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Altered metabolism distinguishes high-risk from stable carotid atherosclerotic plaques

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Aims

Identification and treatment of the rupture prone atherosclerotic plaque remains a challenge for reducing the burden of cardiovascular disease. The interconnection of metabolic and inflammatory processes in rupture prone plaques is poorly understood. Herein, we investigate associations between metabolite profiles, inflammatory mediators and vulnerability in carotid atherosclerotic plaques.

Methods and results

We collected 159 carotid plaques from patients undergoing endarterectomy and measured 165 different metabolites in a targeted metabolomics approach. We identified a metabolite profile in carotid plaques that associated with histologically evaluated vulnerability and inflammatory mediators, as well as presence of symptoms in patients. The distinct metabolite profiles identified in high-risk and stable plaques were in line with different transcription levels of metabolic enzymes in the two groups, suggesting an altered metabolism in high-risk plaques. The altered metabolic signature in high-risk plaques was consistent with a change to increased glycolysis, elevated amino acid utilization and decreased fatty acid oxidation, similar to what is found in activated leucocytes and cancer cells.

Conclusion

These results highlight a possible key role of cellular metabolism to support inflammation and a high-risk phenotype of atherosclerotic plaques. Targeting the metabolism of atherosclerotic plaques with novel metabolic radiotracers or inhibitors might therefore be valid future approaches to identify and treat the high-risk atherosclerotic plaque.

Keywords Atherosclerosis • Carotid plaque • High-risk plaque • Metabolism • Inflammation

Introduction

Atherosclerotic plaques form over a long time by a focal accumulation of lipids, immune cells, and smooth muscle cells in the arterial wall and plaques that rupture can cause acute cardiovascular events, such as myocardial infarction and stroke.[1,2] Rupture-prone, high-risk plaques are associated with clinical symptoms[3,4] and characterized by histological evidence of vulnerability[5,6] and a high inflammatory burden.[7] While this knowledge has advanced considerably over the past few years, our understanding of the metabolic processes within plaques in this inherently metabolic disorder has been lagging behind.

Emerging research has shown that cell metabolism and the inflammatory response are tightly intertwined.[8] Macrophages, abundantly found in atherosclerotic plaques, and other leucocytes, change their metabolism according to their tasks in the immune response. Activated leucocytes change to a predominantly anabolic metabolism

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Translational perspective

Here, we show that high-risk atherosclerotic plaques collected from patients have a distinct metabolic footprint compared with low-risk plaques. This finding highlights a previously unappreciated role of cellular metabolism as a fundamental feature of high-risk plaques. This insight may help to reduce the burden of atherosclerotic cardiovascular disease, as the altered metabolism in high-risk plaques could be targeted with the intention to diagnose or treat high-risk plaques. Metabolic inhibitors, already in development for cancer therapy, might prevent clinical complications caused by high-risk atherosclerotic plaques and novel metabolic radiotracers could be used to discriminate between high- and low-risk atherosclerotic plaques in patients.

by upregulating pathways, such as glycolysis, the pentose-phosphate pathway (PPP), and glutaminolysis, to provide building blocks for nucleic acids, proteins, and lipids as well as the necessary energy to enable their activation and proliferation. In contrast, catabolic pathways, such as fatty acid oxidation (FAO), are downregulated in these cells.[9] Recently, it has been shown that overutilization of glucose is crucial for blood monocytes and *in vitro* differentiated macrophages from patients with coronary artery disease (CAD) to mount a destructive inflammatory response.[10] Yet, it remains to be determined whether such an interconnection between cellular metabolism and the inflammatory response is present in human atherosclerotic plaques.

Recent studies have challenged the established concept of the vulnerable atherosclerotic plaque and call for improved methods for identification of the high-risk plaque.[11] Plaque metabolomics might be able to provide a largely unexplored layer of functional characterization of high-risk lesions and thus add value to future risk stratification strategies and novel therapeutic approaches. Metabolic profiling of atherosclerotic tissues has so far focused on comparing lipid metabolite levels in different parts of the same plaque or to plaque adjacent intimal thickenings without being able to produce clear biological insights of clinical significance.[12,13] A more clinically relevant approach is to distinguish high- from low-risk plaques according to their metabolic profile. Therefore, we assessed metabolite profiles of 159 highly stenotic carotid atherosclerotic plaques isolated from patients with or without symptoms. We show that high-risk plaques, characterized as being symptomatic, vulnerable by histology and inflamed with elevated inflammatory mediators, had a specific metabolite signature, distinct from the metabolite profile of low-risk plaques. These data highlight a previously unappreciated role of cellular metabolism in the high-risk plaque and as a discriminating feature from low-risk plaques, indicating that metabolic pathways could be targeted to treat and identify high-risk atherosclerotic plaques.

Methods

For a detailed description of all methods, see Supplementary material online, Methods.

Patients and plaques

Patients ($n = 159$) undergoing carotid endarterectomy between 2005 and 2010 at the Skåne University Hospital Malmö were consecutively included. Patients, where time from symptom onset to operation exceeded 31 days, were excluded. Carotid plaques were snap-frozen in liquid nitrogen immediately after surgical removal. Serial cryosections were used for histology, while plaque homogenates were used for analysis of metabolite and cytokine content by flow injection analysis-tandem mass spectrometry, enzymatic assays, proximity extension, and Luminex assay, as well as for RNA sequencing. Each plaque was from a different patient and treated independently. Follow-up data on cardiovascular events were acquired from the Swedish Cause of Death and National inpatient Health Registers. All clinical investigations conformed to the Declaration of Helsinki. The study was approved by the Regional Ethical Review Board and all patients included gave informed consent.

