UNICA UNIVERSITÀ DEGLI STUDI DI CAGLIARI

UNICA IRIS Institutional Research Information System

This is the Author's [*accepted*] manuscript version of the following contribution:

Autori: Michele Mussap, Melania Puddu and Vassilios Fanos

Dept. of Surgical Sciences, School of Medicine, University of Cagliari, Italy

Titolo articolo: Metabolic Reprogramming of Immune Cells Following Vaccination: From Metabolites to Personalized Vaccinology

Titolo rivista: Current Medicinal Chemistry

Volume, fascicolo, anno, pagg.

Volume 31, Issue 9, 2024

Published on: 26 June, 2023

Page: [1046 - 1068]

The publisher's version is available at:

http://dx.doi.org/10.2174/0929867330666230509110108

When citing, please refer to the published version.

This full text was downloaded from UNICA IRIS https://iris.unica.it/

Current Medicinal Chemistry, XXXX, *XX*, 1-00

REVIEW ARTICLE

Metabolic Reprogramming of Immune Cells Following Vaccination: From Metabolites to Personalized Vaccinology

Michele Mussap[1,#,*], Melania Puddu[1,#] and Vassilios Fanos[1,#]

[1]*Departmernt of Surgical Sciences, School of Medicine, University of Cagliari, Italy, Cittadella Universitaria S.S. 554, 09042 Monserrato, Cagliari, Italy.*

ARTICLE HISTORY

Received: November 29, 2022
Revised: March 24, 2023
Accepted: March 28, 2023

DOI:
10.2174/0929867330666230509110108

Abstract: Identifying metabolic signatures induced by the immune response to vaccines allows one to discriminate vaccinated from non-vaccinated subjects and decipher the molecular mechanisms associated with the host immune response. This review illustrates and discusses the results of metabolomics-based studies on the innate and adaptive immune response to vaccines, long-term functional reprogramming (immune memory), and adverse reactions. Glycolysis is not overexpressed by vaccines, suggesting that the immune cell response to vaccinations does not require rapid energy availability as necessary during an infection. Vaccines strongly impact lipids metabolism, including saturated or unsaturated fatty acids, inositol phosphate, and cholesterol. Cholesterol is strategic for synthesizing 25-hydroxycholesterol in activated macrophages and dendritic cells and stimulates the conversion of macrophages and T cells in M2 macrophage and Treg, respectively. In conclusion, the large-scale application of metabolomics enables the identification of candidate predictive biomarkers of vaccine efficacy/tolerability.

Keywords : Metabolomics, vaccines, metabolic reprogramming, trained immunity, system vaccinology, immune cells.

Author Proofs
"For Personal Use Only"

1. INTRODUCTION

Over the past decades, the rapid evolution of cutting-edge techniques, *e.g.*, genomics, proteomics, and metabolomics, has considerably expanded our understanding of the immune-mediated protection triggered by vaccines. The activity of the immune cells is orchestrated by two hallmarks, namely epigenetic remodeling and metabolic reprogramming, which are closely related to each other; they are the mainstays for developing effective and safe vaccines [1]. Both innate and adaptive immunity equally contribute to the effectiveness of vaccines. On the one hand, the immune adaptative memory is activated by permanent genetic changes due to gene rearrangement. Activated B cells promptly differentiate into antibody-producing plasma cells or long-lasting memory B cells; similarly, activated $CD8^+$ T lymphocytes differentiate into memory T cells [2].

On the other hand, innate immune cells, including monocytes, macrophages, dendritic cells (DCs), γδT cells, and natural killers (NKs), are mediators of a non-specific immune memory in response to vaccines or pathogens [3]. This innate immune memory, also termed trained immunity, depends on transcription factors, epigenetic reprogramming, and metabolic rewiring but not on permanent genetic changes [4]. Trained immunity mediates the nonspecific protective effects of various live vaccines against infections other than the target diseases; examples include the bacillus Calmette-Guérin (BCG) vaccine, the oral polio vaccine, the smallpox vaccine, and the measles vaccine [5, 6]. In immunocompromised or elderly subjects with impaired antibody production, trained immunity induced by vaccination is crucial. In these subjects, adaptive immunity declined with advancing age, and thus the response to vaccination may be inadequate; conversely, trained immunity can guarantee effective protection in fragile subjects, being less deteriorated [7].

