AUTHOR	Anni Kauko, Joonatan Palmu, Pekka Jousilahti, Aki Havulinna, Veikko Salomaa, Teemu Niiranen
TITLE	Associations between circulating metabolites and arterial stiffness
YEAR	2020, October 22th.
DOI	https://doi.org/10.1038/s41371-020-00434-y
VERSION	Final Draft (AAM)
CITATION	Kauko, A., Palmu, J., Jousilahti, P. *et al.* Associations between circulating metabolites and arterial stiffness. *J Hum Hypertens* (2020). https://doi.org/10.1038/s41371-020-00434-y

Associations between Circulating Metabolites and Arterial Stiffness

Anni Kauko, PhD[1], Joonatan Palmu, MD[1,2,3], Pekka Jousilahti, MD[3],
Aki Havulinna, DSc[3,4], Veikko Salomaa, MD[3], Teemu Niiranen, MD[1,2,3]

[1]Department of Internal Medicine, University of Turku, Finland
[2]Division of Medicine, Turku University Hospital, Turku, Finland
[3]Department of Public Health Solutions, Finnish Institute for Health and Welfare, Turku and Helsinki, Finland
[4]Institute for Molecular Medicine Finland, FIMM-HiLIFE, Helsinki, Finland.

Running Title: Metabolites and Arterial Stiffness

Address for Correspondence:
Anni Kauko, Department of Internal Medicine, Kiinamyllynkatu 4–8, 20014 University of Turku, Finland. email: anirka@utu.fi

Arterial stiffness is a strong predictor of cardiovascular events and the most important contributor to the age-related development of hypertension. Our study suggests that arterial stiffness is connected to elevated circulating concentrations of branched-chain amino acids, aromatic amino acids, glycerol, and the inflammation marker GlycA, all well-known correlates of diabetes and unhealthy lifestyle.

Arterial stiffness predicts cardiovascular morbidities and is a key factor in the development of age-related hypertension.[1] Although challenging, arterial stiffening and ensuing hypertension can be prevented through maintaining a healthy lifestyle throughout life.[1] Serum metabolites, and in particular lipid composition, are in part a proxy for lifestyle, as they reflect variations in diet and metabolism. Earlier studies have demonstrated various associations between serum and urine metabolites and pulse wave velocity (PWV). However, these studies have been limited in sample size,[2,3] have used a targeted case-control design,[3,4] were restricted only to women,[5] or used mass spectrometry for metabolite detection, which often results in a large number of unidentified metabolites.[5,6] We therefore studied the associations between ^{1}H-NMR-determined plasma metabolites and arterial stiffness in a population sample of 461 individuals. Our goal was to elucidate key metabolic risk factors for increased arterial stiffness that could be used as targets for preventing the age-related development of hypertension.

We considered a subsample of 500 individuals who participated in the population-based FINRISK 2007/DILGOM-study.[7] After excluding individuals without valid PWV

and metabolite measurements, our study sample consisted of 461 participants (51% women, mean age 50±14 years, mean BMI 27±5). The participants underwent a health examination, which included blood sampling and measurements for carotid-femoral PWV, as described elsewhere.[7] Nightingale Health Ltd (Helsinki, Finland) determined the concentration of 46 serum metabolites using ^{1}H-NMR spectroscopy (**Figure**). All metabolite variables were normalized. We studied the associations of metabolite concentrations with PWV using linear regression models adjusted for age, sex, BMI, smoking, diabetes, leisure-time exercise, lipid-lowering drugs, and heart rate. We report two-tailed P-values with and without controlling for multiple testing using the false discovery rate (FDR) method. All statistical analyses were performed using R 3.6.3 and the source code for the analyses is available by request. The study was approved by the Coordinating Ethics Committee of the Helsinki University Hospital District and all participants gave written informed consent.

The associations of metabolite concentrations with PWV are reported in the **Figure**. We observed positive associations for several amino acids, the systemic inflammation marker GlycA and glycerol. Of these, leucine and phenylalanine were significant also after FDR correction. A negative association was observed for the fraction of polyunsaturated fatty acids (PUFA) in blood. In addition, our data suggested an association of PWV with lactate, pyruvate and monounsaturated fatty acids (MUFA; P<0.1 for all). The magnitude of the association between GlycA and PWV decreased with age whereas the relation of PWV with phenylalanine, tyrosine, leucine and valine increased with age (FDR-corrected P value < 0.05 for all age-metabolite interaction terms).