Statistical analysis

Consensus clustering with five different clustering algorithms was used to group plaques according to their metabolite profile into two major and robust clusters, where all five algorithms agreed on the cluster allocation, as well as a third smaller cluster in which plaques with less certain cluster allocation were placed. The R package clValid was used to determine the optimal number of clusters for different algorithms based on measures of connectedness, compactness, separation, and stability. Cohen's κ coefficients were used to assess agreement across algorithms and the robustness of the final clusters and their ability to predict new data was evaluated by *k*-fold ($k = 5$) cross-validation. Normality of continuous variables was assessed with a Shapiro–Wilk test. Normally distributed data are presented as the mean ± standard deviation and statistical significance was determined using a two-tailed Student's *t*-test for independent samples. Non-normally distributed data are presented as the median (interquartile range) and significance was assessed with a Wilcoxon rank sum test. The χ^2 test was used for categorical variables. Cardiovascular event-free survival is shown by using Kaplan–Meier curves and the *P*-value was calculated with the log-rank test. Differences between clusters were considered significant if $P \leq 0.05$. Where indicated, the Benjamini–Hochberg procedure was used to control for multiple testing.

Results

Metabolite profiles group carotid plaques into two main clusters

Consensus clustering, applying multiple clustering algorithms, revealed that most plaques were assigned to either one of two major clusters (cluster 1 and cluster 2) with opposing metabolite profiles (*Figure 1*). Only a small number of plaques ($n = 30$, cluster 3) could not be unequivocally assigned to either of the two major clusters due to their intermediate metabolite profile. Principal component analysis revealed a separation between cluster 1 and 2 plaques with cluster 3 plaques lining up in the boundary, confirming the clustering result (*Figure 2A*). As the large majority of plaques could be grouped according to two opposing metabolite signatures, we subsequently focused our analysis on cluster 1 and 2 plaques and the patients from which they were excised. These two opposing signatures were

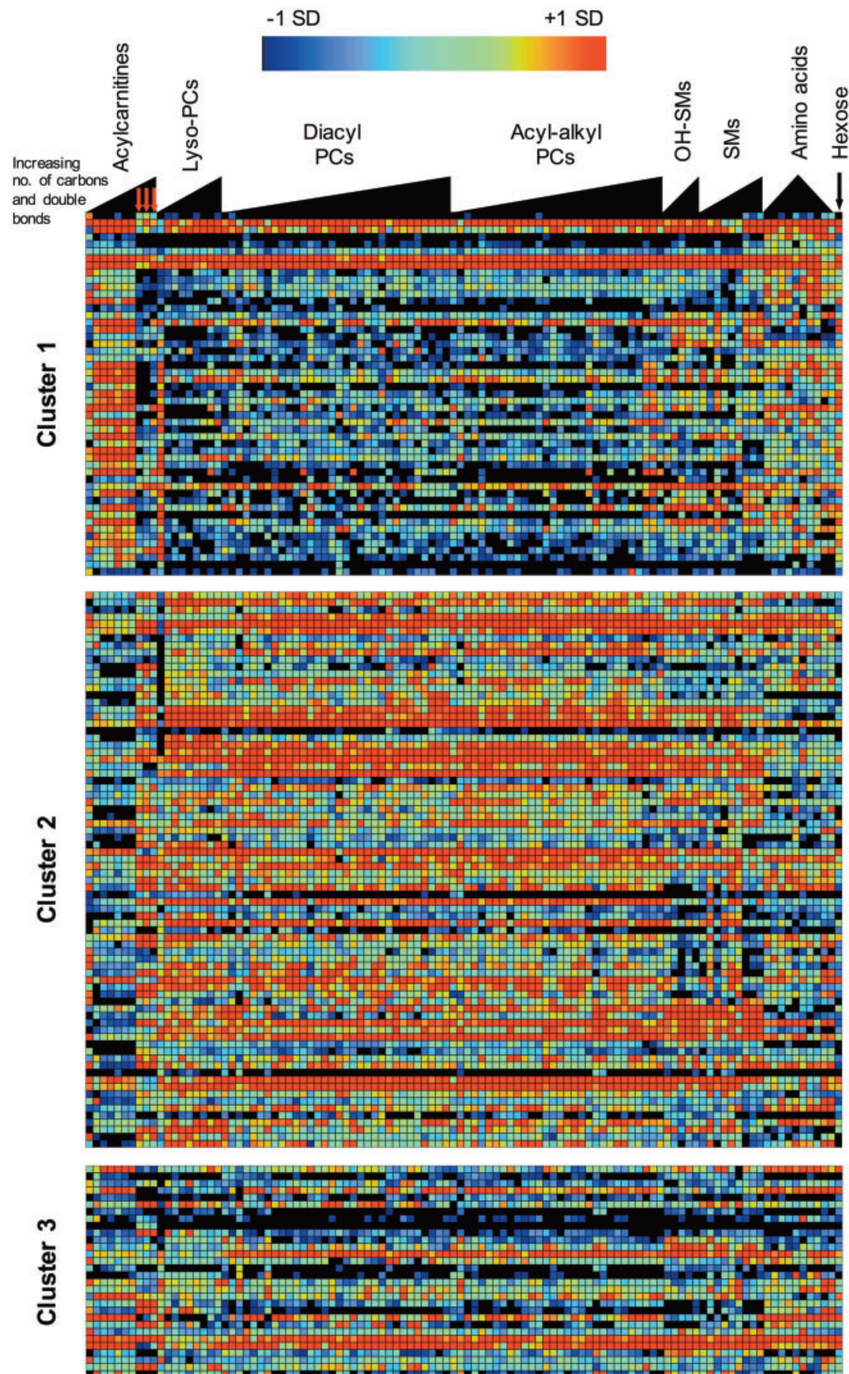


Figure 1 Atherosclerotic plaques can be assigned to either one of two main clusters based on their metabolite profile. Each row represents one plaque and each column one metabolite, where the chain length and number of double-bonds for lipid metabolites increases from left to right. Red arrows depict acylcarnitines C16:0/C18:0/C18:1. *n* = 51 (cluster 1), *n* = 78 (cluster 2), *n* = 30 (cluster 3).

characterized by differing levels of short- and medium-chain (≤C14) acylcarnitines, long-chain (>C14) acylcarnitines, lysophosphatidylcholines (PCs) and diacyl-/acyl-alkyl-PCs (*Figure 1* and Supplementary material online, *Table S4*). Notably, the metabolite levels in plaques were generally not correlated with the levels of metabolites in blood (Supplementary material online, *Figure S1*).