Identifying metabolic and molecular signatures mirroring the immune response to vaccines and infections has raised the interest of researchers, not only to discriminate between vaccinated from non-vaccinated sub-

*Address correspondence to this author at the Department of Surgical Sciences, School of Medicine, University of Cagliari, Italy, Cittadella Universitaria S.S. 554, 09042 Monserrato, Cagliari, Italy. Tel: +39 70 5103403 Mobile: +39 3389352966
E-mail: mumike153@gmail.com
[#] *These authors contributed equally to this work*

0929-8673/XX $65.00+.00 © XXXX Bentham Science Publishers

jects but even to provide new insights into the molecular mechanisms associated with the host immune response [8]. Recent *in vitro* studies based on cell culture and conducted through innovative methods, including extracellular flux analysis (EFA), stable isotope labeling metabolomics, and fluxomic elucidated the mechanistic basis of the immunometabolism [9]; in particular, metabolomics contributes to deciphering the immunometabolism enabling the identification and quantification of hundred metabolites by high-throughput separative techniques, including liquid or gas chromatography-mass spectrometry (LC-MS or GC-MS) and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, in conjunction with sophisticated software tools for multivariate and univariate statistical analysis [10]. Thus, metabolomics may play a crucial role in deciphering molecular mechanisms of vaccine-induced immunity and identifying the individual metabotype. Metabolomics may open new perspectives for developing tailored vaccines in specific categories of individuals, such as patients with autoimmune disorders, children, pregnant women, and aged or very old subjects [11].

This narrative review is focused on currently available literature reporting the use of metabolomics alone or in combination with other omics. Although the body of literature is still limited, as reported in Table **1**, we aimed to identify the most relevant metabolites and metabolic pathways associated with the host response to vaccines and involved in the activation of the innate and adaptive immune response in the long-term functional reprogramming (immune memory) and adverse reactions.

"Author Proofs "For Personal Use Only"

1.1. Immune Cell Metabolic Reprogramming

The immune response is a complex, dynamic process, including cellular activation, immune memory formation, inflammation, and return to the resting state. Each phase requires specific metabolic demands reflected by metabolic phenotypes resulting from the activation of specific metabolic pathways. In innate and adaptative immune cells, the transition from the resting/quiescent stage to the activated stage, as well as the long-term induction of immune memory, implies the rearrangement

of metabolic pathways, termed metabolic reprogramming. Changes in cellular metabolism primarily aim to provide increased energy demand for the immune response to pathogens, vaccines, or environmental challenges. Further key tasks of metabolic reprogramming include the biosynthesis of metabolites acting as signal molecules affecting pro- and anti-inflammatory mechanisms, immune cell differentiation, and post-translational modifications, leading to changes in gene expression and the modulation of cell apoptosis and autophagy [12]. Metabolic pathways mainly involved in the immune cells reprogramming are glycolysis, pentose phosphate pathway (PPP), the tricarboxylic acid (TCA) cycle, oxidative phosphorylation (OXPHOS), fatty acid oxidation (FAO), fatty acid synthesis, and amino acid metabolism. Metabolic reprogramming activates different pathways depending on the type of the immune cell response; during the proinflammatory immune response, metabolism switches toward aerobic glycolysis and PPP, whereas anti-inflammatory immune cell subsets favour oxidative metabolism (OXPHOS and FAO). The former allows prompt ATP availability, increased NADPH production, and nucleotide synthesis, generating, on the other hand, high levels of cytosolic and mitochondrial reactive oxygen species (ROS). The latter allows the prolonged survival of immune cells [13]. Metabolic reprogramming strongly affects epigenetic modifications by at least three mechanisms (Figure 1): (a) enhanced glycolysis, with the increased availability of acetyl-CoA (an acetyl group donor) leading to histone acetylation; (b) perturbation of the homocysteine-methionine cycle with the decrease in S-adenosylmethionine (a methyl group donor) leading to histone hypomethylation; (c) alteration of the TCA cycle with the accumulation of TCA intermediates modulating the activity of epigenetic enzymes [14, 15].

