A metabolomic approach to identify the link between sports activity and atheroprotection

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Received 24 March 2020; revised 15 September 2020; editorial decision 3 November 2020; accepted 10 November 2020

Aims
Physical activity (PA) is a mainstay of cardiovascular prevention. This study aimed to identify metabolic mediators of PA that protect against the development of atherosclerosis.

Methods and results
A total of 2160 participants in the LIFE heart study were analysed with data on PA and vascular phenotyping. In a targeted metabolomic approach, 61 metabolites (amino acids and acylcarnitines) were measured using liquid chromatography–tandem mass spectrometry. We investigated the interactions between PA, metabolites and markers of atherosclerosis in order to uncover possible mediation effects. Intended sports activity, but no daily PA, was associated with a lower degree of atherosclerosis, odds ratio (OR) for total atherosclerotic burden of 0.76 (95% confidence interval 0.62–0.94), carotid artery plaque OR 0.79 (0.66–0.96), and peripheral artery disease OR 0.74 (0.56–0.98). Twelve amino acids, free carnitine, five acylcarnitines were associated with sports activity. Of these, eight metabolites were also associated with the degree of atherosclerosis. In the mediation analyses, a cluster of amino acids (arginine, glutamine, pipecolic acid, taurine) were considered as possible mediators of atheroprotection. In contrast, a group of members of the carnitine metabolism (free carnitine, acetyl carnitine, octadecenoyl carnitine) were associated with inactivity and higher atherosclerotic burden.

Conclusion
Our metabolomic approach, which is integrated into a mediation model, provides transformative insights into the complex metabolic processes involved in atheroprotection. Metabolites with antioxidant and endothelial active properties are believed to be possible mediators of atheroprotection. The metabolomic mediation approach can support the understanding of complex diseases in order to identify targets for prevention and therapy.

Keywords
Atherosclerosis • Sport • Activity • Metabolome • Mediation

Introduction
The positive effect of physical activity (PA) on cardiovascular risk has been shown in many studies.1 Therefore, regular PA is recommended to prevent cardiovascular diseases.2 PA has a positive effect on various cardiovascular risk factors, including hypertension, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein (HDL)-cholesterol, body weight and type 2 diabetes mellitus.3 However, the metabolic mediation of PA has been insufficiently investigated.4 The new high-throughput metabolomics offers a robust, reliable, and inexpensive method for the quantitative profiling of metabolite classes for large sample quantities.5 The integration of metabolomics...
into a systems biological approach therefore has the potential to elucidate mechanisms that play an important role in the development of atherosclerosis.\textsuperscript{5,7} New biomarkers of cardiometabolic disease related to insulin resistance and energy metabolism have already been identified.\textsuperscript{8–10} On the other hand, it has been shown that the circulating metabolite levels depend on the extent of individual activity.\textsuperscript{11–13}

In this study, we combined these observations for the first time in the same human cohort and linking the PA-associated metabolic changes with their potentially preventive effects on atherosclerotic diseases at various anatomical sites, namely coronary, carotid and peripheral atherosclerosis. To this end, we analysed the interrelationships and potential mediation effects between PA, 61 metabolites, which were evaluated with liquid chromatography–tandem mass spectrometry (LC-MS/MS) and the different atherosclerosis characteristics in a large sample of patients with different degrees of atherosclerosis. In a previous study, we introduced a principled approach to analysing MS data that enables the identification and quantification of the effects of clinical and lifestyle factors on metabolite levels. These results are fundamental to understanding the complex relationships between risk factors, mediators, and diseases.\textsuperscript{14}

Methods

Study cohort

The LIFE-Heart study is an observational study of 7000 patients with suspected or confirmed coronary artery disease (CAD) to unravel genetic, molecular and lifestyle-related factors and mechanisms of atherosclerosis.\textsuperscript{15} The study meets the ethical standards of the Declaration of Helsinki, and written informed consent was obtained from all participants.