Patients with plaques belonging to cluster 2 were significantly older than patients with cluster 1 plaques, and they had increased levels of LDL. Importantly, however, neither total cholesterol, HDL, and triglyceride levels, nor the use of statins, or any other baseline characteristic differed between the patients divided into the two groups based on their plaque metabolite profiles (*Table 1*).

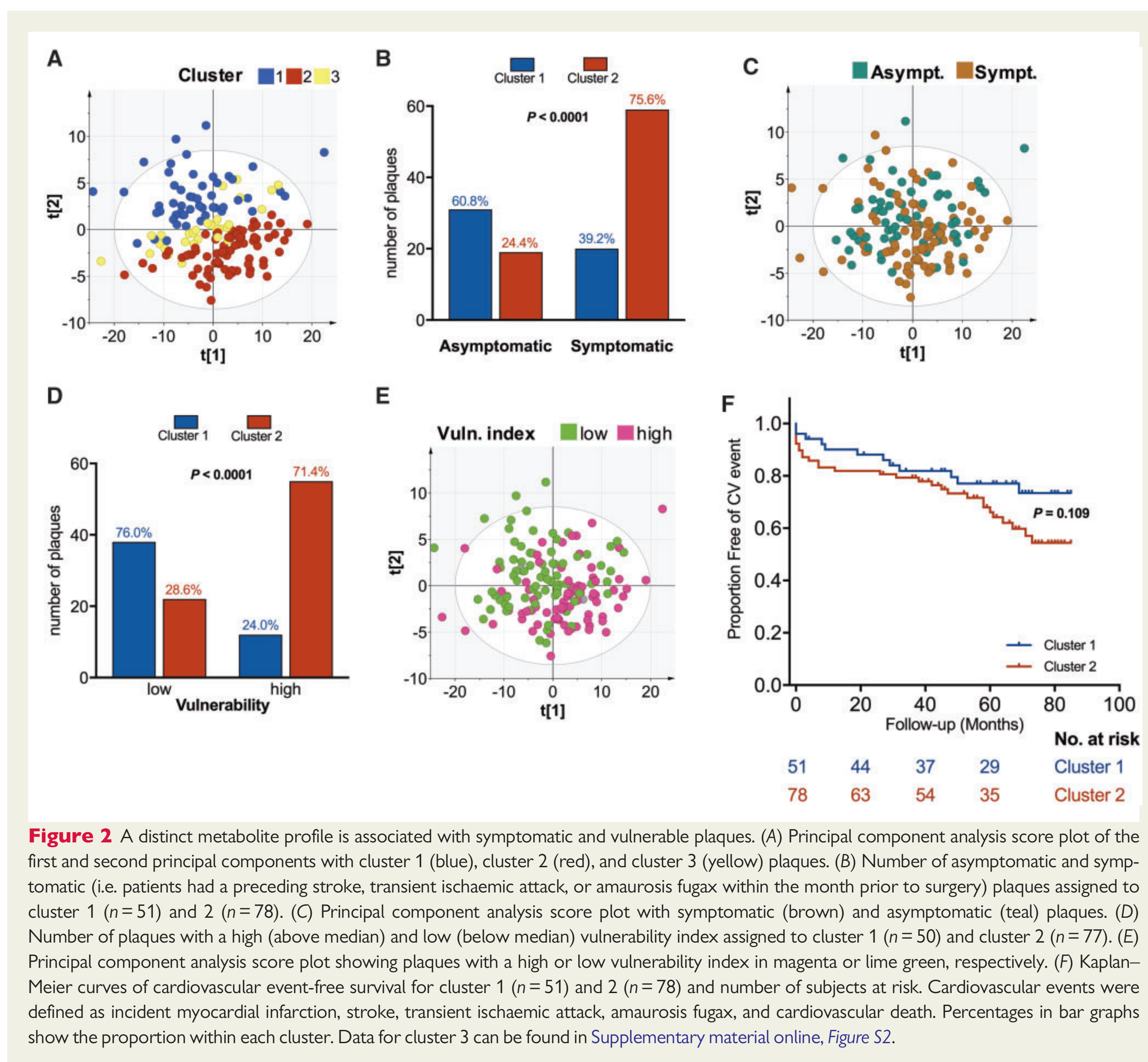


Figure 2 A distinct metabolite profile is associated with symptomatic and vulnerable plaques. (*A*) Principal component analysis score plot of the first and second principal components with cluster 1 (blue), cluster 2 (red), and cluster 3 (yellow) plaques. (*B*) Number of asymptomatic and symptomatic (i.e. patients had a preceding stroke, transient ischaemic attack, or amaurosis fugax within the month prior to surgery) plaques assigned to cluster 1 ($n = 51$) and 2 ($n = 78$). (*C*) Principal component analysis score plot with symptomatic (brown) and asymptomatic (teal) plaques. (*D*) Number of plaques with a high (above median) and low (below median) vulnerability index assigned to cluster 1 ($n = 50$) and cluster 2 ($n = 77$). (*E*) Principal component analysis score plot showing plaques with a high or low vulnerability index in magenta or lime green, respectively. (*F*) Kaplan–Meier curves of cardiovascular event-free survival for cluster 1 ($n = 51$) and 2 ($n = 78$) and number of subjects at risk. Cardiovascular events were defined as incident myocardial infarction, stroke, transient ischaemic attack, amaurosis fugax, and cardiovascular death. Percentages in bar graphs show the proportion within each cluster. Data for cluster 3 can be found in Supplementary material online, *Figure S2*.