The most prominent associations were observed for branched-chain amino acids (BCAA; leucine, isoleucine, valine) and aromatic amino acids (phenylalanine and tyrosine). Leucine is involved in insulin regulation and high concentrations of these five amino-acids in blood are related to higher incidence of diabetes mellitus,[8] one of the important contributors for arterial stiffness.[1] Increased BCAA levels are also connected to downregulation of BCAA catabolism in subcutaneous fat of obese individuals and to various adverse health effects, including inflammation, diabetes and liver fat accumulation.[9] We also observed associations of PWV with the inflammatory marker GlycA and glycerol. Glycerol is a key intermediate between lipid and sugar metabolism and shown to predict type 2 diabetes.[10] Furthermore, low grade inflammatory response has been shown to have a key role in obesity related cardiovascular risk factors, including diabetes and arterial stiffness.[1] Our results indicate that circulating concentrations of BCAAs, aromatic amino acids, glycerol and inflammatory markers are the strongest correlates of arterial stiffness, even after correcting for diabetes and BMI.

In addition to associations with amino acids and inflammatory markers, we observed a negative association between PUFAs and arterial stiffness. Increased PUFA intake is known to improve the HDL/LDL ratio and have a beneficial effect on cardiovascular health, although some controversy exists for omega-6 fatty acids.[11] We also observed a suggestive positive association between MUFA and arterial stiffness. Although somewhat surprising, non-significant associations of MUFA intake with arterial stiffness and cardiovascular outcomes have been reported.[12]

Our findings on the association of PWV with amino acids and fatty acids were in accordance with three prior studies.[2,3,6] However, due to differences in study designs, these results may not be directly comparable. Although our investigation had a larger sample size than most earlier studies, it was still relatively small and covered only small proportion of metabolome. This resulted in our analyses being somewhat underpowered, particularly if corrected for multiple testing. However, as several of the examined lipid metabolites are highly correlated, any corrections for multiple testing may be too conservative.

In summary, our study demonstrates associations between arterial stiffness and increased levels of BCAAs, aromatic amino acids and inflammation marker GlycA in blood, even after adjusting for BMI and diabetes. In addition, we observed negative relation between PUFA and arterial stiffness. Although cross-sectional, our results suggest that these circulating metabolites could be used for estimating risk of arterial stiffness. In addition, these metabolites are known to associate with obesity and dietary factors, emphasizing the importance of healthy lifestyles in the prevention of arterial stiffening and hypertension.

Conflict of Interest

We have no conflicts of interests to disclose.

Funding

T. Niiranen was funded by the Academy of Finland (grant no. 321351), the Urmas Pekkala Foundation, the Paavo Nurmen Säätiö, the Suomen Lääketieteen Säätiö, the Emil Aaltosen Säätiö, and the Hospital District of Southwest Finland.

References

1. Niiranen TJ, Lyass A, Larson MG, Hamburg NM, Benjamin EJ, Mitchell GF, et al. Prevalence, Correlates, and Prognosis of Healthy Vascular Aging in a Western Community-Dwelling Cohort. Hypertension. 2017;70(2):267–74.
2. Anderson SG, Sanders TAB, Cruickshank JK. Plasma fatty acid composition as a predictor of arterial stiffness and mortality. Hypertension. 2009;53(5):839–45.
3. Zagura M, Kals J, Kilk K, Serg M, Kampus P, Eha J, et al. Metabolomic signature of arterial stiffness in male patients with peripheral arterial disease. Hypertens Res. 2015;38(12):840–6.
4. Jung S, Kim M, Lee YJ, Lee SH, Lee JH. Associations between metabolomic-identified changes of biomarkers and arterial stiffness in subjects progressing to impaired fasting glucose. Clin Endocrinol (Oxf). 2015;83(2):196–204.
5. Menni C, Mangino M, Cecelja M, Psatha M, Brosnan MJ, Trimmer J, et al. Metabolomic study of carotid-femoral pulse-wave velocity in women. J Hypertens. 2015;33(4):791–6.
6. Li C, He J, Li S, Chen W, Bazzano L, Sun X, et al. Novel metabolites are associated with augmentation index and pulse wave velocity: Findings from the Bogalusa Heart Study. Am J Hypertens. 2019;32(6):547–56.
7. Johansson JK, Puukka PJ, Jula AM. Interarm blood pressure difference and target organ damage in the general population. J Hypertens. 2014;32(2):260–6.
8. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, et al.