In the present analysis, we considered a subset of 2552 patients with available PA data and with suspected CAD. We excluded 231 cases in which documentation of their medical history or information of PA were incomplete and 161 cases in which atherosclerotic imaging or metabolic data set were incomplete or with insufficient quality. Thus, a total of 2160 subjects were included into the analyses. Classical cardiovascular risk factors were presented as combined points: Hypertension—systolic blood pressure $\geq 140$ mmHg, diastolic blood pressure $\geq 90$ mmHg, or anti-hypertensive medication. Diabetes mellitus: HbA1c $\geq 6.5\%$ or anti-diabetic medication. Dyslipidaemia: total cholesterol $>5.0$ mmol/L, HDL-cholesterol <1.0 mmol/L or lipid-lowering medication.

Assessment of physical activity

The Paffenbarger Physical Activity Questionnaire (PPAQ) was used to obtain data on PA within the last 12 months.\textsuperscript{16} Using subject responses to these questions, we estimate energy expenditure by calculating a total PA index (total-PAI) and an index of sports activity (sport-PAI) according to Paffenbarger et al.\textsuperscript{17} A more detailed description of processing the PPAQ is presented in the Supplementary material online. Subjects who engaged regularly, at least once a week for a minimum of 30 min, were classified as regular. Subjects with no regular activity but at least ten activities with a minimum of 60 min (per session) within the last 12 months were classified as sporadic active. Subjects who did not fulfill the criteria for regular or sporadic sports activity were valuated as inactive.

Evaluation of atherosclerosis

A detailed description of the assessment of coronary and non-coronary atherosclerosis has been published before.\textsuperscript{18,19} In brief, CAD was assessed by invasive coronary angiography. According to visual assessment of the coronary arteries, patients were classified in subsets of normal angiogram and CAD with at least one stenosis $\geq 25\%$ in a major coronary vessel. The presence of carotid artery plaque was determined by carotid ultrasound applying the definition of the American Society of Echocardiography Intima-Media Thickness Task Force;\textsuperscript{20} echogenic thickening of intimal reflection that extends into the arterial lumen at least 0.5 mm or 50\% of the surrounding intima-media-complex or an intimal + medial thickness of $>1.5$ mm. As the extracranial length of internal carotid artery and the quality of its imaging is lower, we restricted plaque scoring to the common part and the bulb.\textsuperscript{19} Peripheral artery disease (PAD) was defined by an ankle-brachial-index $<0.9$ for one of the legs or a revascularization of a peripheral artery in the patient history. The total atherosclerotic burden was defined when presenting a pathological finding for at least one of the locations (CAD or/and carotid plaque or/and PAD).

Metabolic analysis and data processing

A detailed description of the metabolomic approach has been published previously.\textsuperscript{21,22} In brief, venous blood samples were spotted on filter paper WS 903 (Schleicher and Schuell, Germany). Blood spots were stored at $-80^\circ C$ after 3 h of drying until mass spectrometric analysis. Specific 3.0 mm diameter dried blood spot punches (containing 3 $\mu$L whole blood) were extracted with methanol containing isotope labelled standards. After sample extraction and derivatization, analysis was performed on an API 2000 tandem mass spectrometer (Applied Biosystems, Germany). In a targeted metabolomic approach the quantification of 26 amino acids, free carnitine and 34 acylcarnitines including related metabolites was performed using ChemoView 1.4.2 software (Applied Biosystems, Germany). Furthermore, using these 61 directly measured analytes, we derived a number of biologically relevant sums (total acylcarnitine) and ratios ($n=34$) to assess reaction equilibria within physiological pathways and processes (e.g. Fischer’s ratio).\textsuperscript{23} Metabolites with more than 20\% of values below detection limit were excluded from consideration. This applies for the metabolites C5:1, C6DC, C14OH, C16OH, MeGlut, C18:1OH, C18:2OH, C18OH, C20:3, and the derived quantity Q18 ([C5:1]/[Leu/Ile]). Consequently, a total of 86 features were considered. A list of these metabolites with their abbreviations and derived quantities is presented in Supplementary material online, Table S1.

