











The metabolic signature of cardiorespiratory fitness: a protocol for a systematic review and meta-analysis

Justin Carrard ¹, Chiara Guerini,¹ Christian Appenzeller-Herzog ², Denis Infanger ¹, Karsten Königstein ¹, Lukas Streese ¹, Timo Hinrichs ¹, Henner Hanssen ¹, Hector Gallart-Ayala ³, Julijana Ivanisevic ³, Arno Schmidt-Trucksäss ¹

To cite: Carrard J, Guerini C, Appenzeller-Herzog C, *et al*. The metabolic signature of cardiorespiratory fitness: a protocol for a systematic review and meta-analysis. *BMJ Open Sport & Exercise Medicine* 2021;**7**:e001008. doi:10.1136/bmjsem-2020-001008

► Additional material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/bmjsem-2020-001008>).

Jl and AS-T are joint senior authors.

Accepted 7 January 2021



© Author(s) (or their employer(s)) [year]. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

¹Division of Sports and Exercise Medicine, Department of Sport, Exercise and Health, University of Basel, Basel, Switzerland

²University Medical Library, University of Basel, Basel, Switzerland

³Metabolomics Platform, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland

Correspondence to

Dr Justin Carrard;
justin.carrard@unibas.ch and

Prof Dr Arno Schmidt-Trucksäss;
arno.schmidt-trucksass@unibas.ch

ABSTRACT

Introduction A low cardiorespiratory fitness (CRF) is a strong and independent predictor of cardiometabolic, cancer and all-cause mortality. To date, the mechanisms linking CRF with reduced mortality remain largely unknown. Metabolomics, which is a powerful metabolic phenotyping technology to unravel molecular mechanisms underlying complex phenotypes, could elucidate how CRF fosters human health.

Methods and analysis This study aims at systematically reviewing and meta-analysing the literature on metabolites of any human tissue sample, which are positively or negatively associated with CRF. Studies reporting estimated CRF will not be considered. No restrictions will be placed on the metabolomics technology used to measure metabolites. PubMed, Web of Science and EMBASE will be searched for relevant articles published until the date of the last search. Two authors will independently screen full texts of selected abstracts. References and citing articles of included articles will be screened for additional relevant publications. Data regarding study population, tissue samples, analytical technique, quality control, data processing, metabolites associated to CRF, cardiopulmonary exercise test protocol and exercise exhaustion criteria will be extracted. Methodological quality will be assessed using a modified version of QUADOMICS. Narrative synthesis as well as tabular/charted presentation of the extracted data will be included. If feasible, meta-analyses will be used to investigate the associations between identified metabolites and CRF. Potential sources of heterogeneity will be explored in meta-regressions.

Ethics and dissemination No ethics approval is required. The results will be published in a peer-reviewed journal and as conference presentation.

PROSPERO registration number CRD42020214375.

INTRODUCTION

Cardiorespiratory fitness (CRF), defined as the maximal oxygen uptake, is considered to be such a powerful health marker that the American Heart Association recommends assessing it as a vital sign in clinical routine.¹ Indeed, CRF has been inversely correlated

Strengths and limitations of this study

- To the best of authors' knowledge, this will be the first systematic review summarising associations between metabolites of any human tissue sample and cardiorespiratory fitness (CRF).
- Identifying metabolites associated with high and low CRF could help elucidating how CRF fosters human health.
- A possible limitation is the inclusion of studies written in English, French, German, Spanish and Italian only.
- A second possible limitation is the restriction to studies using metabolomics approaches.

with incidence of cancer, cardiometabolic diseases as well as with all-cause mortality.^{2–7} Even more importantly, improvement in CRF is associated with reduced incidence of stroke, type 2 diabetes, dementia and lowered all-cause mortality.^{8–13}

CRF reflects the capacity of the body to transport oxygen from its uptake in the air to its delivery to the mitochondria in order to carry out physical work.¹ If the heritability of both CRF and gains in CRF has been shown to be around 50%, the biological mechanisms linking CRF with reduced morbidity and mortality remain largely unknown.^{14–16} Since CRF is considered a better morbidity and mortality predictor than physical activity level itself, it can be deduced that the mechanisms underlying responses to exercise are not sufficient to explain the link between CRF and mortality.^{17–19} Understanding through which metabolic pathways CRF mitigates morbidity and mortality might pave the way for novel fitness-enhancing strategies in clinical routine.²⁰

Metabolomics is a powerful metabolic phenotyping technology to investigate biochemical mechanisms underlying complex phenotypes.²¹ Indeed, the metabolome

**Table 1** The Population, Exposure, Comparison, Outcome, Study design process

Item	Specification
Population or participants and conditions of interest	Humans (any age, any sex and any health condition).
Exposure	Metabolites derived from metabolomics or multiomics studies applying metabolomics approaches.
Comparisons or control groups	NA
Outcomes of interest	CRF measured by means of a cardiopulmonary exercise test (spiroergometry).
Study designs	Any study design, only published studies, no editorials, letters, reviews, meta-analyses, case reports or conference abstracts.

