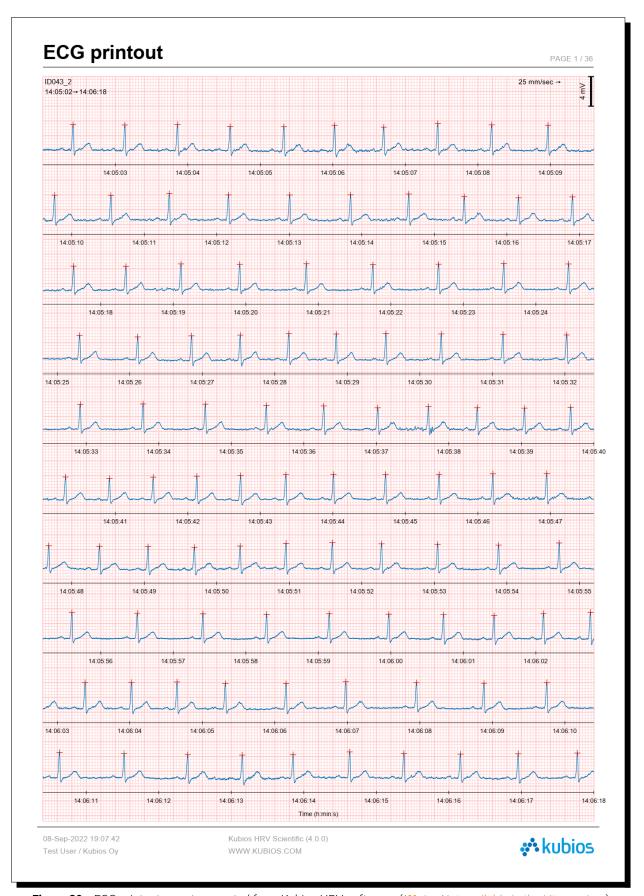


Figure 29: ECG printout report generated from Kubios HRV software. (\*Note: Not available in the Lite version.)

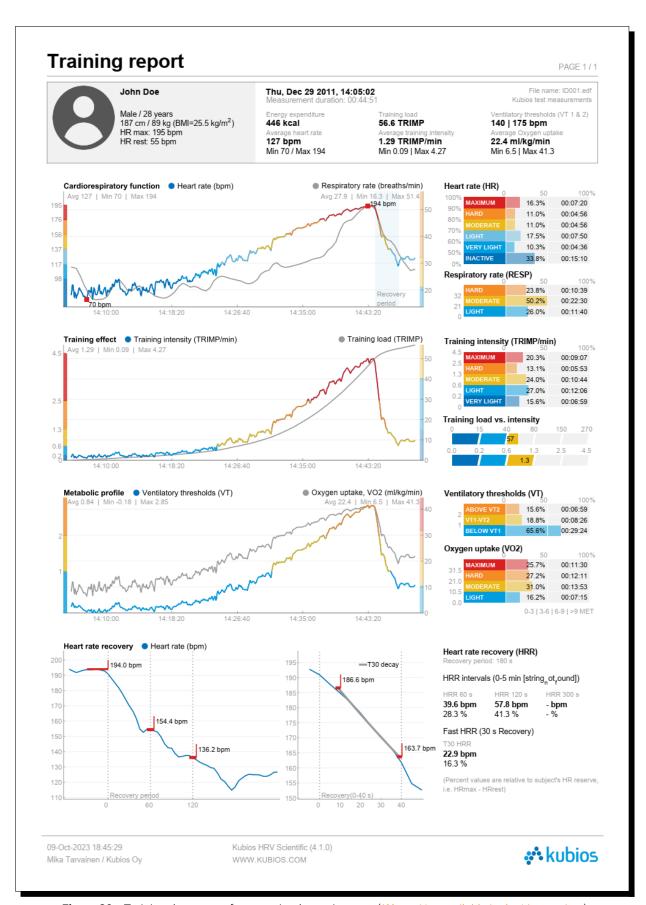
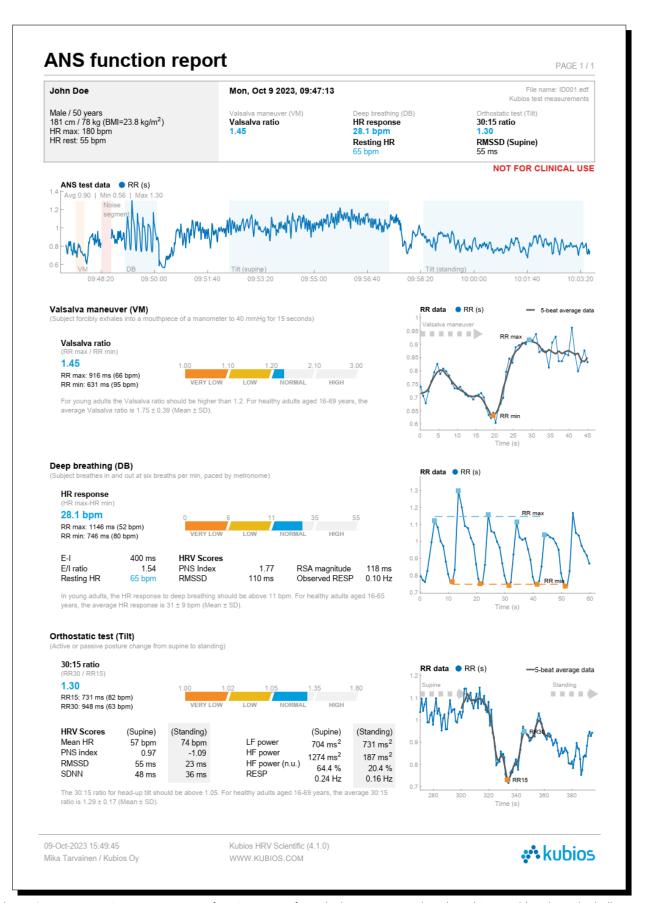


Figure 30: Training data report for a maximal exercise test. (\*Note: Not available in the Lite version.)



**Figure 31:** Autonomic nervous system function report for Valsalva maneuver, deep breathing, and head-up tilt challenges. (\*Note: Not available in the Lite version.)

References 48

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