Statistical analyses

Categorical data are presented as numbers and percentages. For continuous variables, we present arithmetic mean ± standard deviation for normally distributed variables, median and interquartile ranges for non-normally distributed variables.

Analysis of associations between PA, metabolites and atherosclerosis traits was performed in four steps. In Steps 1–3, we separately studied all bivariate associations, in Step 4, we combined significant findings in a mediation model (Figure 1). In detail: in Step 1, we analysed the association of PA parameters with prevalent atherosclerosis. Odds ratios (ORs) of total-PAI (per 10 000 kJ/week) and sport-PAI (per 1000 kJ/week) were obtained from logistic regression analyses. In case of significance (sport-PAI), we use receiver operating characteristic (ROC) analysis to identify...
**Figure 1** Study design of the mediation model.

**Table 1** General characteristics

| Overall | Inactive Sport-PAI <600 kJ/week | Active Sport-PAI >600 kJ/week | P-value |
|---------|---------------------------------|-------------------------------|---------|
| N       | 2160                            | 846                           | 1314    |
| Age, years | 62 ± 11                          | 64 ± 11                       | 62 ± 11 | 1.7*10^-7 |
| Male, % (n) | 64.1 (1385)                      | 61.9 (524)                    | 65.5 (861) | 0.098 |
| Diabetes, % (n) | 29.1 (629)                      | 32.2 (272)                    | 27.2 (357) | 0.013 |
| Current smoker, % (n) | 16.7 (365)                      | 21.7 (184)                    | 13.8 (181) | 8.0*10^-4 |
| Systolic blood pressure, mmHg | 138 ± 19                        | 138 ± 20                      | 138 ± 19 | 0.976 |
| Diastolic blood pressure, mmHg | 84 ± 11                         | 83 ± 12                       | 84 ± 11 | 0.018 |
| Body mass index, kg/m² | 29.8 ± 5.0                      | 30.0 ± 5.3                    | 29.7 ± 4.8 | 0.223 |
| IMTmean, mm | 0.79 ± 0.15                     | 0.80 ± 0.15                   | 0.79 ± 0.15 | 0.030 |
| IMTmax, mm | 0.95 ± 0.17                     | 0.96 ± 0.17                   | 0.95 ± 0.17 | 0.048 |
| Carotid artery plaque, % (n) | 55.8 (1208)                      | 61.6 (521)                    | 52.1 (685) | 1.7*10^-5 |
| CAD > 25% stenosis, % (n) | 62.4 (1347)                      | 66.4 (562)                    | 59.7 (785) | 0.002 |
| CAD with revascularization, % (n) | 31.6 (682)                      | 34.0 (288)                    | 30.0 (394) | 0.052 |
| PAD, % (n) | 12.0 (260)                       | 16.1 (136)                    | 9.4 (124)  | 5.0*10^-4 |
| Total atherosclerotic burden, % (n) | 67.5 (1457)                    | 72.9 (617)                    | 63.9 (840) | 1.2*10^-5 |
| LV-EF <55%, % (n) | 23.1 (498)                       | 26.0 (220)                    | 21.2 (278) | 0.009 |
| Sport-PAI (kJ/week) | 124 (78-3901)                    | 0 (0-210)                     | 3012 (1561–6254) | 5.2*10^-4 |
| Total-PAI (kJ/week) | 12 500 (7740–19 675)             | 9224 (5209–15 460)            | 14 577 (9518–22 091) | 5.2*10^-49 |
| Apolipoprotein A, mmol/L | 1.46 (1.29–1.66)                | 1.46 (1.29–1.67)              | 1.46 (1.29–1.66) | 0.657 |
| Apolipoprotein B, mmol/L | 0.94 (0.77–1.13)                | 0.95 (0.76–1.13)              | 0.93 (0.78–1.13) | 0.766 |
| Total cholesterol, mmol/L | 5.34 (4.62–6.20)                | 5.38 (4.62–6.19)              | 5.32 (4.63–6.22) | 0.747 |
| LDL cholesterol, mmol/L | 3.25 (2.56–3.98)                | 3.21 (2.52–3.95)              | 3.28 (2.60–3.99) | 0.212 |
| HDL cholesterol, mmol/L | 1.25 (1.04–1.52)                | 1.24 (1.04–1.54)              | 1.26 (1.04–1.51) | 0.805 |
| Lipoprotein (a), µmol/L | 0.18 (0.08–0.48)                | 0.19 (0.08–0.49)              | 0.17 (0.08–0.47) | 0.315 |
| Triglyceride, mmol/L | 1.69 (1.22–2.45)                | 1.70 (1.22–2.46)              | 1.68 (1.22–2.43) | 0.715 |
| HbA1c, mmol/L | 5.81 (5.49–6.33)                | 5.89 (5.54–6.45)              | 5.78 (5.47–6.24) | 4.6*10^-5 |
| White blood cell count, /µL | 7.0 (5.8–8.4)                   | 7.2 (6.0–8.6)                | 6.9 (5.7–8.3) | 0.001 |
| High-sensitive CRP, mg/L | 2.24 (1.10–4.83)                | 2.48 (1.20–5.51)              | 2.09 (1.05–4.34) | 4.5*10^-4 |
| Creatinine, µmol/L | 77 (66–88)                      | 76 (65–87)                    | 77 (67–90) | 0.057 |
| Antihypertensive medication, % (n) | 81.1 (1731)                    | 84.0 (711)                    | 79.1 (1040) | 0.005 |
| Hypertension, % (n) | 84.2 (1818)                      | 87.0 (736)                    | 82.2 (1082) | 0.012 |
| Statin use, % (n) | 36.6 (791)                      | 36.1 (305)                    | 36.9 (486) | 0.660 |
| Dyslipidaemia, % (n) | 82.7 (1787)                      | 83.5 (706)                    | 82.1 (1081) | 0.477 |

