Journal of Nuclear Medicine, published on May 7, 2021 as doi:10.2967/jnumed.120.245407

Imaging Immunometabolism in Atherosclerosis

Philip Z. Mannes[1] & Sina Tavakoli[1,2,3]

[1] Departments of Radiology and [2] Medicine, University of Pittsburgh; and [3] Heart, Lung, Blood, and Vascular Medicine Institute, UPMC, Pittsburgh, Pennsylvania

Corresponding Author: Sina Tavakoli, UPMC Presbyterian Hospital, 200 Lothrop Street, Suite E200, Pittsburgh, PA 15213
Phone: 412-647-7288
Fax: 412-647-2601
Email: sit23@pitt.edu

First Author: Philip Z. Mannes (MSTP student), UPMC Hospital, 200 Lothrop Street, Suite E200, Pittsburgh, PA 15213
Phone: 412-467-7288
Fax: 412-647-2601
Email: pzm2@pitt.edu

Sources of Funding: Sina Tavakoli is supported by NIH-NHLBI K08-HL144911 and NIH-NIBIB R21-EB027871.

Running Title: Immunometabolism and atherosclerosis

Word Count: 4,621

ABSTRACT

Over the past decade, there has been a growing recognition of the links between intracellular metabolism and immune cell activation, i.e., immunometabolism, and its consequences in atherogenesis. However, the majority of immunometabolic investigations have been conducted in cultured cells through pharmacologic and/or genetic manipulations of selected immunologic or metabolic pathways, limiting their extrapolation to the complex microenvironment of plaques. *In vivo* metabolic imaging is ideally situated to address this gap and to determine the clinical implications of immunometabolic alterations for diagnosis and management of patients. Indeed, ^{18}F-FDG has been widely used in clinical studies with promising results for risk stratification of atherosclerosis and monitoring the response to therapeutic interventions, though the biological basis of its uptake in plaques has been evolving. Herein, we describe recent advances in understanding of immunometabolism of atherosclerosis with an emphasis on macrophages, and we review promising metabolic imaging approaches using ^{18}F-FDG and other PET radiotracers.

Key Words: atherosclerosis, immunometabolism, inflammation, macrophage, metabolic imaging

Through determining the severity of luminal stenosis and compromised blood flow, conventional imaging, e.g., angiography and perfusion imaging, has revolutionized the diagnosis and management of flow-limiting atherosclerotic plaques causing chronic ischemia (*1*). However, these approaches are limited for identification of lesions at risk of acute complications (e.g., plaque rupture or erosion) leading to acute myocardial infarction (MI) or other thromboembolic events as well as in the assessment of the response to therapeutic interventions (*1*). Imaging pathophysiological processes occurring within the plaques, rather than their late structural/physiological sequelae on luminal patency/perfusion, promotes a precision approach to atherosclerosis by improving the risk stratification of patients and early monitoring of disease progression or response to therapy (*1*).

Inflammation as a key pathogenic driver of atherosclerosis has been a major target for molecular imaging (*1*), a role which has been further highlighted with the emergence of novel immunomodulatory interventions. The versatility of positron emission tomography (PET) to provide insights into various metabolic pathways along with the growing discoveries in immunometabolism, i.e., the interplay between cell metabolism and immune response, have placed it at the forefront of molecular imaging of atherosclerosis.

Here, we will review recent immunometabolic discoveries in atherosclerosis and discuss promising strategies to non-invasively image plaque immunometabolism.

INFLAMMATION AND ATHEROGENESIS

Atherosclerosis is a chronic inflammatory disease of the arterial wall, which starts by subendothelial deposition of lipoproteins in areas of perturbed blood flow and endothelial activation (*2*). Enzymatic and non-enzymatic modifications of the deposited lipoproteins generate pro-inflammatory derivatives which trigger innate and adaptive immune responses, resulting in recruitment of leukocytes and migration/proliferation of resident cells within the vessel wall, e.g., vascular smooth muscle cells (VSMCs), leading to plaques' expansion (*1,2*). The balance between pro-inflammatory and inflammation-resolving processes within the plaque determines if a lesion stabilizes/regresses or further expands, eventually forming a core largely composed of lipids and necrotic cells (*1,2*). Formation of a fibrous cap rich in collagen and VSMCs is another critical process, which contributes to plaque stability.

Vulnerable plaques, characterized by thin fibrous caps, large necrotic cores, and abundant inflammatory cells, are prone to fibrous cap rupture and have been historically the most common cause of acute complications of atherosclerosis (*3*). However, improved control of cardiovascular

risk factors, particularly hyperlipidemia, over the past decades has led to significant declines in the prevalence of rupture-prone plaques with concomitant increases in the prevalence of endothelial erosion as the cause of acute complications (*3*). These plaques often contain smaller necrotic cores and fewer inflammatory cells. Therefore, imaging strategies to identify erosion-prone from rupture-prone plaques may be distinct.

IMMUNOMETABOLISM AND ATHEROSCLEROSIS

Metabolic adaptations are critical for leukocytes to alter their functions in response to various pro-inflammatory and inflammation-resolving stimuli. However, immunometabolism has been largely studied *ex vivo* using metabolic tracing in conjunction with pharmacologic and/or genetic manipulations of specific metabolic and immunological pathways, which do not recapitulate the complexities of the plaque microenvironment. Metabolic imaging is well positioned to bridge this gap and to translate immunometabolic discoveries into clinical applications.