A metabolite profile that is associated with high-risk plaques

Next, we sought to determine the clinical and pathophysiological relevance of the two clusters. Cluster 2 plaques were significantly more symptomatic, whereas cluster 1 plaques were less likely to be associated with symptoms (*Figure 2B*). Principal component analysis confirmed these results by showing a clear separation of symptomatic and asymptomatic plaques along the path that also separated cluster 1 and 2 plaques (*Figure 2C*).

In order to assess the vulnerability of the plaques, we used a previously described index[14] based on the histopathological content of neutral lipids (Oil Red O), macrophages (CD68), haemorrhage (glycophorin A), smooth muscle cells (α-actin), and collagen (Masson-Trichrome) in plaque sections (Supplementary material online, *Figure S3*). Highly vulnerable plaques, with an above median vulnerability index, were significantly overrepresented within cluster 2, whereas the majority of cluster 1 plaques had a low vulnerability index (*Figure 2D*). Furthermore, cluster 2 plaques were bigger (greater wet weight), although the pre-operative ultrasound showed no difference in the degree of stenosis (Supplementary material online, *Table S2*). Separate analysis of symptomatic and asymptomatic plaques ruled out that the overrepresentation of highly vulnerable plaques in cluster 2 was merely a result of a preceding cerebrovascular event (i.e. symptomatic plaque) (Supplementary material online, *Figure S4*). Additionally, the time from the onset of symptoms to endarterectomy was similar in the two clusters (17 ± 8 days for cluster 1, 14 ± 9 days for cluster 2; $P = 0.29$). Principal component analysis also revealed a separation of the plaques with a high and low vulnerability index similar to, and overlapping with, the metabolite clusters and symptoms (*Figure 2E*).

Table 1 Patients characteristics

	Cluster 1 (*n* = 51 patients)	Cluster 2 (*n* = 78 patients)	*P*-value
Sex (female)	15 (29%)	29 (37%)	0.363
Age (years)	68 (64–71)	73 (67–78)	0.001
BMI (kg/m^2)	26.8 ± 3.9	26.8 ± 3.7	0.938
Current smokers	20 (39%)	20 (26%)	0.103
Hypertension	37 (73%)	58 (74%)	0.820
Diabetes	22 (43%)	35 (45%)	0.846
HbA1c (mmol/mol)*	56.3 (50.0–65.7)	55.2 (45.8–67.5)	0.907
Total cholesterol (mmol/L)	4.2 (3.5–5.1)	4.4 (3.6–5.1)	0.531
LDL (mmol/L)	2.1 (1.6–3.1)	2.6 (2.1–3.4)	0.042
HDL (mmol/L)	1.2 (0.9–1.6)	1.1 (0.9–1.3)	0.186
Triglycerides (mmol/L)	1.2 (0.9–1.9)	1.3 (1.0–1.7)	0.892
hsCRP (mg/L)	4.3 (2.2–7.0)	4.0 (2.1–7.2)	0.975
WBC (10^8/L)	8.2 ± 2.0	7.8 ± 2.0	0.264
Creatinine (μmol/L)	82.0 (71.0–96.0)	90.5 (76.7–103.5)	0.104
Statins	45 (88%)	69 (88%)	0.969

For all measurements $n \geq 50$ (cluster 1), $n \geq 65$ (cluster 2) except for *$n = 19$ (cluster 1), $n = 32$ (cluster 2). Data for cluster 3 can be found in Supplementary material online, *Table S1*.
WBC, white blood cell count; hsCRP, high-sensitive C-reactive protein.

Atherosclerosis is a systemic disease and affected vessels and plaques in one location might reflect the disease process in plaques at another location.[5] Over a follow-up period of up to 7 years, patients with cluster 1 plaques experienced 12 new cardiovascular events (24% of the cluster 1 patients), whereas patients with cluster 2 plaques had 29 events (37% of the cluster 2 patients). Survival curves, however, showed no significant difference ($P = 0.109$) in incident cardiovascular events of patients belonging to cluster 1 or 2 (*Figure 2F*).

Inflammatory mediators are elevated in high-risk plaques

To answer whether the metabolite profile of plaques was associated with increased inflammation, a known factor contributing to plaque vulnerability, we analysed protein levels of inflammatory mediators and cytokines in plaque homogenates. The levels of the pro-inflammatory cytokines interleukin-6 (IL-6), IL-18, and IL-1β were significantly higher in homogenates of cluster 2 plaques than in the homogenates of the low-risk cluster 1 plaques (*Figure 3*). Unexpectedly, interferon-γ (IFN-γ) levels were higher in the plaque homogenates from cluster 1. In contrast, several chemokines, including monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1β (MIP-1β) were significantly elevated in cluster 2 plaques compared with cluster 1 (*Figure 3* and Supplementary material online, *Figure S5*). Interestingly, tumour necrosis factor-α (TNF-α) levels in the homogenates did not differ between the plaques of cluster 1 and 2. Taken together, these results indicate that the distinct metabolite profile of high-risk plaques associates with high levels of inflammatory mediators.