Metabolic reprogramming has been extensively investigated in cells of the innate immune system, especially in macrophages and DCs. Metabolic rewiring manages cell functions, such as cytokine synthesis and phagocytosis. The rapid pro-inflammatory response to lipopolysaccharides (LPS) in combination with interferon-γ (IFN-γ) depicts the so-called macrophage phenotype M1 (LPS- or IFN-γ macrophages), marked by the upregulation of aerobic glycolysis and PPP in conjunction with the downregulation of FAO and OXPHOS and the activation of two breakpoints in the TCA cycle. The resolution of inflammation and the repair of tissue injuries leads to the anti-inflammatory phenotype M2 (interleukin-4 macrophages), marked by the TCA cycle and OXPHOS upregulation.

1.2. Glycolysis, Tricarboxylic Acid (TCA) Cycle, and Oxidative Phosphorylation (OXPHO)

The activation of immune cells requires a rapid bioavailability of energy; therefore, activated cells utilize glycolysis as the main metabolic pathway instead of OXPHOS; the latter is conversely employed mainly in the quiescent phase [12]. Glycolysis supports the

response of the innate immune cells, memory formation, and recall response. Despite the considerable difference in energy production between glycolysis and OXPHOS (36 and 2 ATP molecules respectively), the former is preferred during intense cell proliferation and activation because of the instant availability of ATP, even though in a small amount. Upon activation, glycolysis predominates even in the presence of oxygen availability (Warburg effect), providing biosynthetic intermediates for the synthesis of nucleotides, amino acids, and fatty acids, which facilitate rapid cell growth. Finally, glycolysis reduces NAD^+ to NADH, an essential cofactor for many enzymes.

Table 1. Characteristics of the metabolomics-based studies included in this review.

Vaccine	Vaccine Type	Subjects (n)	Administration Route	Dose Injection (n)	Dose Timing	Sample Timing (Basal and After Injection)	Analytical Platform	Matrix	References
Zostavax® (Herpes Zoster Virus)	Live attenuated	10	subcutaneous	1	==	T0 (basal), T1, T3, T7, T14 days after	LC-MS	Plasma	[30]
Hantavax (Yellow fever Virus)	Live attenuated	19	intramuscular	4	0-1-2-14 months	T0 (basal), 72h both from the 1st and the 2nd dose	LC-MS	Serum	[29]
DVC-LVS® (*Francisella tularensis*)	Live strain	10	scarification	1	==	T0 (basal), T1, T2, T7, T14 days after	LC-MS LC-MS/MS	Plasma	[28, 49]
CoronaVac (Sinovac®) (SARS--COv2)	Live attenuated	50	intramuscular	2	0-21 days	T0 (basal), T21, T35 days after	LC-MS	Plasma	[31]
CoronaVac (Sinovac®) (SARS--COv2)	Live attenuated	30	intramuscular	2	0-30 days	44 days after	LC-MS/MS	Plasma	[32]
BTN162b2® (SARS-COv2)	Viral mRNA	58	intramuscular	2	0-21 days	T0 (basal), T22, T90 days after	LC-MS ^1H NMR	Plasma	[55]
BTN162b2® (SARS-COv2)	Viral mRNA	20	intramuscular	2	0-21 days	T0 (basal), T7, T14, T21, T28, T51 days after	^1H NMR	Serum	[56]
Fluzone® (Seasonal trivalent flu 2014-2015)	Live attenuated trivalent	11	intramuscular	1	==	T0 (basal), T1, T3, T7 days after	LC-MS	Plasma	[27]
Seasonal trivalent flu (2011-2012	Live attenuated trivalent	33	intramuscular	1	==	T0 (basal), T2, T7, T28 days after	LC-MS	Plasma	[48]
BCG-SSI® (Statens Serum Inst. Copenhagen)	Live strain	60	intradermal	1	1	4 weeks after	UPLC-MS/MS, GC-MS	Plasma	[50]

Author Proofs
"For Personal Use Only"