In this review, we will focus on macrophages, as the most abundant leukocytes in plaques with critical functions throughout different stages of atherogenesis (*4*). Despite the contribution of other immune cells, including neutrophils, dendritic cells, and lymphocytes, to atherogenesis (*2*), these cells are present in smaller numbers compared to macrophages (and other resident cells within the vessel wall, such as VSMCs), which challenges their metabolic assessment by PET.

Macrophage metabolism has been largely studied in the context of “M1” (pro-inflammatory) and “M2” (inflammation-resolving) polarization states, representing the extreme ends of the functional diversity of macrophages in response to *ex vivo* activation with selected pro-inflammatory (e.g., interferon-γ (IFN-γ) and/or lipopolysaccharide) or anti-inflammatory (e.g., interleukin (IL)-4) stimuli. However, this dichotomization is not a physiologic phenomenon and development of less reductive approaches is required to delineate the immunometabolic heterogeneity of macrophages in atherosclerosis. Within this constraint, observational studies suggest that macrophages resembling M1 *vs.* M2 polarization states play opposing effects in atherogenesis and contribute to plaque progression/vulnerability *vs.* regression/stability, respectively (*5*).

<u>M1 polarization:</u> Metabolic reprogramming of M1 polarized macrophages is mostly studied after activation with lipopolysaccharide, as a classical pathogen-derived stimulus (*4*). Enhanced glucose uptake and glycolysis along with a suppressed tricarboxylic acid (TCA) cycle and reduced

oxidative phosphorylation (OxPhos) are hallmarks of lipopolysaccharide-stimulated macrophages (*4,6*). Activation of hypoxia-inducible factor-1α (HIF-1α) is a key regulatory mechanism of lipopolysaccharide-induced metabolic reprogramming, which mimics a pseudo-hypoxic state manifested by increased expression of glucose transporters (GLUT) and critical rate-limiting glycolytic enzymes, including hexokinases (*4*). Moreover, lipopolysaccharide causes functional breaks in the TCA cycle by suppressing isocitrate dehydrogenase and succinate dehydrogenase activities (*6*). These breaks lead to the accumulation of citrate and succinate, which contribute to other immunometabolic changes of macrophages, including increased *de novo* synthesis of fatty acids, suppression of OxPhos and the electron transport chain, stabilization of HIF-1α, and production of inflammatory mediators, such as IL-1β (*4*). Upregulation of the oxidative phase of the pentose phosphate pathway (PPP) is another key metabolic feature of lipopolysaccharide stimulation, which provides macrophages with the 5-carbon sugars and NADPH needed for the biosynthesis of fatty acids and sterols, and redox regulation (*4*).

Emerging evidence supports the role of HIF-1α-mediated metabolic reprogramming in atherogenesis. Notably, myeloid cells expression of HIF-1α is critical in high-fat diet induced atherosclerosis and development of the necrotic core in a mouse model (*7*). Additionally, hematopoietic deficiency of GLUT1 reduces both monocyte influx into plaques and progression of atherosclerosis (*8*).

M2 polarization: The conventional M2 polarizing stimulus IL-4 induces a markedly different metabolic signature, compared to that of lipopolysaccharide, and is regulated through different transcriptional machinery, including Akt, STAT-6, and PGC1-β (*4*). Moreover, IL-4-induced metabolic changes are relatively delayed and require mitochondrial biogenesis, a key driver of enhanced OxPhos, TCA cycle activity, and fatty acid oxidation (*4*). Therefore, mitochondrial damage and compromised respiration present in atherosclerotic plaques may underlie the impaired inflammation-resolving capacity of plaque macrophages, particularly in the fibrous cap and necrotic core (*9*). Interestingly, restoration of plaques' mitochondrial respiration leads to smaller necrotic cores and larger fibrous caps, supporting the plaque-stabilizing role of mitochondrial respiration (*9*).

It should however be noted that metabolic reprogramming of macrophages is stimulus-specific and various pro-inflammatory (e.g., oxidized phospholipids and granulocyte-macrophage colony-stimulating factor (GM-CSF)) and anti-inflammatory (e.g., macrophage colony-stimulating factor (M-CSF)) stimuli result in overlapping metabolic alterations (Fig. 1A). Moreover, the

metabolic profiles of additional plaque-associated macrophage phenotypes outside the M1 and M2 dichotomy, including Mox and Mhem (*5*), have not been well established.

IMMUNOMETABOLIC IMAGING

In the following sections, promising PET tracers for imaging the immunometabolism of atherosclerosis are briefly reviewed (Fig. 1B and Table 1).

^{18}F-fluorodeoxyglucose (^{18}F-FDG)

Following the initial retrospective reports of ^{18}F-FDG uptake in atherosclerosis, several studies demonstrated higher ^{18}F-FDG uptake in plaques with features of vulnerability (e.g., large lipid core and intra-plaque hemorrhage) (*10*) and in symptomatic patients (e.g., recent transient ischemic attack (TIA) or stroke), compared to stable/asymptomatic lesions (*11*). Further investigations revealed that plaques' ^{18}F-FDG uptake correlates with its inflammatory burden, particularly macrophage content, and can be reproducibly quantified (*12*), suggesting the potential of ^{18}F-FDG PET for non-invasive monitoring of plaque inflammation and risk of vulnerability. Moreover, ^{18}F-FDG uptake in large (e.g., aorta, carotid and iliofemoral) arteries was shown to correlate with various cardiovascular risk factors and improve the prediction of future cardiovascular events, including stroke and MI, beyond conventional risk stratification tools, such as Framingham risk score and severity of carotid artery stenosis (*13,14*). A dose-dependent decline in ^{18}F-FDG uptake of aorta and carotid arteries was also shown as early as 4 weeks after treatment with atorvastatin (*15*), indicating its role in monitoring the therapeutic response. However, subsequent studies with novel lipid-lowering or anti-inflammatory drugs showed no significant decline in arterial uptake of ^{18}F-FDG (*16*), indicating that the role of ^{18}F-FDG in monitoring the therapeutic response needs to be validated with individual drugs.