CRF, cardiorespiratory fitness; NA, not available.

readout offers a sensitive and dynamic measure of phenotypes at the molecular level, reflecting the underlying biochemical activity in different physiological conditions or following the exposure to diverse external or internal stimuli.^{21 22} In contrast to genomics and proteomics, metabolomics provides insights on what has happened.²¹ The changes recorded in the metabolome reflect the influence of both, the genome and the exposome.²¹ In addition to having a structural function as building blocks of cell components and fuels in cellular energetics, metabolites are important signalling molecules and a potential driving force in the pathophysiology of human diseases.^{23 24}

Why is it important to do this review?

In the light of the high clinical relevance of CRF, it is of utmost importance to better understand the mechanisms linking CRF with reduced morbidity and mortality. Reviewing and meta-analysing the literature to identify metabolites associated with high and low CRF levels represents a first step to reveal biological mechanisms connecting CRF to health benefits.

Aim and review question

This study aims at systematically reviewing and meta-analysing the current literature on metabolites in human body tissues, fluids, or excretions that are positively or negatively associated with CRF.

The research question of this review is the following: which metabolites in human body tissues, fluids or excretions are positively or negatively associated with CRF?

METHODS AND ANALYSIS

This systematic review protocol follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.²⁵ The initial preliminary search was conducted on 17 August 2020. The protocol was submitted for registration in International Prospective Register of Systematic Reviews on 14 October 2020 and registered on 14 November 2020. The anticipated completion date is 1 November 2021. The research question was formulated according to the Population, Exposure, Comparison, Outcome, Study Type framework (table 1).²⁶

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Eligibility criteria

Inclusion criteria

- ▶ All clinical studies involving humans, which were published until the date of the last search.
- ▶ Metabolomics studies, or multiomics studies applying metabolomics approaches, reporting metabolites of any tissue, associated, positively or negatively, with CRF.
- ▶ Studies measuring CRF by means of a cardiopulmonary exercise test (spiroergometry).

Exclusion criteria

- ▶ Studies reporting estimated CRF.
- ▶ Studies published in languages other than English, German, French, Italian and Spanish.
- ▶ Non-original articles (ie, editorials, letters, reviews), meta-analyses, case reports, conference abstracts.

Methodological considerations

Due to the fact that estimated CRF has been shown to be only moderately correlated with measured CRF studies reporting estimated CRF will not be considered.²⁷

Information sources and search strategy

Search strategies were developed in collaboration with an information specialist (CA-H) using the Peer Review of Electronic Search Strategies framework.²⁸ PubMed, Web of Science and EMBASE will be searched. Database-specific subject headings and text word synonyms around the concepts metabolomics and CRF will be used. The searches will be rerun immediately prior to the final analysis. Search results will be exported to EndNote X9 (Clarivate, London, UK) and deduplicated. The detailed search strings can be found in online supplemental document.

Table 2 Data that will be extracted from every study included in the review

No	Description
1	Authors and year of publication.
2	Country of study.
3	Study design.
4	Study population.
5	Study population demographics (n, age, sex, body mass index, body fat percentage, physical activity levels, cardiorespiratory fitness, medication).
6	Study completion rate.
7	Potential health conditions.
8	Tissue sample.
9	Sample collection and storage.
10	Sampling time and nutritional protocol before sampling.
11	Metabolite extraction method.
12	Metabolomics analytical technique.
13	Quality control used to assess data quality.
14	Data processing and metabolite annotation.
15	Metabolites associated with CRF.
16	CPET protocol and exercise exhaustion criteria.

CPET, cardiopulmonary exercise testing; CRF, cardiorespiratory fitness.