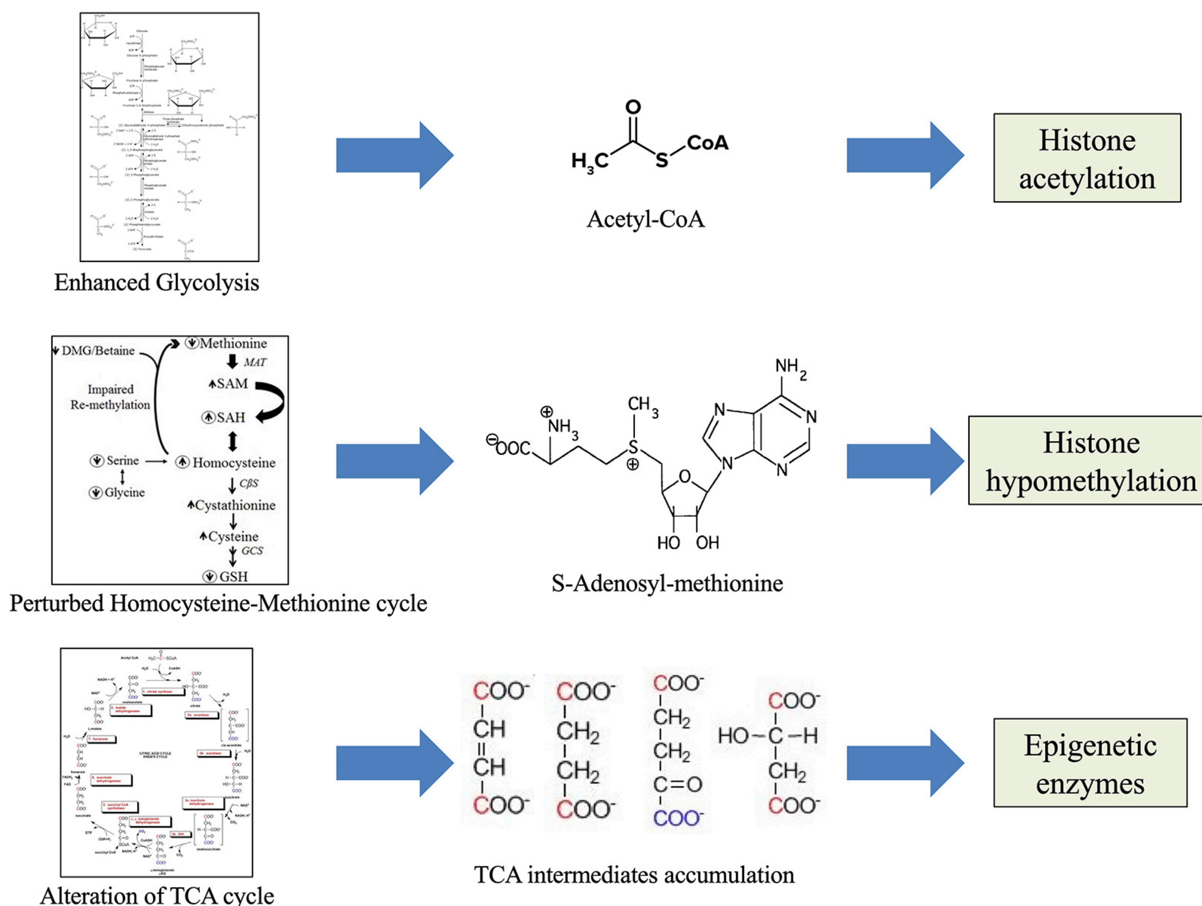


Fig. (1). Relationships between the metabolic reprogramming of immune cells and epigenetic modifications. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Several signaling pathway molecules promote glycolysis, including the hypoxia-inducible factor-1α (HIF-1α), a key transcriptional factor inducing the gene expression of interleukin 1 (IL-1) [16, 17] and the expression of the induced nitric oxide synthase (i-NOS). The latter is an enzyme generating nitric oxide (NO) from arginine metabolism. The induction of trained immunity by *Candida albicans*, the cell wall component β-glucan, and the BCG activates glycolysis, glutaminolysis, and oxidative phosphorylation in trained and LPS-activated macrophages (M1 macrophages) with the accumulation of fumarate [18]; interestingly, some intermediate metabolites act as cofactors by the epigenetic reprogramming of the macrophages. The overexpression of glycolytic enzymes is mediated by histone modifications to unfold the chromatin and facilitate the transcription of proinflammatory factors [19, 20].