Metabolite levels in high-risk plaques reveal an altered energy metabolism

The decrease of short-chain acylcarnitines and in particular the accumulation of C16:0/C18:0/C18:1 acylcarnitines (arrows in *Figure 1*) seen in the high-risk cluster 2 plaques may be indicative of a dysfunctional FAO,[15,16] one of the traits of the reprogrammed metabolism in activated leucocytes. In addition, a cytokine profile similar to the one we observed in cluster 2 plaques (elevated IL-6 and IL-1β, but not TNF-α) has been linked with increased glucose uptake and glycolytic flux in lipopolysaccharide/IFN-γ stimulated monocytes and macrophages of CAD patients.[10] In support of an altered energy metabolism, hexoses, mainly comprised of glucose,[17] were reduced, whereas the lactate concentration was higher in cluster 2 plaques than in the low-risk plaques of cluster 1 (*Table 2*). Notably, blood hexose levels were not correlated with plaque hexose levels (Supplementary material online, *Figure S1*) and did not differ in patients with plaques belonging to cluster 1 or 2 [5.3 mM (5.0–6.9) vs. 5.8 mM (5.0–7.4), $P = 0.36$], indicating a locally higher utilization of glucose towards lactate in cluster 2 plaques. Despite the different levels of glucose, the net energy content of cluster 1 and 2 plaques was not different, as judged by adenosine triphosphate (ATP) levels (*Table 2*).

In activated or pro-inflammatory leucocytes a shift towards a more glycolytic metabolism is commonly accompanied by anaplerosis, a process that replenishes tricarboxylic acid cycle intermediates that have been removed for biosynthesis.[9] The levels of amino acids that can serve as anaplerotic substrates,[18] including glutamine and serine, were lower in homogenates of cluster 2 plaques relative to cluster 1, suggesting increased anaplerosis (*Table 2*). Collectively, the metabolite levels in high-risk plaques are consistent with a changed usage of metabolic pathways and an altered energy metabolism compared to low-risk plaques.

Expression of glucose metabolism genes is elevated in high-risk plaques

To explore if plaque metabolite profiles were accompanied by altered activity of genes involved in glucose catabolism,

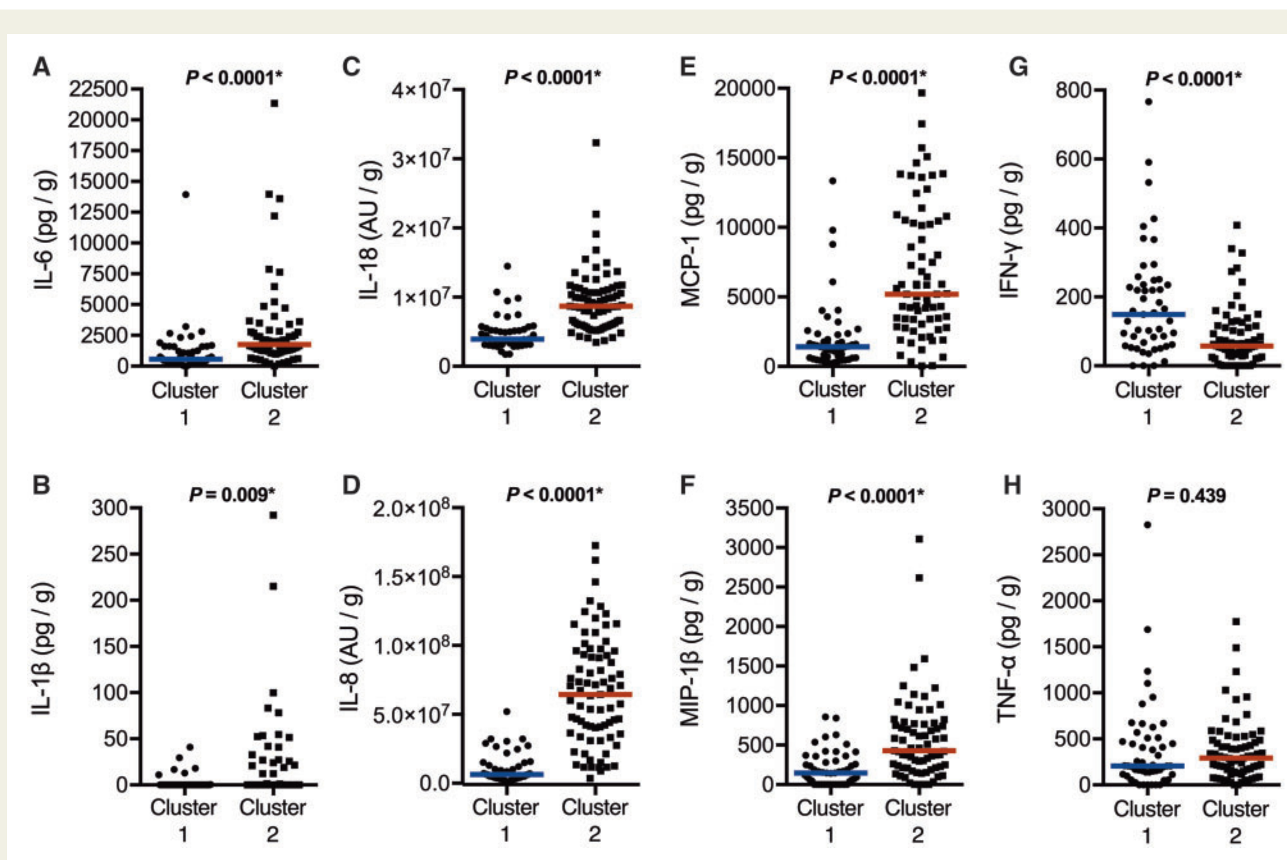


Figure 3 Several pro-inflammatory mediators are elevated in cluster 2 plaques. Levels of the pro-inflammatory cytokines (*A*) interleukin-6, (*B*) interleukin-1β, (*C*) interleukin-18, (*D*) interleukin-8 and of the chemokines (*E*) monocyte chemoattractant protein-1 and (*F*) macrophage inflammatory protein-1β as well as of (*G*) interferon-γ and (*H*) tumour necrosis factor-α in homogenates of cluster 1 and 2 plaques. Vertical lines show the median, dots depict single plaques. Significant *P*-values after controlling for multiple testing are marked with an asterisk (*). $n \geq 45$ (cluster 1) and $n \geq 70$ (cluster 2). Data for cluster 3 can be found in Supplementary material online, *Figure S6*. AU, arbitrary units.