Despite extensive clinical investigations, the biological basis of ^{18}F-FDG uptake in atherosclerosis has been evolving. While ^{18}F-FDG uptake was largely attributed to its enhanced uptake by pro-inflammatory macrophages, recent evidence supports that other components of plaques, particularly VSMCs, may have similar or even higher levels of ^{18}F-FDG uptake (*17,18*). Moreover, increasing evidence supports that ^{18}F-FDG uptake, as a stand-alone marker, fails to discriminate the metabolic reprogramming of macrophages in response to different pro-inflammatory and inflammation-resolving stimuli (*19-21*). For example, enhanced glucose uptake is not a ubiquitous feature of pro-inflammatory macrophages and may be absent in macrophages

stimulated with IFN-γ and TNF-α. Additionally, inflammation-resolving stimuli, such as M-CSF (*20*), and other microenvironmental factors, e.g., hypoxia (*21*) and oxidized phospholipids (*22*), increase ^{18}F-FDG uptake in macrophages and atherosclerotic plaques.

Hypoxia

Intra-plaque hypoxia, particularly in advanced lesions, results from excessive oxygen consumption by metabolically active cells and insufficient oxygen delivery from the lumen and vaso vasorum (*7*). Hypoxia contributes to plaque's advancement and vulnerability through expansion of the necrotic core, impairment of efferocytosis, and accentuation of inflammation (*4,7,23*). Moreover, hypoxia induces the expression of HIF-1α which is essential for macrophage migration (*24,25*) and shifts their metabolism towards glycolysis, allowing for the buildup of TCA intermediates necessary to support a pro-inflammatory response, e.g., production of IL-1β (*4,23*).

Two major classes of hypoxia imaging agents, including nitroimidazole (e.g., ^{18}F-FMISO and ^{18}F-HX4) and copper complexed with diacetyl-bis(N4-methylthiosemicarbazone) (Cu-ATSM) analogs, have been used to image atherosclerosis in small clinical studies. Localized ^{18}F-HX4 uptake in symptomatic carotid plaques was shown to correlate with arterial wall thickness and ^{18}F-FDG uptake (*26*). ^{18}F-FMISO uptake was also found to be higher in symptomatic, compared to the contralateral, carotid plaques in patients with a recent stroke or TIA (*27*). Histologic analysis of endarterectomy specimens trended toward a positive correlation between ^{18}F-FMISO and macrophage content, but not with HIF-1α expression or VSMC content (*27*). Both studies found positive correlations between the uptake of ^{18}F-FDG and either ^{18}F-HX4 or ^{18}F-FMISO (*26,27*) (Fig. 2A), which is consistent with the major role of hypoxia in promoting glycolysis (*21*). Encouragingly, a recent paper using ^{64}Cu-ATSM in asymptomatic patients with carotid stenosis >50% reported that HIF-1α expression colocalized with CD68^{+}-expressing macrophages in PET positive plaques (*28*).

One limitation of hypoxia radiotracers is that barriers to diffusion, such as poor vascularization and fibrosis, reduce tracer uptake in hypoxic tissue, likely contributing to the modest target-to-background signal observed with these agents in atherosclerosis. Future work is required to determine if imaging hypoxia allows for prospective identification of plaques at risk of acute complications or response to medical interventions.

Acetate

Acetate, a two-carbon intermediate used in energy-generating and biosynthetic pathways, is transported into cells by monocarboxylate transporters (MCT) (*29*) and is converted to acetyl-

CoA by cytosolic or mitochondrial acetyl-CoA synthases (*29*). Cytosolic acetyl-CoA is used for fatty acid synthesis and protein acetylation. Mitochondrial acetyl-CoA enters the TCA cycle by condensation with oxaloacetate to form citrate, which can be used to generate ATP through oxidative phosphorylation or exported to the cytoplasm for fatty acid synthesis, intimately linking acetate and fatty acid metabolism. Acetate metabolism is therefore an attractive imaging target, which provides insight into cellular catabolism and anabolism.

The feasibility of imaging ^{11}C-acetate uptake by plaques along the large (e.g., aorta, iliac and carotid) arteries has been shown in patients undergoing PET/CT for oncologic indications (Fig. 2B) (*30*). High-resolution autoradiography of murine brachiocephalic arteries has also demonstrated that arterial uptake of acetate is primarily localized to macrophage-rich regions of plaques (*31*). Moreover, it was shown that IFN-γ plus lipopolysaccharide markedly suppressed *ex vivo* acetate uptake by murine macrophages and human carotid endarterectomy specimens, while IL-4 increased its uptake (*31*), suggesting a potential role of ^{11}C-acetate PET in distinction of pro-inflammatory and inflammation-resolving macrophages. However, the role of ^{11}C-acetate imaging in risk stratification of patients or monitoring the response to anti-inflammatory interventions remains to be further explored in clinical studies. Moreover, despite the promise of ^{11}C-acetate, less attention has been given to plaque imaging using tracers derived from longer chain fatty acids.

