**1. ABOUT THE DATASET**

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Title: Dataset supporting paper ‘Circulating extracellular vesicles are strongly associated with cardiovascular risk markers’

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Rights-holders: University of Reading and Esra Bozbas

Publication Year: 2022

Description: This dataset includes all reports and summaries of raw data supporting the results presented in the paper ‘Circulating extracellular vesicles are strongly associated with cardiovascular risk markers’. The objective of this paper is to investigate the relationships between circulating extracellular vesicles (EVs), conventional cardiovascular risk markers and thrombogenic markers in subjects with moderate risk of cardiovascular diseases (CVDs), which is the part aim of a larger study investigating the effect of fish oil-derived n-3 polyunsaturated fatty acids on the generation and functional activities of EVs based on a randomised, double-blind, placebo-controlled crossover intervention. The results and findings involved in this dataset relate only to the baseline data prior to any intervention. A total of 40 subjects (aged 40~70yrs) with moderate risk of CVDs were recruited and involved in the study. Approximately 100ml of blood was collected from individuals after overnight fasting for all analysis, in which 35ml was processed for the analysis of circulating EVs, 9ml was taken for the analysis of blood lipids and glucose levels for the conventional cardiovascular markers evaluation and the rest was operated for the thrombogenic markers evaluation. Data about EVs parameters include: i. the numbers and size of circulating total EVs measured by Nanoparticle Tracking Analysis (NTA); ii. the numbers of EV subpopulations (i.e. phosphatidylserine-positive EVs (PS+EVs), platelet-derived EVs (PDEVs) and endothelial-derived EVs (EDEVs)) measured by flow cytometry (FCM); iii. the procoagulatory activity of circulating EVs (*in vitro* thrombogenic potential in activating tissue factor-dependent thrombin generation) measured by thrombin generation assay. Data about conventional cardiovascular risk markers include i. body mass index (BMI) measured by Tanita; ii. blood pressure measured by upper arm blood pressure monitor; iii. plasma lipid profile (i.e. triacylglycerol (TAG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C)), and glucose concentrations measured by iLab; iv. 10-year CVD risk detected by QRISK2 scoring system. Data about thrombogenic markers include: i. plasma platelet aggregation measured by 96-well high-throughput aggregometry; ii. plasma thrombin generation measured by thrombin generation assay; iii. plasma clot growth and fibrinolysis measured by thrombodynamics analyser.

Cite as: Zhou, Ruihan and Bozbas, Esra (2022): Dataset supporting paper ‘Circulating extracellular vesicles are strongly associated with cardiovascular risk markers’. University of Reading. Dataset. https://doi.org/10.17864/1947.000366.

Related publication: Zhou, Ruihan, Bozbas, Esra, Allen-Redpath, Keith and Yaqoob, Parveen (2022). Circulating extracellular vesicles are strongly associated with cardiovascular risk markers (In preparation).

**2. TERMS OF USE**

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**3. PROJECT AND FUNDING INFORMATION**

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Title: Effects of Fish Oil-derived N-3 Polyunsaturated Fatty Acids on the Generation and Functional Activities of Extracellular Vesicles

Dates: February 2018-March 2021

Funding organisation: Biotechnology and Biological Sciences Research Council (BBSRC)

Grant no.: BB/N021185/1

**4. CONTENTS**

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**1. Data\_Summary.csv (file)**

 A summary of all data related to EVs parameters, conventional cardiovascular risk markers and thrombogenic markers.

\*HF03 has dropped out

\*Missing results for HF02 in EV thrombogenic potential

Abbreviation: *ADP, adenosine diphosphate; BMI, body mass index; CRP-XL, cross-linked collagen-related peptide; DBP, diastolic blood pressure; EC50,* *half maximal effective concentration; EDEV, endothelial-derived extracellular vesicles; EPI, epinephrine; ETP, endogenous thrombin potential; FCM, flow cytometry; HDL-C, high-density lipoprotein cholesterol; NTA, nanopartic le tracking analysis; PDEVs, platelet-derived extracellular vesicles; PS+EVs, phosphatidylserine positive extracellular vesicles; SBP, systolic blood pressure; TAG, triacylglycerol; TC, total cholesterol; TEVs, total extracellular vesicles; TRAP-6, thrombin receptor-activated peptide 6.*

**2. NTA\_data (zip)**

**2.1 NTA\_data\_report (folder)**

Raw data exported by NTA 3.20 instrument software illustrating the numbers of total events and events ranging 70nm-1000nm, and mean/mode size of events detected by NTA. In each HFXX folder, file ‘FraX (fractions isolated by size exclusion chromatography before NTA analysis)\_Dilute factor (diluted with PBS)\_total\_events\_and\_size’ provides the results of total events numbers and mean/mode size in each fraction; file ‘FraX (fractions isolated by size exclusion chromatography before NTA analysis)\_Dilute factor (diluted with PBS)\_events\_(70nm-1000nm)’ provides the results of events ranging 70nm-1000nm numbers in each fraction.

\*HF03 has dropped out

**2.2 NTA\_data.xlsx (file)**

A spreadsheet illustrating how raw data exported by NTA 3.20 instrument software were calculated to final results of TEV numbers. The Total events columns, Events (70nm-1000nm) columns and Dilution factor columns were recorded based on the corresponding reports mentioned above. Total particles /TEVs per ml PFP was calculated as

Total events/Events (70nm-1000nm) X Dilution factor = Total particles /TEVs per ml PFP

Then all frac of Total particles /TEVs per ml PFP were summed in Total row. Total particles /TEVs per ml blood was calculated as

Total particles /TEVs per ml PFP $×$ $\frac{Total volume of blood}{Total volume of plasma}$= Total particles /TEVs per ml blood

The mean size/mode size of EVs was averaged by the Mean size/Mode size of all frac.