Sport-PAI, physical activity index including only sport activities; Total-PAI, physical activity index including all energy expenditure.
the cut-off of best discrimination between subjects with and without atherosclerosis (Youden Index maximum at 600 kJ/week). Based on this result, we classified subjects in inactive (sport-PAI < 600 kJ/week) and active subjects. Active subjects were grouped in two sets with equal numbers: (i) low-moderate sports activity (600–3000 kJ/week) and (ii) higher sports activity (>3000 kJ/week). ORs were obtained from logistic regression analyses testing for several cut-off categories and for the different atherosclerotic subphenotypes (cIMT, carotid plaque, PAD, CAD >25% stenosis, CAD with revascularization, and the total atherosclerotic burden). Analyses were adjusted for traditional risk factors: age, sex, diabetes, hypertension, LDL-C, HDL-cholesterol, smoking status. In Step 2, we associated analyses between intended sports activity and the metabolic parameters by linear regression analyses treating the metabolites as outcome. Effect sizes are presented as standardized Beta-regression coefficient. In Step 3, we considered the metabolites identified in Step 2 (P < 0.05) and performed logistic regression analyses of associations with atherosclerotic phenotypes. In Step 4, we performed mediation analysis by testing the hypotheses that the metabolites identified in Step 3 mediate the effects of PA (dichotomous: sport-PAI < 600 kJ/week versus ≥600 kJ/week) towards atherosclerosis phenotypes. Identifying the strongest association for carotid plaque (dichotomous dependent variable) and showing robust correlation to the total atherosclerotic burden (R² = 0.78), we focused the mediation analysis only on carotid plaque. Monte-Carlo simulations with 10 Mio. replicates were used to determine size and significance of the mediation effect.25,26 For analyses, we used the statistical software packages IBM SPSS Statistics 23 and R.