Choline

Choline is a quaternary amine important for the biosynthesis of membrane lipids and acetylcholine as well as hepatic one-carbon metabolism. Several choline transport systems have been identified with choline transporter-like proteins (CTL) and organic cation transporters being widely expressed (*32*). Once in the cell, choline and choline-based radiotracers are incorporated into lipids (*32*), like phosphatidylcholine, which is a major constituent of the outer cell membrane.

Choline has thus emerged as a promising marker of cells with high rates of phospholipid biosynthesis, including proliferating cells. Lipopolysaccharide stimulation of macrophages increases CTL1 mediated transport of choline, which is required for their pro-inflammatory activation (*33,34*). Reducing choline uptake through blocking CTL1 or culturing in choline-deficient media impairs macrophage activation, alters the secretion of inflammatory cytokines, and inhibits the residual mitochondrial activity (*33,34*). Multiple PET radiotracers, including ^{11}C-choline, ^{18}F-fluorocholine (FCH), and ^{18}F-fluoroethylcholine (FEC) have been developed (Fig. 2C), though the fluorocholine-based radiotracers may be substrates for a distinct set of transporters (*32,35*).

The feasibility of choline-derived radiotracers for imaging calcified and noncalcified plaques within the aorta, iliac and carotid arteries was shown in men undergoing ^{18}F-FMC, ^{11}C-choline, and ^{18}F-FEC PET for prostate cancer (*36-38*). Additionally, plaques' uptake of ^{18}F-FMC strongly correlates with their macrophage content in patients with severe carotid stenosis (*39*). These early choline-based imaging studies are promising, although additional studies are needed to examine the association between choline uptake and various macrophage activation states or plaques' risk of vulnerability.

Glutamine

Glutamine is an attractive imaging target given its essential roles in major biosynthetic and metabolic pathways (*6*). For instance, glutamine replenishes TCA cycle intermediates, by conversion to glutamate and α-ketoglutarate, which serve as carbon sources for glucose, fatty acid, and protein synthesis. Additionally, glutamine metabolism is used for NADPH production, required for nitric oxide production, fatty acid synthesis, and glutathione regeneration (*6*). Glutamine also serves as a nitrogen source for purine and pyrimidine synthesis.

Macrophages have high levels of glutamine utilization (*6*). Alterations in glutamine metabolism influence macrophage function in both pro-inflammatory and inflammation-resolving states. Glutamine metabolism is required for IL-1β production by lipopolysaccharide-stimulated macrophages (*23*). This pro-inflammatory function is driven through glutamine entering a functionally broken TCA cycle and contributing to increased intracellular succinate levels and HIF-1α stabilization (*40*). In contrast, IL-4 causes glutamine shuttling into a functional TCA cycle, which reinforces an M2-like macrophage phenotype through epigenetic modification of M2-specific gene promoters, increased OxPhos and fatty acid oxidation, and destabilization of HIF-1α through an increased α-ketoglutarate-to-succinate ratio (*24,40*). Interestingly, hyperinflammatory macrophages stimulated with lipopolysaccharide and oxidized phospholipids use glutamine along with mitochondrial respiration to support IL-1β production (*22*).

Distinct patterns of ^{18}F-FDG and ^{14}C-glutamine uptake by aortic atherosclerotic plaques have been shown using high-resolution autoradiography in a mouse model of atherosclerosis, suggesting that combined imaging of glutamine and glucose metabolism may better delineate the immunometabolic heterogeneity of plaques (*19*). Consistently, combined measurement of glucose and glutamine uptake improves the identification of different activation states of macrophages induced by conventional polarizing agents (*19*). Despite the availability of glutamine-derived PET-radiotracers, e.g., ^{18}F-(2S,4R)-4-fluoroglutamine and ^{11}C-glutamine, the

feasibility of *in vivo* imaging of glutamine metabolism in atherosclerosis has remained to be determined.

LIMITATIONS

Despite the unique role of PET to delineate metabolism *in vivo*, the immunometabolic complexity of plaques is incompletely captured using individual metabolic substrates as the targeted pathways are exploited by a variety of cells, sometimes near-ubiquitously. Moreover, available radiotracers primarily assess the first steps of metabolism, i.e., membrane transport and/or intra-cellular trapping, and cannot discern the downstream divergence of intra-cellular utilization of substrates. Overlapping metabolic signatures of (activated) immune cells further complicate the assessment of inflammatory states of plaques using a single metabolic pathway. Therefore, unraveling the biological correlates of radiotracer uptake in atherosclerosis requires meticulous correlations of high-resolution PET, autoradiography, and histological profiling of plaques' immune cells. Moreover, current metabolic imaging approaches to atherosclerosis are mostly focused on detection of rupture-prone plaques and may be ineffective for identification of plaques at risk of erosion.