Together with glycolysis, the PPP is a major pathway for glucose catabolism. By the oxidative branch, PPP generates NADPH, necessary for producing ROS and anti-oxidant metabolites, such as glutathione; by the non-oxidative branch, PPP generates the five-carbon sugar ribose-5 phosphate, which can be reversibly converted into glycolytic intermediates. PPP is involved in the synthesis of fatty acids (FAs) through the production of NADPH and the formation of glycerol 3-phosphate from fructose 6-phosphate. PPP is controlled by the enzyme carbohydrate kinase-like protein (CARKL), which is highly expressed in M2 macrophages; the suppression of CARKL is linked to the M1 macrophage phenotype [21].

The TCA cycle is a highly efficient metabolic pathway generating ATP from pyruvate and FAs; in $CD8^+$ T memory cells, this metabolic pathway is strategic [22]. In activated immune cells, such as M1 macrophages, the TCA cycle fragmentation is followed by the accumulation of citrate and succinate. In addition, the TCA cycle provides intermediary metabolites for other metabolic pathways generating glucose *via* glycolysis, amino acids, and lipids. However, inter-

mediates must be replaced to ensure a seamless TCA cycle; this activity is termed anaplerosis [23]. The major anaplerotic enzyme is pyruvate carboxylase which generates oxalacetate. Glutamine provides α-ketoglutarate (α-KG) *via* glutaminolysis, and FAs provide acetyl-CoA *via* -oxidation [24]. Glutaminolysis anaplerosis is crucial in trained immunity induction by histone modification, as demonstrated by studies on macrophages treated with -glucan [18] and by the fact that inhibition of glutaminolysis or α-KG generation blocks trained immunity. Anaplerosis is associated with the removal of intermediates from the cycle, termed cataplerosis; in this way, the TCA cycle is balanced between the inflow and output of intermediates.

1.3. Lipids and Lipid Metabolism

Lipid metabolism results from the balance between lipogenesis (synthesis of lipid species, such as cholesterol, fatty acids, and phospholipids) and lipolysis (the breakdown of lipid species *via* FAO). Lipids play a key role in the life cycle of immune cells as structural components of biological membranes, sources of energy, and bioactive signaling molecules, also known as lipid mediators. Thus, perturbations in lipid metabolic pathways may induce detrimental effects on innate and adaptative immune response, immune memory, cell proliferation and differentiation, and survival. Lipid mediators can be categorized into three main classes, namely class 1 eicosanoids, class 2 phospholipids and sphingolipids, and class 3 omega-3 and omega-6 polyunsaturated fatty acids (PUFAs). Recent studies have elucidated and named a new class of molecules that inhibit and resolve inflammation, the specialized pro-resolving mediators (SPMs) [25]. SPMs include lipoxins and resolvins; during the acute inflammatory response, they are enzymatically generated from essential FAs, namely arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid. After the activation of the immune cell, lipid mediators are generated within lipid droplets by either oxidative fragmentation of PUFAs or enzymatic phosphorylation/dephosphorylation of glycerophospholipids. Lipid droplets may be considered the first-line intracellular defense against pathogens, such as SARS-CoV-2, being critical components of the metabolic reprogramming in innate immune cells [26]. In particular, lipogenesis is an essential arm of the metabolic reprogramming in M1 macrophages and activated DCs, with the up-regulation of lipogenic transcriptional factors, such as peroxisome proliferator-activated receptor γ (PPARγ), CD36, diacylglycerol o-acyltransferase-1 (DGAT-1), and sterol regulatory element-binding protein-1 (SREBP-1).