Table 2 Non-lipid metabolites in plaques

	Cluster 1	Cluster 2	*P*-value
Hexose (μmol/g)	51.8 (40.0–81.1)	28.9 (21.7–39.6)	<0.0001*
Lactate (μmol/g)†	2.21 (1.69–3.04)	2.81 (2.22–3.48)	0.013*
ATP (pmol/g)‡	161.9 (58.2–532.9)	172.5 (55.7–414.3)	0.783
Arginine (nmol/g)	114.7 (79.9–134.8)	108.4 (80.8–129.0)	0.488
Glutamine (nmol/g)	378.3 (314.7–424.4)	322.1 (248.1–402.3)	0.003*
Glycine (nmol/g)	457.9 (380.9–531.8)	434.1 (354.7–510.6)	0.316
Histidine (nmol/g)	65.2 (52.2–82.0)	62.8 (46.1–74.7)	0.360
Phenylalanine (nmol/g)	47.9 (43.7–58.1)	51.1 (42.0–57.8)	0.946
Proline (nmol/g)	120.1 (106.0–137.7)	114.2 (99.9–142.3)	0.432
Serine (nmol/g)	146.9 (115.8–178.2)	130.3 (104.8–148.3)	0.006*
Threonine (nmol/g)	126.2 (98.1–147.1)	101.0 (81.0–120.5)	0.0006*
Valine (nmol/g)	193.6 (165.7–222.4)	189.0 (164.9–227.0)	0.791
Leucine/Isoleucine (nmol/g)	189.4 (172.2–217.4)	194.8 (164.8–225.4)	0.544

Significant *P*-values after controlling for multiple testing are marked with an asterisk (*). $n = 51$ (cluster 1), $n = 78$ (cluster 2) except for †$n = 39$ (cluster 1), $n = 71$ (cluster 2) and ‡$n = 15$ (cluster 1), $n = 27$ (cluster 2). Data for cluster 3 can be found in Supplementary material online, *Table S3*.

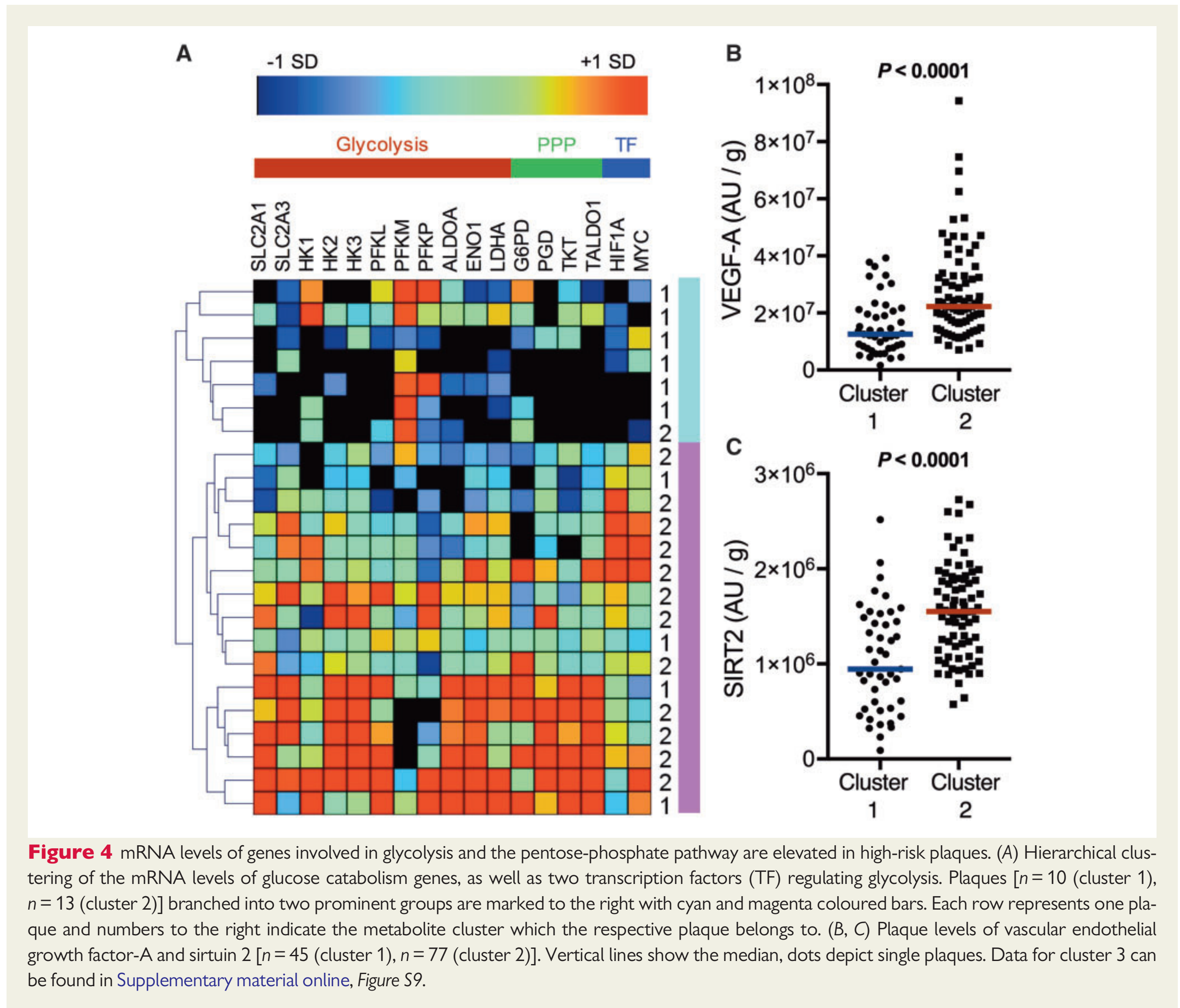
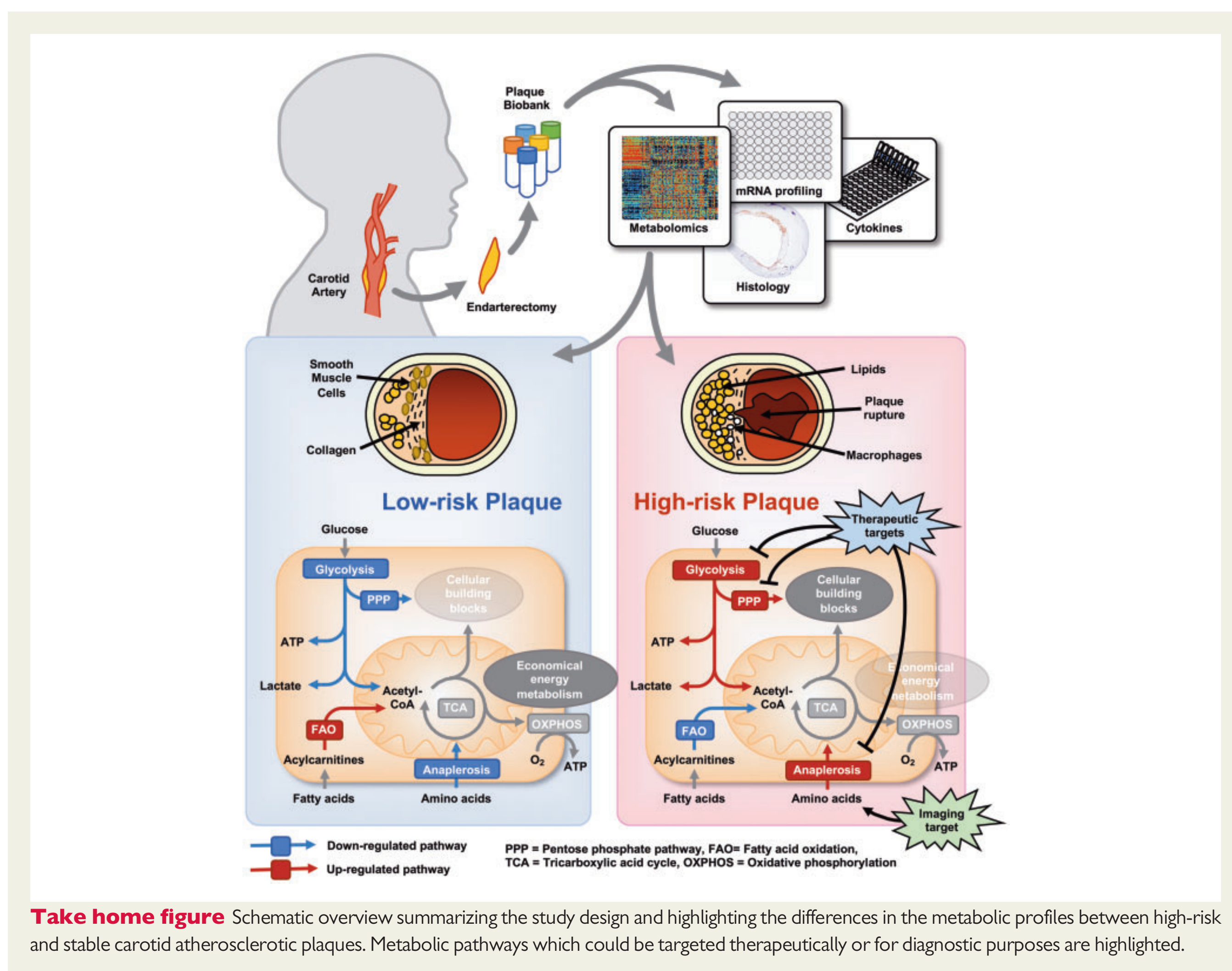