FUTURE DIRECTIONS

Recent state-of-the-art immunometabolic discoveries have advanced our understanding of the biological basis of ^{18}F-FDG uptake in atherosclerosis and highlighted the promise of imaging other metabolic pathways, described herein, to improve the non-invasive characterization of plaque inflammation. However, there is a dire need to transition towards *in vivo* immunometabolic phenotyping of atherosclerosis. Combined imaging of two or more metabolic targets/pathways is a direction which improves the distinction of different inflammatory states of immune cells (*19*) and may partially address the limited cell-specificity of metabolic radiotracers and the overlapping metabolic signatures of different activation states of immune cells (and other cells residing in the inflamed tissues). However, this approach is logistically complex in clinical settings. Alternatively, development of novel metabolic imaging tracers which are simultaneously targeted towards, or delivered to, specific cell types may address this limitation.

A multi-dimensional approach extending from pre-clinical investigations to large-scale longitudinal clinical cohort studies and clinical trials is required to establish the role of

immunometabolic imaging as a precision medicine tool in non-invasive plaque characterization and prediction of atherosclerosis outcomes using clinically-established endpoints (e.g., acute coronary syndrome, embolic stroke, and progressive stenosis or calcification) or monitoring the response to therapeutic interventions.

DISCLOSURE: No potential conflicts of interest.

REFERENCES

1. Tavakoli S, Vashist A, Sadeghi MM. Molecular imaging of plaque vulnerability. *J Nucl Cardiol.* 2014;21:1112-1128; quiz 1129.

2. Back M, Yurdagul A, Jr., Tabas I, Oorni K, Kovanen PT. Inflammation and its resolution in atherosclerosis: mediators and therapeutic opportunities. *Nat Rev Cardiol.* 2019;16:389-406.

3. Libby P, Pasterkamp G, Crea F, Jang IK. Reassessing the mechanisms of acute coronary syndromes. *Circ Res.* 2019;124:150-160.

4. Tabas I, Bornfeldt KE. Intracellular and intercellular aspects of macrophage immunometabolism in atherosclerosis. *Circ Res.* 2020;126:1209-1227.

5. Chinetti-Gbaguidi G, Colin S, Staels B. Macrophage subsets in atherosclerosis. *Nat Rev Cardiol.* 2015;12:10-17.

6. O'Neill LA, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nat Rev Immunol.* 2016;16:553-565.

7. Aarup A, Pedersen TX, Junker N, et al. Hypoxia-inducible factor-1alpha expression in macrophages promotes development of atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2016;36:1782-1790.

8. Sarrazy V, Viaud M, Westerterp M, et al. Disruption of Glut1 in hematopoietic stem cells prevents myelopoiesis and enhanced glucose flux in atheromatous plaques of apoE(-/-) mice. *Circ Res.* 2016;118:1062-1077.

9. Yu EPK, Reinhold J, Yu H, et al. Mitochondrial respiration is reduced in atherosclerosis, promoting necrotic core formation and reducing relative fibrous cap thickness. *Arterioscler Thromb Vasc Biol.* 2017;37:2322-2332.

10. Saito H, Kuroda S, Hirata K, et al. Validity of dual MRI and F-FDG PET imaging in predicting vulnerable and inflamed carotid plaque. *Cerebrovasc Dis.* 2013;35:370-377.

11. Rudd JH, Warburton EA, Fryer TD, et al. Imaging atherosclerotic plaque inflammation with [18F]-fluorodeoxyglucose positron emission tomography. *Circulation.* 2002;105:2708-2711.

12. Rudd JH, Myers KS, Bansilal S, et al. (18)Fluorodeoxyglucose positron emission tomography imaging of atherosclerotic plaque inflammation is highly reproducible: implications for atherosclerosis therapy trials. *J Am Coll Cardiol.* 2007;50:892-896.

13. Figueroa AL, Abdelbaky A, Truong QA, et al. Measurement of arterial activity on routine FDG PET/CT images improves prediction of risk of future CV events. *JACC Cardiovasc Imaging.* 2013;6:1250-1259.

14. Marnane M, Merwick A, Sheehan OC, et al. Carotid plaque inflammation on 18F-fluorodeoxyglucose positron emission tomography predicts early stroke recurrence. *Ann Neurol.* 2012;71:709-718.

15. Tawakol A, Fayad ZA, Mogg R, et al. Intensification of statin therapy results in a rapid reduction in atherosclerotic inflammation: results of a multicenter fluorodeoxyglucose-positron emission tomography/computed tomography feasibility study. *J Am Coll Cardiol.* 2013;62:909-917.

16. Zhao TX, Mallat Z. Targeting the Immune System in Atherosclerosis: JACC state-of-the-art review. *J Am Coll Cardiol.* 2019;73:1691-1706.

17. Al-Mashhadi RH, Tolbod LP, Bloch LO, et al. (18)Fluorodeoxyglucose accumulation in arterial tissues determined by PET signal analysis. *J Am Coll Cardiol.* 2019;74:1220-1232.

18. Guillermier C, Doherty SP, Whitney AG, et al. Imaging mass spectrometry reveals heterogeneity of proliferation and metabolism in atherosclerosis. *JCI Insight.* 2019;4:e128528.

19. Tavakoli S, Downs K, Short JD, et al. Characterization of macrophage polarization states using combined measurement of 2-deoxyglucose and glutamine accumulation: Implications for imaging of atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2017;37:1840-1848.

20. Tavakoli S, Short JD, Downs K, et al. Differential regulation of macrophage glucose metabolism by macrophage colony-stimulating factor and granulocyte-macrophage colony-stimulating factor: Implications for (18)F-FDG PET imaging of vessel wall iInflammation. *Radiology.* 2017;283:87-97.