2. EXPLORING THE METABOLIC RESPONSE TO VACCINES BY METABOLOMICS

Vaccines induce metabolic changes due to the interplay between the vaccine, the associated immune response, and the gut microbiota. The latter is a powerful driver of the immune response to vaccines by direct interaction with immune cells and indirect effects mediated by critical metabolites generated by the overgrowth of such gut bacterial strains [27]. When the gut microbial ecosystem is imbalanced, *e.g.*, following antibiotic therapy, the metabolic response to vaccination is significantly altered. This effect was demonstrated in adults vaccinated with the trivalent inactivated influenza vaccine (Fluzone®) by comparing the serum metabolome between adults treated with antibiotics from 3 days before vaccination to one day after vaccination with the serum metabolome of untreated individuals [27]. Perturbations in serum metabolome induced by vaccination peaked after one day, returning closely comparable to that before vaccination within a very short time. In antibiotic-treated adults, metabolic changes stayed longer and were also recognized seven days (and more) after vaccination. To date, a limited number of metabolomics-based studies on vaccinated individuals have been published; their main results, consisting of the over- or under-expression of metabolites and differentially abundant pathways at different time points before and after vaccination, are summarized in Tables **2-5**.

2.1. Pyruvate and Lactate

Pyruvate is generated from extracellular glucose through glycolysis. In M1 macrophages, pyruvate deriving from glycolysis does not enter the mitochondria to undergo oxidation; instead, it is metabolized to lactate. Data from the literature point out that various vaccines induce the predominance of the TCA over glycolysis in conjunction with the depletion of pyruvate and to a lesser extent, lactate (Table **2**).

A reduction in pyruvate and lactate was observed in subjects vaccinated against *Francisella tularensis* with the DVC-LVS® vaccine [28]. Using an LC-MS technique and a targeted metabolomic approach, the authors compared the metabolome of samples collected before vaccination (baseline) with that of samples collected 1, 2, 7, and 14 days post-vaccination. Pyruvate decreased in samples collected after vaccination; however, the depletion was statistically significant ($p < 0.05$) only on the day after vaccination. The metabolism of 2-oxocarboxylic acid is associated with the most differentially abundant pathways; several metabolites are involved in this pathway, such as pyru-

Author Proofs "For Personal Use Only"

vate, 2-oxobutanoate, oxaloacetate, and 2-oxoglutarate. Since 2-oxocarboxylic acid metabolism represents a link between glycolysis and the TCA cycle, this result confirms the TCA predominance at the expense of glycolysis. Notably, several metabolites were found to be linked to genes of innate and adaptive immunity [28]. The assumption that TCA prevails over glycolysis after vaccination is supported by results obtained in a study on 20 healthy subjects vaccinated with four doses of inactivated hantaviruses (Hantavax®) against hemorrhagic fever with renal syndrome during 13 months [29]. Hence, the 2-oxocarboxylic acid pathway was the third-best-ranked pathway among the top twenty enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

A study investigated the metabolic phenotype of healthy adults vaccinated with the live attenuated virus vaccine against Herpes Zosters (Zostavax®) using an untargeted high-resolution metabolomic approach [30]. After one day from the subcutaneous injection of the vaccine, among the top 14 enriched KEGG pathways, glycolysis was ranked 10th, whereas TCA was the first. This finding supports the conclusion that even in this study, TCA was found to prevail over glycolysis.

The reduction in various TCA intermediates was observed in blood samples of 50 healthy subjects vaccinated with CoronaVac (Sinovac®), an inactivated vaccine against Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) [31]. After the second dose, pyruvate, lactate, and malate blood levels significantly decreased. The depletion of pyruvate and lactate was negatively correlated with IgG levels 21 days after the first dose and 14 days after the second dose (Table **2**). These findings suggested the primary role of vaccination in promoting changes in energy metabolism. Interestingly, a further study involving 30 healthy individuals vaccinated with CoronaVac (Sinovac®) found a significant increase in serum levels of γ-aminobutyric acid (GABA) and its precursor glutamic acid after vaccination [32]. In particular, the significant increase in GABA combined with the significant decrease in indole exhibited a high diagnostic accuracy for discriminating non-vaccinated individuals from those receiving two doses of vaccine. After the second dose, serum levels of GABA and indole closely correlated with IgG serum levels; GABA was positively correlated, and indole negatively.