Results

A total of 2160 study participants (1385 men; 64.1%) contributed to this analysis. The average age of the study participants was 62 ± 11 years. Among the study population classic risk factors were frequent: hypertension 84.2%, diabetes 29.1%, dyslipidaemia 82.7%, and active smoking 16.7% (Table 1). We classified 39.2% as athletically inactive (sport-PAI < 600 kJ/week). Another 46.9% reported they were intentionally active and exercising sporadically and 28.9% exercised regularly (sport PAI ≥ 600 kJ/week, respectively). People with regular sports activity frequently performed health-related fitness and sport programmes (32%), home exercise machinery (17%), jogging/walking (13%), aerobic/fitness (12%), and swimming (11%). Swimming (50%), cycling (42%), and walking (19%) were most common in the group with sporadic activity.

Among the study population, 32.5% showed no signs of atherosclerosis in the vascular examinations. In the remaining participants, CAD and carotid plaque were most common (62% and 56%, respectively). Atherosclerosis was more common in patients with conventional risk factors such as high blood pressure, the presence of diabetes, and dyslipidaemia (Supplementary material online, Table S2).

**Step 1: Association of sports activity and degree of atherosclerosis**

Total physical activity (total-PAI) was not associated with the extent of atherosclerosis (Table 2). In contrast, intended sports activity (sport-PAI) was significantly associated with a lower level of atherosclerosis [sport-PAI OR 0.98 per 1000 kJ/week, 95% confidence interval (0.97–1.00), Table 2]. The ROC determined an average weekly exercise intensity of 600 kJ that best distinguished between subjects with and without atherosclerosis (Youden Index). A very low sports activity level (sport-PAI < 600 kJ/week) had a comparable atherosclerotic burden as completely inactive subjects [OR 0.96 (0.70–1.31)]. A low to moderate sports activity level (600–3000 kJ/week) was associated with a significantly lower prevalence of atherosclerosis [OR 0.66 (0.46–1.00)]. A higher sports activity level (>3000 kJ/week) increased this benefit insignificantly [OR 0.64 (0.50–0.82)]. In comparison to sporadic sports activity, the protective effect of regular sports activity was stronger [OR sporadic = 0.76 (0.61–0.97); OR<sub>regular</sub> = 0.60 (0.47–0.77), Figure 2A].

### Table 2  Atheroprotective potential of sports activity, odds ratio (95% CI)

| Condition                                      | Unadjusted       | Adjusted        |
|------------------------------------------------|------------------|-----------------|
| **Total atherosclerotic burden**               |                  |                 |
| Total-PAI per 10 000 (kJ/week)                | 1.08 (0.98–1.18) | 1.00 (0.91–1.11)|
| Sport-PAI per 1000 (kJ/week)                  | **0.98 (0.97–1.00)** | 0.99 (0.97–1.01) |
| <600 kJ/week<sup>a</sup>                       | 0.96 (0.70–1.31) | 1.01 (0.74–1.37) |
| 600–3000 kJ/week<sup>a</sup>                   | 0.66 (0.51–0.84) | 0.82 (0.64–1.05) |
| >3000 kJ/week<sup>a</sup>                      | **0.64 (0.50–0.82)** | **0.77 (0.59–1.00)** |
| Frequency * sporadic                           | **0.76 (0.61–0.97)** | 0.86 (0.67–1.12) |
| **Regular**                                    | **0.60 (0.47–0.77)** | 0.77 (0.58–1.02) |
| **Active lifestyle (Sport-PAI ≥ 600 kJ/week)** | **0.65 (0.54–0.79)** | **0.76 (0.62–0.94)** |
| **Atherosclerotic subphenotypes, OR for sport-PAI ≥ 600 kJ/week** |                  |                 |
| cIMT—carotid intima-media thickness ≥0.9 mm    | 0.86 (0.70–1.06) | 1.00 (0.80–1.25) |
| Carotid plaque                                 | **0.68 (0.57–0.81)** | **0.79 (0.66–0.96)** |
| PAD                                           | **0.54 (0.42–0.71)** | **0.74 (0.56–0.98)** |
| CAD >25% stenosis                              | **0.75 (0.63–0.89)** | 0.87 (0.71–1.06) |

*Reference: subjects without intended sports activity.