\*Bold values were finally recorded in Data\_Summary.csv and applied in statistical analysis.

\*HF03 has dropped out

Abbreviation: *PFP, platelet-free plasma; TEVs, total extracellular vesicles.*

**3. FCM\_data (zip)**

**3.1 FCM\_data\_report (folder)**

Raw data exported by BD FACSDiva™ Software illustrating the numbers of PS+EVs, PDEVs and EDEVs detected by FCM. In each pdf report, top left figure refers to all particles detected within an initial EV size gate on side scatter mode; top right figure refers to all Allophycocyannin (APC) fluorescently labelled particles detected on fluorescence mode; bottom left figure refers to all APC and/or Phycoerythrin (PE) fluorescently labelled particles detected on fluorescence mode; bottom right figure refers to all APC and/or Pacific Blue (PB) fluorescently labelled particles detected on fluorescence mode. In each HFXX folder, file ‘HFX\_AnnV\_control\_APC’ provides results of particles numbers (P1 and top right figure) displayed in AnnV negative control; file ‘HFXX\_AnnV+events\_and\_AnnV+CD41+events\_APC’ provides results of all AnnV+particles numbers (P1 and top right figure) and AnnV+CD41+ particles (i.e. PDEVs) numbers (P4 and bottom left figure) displayed in main sample tube; file ‘HF01\_AnnV+CD105+events\_PB’ provides results of AnnV+CD105+particles (i.e. EDEVs) numbers (Q2-1 and bottom right figure) displayed in the main sample tube.

**3.2 FCM\_data****.xlsx (file)**

A spreadsheet illustrating how raw data exported by BD FACSDiva™ Software were calculated to final results of PS+EV, PDEVs and EDEVs numbers. AnnV+particles was obtained by All AnnV+particles subtracting AnnV negative control. AnnV+CD41+particles and AnnV+CD105+particles were recorded based on the corresponding report mentioned above. FCM-analysed volume was calculated as:

$\frac{[(Trucount morning events+Trount evening events)/2]×500μL}{Trucount beads}$ = FCM-analysed volume (μL)

PS+EVs/PDEVs/EDEVs per ml PFP were calculated as:

$\frac{AnnV+particles/AnnV+CD41+particles/AnnV+CD105+particles×250μL}{FCM-analysed volume}$ x 200 x PFP volume = PS+EVs/PDEVs/EDEVs per ml PFP

PS+EVs/PDEVs/EDEVs per ml blood were calculated as:

PS+EVs/PDEVs/EDEVs per ml PFP $×$ $\frac{Total volume of blood}{Total volume of plasma}$= PS+EVs/PDEVs/EDEVs per ml blood

\*Bold values were finally recorded in Data\_Summary.csv and applied in statistical analysis.

\*HF03 has dropped out

Abbreviation: *AnnV, Annexin V; EDEV, endothelial-derived extracellular vesicles; EPI, epinephrine; ETP, endogenous thrombin potential; FCM, flow cytometry; HDL-C, high-density lipoprotein cholesterol; NTA, nanoparticle tracking analysis; PFP, platelet-free plasma; PDEVs, platelet-derived extracellular vesicles; PS+EVs, phosphatidylserine positive extracellular vesicles; SBP, systolic blood pressure; TAG, triacylglycerol; TC, total cholesterol; TEVs, total extracellular vesicles; TRAP-6, thrombin receptor-activated peptide 6.*

**4. Thrombodynamics\_data\_report (zip)**

PDFs exported by thrombodynamics instrument software include fibrin dynamics parameters and representative images of the coagulation process and fibrinolysis. Each HFXX pdf file provides the results of the rate of clot growth, lag time, initial of clot growth, clot size at 30 min, clot density, lysis onset time and lysis progression presented in Data\_Summary.csv.

\*HF03 has dropped out

**5. Thrombin\_generation\_data.xlsx (file)**

A spreadsheet illustrating the results of fluorescent intensity was detected by the plate reader (FlexStation 3, United State) at 360 nm for excitation and at 460 nm for emission to calculate thrombin generation in the unit of RFU. Raw data were then analysed by the TGA Evaluation Software manually to convert the unit of thrombin generation from RFU to nM and presented as five variables: lag time, time to the peak, the concentration of thrombin (nM), velocity-index and area under curve in Data\_Summary.csv.

\*HF03 has dropped out

**6. Platelet\_aggregation\_data. xlsx (file)**

A spreadsheet illustrates the results of platelet aggregation included by ADP, CRP-XL, EPI, TRAP-6 and U46619. Raw absorbance data were exported by a plate-reader (Tecan Microplate Reader Spark, Switzerland) and converted to a percent aggregation by reference to the absorbance of platelet-rich plasma and platelet-poor plasma as 0% and 100% aggregation controls, respectively. Dose-response curves in response to each agonist were obtained and curves were fitted by a four-parameter logistic non-linear regression using Prism (8.2; GraphPad Software, Inc., San Diego, CA). These dose-response curves of platelet aggregation were used to calculate relative parameters including EC50 and/or LogEC50 values.

Calculate percentage aggregation:

% aggregation = 100 - 100\* $\frac{absorbance well – average absorbance wells A1-D1}{Average absorbance wells E1-G1 - average absorbance wells }$

\*HF03 has dropped out

Abbreviation: *ADP, adenosine diphosphate; CRP-XL, cross-linked collagen-related peptide; EC50,* *half maximal effective concentration; EPI, epinephrine; TRAP-6, thrombin receptor-activated peptide 6; U4,* *U46619.*

**5. METHODS**

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Detailed information about methods could be found in the paper ‘Circulating extracellular vesicles are strongly associated with cardiovascular risk markers’.