21. Folco EJ, Sheikine Y, Rocha VZ, et al. Hypoxia but not inflammation augments glucose uptake in human macrophages: Implications for imaging atherosclerosis with 18fluorine-labeled 2-deoxy-D-glucose positron emission tomography. *J Am Coll Cardiol.* 2011;58:603-614.

22. Di Gioia M, Spreafico R, Springstead JR, et al. Endogenous oxidized phospholipids reprogram cellular metabolism and boost hyperinflammation. *Nat Immunol.* 2020;21:42-53.

23. Koelwyn GJ, Corr EM, Erbay E, Moore KJ. Regulation of macrophage immunometabolism in atherosclerosis. *Nat Immunol.* 2018;19:526-537.

24. Viola A, Munari F, Sanchez-Rodriguez R, Scolaro T, Castegna A. The metabolic signature of macrophage responses. *Front Immunol.* 2019;10:1462.

25. Cramer T, Yamanishi Y, Clausen BE, et al. HIF-1alpha is essential for myeloid cell-mediated inflammation. *Cell.* 2003;112:645-657.

26. van der Valk FM, Sluimer JC, Voo SA, et al. In vivo imaging of hypoxia in atherosclerotic plaques in humans. *JACC Cardiovasc Imaging.* 2015;8:1340-1341.

27. Joshi FR, Manavaki R, Fryer TD, et al. Vascular imaging with (18)F-fluorodeoxyglucose positron emission tomography is influenced by hypoxia. *J Am Coll Cardiol.* 2017;69:1873-1874.

28. Nie X, Elvington A, Laforest R, et al. (64)Cu-ATSM positron emission tomography/magnetic resonance imaging of hypoxia in human atherosclerosis. *Circ Cardiovasc Imaging.* 2020;13:e009791.

29. Bose S, Ramesh V, Locasale JW. Acetate metabolism in physiology, cancer, and beyond. *Trends Cell Biol.* 2019;29:695-703.

30. Derlin T, Habermann CR, Lengyel Z, et al. Feasibility of 11C-acetate PET/CT for imaging of fatty acid synthesis in the atherosclerotic vessel wall. *J Nucl Med.* 2011;52:1848-1854.

31. Demirdelen S, Mannes PZ, Aral AM, Haddad J, Leers SA, Gomez D, Tavakoli S. Divergence of acetate uptake in proinflammatory and inflammation-resolving macrophages: Implications for imaging atherosclerosis. *Journal of Nuclear Cardiology* 2021;In Press.

32. Hedtke V, Bakovic M. Choline transport for phospholipid synthesis: An emerging role of choline transporter-like protein 1. *Exp Biol Med (Maywood).* 2019;244:655-662.

33. Snider SA, Margison KD, Ghorbani P, et al. Choline transport links macrophage phospholipid metabolism and inflammation. *J Biol Chem.* 2018;293:11600-11611.

34. Sanchez-Lopez E, Zhong Z, Stubelius A, et al. Choline uptake and metabolism modulate macrophage IL-1beta and IL-18 production. *Cell Metab.* 2019;29:1350-1362 e1357.

35. Visentin M, van Rosmalen BV, Hiller C, et al. Impact of organic cation transporters (OCT-SLC22A) on differential diagnosis of intrahepatic lesions. *Drug Metab Dispos.* 2017;45:166-173.

36. Bucerius J, Schmaljohann J, Bohm I, et al. Feasibility of 18F-fluoromethylcholine PET/CT for imaging of vessel wall alterations in humans--first results. *Eur J Nucl Med Mol Imaging.* 2008;35:815-820.

37. Kato K, Schober O, Ikeda M, et al. Evaluation and comparison of 11C-choline uptake and calcification in aortic and common carotid arterial walls with combined PET/CT. *Eur J Nucl Med Mol Imaging.* 2009;36:1622-1628.

38. Forster S, Rominger A, Saam T, et al. 18F-fluoroethylcholine uptake in arterial vessel walls and cardiovascular risk factors: correlation in a PET-CT study. *Nuklearmedizin.* 2010;49:148-153.

39. Voo S, Kwee RM, Sluimer JC, et al. Imaging intraplaque inflammation in carotid atherosclerosis with 18F-fluorocholine positron emission tomography-computed tomography: Prospective study on vulnerable atheroma with immunohistochemical validation. *Circ Cardiovasc Imaging.* 2016;9.