Metabolite profiles and the risk of developing diabetes. Nat Med. 2011;17(4):448–53.

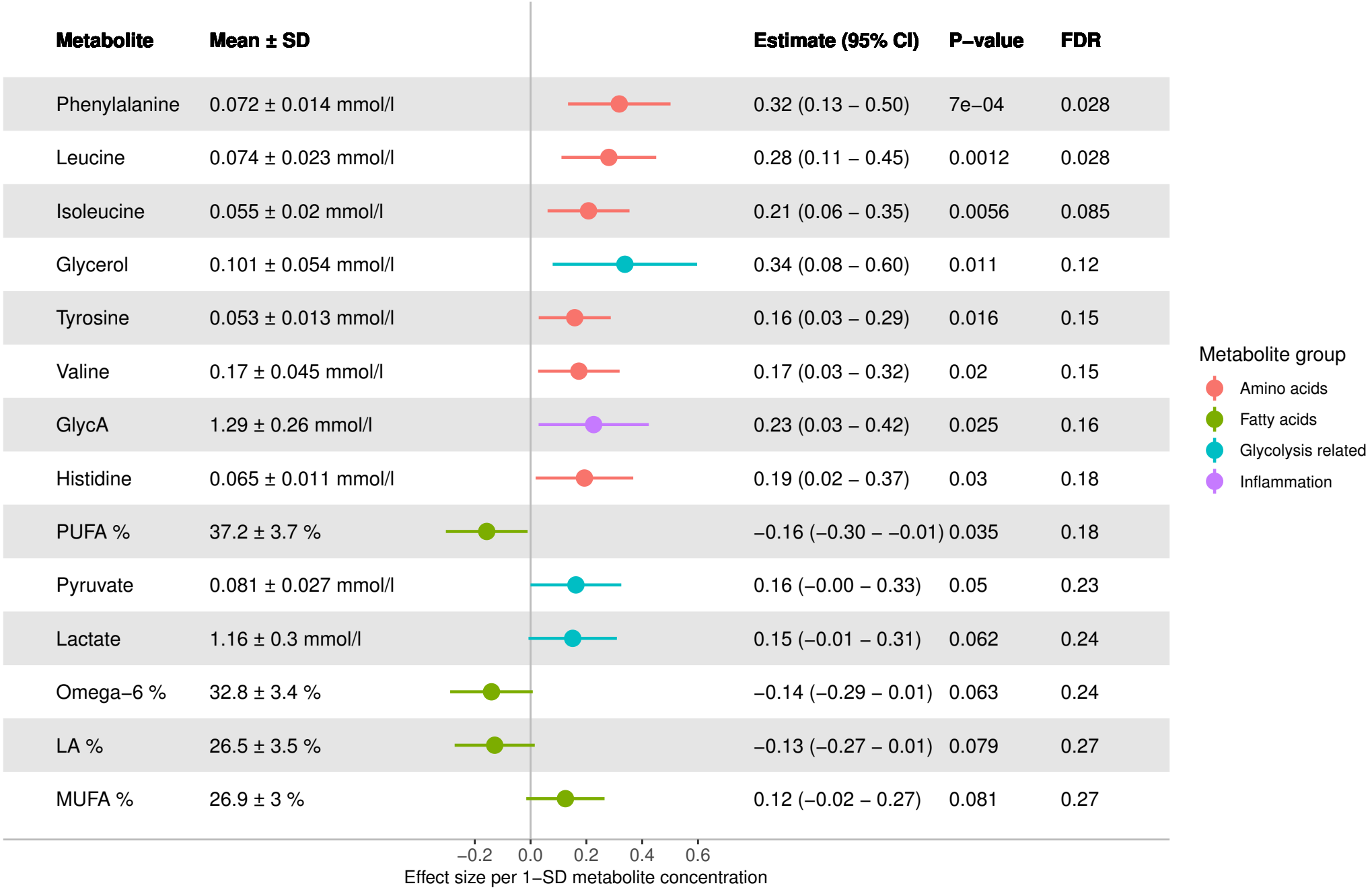
9. Pietiläinen KH, Naukkarinen J, Rissanen A, Saharinen J, Ellonen P, Keränen H, et al. Global transcript profiles of fat in monozygotic twins discordant for BMI: Pathways behind acquired obesity. PLoS Med. 2008;5(3):0472–83.