Figure 4 mRNA levels of genes involved in glycolysis and the pentose-phosphate pathway are elevated in high-risk plaques. (*A*) Hierarchical clustering of the mRNA levels of glucose catabolism genes, as well as two transcription factors (TF) regulating glycolysis. Plaques [$n = 10$ (cluster 1), $n = 13$ (cluster 2)] branched into two prominent groups are marked to the right with cyan and magenta coloured bars. Each row represents one plaque and numbers to the right indicate the metabolite cluster which the respective plaque belongs to. (*B*, *C*) Plaque levels of vascular endothelial growth factor-A and sirtuin 2 [$n = 45$ (cluster 1), $n = 77$ (cluster 2)]. Vertical lines show the median, dots depict single plaques. Data for cluster 3 can be found in Supplementary material online, *Figure S9*.

we examined mRNA expression patterns. Several glycolysis and PPP genes, such as *SLC2A3*, *HK2*, *HK3*, and *PGD* were more highly expressed in cluster 2 plaques compared to cluster 1 plaques (Supplementary material online, *Figure S7*). In addition, the two master regulators of glycolysis, *MYC* and *HIF1A*, were expressed at significantly higher levels in cluster 2 plaques (Supplementary material online, *Figure S7P, Q*). Hierarchical clustering of the genes revealed two major groups of plaques; one characterized by increased expression levels of glucose catabolism genes and a statistically significant predominance of plaques assigned to cluster 2 based on metabolite profile, and another group with reduced expression of these genes and a significant predominance of cluster 1 plaques (χ^2 test $P = 0.019$; *Figure 4A*). In order to validate our findings of the glucose metabolism genes we examined the association of gene expression levels and presence of symptoms in an independent cohort, the Biobank of Karolinska Endarterectomies (BiKE). The expression of glycolysis and PPP genes, including *SLC2A1*, *SLC2A3*, *HK2*, *HK3*, *ALDOA*, *ENO1*, *PGD*, *TKT*, and *TALDO1*, as well as the master regulators *HIF1A* and *MYC*, was significantly higher in plaques from patients displaying symptoms compared with those without (Supplementary material online, *Figure S8*).