40. Jha AK, Huang SC, Sergushichev A, et al. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity.* 2015;42:419-430.

FIGURE LEGENDS

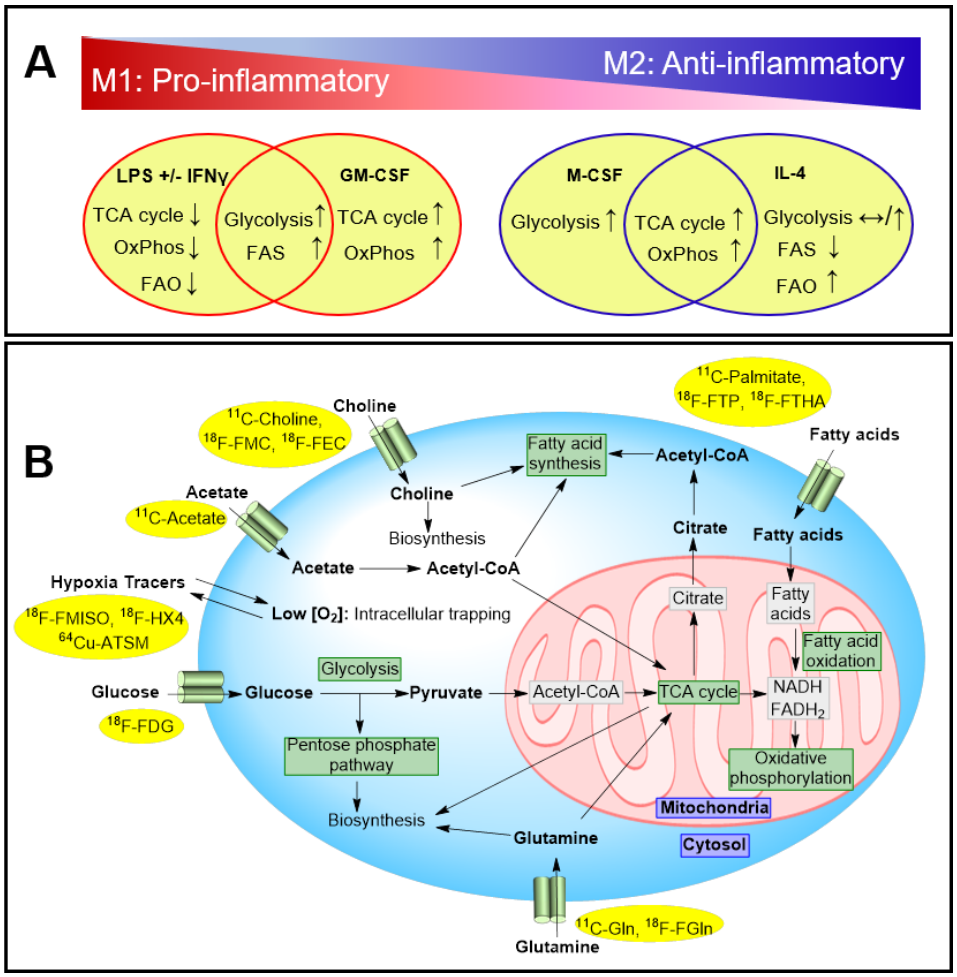

Figure 1. Overlapping and stimulus-specific metabolic reprogramming of macrophages polarized into pro-inflammatory (M1) and inflammation-resolving (M2) states **(A)**. Metabolic changes in response to lipopolysaccharide (LPS) +/- IFN-γ and GM-CSF, as examples of M1-polarizing stimuli, *vs.* IL-4 and M-CSF, as examples of M2-polarizing stimuli, are summarized. Immunometabolic profiles of macrophages within the plaque microenvironment are far more complex and cannot be accurately extrapolated from M1/M2 polarization scheme. **(B)** Major metabolic pathways targeted by PET imaging and examples of available radiotracers.

^{18}F-FDG: ^{18}F-fluorodeoxyglucose; ^{18}F-FMISO: ^{18}F-fluoromisonidazole; ^{64}Cu-ATSM: ^{64}Cu-Copper(II)diacetyl-di(N4-methylthiosemicarbazone); ^{18}F-HX4: ^{18}F-(3-fluoro-2-{4-[(2-nitro-1H-imidazol-1-yl)methyl]-1H-1,2,3-triazol-1-yl}propan-1-ol); ^{18}F-FMC: ^{18}F-fluoromethylcholine; ^{18}F-FEC: ^{18}F-fluoroethylcholine; ^{18}F-FTP: ^{18}F-fluoro-4-thia-palmitate; ^{18}F-FTHA: ^{18}F-fluoro-6-thia-heptadecanoic acid; ^{11}C-Gln: L-5-^{11}C-glutamine; ^{18}F-FGln: ^{18}F-(2S,4R)-4-fluoro-L-glutamine.