Study records: data management, selection process and data collection process

The titles and abstracts of retrieved records will be reviewed independently by two authors (CG and JC). Articles will be deemed as 'include', 'exclude' or 'uncertain' by following the prespecified eligibility criteria. For articles deemed 'include' or 'uncertain' the full text will be retrieved and independently reviewed for eligibility by two authors (CG and JC). If discrepancies arise during title/abstract or full text screening, they will be resolved by discussion between the two screening authors. A third party will make a final judgement in case no resolution can be found (LS). To complement the results of direct database searching, the bibliographic references of all included articles (backward citation tracking), as well as the citing articles of those that are indexed in Scopus or the Web of Science will be screened (forward citation tracking). Data will be extracted from the full texts and entered into a standardised Excel form. One author will extract the data (CG), and a second author will independently check the extractions (JC). Discrepancies will be resolved through discussion (with a third party if necessary, LS). Corresponding authors will be contacted twice by email in case any data are missing or unclear. In the absence of response or if required data cannot be provided, publications will be excluded from meta-analysis. The information to be extracted are shown in [table 2](#).

Outcome and prioritisation

The main outcome will be:

Metabolites which are either positively or negatively associated with measured CRF.

Risk of bias in individual studies

The quality of the following metabolomics workflow key steps will be assessed at the study level: sample collection and storage, sampling time and nutritional protocol before sampling, metabolite extraction method, analytical technique, quality control used to assess data quality, data processing and metabolite annotation. Additionally, a custom modified version of QUADOMICS will be used for study quality rating (online supplemental table S1).^{29 30} The risk of bias at the study level will be assessed by two authors independently (CG and JC). Discrepancies will be resolved through discussion (with a third party if necessary, LS).

Data synthesis

Data issued from untargeted (relative quantification) and targeted (absolute quantification) metabolomics studies will be analysed separately.³¹ Quantitative data describing associations between metabolites and CRF levels will be extracted from all selected studies. The data will be presented in tabular/charted format. The adequate summary measure will be determined according to the nature of the collected outcomes (likely correlation or regression coefficients).³² Effect sizes will be converted into the chosen summary measure as previously described.³³

In case of longitudinal data, information available for several time points will be extracted, too. Only metabolites, which are reported in at least three different studies and are identified on a level 1 identification according to the Metabolomics Standards Initiative, will be meta-analysed.³⁴ In other words, three data points will be the minimum threshold for conducting a meta-analysis.

Summary measures will be calculated using a random effects model. Restricted maximum likelihood will be used to estimate between-study variance.³⁵ Forest plots will be used to display and compare estimates across studies. Heterogeneity among studies will be estimated by the Cochran Q test and quantified by the I² statistic.³⁶

Additional analyses

Potential sources of heterogeneity will be explored in meta-regressions, such as age, sex, health conditions and physical activity levels, study design (case-control, nested case-control, cohort), biological sample and analytical technique (gas or liquid chromatography coupled with mass spectrometry; or proton nuclear magnetic resonance).

Meta-bias(es)

If at least five studies report on the same outcome parameter, publication bias will be assessed using funnel plots, displaying effect estimates against sample sizes.³⁷ Plot asymmetry will be assessed using Egger's regression test,

where a regression intercept of zero indicates an absence of publication bias.

Confidence in cumulative evidence

The confidence in evidence will be evaluated with the Grading of Recommendations Assessment, Development and Evaluation system, which is a tool classifying evidence into one of four categories ranging from very low to high.³⁸

Ethics and dissemination

The present work is a systematic review and meta-analysis protocol. No human participants will be involved; therefore, no ethics approval is required. It is planned to communicate the study results in a peer-reviewed journal and as a conference presentation.

CONCLUSION

The biological mechanisms linking CRF with reduced morbidity and all-cause mortality remain largely unknown. Conducting a systematic review to identify metabolites associated, positively or negatively, with CRF could be a first step to reveal metabolic pathways mediating the protective effect of high CRF level. Finally, understanding through which pathways CRF mitigates morbidity and mortality might pave the way for novel fitness-enhancing strategies in clinical routine.

Twitter Justin Carrard @CarrardJustin, Karsten Königstein @KarstenKnigste1, Lukas Streese @StreeseL, Henner Hanssen @HanssenHenner, Hector Gallart-Ayala @HGallartAyala and Julijana Ivanisevic @JuliJivanisevic

Contributors The following work has been developed in contribution of each coauthor. The manuscript underwent several revisions with substantial contributions provided by each coauthor. CG, JC and CA-H designed the search string. CG and JC wrote the protocol. CA-H was responsible for the search strategy. DI and JC designed the data synthesis strategy. CA-H reviewed the protocol, while LS, KK, TH, HH, HG-A, JI and AS-T critically revised it. JC registered the protocol in PROSPERO. JC, CG and LS will conduct the systematic review and meta-analysis. All authors provided critical feedback, have read and approved the final manuscript.