Adjusted for age, sex, hypertension, diabetes, LDL-C, HDL-C, smoking status. The boldface values highlight statistical significant values.

Sport-PAI, physical activity index including only sport activities; Total-PAI, physical activity index including all energy expenditure.

<sup>a</sup>Reference: subjects without intended sports activity.
When analysing atherosclerotic subphenotypes, we found strong associations of sports activity $\geq 600$ kJ/week with lower extent of carotid plaque ($\text{OR}_{\text{carotid plaque}} 0.68 (0.57–0.81)$), PAD ($\text{OR}_{\text{PAD}} 0.54 (0.42–0.71)$), angiographically CAD with $\geq 25$% stenosis ($\text{OR}_{\text{CAD}} 0.75 (0.63–0.89)$). Table 2. In contrast to the strong association of carotid plaque, cIMT was not significant. Adjusted for the conventional risk factors age, sex, hypertension, diabetes, dyslipidaemia, and smoking, the beneficial effect of sports activity remained significance in carotid...
plaque [OR 0.79 (0.66–0.96)], PAD [OR 0.74 (0.56–0.98), Table 2] and total atherosclerotic burden [OR 0.76 (0.62–0.94), Table 2].

Step 2: Association of sports activity and metabolic profile
Since we observed the strongest atherosclerotic differences between subjects with <600 kJ/week (n = 846) and active subjects with an average weekly sports activity load ≥600 kJ/week (n = 1314), we analysed the metabolic differences between these two groups.

Of the 96 metabolites and ratios, 12 amino acids, free carnitine, 5 acylcarnitines, and 11 metabolite ratios were significantly associated with the active lifestyle. While free carnitine, five acylcarnitines and two amino acids (citrulline, glutamate) correlated negatively with sports activity, the majority of the amino acids, namely 10, showed a positive correlation (arginine, glutamine, leucine/isoleucine, methionine, phenylalanine, pipolic acid, hydroxyproline, taurine, tyrosine, and valine, Supplementary material online, Table S2 and Figure 2B). Lipid metabolism markers LDL-C, HDL-cholesterol, apolipoprotein A, apolipoprotein B, and triglycerides showed no difference between active and inactive people (data not shown).

Step 3: Association of metabolome and atherosclerotic phenotypes
We analysed the metabolites identified in Step 2 for their association with carotid plaque, PAD, CAD >25% and the total atherosclerotic burden. We found differences in the number of associated metabolites for certain atherosclerotic subtypes. We identified 11 metabolites associated with carotid plaque and the total atherosclerotic burden (10 that overlap). Six metabolites were associated with PAD (four crossed with carotid plaque and total atherosclerotic exposure, Supplementary material online, Table S3). For CAD >25%, only four metabolomic associations without significant overlap with other phenotypes were found. Of the metabolites identified in Step 2, four amino acids (arginine, glutamine, pipolic acid, taurine), three metabolites of carnitine metabolism (free carnitine, acetylcarnitine, tetradecenoylcarnitine, octadecenoylcarnitine) and three metabolite ratios (Q5: glutamine/glutamic acid, Q11: alanine/acetylcarnitine, Q13: arginine/citrulline) were associated with sports activity and the degree of atherosclerosis, especially carotid plaque. In this context, arginine, glutamine, pipolic acid, taurine and metabolite ratios of Q5, Q11, and Q13 were significantly associated with sports activity and lower extent of carotid plaque. On the other hand, free carnitine, acetylcarnitine, tetradecenoylcarnitine, and octadecenoylcarnitine were significantly associated with inactivity and higher extent of carotid plaque (Figure 2C).