In search of additional support for an altered metabolism in cluster 2 plaques, we measured protein levels of vascular endothelial growth factor A (VEGF-A), a target of HIF-1α, and of the deacetylase sirtuin 2 (SIRT2), which is required for the function of glucose-6-phosphate dehydrogenase, the rate-limiting enzyme of the PPP. Notably, VEGF-A and SIRT2 levels were significantly elevated in homogenates from cluster 2 plaques compared with cluster 1 plaques (*Figure 4B*, *C*). Taken together, the expression profile of metabolic genes is consistent with metabolite levels in plaques belonging to cluster 1 and 2 and supports the notion that high-risk plaques have an altered metabolism compared to low-risk plaques.



Take home figure Schematic overview summarizing the study design and highlighting the differences in the metabolic profiles between high-risk and stable carotid atherosclerotic plaques. Metabolic pathways which could be targeted therapeutically or for diagnostic purposes are highlighted.

Discussion

Cellular metabolism is innately connected to the ability of leucocytes to mount an immune response. Evidence for a link between cellular metabolism and inflammation in human atherosclerotic plaques and its relation to plaque stability has been missing. Herein, we identify a distinct metabolite signature in carotid atherosclerotic plaques that associates with the presence of symptoms, histologically evaluated markers of vulnerability and the content of inflammatory mediators in plaque homogenates. The metabolite signature in these high-risk plaques is consistent with an increased glucose utilization, a decreased FAO flux and an increased amino acid anaplerosis similar to the reprogrammed metabolism seen in activated T lymphocytes and classically activated M1 macrophages.[9]

Although HIF-1α and c-myc mRNA levels were increased in high-risk plaques, in line with their role as important metabolic regulators, HIF-1α is also a key transcriptional regulator of responses to hypoxia, which has been extensively discussed in atherosclerotic plaques.[19] Thus, we cannot draw conclusions as to whether the increase in *HIF1A* reflects metabolism under more extensive hypoxic conditions or metabolism in a normoxic, more inflammatory milieu, in which HIF-1α is essential as well.[20] It is conceivable that cluster 2 plaques have a more hypoxic environment, particularly since the higher weight of the cluster 2 endarterectomy specimens could result in increased hypoxia purely because a larger plaque size may impede oxygenation. However, without taking into account the intimal thickness, the oxygen diffusion distance cannot be determined. Moreover, considering that neither the degree of carotid stenosis nor the ATP levels, which are reported to be lowered in hypoxic plaques,[21] were different between the two clusters, a potential different level of hypoxia is unlikely to account for the gross metabolic differences seen in cluster 2 plaques. Interestingly, it has been suggested that not alterations in the oxygen supply but rather the higher oxygen demand caused by the inflammatory activity is the main determinant of plaque hypoxia.[19]

A limitation of the current study, and something that needs to be addressed in the future, is that we cannot identify the cellular source of the altered metabolism in the high-risk plaques. Although the metabolic profile we found fits the described metabolism of activated leucocytes, other potential sources could be red blood cells, endothelial cells or vascular smooth muscle cells. Importantly, however, it was recently shown that *in vitro* differentiated macrophages from patients with CAD require a reprogrammed metabolism to mount a potent inflammatory response.[10] Notably, unchanged TNF-α levels,

despite elevated levels of IL-1β and IL-6, released by patient macrophages in this study, is similar to the cytokine pattern we observed in the homogenates of high-risk plaques. In addition, IFN-γ levels were unexpectedly lower in the high-risk plaques. It has, however, been shown in mice that HIF-1α regulates the expansion of IFN-γ positive Th1 cells and IFN-γ mRNA expression in the aorta.[22,23] Thus, it is possible that the increased HIF-1α mRNA levels found in high-risk plaques could repress IFN-γ release also in human plaques.

All patients included in this study were judged to be at risk of clinical complications if the carotid plaque had not been removed surgically and the pre-operative ultrasound did not show a different degree of stenosis in the high- and low-risk plaque clusters, stressing that the metabolic phenotype of plaques could be of interest for risk stratification. Our study indicates that, in addition to positron emission tomography with the glucose analogue ^{18}F-fluorodeoxyglucose, whose uptake has been shown to correlate with inflammation in human atherosclerotic plaques and to associate with incident cardiovascular events,[24] several other metabolites could be used for metabolic imaging of high-risk plaques. Notably, ^{18}F-(2S, 4R)4-fluoroglutamine (^{18}F-FGln) is already being tested in a clinical trial for imaging of glioblastomas, as cancer cells show a metabolism comparable to the one we found in the high-risk plaques.[25] The use of ^{18}F-FGln could also significantly improve metabolic imaging of coronary artery plaques, which is hampered by the high basal glucose uptake of myocardial cells. The reprogrammed metabolism in leucocytes and cancer cells is also an attractive therapeutic opportunity and several oncological trials are underway.[26]

Conclusion

Our data highlight a possible role of cellular metabolism in high-risk plaques, that are accountable for the high global burden of cardiovascular disease. The metabolic signature found in high-risk plaques coincides largely with other measures used to describe a high-risk phenotype of plaques including increased inflammation, haemorrhage, neutral lipid deposition, macrophage content and reduced smooth muscle cell content, and consequently this study does not, *per se*, offer a leap in our understanding of the sequence of events or causes leading to the development of the high-risk plaque. To understand cause-effect relationships, additional studies validating metabolite signatures and mechanistic studies in animal models are needed. Although the direct clinical application is limited, our data provides important biological insight with clear clinical implications for the future. The current study does for the first time provide evidence for a link between an altered cellular metabolism and inflammation in human high-risk plaques. Knowledge of this largely unexplored disease mechanism could be exploited to identify and treat high-risk plaques.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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