Funding This study was funded by the Department of Sport, Exercise and Health of the University of Basel, Switzerland.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data sharing not applicable as no datasets generated and/or analysed for this study. All data relevant to the study are included in the article or uploaded as online supplemental information.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iDs

Justin Carrard <http://orcid.org/0000-0002-2380-105X>
 Christian Appenzeller-Herzog <http://orcid.org/0000-0001-7430-294X>
 Denis Infanger <http://orcid.org/0000-0001-9028-7110>
 Karsten Königstein <http://orcid.org/0000-0002-2994-8570>
 Lukas Streese <http://orcid.org/0000-0003-3920-8610>
 Timo Hinrichs <http://orcid.org/0000-0001-6200-307X>
 Henner Hanssen <http://orcid.org/0000-0001-5501-4205>

Hector Gallart-Ayala <http://orcid.org/0000-0003-2333-0646>
 Julijana Ivanisevic <http://orcid.org/0000-0001-8267-2705>
 Arno Schmidt-Trucksäss <http://orcid.org/0000-0002-4662-3911>

REFERENCES

- Ross R, Blair SN, Arena R, *et al*. Importance of assessing cardiorespiratory fitness in clinical practice: a case for fitness as a clinical vital sign: a scientific statement from the American heart association. *Circulation* 2016;134:e653–99.
- Ahmed HM, Al-Mallah MH, McEvoy JW, *et al*. Maximal exercise testing variables and 10-year survival: fitness risk score derivation from the fit project. *Mayo Clin Proc* 2015;90:346–55.
- Robsahm TE, Falk RS, Heir T, *et al*. Measured cardiorespiratory fitness and self-reported physical activity: associations with cancer risk and death in a long-term prospective cohort study. *Cancer Med* 2016;5:2136–44.
- Farrell SW, Finley CE, Radford NB, *et al*. Cardiorespiratory fitness, body mass index, and heart failure mortality in men: Cooper center longitudinal study. *Circ Heart Fail* 2013;6:898–905.
- Gander JC, Sui X, Hébert JR, *et al*. Association of cardiorespiratory fitness with coronary heart disease in asymptomatic men. *Mayo Clin Proc* 2015;90:1372–9.
- Juraschek SP, Blaha MJ, Whelton SP, *et al*. Physical fitness and hypertension in a population at risk for cardiovascular disease: the Henry Ford exercise testing (fit) project. *J Am Heart Assoc* 2014;3:e001268.
- Williams PT. Changes in vigorous physical activity and incident diabetes in male runners. *Diabetes Care* 2007;30:2838–42.
- Prestgaard E, Mariampillai J, Engeseth K, *et al*. Change in cardiorespiratory fitness and risk of stroke and death. *Stroke* 2018;Strokeaha118021798.
- de Lannoy L, Sui X, Lavie CJ, *et al*. Change in submaximal cardiorespiratory fitness and all-cause mortality. *Mayo Clin Proc* 2018;93:184–90.
- Tarp J, Støle AP, Blond K, *et al*. Cardiorespiratory fitness, muscular strength and risk of type 2 diabetes: a systematic review and meta-analysis. *Diabetologia* 2019;62:1129–42.
- Ehrman JK, Brawner CA, Al-Mallah MH, *et al*. Cardiorespiratory fitness change and mortality risk among black and white patients: Henry Ford exercise testing (FIT) project. *Am J Med* 2017;130:1177–83.
- Tari AR, Nauman J, Zisko N, *et al*. Temporal changes in cardiorespiratory fitness and risk of dementia incidence and mortality: a population-based prospective cohort study. *Lancet Public Health* 2019;4:e565–74.
- Imboden MT, Harber MP, Whaley MH, *et al*. The association between the change in directly measured cardiorespiratory fitness across time and mortality risk. *Prog Cardiovasc Dis* 2019;62:157–62.
- Bouchard C, Daw EW, Rice T, *et al*. Familial resemblance for VO₂max in the sedentary state: the heritage family study. *Med Sci Sports Exerc* 1998;30:252–8.
- Bouchard C, Sarzynski MA, Rice TK, *et al*. Genomic predictors of the maximal O₂ uptake response to standardized exercise training programs. *J Appl Physiol* 2011;110:1160–70.
- Ross R. Knowing that cardiorespiratory fitness should be a vital sign is not enough. *Mayo Clin Proc* 2019;94:1395–7.
- Williams PT. Physical fitness and activity as separate heart disease risk factors: a meta-analysis. *Med Sci Sports Exerc* 2001;33:754–61.
- Myers J, Kaykha A, George S, *et al*. Fitness versus physical activity patterns in predicting mortality in men. *Am J Med* 2004;117:912–8.
- Lee D-C, Sui X, Ortega FB, *et al*. Comparisons of leisure-time physical activity and cardiorespiratory fitness as predictors of all-cause mortality in men and women. *Br J Sports Med* 2011;45:504–10.
- Sanford JA, Nogiec CD, Lindholm ME, *et al*. Molecular transducers of physical activity Consortium (MoTrPAC): mapping the dynamic responses to exercise. *Cell* 2020;181:1464–74.
- Ivanisevic J, Thomas A. Metabolomics as a Tool to Understand Pathophysiological Processes. In: Giera M, ed. *Clinical metabolomics: methods and protocols*. New York, NY: Springer New York, 2018: 3–28.
- Hoffman JM, Lyu Y, Pletcher SD, *et al*. Proteomics and metabolomics in ageing research: from biomarkers to systems biology. *Essays Biochem* 2017;61:379–88.
- Rinschen MM, Ivanisevic J, Giera M, *et al*. Identification of bioactive metabolites using activity metabolomics. *Nat Rev Mol Cell Biol* 2019;20:353–67.
- Wishart DS. Metabolomics for investigating physiological and pathophysiological processes. *Physiol Rev* 2019;99:1819–75.