Step 4: Linkage of sports activity, metabolic parameters and non-occurrence of carotid plaque using a mediation model
Eleven triangles could be defined according to our criteria (Figure 3). For ten of them, we were able to show a mediating effect of metabolites, namely arginine, glutamine, pipolic acid, taurine, free carnitine, acetylcarnitine, octadecenoylcarnitine, Q5, Q11, and Q13 (P < 0.05). For tetradecenoylcarnitine, there was only one trend, but no significant mediation effect (Table 3).

Discussion
This study contributes to the metabolic understanding of the athero-protective effects of sports activities by identifying blood metabolites as potential mediators. What is special about the study is that the triangular connection between sports activity, metabolome, and atherosclerosis was analysed for the first time within a cohort. Therefore, we integrated a targeted metabolomic approach into a mediation model and identified clusters of metabolites that may influence atherogenesis.
Association of sports activity and lower extent of atherosclerosis

The relationship between PA and reduced risk of CAD has been extensively studied in prior studies. A meta-analysis indicated that the biggest bang for reducing CAD risk occurs at the lower end of the activity spectrum. The effect is comparable in middle-aged and older adults. This is in line with results of our study, which shows that a low to moderate level of sports activity is already associated with a lower extent of atherosclerosis and a higher level of physical exertion does not essentially increase the benefit compared to moderate. In particular, regular sports activity seems to be more effective than sporadic.

Our study reports on the first parallel analyses of the relationship between sports activity and vascular phenotypes of coronary arteries, carotid arteries and lower extremities. We found the strongest association between sports activity and lower extent of non-coronary atherosclerosis, but there was also a trend for coronary atherosclerosis. This finding is consistent with prospective data of the MESA, which reported a robust protective effect of intended activity for PAD (RR 0.82 (0.71–0.94)), but only a trend for the calcium score of coronary arteries (RR 0.96 (0.90–1.03)). In a large cross-sectional study with more than 3.2 million women and men in the USA, a significantly lower prevalence of PAD and carotid artery stenosis (CAS) was found when participating in intensive leisure PA [OR_{PAD, intensive} = 0.64 (0.63–0.65) and OR_{CAS} = 0.80 (0.79–0.81)]. We report comparable results for PAD [OR = 0.74 (0.56–0.98)]. Interestingly, the association between PA and lower extent of carotid artery disease was also comparable [OR = 0.79 (0.66–0.96)], although we used plaque as a more refined and common feature compared to CAS.

Association between sports activity and metabolome as a possible link to atherogenesis

Interpretation of metabolic data and its comparison with published work is difficult due to different analytic approaches [nuclear magnetic resonance (NMR)-based vs. mass-spectrometry] and the lack of standards regarding blood samples (plasma, serum, whole blood). While most published vascular studies performed NMR from plasma, we analysed whole blood samples using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). This validated approach allows measurements in high-throughput resulting in sufficiently large sample sizes required for mediation analyses.

As a major finding, the metabolomic mediation approach used in this study was able to provide a potential mechanistic link between sports activity and atheroprotection. We find clusters of metabolites with multiple vasoactive properties. In detail, a cluster of four amino acids (arginine, glutamine, pipecolic acid, and taurine) has been identified with promising candidates for mediation of the atheroprotective effect. Another cluster of members of the carnitine metabolism and beta oxidation (free carnitine, acetylcarnitine, octadecenoylcarnitine, tetradeconoylcarnitine) were significantly associated with low activity and the occurrence of atherosclerosis. In addition, three metabolite ratios that characterize ammonia recycling (Q5), energy metabolism (Q11), and the urea cycle (Q13) were significantly associated with sports activity and a lower presence of atherosclerosis. However, the assignment to certain pathways and their meaning in the context of atherogenesis remains uncertain.