10. Mahendran Y, Cederberg H, Vangipurapu J, Kangas AJ, Soininen P, Kuusisto J, et al. Glycerol and fatty acids in serum predict the development of hyperglycemia and type 2 diabetes in Finnish men. Diabetes Care. 2013;36(11):3732–8.

11. Harris WS, Mozaffarian D, Rimm E, Kris-Etherton P, Rudel LL, Appel LJ, et al. Omega-6 fatty acids and risk for cardiovascular disease: A science advisory from the American Heart Association nutrition subcommittee of the council on nutrition, physical activity, and metabolism; council on cardiovascular nursing; and council on epidem. Circulation. 2009;119(6):902–7.

12. Livingstone KM, Givens DI, Cockcroft JR, Pickering JE, Lovegrove JA. Is fatty acid intake a predictor of arterial stiffness and blood pressure in men? Evidence from the Caerphilly Prospective Study. Nutr Metab Cardiovasc Dis. 2013;23(11):1079–85.

Figure 1. The association between circulating metabolites and pulse wave velocity. All metabolites were adjusted for age, sex, BMI, smoking, diabetes, leisure-time exercise, lipid-lowering drugs, and heart rate. Estimates are expressed as an effect of 1-SD change in metabolite concentration on pulse wave velocity (m/s). The error bars indicate 95 % confidence intervals. Both unadjusted and FDR-corrected p-values are reported. The results are shown for all metabolites with $p<0.10$. N=461 for all metabolites, except for glycerol (n=460) and glutamine (n=309). Results are not shown for following metabolites: 3-hydroxybutyrate, acetate, acetoacetate, alanine, albumin, apolipoprotein A1 and B, citrate, creatinine, docosahexaenoic acid %, glucose, glutamine, glycine, HDL-TG, HDL cholesterol, HDL size, LDL-TG, LDL cholesterol, LDL size, omega-3 %, phosphatidylcholines, phosphoglycerides, saturated fatty acid %, sphingomyelins, total cholesterol, total cholines, total fatty acids, total triglycerides, unsaturation, VLDL-TG, VLDL cholesterol and VLDL size. Abbreviations: GlycA: glycoprotein acetylation, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid, LA: linoleic acid, HDL: high density lipoprotein particle, LDL: low density lipoprotein particle, TG: triglyceride, PG: phosphoglyceride, VLDL: very low density lipoprotein particle.


Metabolite
Mean ± SD
Estimate (95% CI)
P–value
FDR
Phenylalanine
0.072 ± 0.014 mmol/l
0.32 (0.13 – 0.50)
7e–04
0.028
Leucine
0.074 ± 0.023 mmol/l
0.28 (0.11 – 0.45)
0.0012
0.028
Isoleucine
0.055 ± 0.02 mmol/l
0.21 (0.06 – 0.35)
0.0056
0.085
Glycerol
0.101 ± 0.054 mmol/l
0.34 (0.08 – 0.60)
0.011
0.12
Tyrosine
0.053 ± 0.013 mmol/l
0.16 (0.03 – 0.29)
0.016
0.15
Valine
0.17 ± 0.045 mmol/l
0.17 (0.03 – 0.32)
0.02
0.15
GlycA
1.29 ± 0.26 mmol/l
0.23 (0.03 – 0.42)
0.025
0.16
Histidine
0.065 ± 0.011 mmol/l
0.19 (0.02 – 0.37)
0.03
0.18
PUFA %
37.2 ± 3.7 %
–0.16 (–0.30 – –0.01)
0.035
0.18
Pyruvate
0.081 ± 0.027 mmol/l
0.16 (–0.00 – 0.33)
0.05
0.23
Lactate
1.16 ± 0.3 mmol/l
0.15 (–0.01 – 0.31)
0.062
0.24
Omega–6 %
32.8 ± 3.4 %
–0.14 (–0.29 – 0.01)
0.063
0.24
LA %
26.5 ± 3.5 %
–0.13 (–0.27 – 0.01)
0.079
0.27
MUFA %
26.9 ± 3 %
0.12 (–0.02 – 0.27)
0.081
0.27
–0.2
0.0
0.2
0.4
0.6
Effect size per 1–SD metabolite concentration
Metabolite group
Amino acids
Fatty acids
Glycolysis related
Inflammation