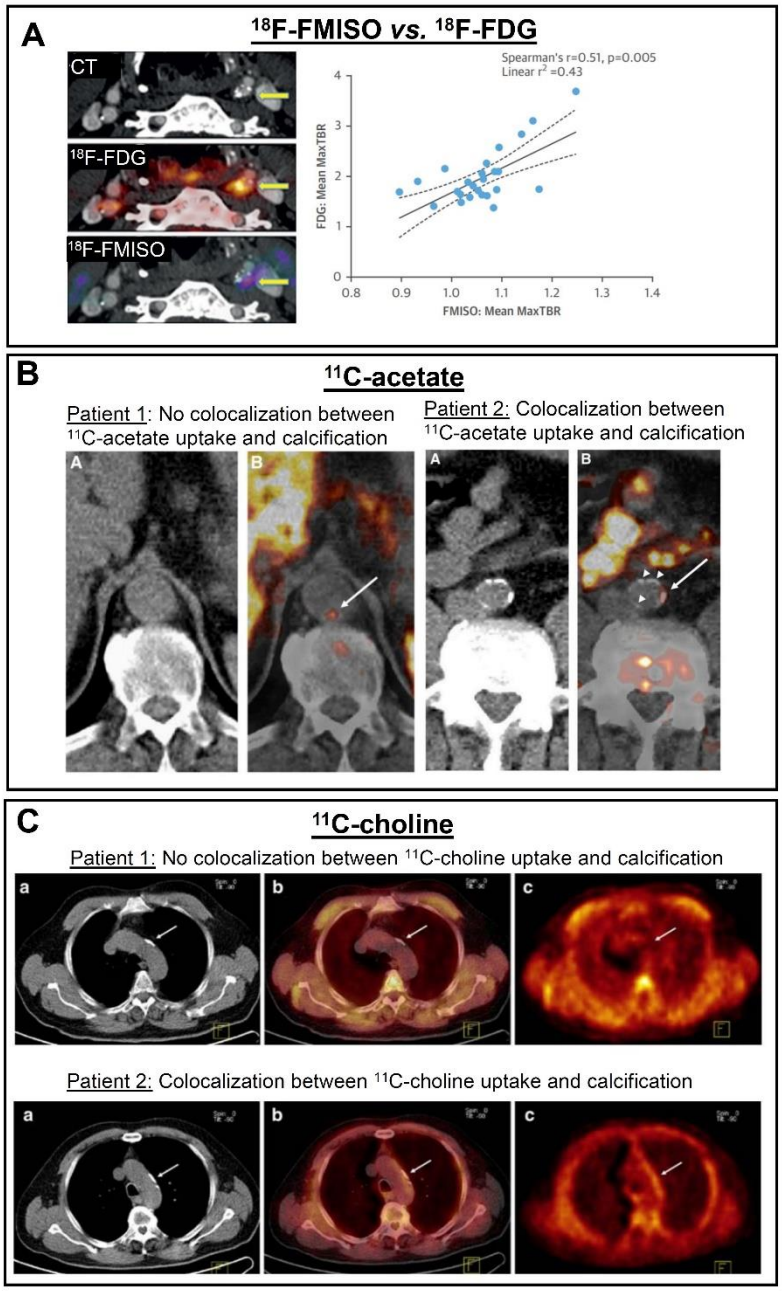

Figure 2. PET metabolic imaging of atherosclerosis. **(A)** Examples of ^{18}F-FMISO and ^{18}F-FDG PET/CT in a patient with carotid artery atherosclerosis (left panel) demonstrate uptake of both tracers in the left carotid artery plaque (yellow arrows). ^{18}F-FMISO uptake correlates with ^{18}F-FDG uptake (right panel). **(B)** Examples of ^{11}C-acetate PET/CT of the aorta in patients receiving whole body scans for oncologic indications. Images from patient 1 (left panel) indicate ^{11}C-acetate uptake in an aortic region without calcified plaques, whereas images from patient 2 (right panel) demonstrate colocalization of ^{11}C-acetate uptake with aortic calcifications (white arrows point to areas of arterial ^{11}C-acetate uptake). **(C)** Examples of ^{11}C-choline PET/CT of the aortic arch in

men receiving whole body scans for assessment of prostate cancer. Colocalization of ^{11}C-choline uptake with aortic calcifications is observed in patient 2 (lower panel), but not in patient 1 (upper panel) (white arrows indicate areas of aortic arch calcification). Reproduced from Joshi *et al.* (27), Derlin *et al.* (30), and Kato *et al.* (37) with permission from the publishers.
^{18}F-FDG: ^{18}F-fluorodeoxyglucose; ^{18}F-FMISO: ^{18}F-fluoromisonidazole.

Table 1. Summary of metabolic radiotracers used for PET/CT imaging of Atherosclerosis

Radiotracer	Metabolic targets	Advantages and key findings	Limitations	Ref.
^{18}F-FDG	Glucose transport and phosphorylation	Readily available Most extensively validated radiotracer in preclinical and clinical studies Uptake correlates with overall inflammatory burden of plaques, e.g., macrophage content Early detection of response to statins	Targets a nearly ubiquitous metabolic process with limited specificity for individual cell type or phenotype	(*12-15*)
^{18}F-FMISO	Cellular hypoxia	Higher uptake in symptomatic carotid plaques Positive correlation between ^{18}F-FMISO and ^{18}F-FDG and uptake	Diffusion barrier limits uptake Lack of cell-specificity	(*27*)
^{18}F-HX4	Cellular hypoxia	Positive correlation between ^{18}F-HX4 and ^{18}F-FDG	Diffusion barrier limits uptake Lack of cell-specificity	(*26*)
^{64}Cu-ATSM	Cellular hypoxia	Uptake correlates with plaque hypoxia and macrophage content Higher hypoxic cell uptake and faster washout from normoxic tissues than ^{18}F-FMISO	Diffusion barrier limits uptake Lack of cell-specificity	(*28*)
^{11}C-Acetate	TCA, FAO, FAS, OxPhos	Focal uptake in calcified plaques and arterial segments without calcifications	Short half-life, limiting the availability	(*30*)
^{11}C-Choline	FAS, Biosynthesis	Uptake is mostly in arterial segments with thickening and increased lipid content Lower cardiac uptake than that of ^{18}F-FDG	Short half-life, limiting the availability	(*37*)
^{18}F-FMC	FAS, Biosynthesis	Uptake is mostly in arterial segments with thickening and increased lipid content	May be a substrate for a distinct set of transporters from those used to transport choline	(*36*)
^{18}F-FEC	FAS, Biosynthesis	Uptake correlates with cardiovascular risk factors No association between uptake and prior cerebrovascular or cardiovascular events	May be a substrate for a distinct set of transporters from those used to transport choline	(*38*)