2.2. Citrate

Citrate is generated within the TCA by citrate synthase from the aldol condensation of oxaloacetate and acetyl-CoA. Due to the first break of the TCA cycle in M1 macrophages and activated DCs, citrate accumulates by the transcriptional repression of isocitrate dehydrogenase. A citrate transporter exports citrate from the mitochondria to the cytoplasm, cleaving it into acetyl-CoA and oxalacetate. Acetyl-CoA is utilized for *de novo* lipogenesis, including FAs and cholesterol. FAs are key components of cell membranes and precursors of prostaglandin E2 (PGE2); cholesterol is essential for cell proliferation and trained immunity [33, 34]. In addition, acetyl-CoA is utilized for the acetylation of histones and non-histone proteins. Oxaloacetate is a source of NADPH for the enzymes NADPH oxidase and NO synthase, implicated in the production of ROS and NO, both important against pathogens. Citrate also generates itaconic acid, a metabolite having potent antimicrobial and bactericidal activities, antagonizing oxidative stress and transcriptional responses to LPS (anti-inflammatory phenotype) [35] and inhibiting nucleotide-binding oligomerization domain-like receptor protein 3(NLRP3) inflammasome [36, 37].

Author Proofs "For Personal Use Only"

Table 2. Effects of vaccinations on energy metabolism and related metabolites (TCA, Tricarboxylic Acid Cycle; TNR, timing not reported).

Metabolites and Pathways	Zostavax® [30]	Hantavax® [29]	DVC-LVS® [28]	CoronaVac (Sinovac®) [31]	CoronaVac (Sinovac®) [32]
Piruvate	↑ T1	-	↓ T1, T2, T7, T14	↓ T21	-
Glycolysis	↑ T1	-	-	-	-
Lactate	-	-	↓ T1, T2, T7, T14	↓ T21	-
TCA cycle	↑ T1, T3	↑ TNR	-	-	-
Citrate	↑ T1	-	↑ T1, T2, T7, T14	↑ T1; ↓ T21	-
Succinate	-	-	-	↓ T1	↑ T1 *
Malate	-	-	-	-	-

* After the second dose

In Zostavax®-vaccinated subjects, the TCA cycle was the most significantly enriched pathway discriminating the metabolic phenotype between pre- and post--vaccination. TCA involvement was confirmed by transcriptomics performed in peripheral blood mononuclear cells after three days from vaccination; TCA was still upregulated after three days ($p < 0.05$) [30]. Although not statistically significant, an increase in citrate was found in DVC-LVS® vaccine recipients starting at time point 1/0 and reaching a maximum14 days after vaccination [28] (Table **2**).

In subjects vaccinated with CoronaVac (Sinovac®), blood citrate levels were significantly different between pre- and post-vaccination. In particular, citrate significantly increased 14 days after the first dose ($p < 0.001$) and then decreased 21 days after the second dose [31]. Regrettably, as observable in the plot reported in that study, the broad data dispersion within and outside the interquartile range after vaccination limits the strength and the value of the result. In immunized individuals, citrate was found to be significantly higher than in individuals infected with SARS-CoV-2. This finding was recently confirmed in a study on 30 healthy adults vaccinated with CoronaVac (Sinovac®) [32] (Table **2**).

2.3. Succinate

Succinate has long been known as a marker of inflammation and oxidative and metabolic stress. Due to the second breakpoint in the TCA cycle of M1 macrophages and activated DCs, succinate accumulates within mitochondria, and then it is exported into the cytoplasm by the decarboxylate carrier SLC25A10, wherein it stimulates the production of IL-1β through the stabilization of HIF1α. Furthermore, the oxidation of succinate by the enzyme succinate dehydrogenase is associated with the production of ROS, which further stabilizes HIF1α [38, 39]. However, other metabolic pathways can promote the accumulation of succinate, including glutaminolysis anaplerosis *via* the GABA shunt. Even itaconate induces succinate accumulation by inactivating the enzyme succinate dehydrogenase. Succinate, α-KG, 2-hydroxyglutarate, and fumarate are crucial for the induction of trained immunity-regulating genes implicated in epigenetic remodeling. The ratio succinate/α-KG is important for determining the macrophage phenotype: M1 (pro-inflammatory) when the ratio is high and M2 (anti-inflammatory) when low [39, 40]. In DCs, succinate cooperates with Toll-Like Receptors (TLRs) to synthesize cytokines and enhances the ability of DCs to present antigens to the TLR, positively affecting adaptive immunity as well. The pro-inflammatory effects of succinate appear to be associated with HIF1α in DCs as well [41].