- 25 Shamseer L, Moher D, Clarke M, *et al.* Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ* 2015;349:g7647.
- 26 Methley AM, Campbell S, Chew-Graham C, *et al.* PICO, PICOS and spider: a comparison study of specificity and sensitivity in three search tools for qualitative systematic reviews. *BMC Health Serv Res* 2014;14:579.
- 27 Loprinzi PD, Edwards MK, Addoh O, *et al.* Evaluation of the convergent validity of an estimated cardiorespiratory fitness algorithm. *Eur J Appl Physiol* 2018;118:629–36.
- 28 McGowan J, Sampson M, Salzwedel DM, *et al.* PRESS Peer Review of Electronic Search Strategies: 2015 Guideline Statement. *J Clin Epidemiol* 2016;75:40–6.
- 29 Lumbreras B, Porta M, Márquez S, *et al.* QUADOMICS: an adaptation of the Quality Assessment of Diagnostic Accuracy Assessment (QUADAS) for the evaluation of the methodological quality of studies on the diagnostic accuracy of '-omics'-based technologies. *Clin Biochem* 2008;41:1316–25.
- 30 Parker LA, Gómez Saez N, Lumbreras B, *et al.* Methodological deficits in diagnostic research using '-omics' technologies: evaluation of the QUADOMICS tool and quality of recently published studies. *PLoS One* 2010;5:e11419.
- 31 Lu W, Su X, Klein MS, *et al.* Metabolite measurement: pitfalls to avoid and practices to follow. *Annu Rev Biochem* 2017;86:277–304.
- 32 Borenstein M, Hedges LV, Higgins JPT, *et al.* Effect sizes based on correlations. In: *Introduction to Meta-Analysis*, 2009: 41–3.
- 33 Borenstein M, Hedges LV, Higgins JPT, *et al.* Converting among effect sizes. In: *Introduction to Meta-Analysis*, 2009: 45–9.
- 34 Sumner LW, Amberg A, Barrett D, *et al.* Proposed minimum reporting standards for chemical analysis chemical analysis Working Group (CAWG) metabolomics standards initiative (MSI). *Metabolomics* 2007;3:211–21.
- 35 Langan D, Higgins JPT, Jackson D, *et al.* A comparison of heterogeneity variance estimators in simulated random-effects meta-analyses. *Res Synth Methods* 2019;10:83–98.
- 36 Borenstein M, Hedges LV, Higgins JP. *Introduction to meta-analysis*. John Wiley & Sons, 2011.
- 37 Sterne JA, Egger M. Funnel plots for detecting bias in meta-analysis: guidelines on choice of axis. *J Clin Epidemiol* 2001;54:1046–55.
- 38 Guyatt GH, Oxman AD, Vist GE, *et al.* Grade: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* 2008;336:924–6.