











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

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## 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.<sup>1</sup> 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.<sup>2–7</sup> Even more importantly, improvement in CRF is associated with reduced incidence of stroke, type 2 diabetes, dementia and lowered all-cause mortality.<sup>8–13</sup>

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.<sup>1</sup> 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.<sup>14–16</sup> 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.<sup>17–19</sup> Understanding through which metabolic pathways CRF mitigates morbidity and mortality might pave the way for novel fitness-enhancing strategies in clinical routine.<sup>20</sup>

Metabolomics is a powerful metabolic phenotyping technology to investigate biochemical mechanisms underlying complex phenotypes.<sup>21</sup> 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.<sup>21 22</sup> In contrast to genomics and proteomics, metabolomics provides insights on what has happened.<sup>21</sup> The changes recorded in the metabolome reflect the influence of both, the genome and the exposome.<sup>21</sup> 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.<sup>23 24</sup>

### 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.<sup>25</sup> 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).<sup>26</sup>

### 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.<sup>27</sup>

### 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.<sup>28</sup> 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).<sup>29 30</sup> 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.<sup>31</sup> 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).<sup>32</sup> Effect sizes will be converted into the chosen summary measure as previously described.<sup>33</sup>

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.<sup>34</sup> 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.<sup>35</sup> 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 I<sup>2</sup> statistic.<sup>36</sup>

### 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.<sup>37</sup> 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.<sup>38</sup>

### 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.

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**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.

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