Several in this study identified metabolites that were associated with lower extent of atherosclerosis are well assumed candidates for atheroprotective activity. Arginine is a precursor of nitric oxide (NO), therefore arginine has a central role in endothelial function. Mediated by vasodilatation, inhibition of leucocyte adhesion, fibrous plaque formation and vascular inflammation, arginine conveys numerous vasoactive and anti-atherosclerotic effects. Glutamine is a precursor of nitric oxide (NO), therefore it is a key player in the oxidation/anti-oxidation cascade. Moreover, glutamine increased NO formation in vascular cells and improved relaxation responses of the vascular wall. Divers studies demonstrated that glutamine beneficially affects glucose tolerance in subjects with and without diabetes mellitus and has multiple other vascular protective effects. Investigations showed that taurine decreases serum lipids, especially LDL, and can inhibit hyperglycaemia and oxidized LDL-induced enhancement in apoptosis, reactive oxygen species, intracellular calcium and adhesion.
molecule-1 expression, which is important for the adhesion of circulating leukocytes to endothelial cells at the beginning of the atherosclerosis process. Taurine also reduces pro-inflammatory cytokines like tumour necrosis factor-alpha and interleukin-6. Interestingly, our results showed that members of the carnitine-carrier-system and beta oxidation (free-carnitine, acetyl-carnitine, and octadecenoic-carnitine) were co-associated with physical inactivity and prevalent atherosclerosis. In all mammalian tissues, the carnitine-acylcarnitine transporter system is a key player in the mitochondrial beta-oxidation and ATP production. Although it is known that carnitine metabolism changes during PA, studies are rare and there is no detailed knowledge about the persistence of this effect. Therefore, this issue needs to be further explored for a more accurate estimate of the role of carnitine metabolism and betaoxidation in atherogenesis.

Several potential limitations have to be addressed. First, it is difficult to elucidate the specific role of sports activity as part of a healthy lifestyle which is typically a mixture of diet, body weight control and reduction or lack of addictive behaviours, such as cigarette smoking. Adjusting for these factors in multivariate statistical analysis has been performed as far as possible but is inevitably incomplete. Second, we used self-reported PA data which could be improved by actometric devices not available at the time of study enrolment. We have therefore divided the study cohort into two groups: inactive (no or very little intended sports activity) and active (intended sports activity >600 kJ/week), according to the ROC threshold for the best differentiation between subjects with and without atherosclerosis. Third, results of this study are based on blood sampling at rest implicating that only persistent effects of sports activity on metabolic parameters could be studied.

Based on our results from this cross-sectional study, prospective sports activity studies should be carried out. On the one hand, the prevention potential and on the other hand, the therapeutic potential for an existing atherosclerosis could be of interest. Are metabolites and mediation in the de novo development of atherosclerosis the same as in the progression of an existing disease, and how do they relate to regression? In relation to the different stages of atherosclerosis, it would make sense to include functional markers of early atherosclerosis such as flow-mediated dilation or measurements of arterial stiffness.

In summary, this is a pilot study which, in a comprehensive analysis of metabolic parameters, was able to identify probable atheroprotective mediators of sports activity on atherosclerosis. Metabolites with antioxidant and endothelial effects as well as metabolites that are involved in impaired insulin sensitivity seem to be important here. The metabolic approach opens up additional possibilities to unravel the complex and metabolite-mediated process of atherogenesis and to identify targets for preventive and therapeutic interventions.

Supplementary material

Supplementary material is available at European Journal of Preventive Cardiology online.

Acknowledgements

We thank Annegret Unger and Kai Olischer for their technical assistance.

Funding

The LIFE-Heart study is funded by the Leipzig Research Center for Civilization Diseases (LIFE). LIFE is funded by means of the European Union, the European Regional Development Fund and the Free State of Saxony. Initial funding of LIFE-Heart was supported by the Roland-Ernst Foundation.

Conflict of interest: M.S. receives funding from Pfizer and U.C. from Roche, both for projects not related to this research. M.S. receives funding from Pfizer and Uta Ceglarek from Roche, both for projects not related to this research.

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