In subjects vaccinated with CoronaVac (Sinovac®), succinate blood level decreased after the first dose and returned to the baseline level after the second dose [31]. Conversely, succinate and succinate semialdehyde were found to increase after vaccination in the second study on CoronaVac (Sinovac®) [32]. However, it must be emphasized that in the study by Wang *et al.* [31], the presence of outliers in samples collected before and after vaccination makes questionable the conclusion that the CoronaVac (Sinovac®) vaccination induces significant changes in succinate concentration; rather, the elimination of outliers, clearly evident on the graph reported in that paper, invalidate the significant differences between time points. Similarly, in the study of He *et al.* [32], data are widely spread outside the interquartile range, as clearly recognizable in the plot reported in that study. It is reasonable to assume that in the absence of robust data, succinate cannot be significantly influenced by the CoronaVac (Sinovac®) vaccine. In subjects vaccinated against *F. tularensis* with DVC-LVS®, no difference was observed after vaccination both for malate and succinate [28] (Table **2**).

2.4. Fatty Acids

FAs are the primary fuel source for immune cell growth and differentiation and for building the cell membrane structure. Both effector T cells and macrophages utilize FAs for the activation of processes triggering adaptive and innate immunity. In M1 macrophages and DCs, FAs synthesis is upregulated upon stimulation of TLRs; the up-regulation is basic for cell proliferation, cytokine synthesis, and specific functions of DCs, including that of sentinel cell and antigen presentation [42]. On the other hand, FAO supports the anti-inflammatory macrophage phenotype M2. The differentiation of naïve T cells into $CD8^+$ lymphocytes and $CD4^+$ T helpers (Th1, Th2, and Th17) is associated with the rapid downregulation of FAO and the upregulation of FAs synthesis [43]; both pathways are mediated by the mammalian target of rapamycin (mTOR) pathway [44]. Conversely, the differentiation of naïve T cells into $CD8^+$ memory T cells and $CD4^+$ T regulatory (T_{reg}) lymphocytes is associated with the activation of FAO and the inhibition of FA synthesis [45]. Arachidonic acid, an essential FA, is the precursor of prostaglandins (PGs), a family of mediators participating in the modulation of multiple biological processes in healthy and disease states. In particular, PGE2 exerts anti-inflammatory and immunosuppressive effects by inhibiting DCs, NKs, $CD8^+$ cytotoxic

Author Proofs
"For personal use only"

Table 3. Effects of vaccination on fatty acids metabolism at various time points.

Metabolites and Metabolic Pathways	Zostavax® [30]	Hantavax® [29]	DVC-LVS® [28, 49]	CoronaVac (Sinovac®) [31]	BNT162b2 COVID-19 [55]	BNT162b2 COVID-19 [56]	Fluzone® (Trivalent flu) [27]	Seasonal Trivalent flu [48]	BCG-SSI (DK) [50]
Fatty Acids synthesis	-	↑ T1, T2, T3	-	↑ T1 [>]	-	-	↑ T1, T7	-	↑ 4 weeks after vaccine [§]
Fatty Acids 20:0, 22:2, 22:4, 24:0	-	-	-	-	-	-	-	-	↑ 4 weeks after vaccine [§]
Polyunsaturated fatty acids (PUFAs)	-	-	-	-	↑ T0 *.	-	-	↑ T7 [†], ↓ T28 ^	-
Linoleic acid	↑ T1	↑ T1, T2, T3	-	↑ T1 ↓ T21	-	-	↑ T7	-	-
Arachidonic Acid	-	↑ T1, T2, T3	-	-	-	-	↑ T7	↑ T7 [†], ↓ T28 ^	-
Prostaglandins	-	-	-	-	-	↑ T7	-	-	-
Oleoylethanolamine (by oleic acid)	-	-	↓ T2; ↑ T14 [f]	-	-	-	-	-	-
Arachidonoyl-ethanolamine (by arachidonic acid)	-	-	↓ T2; ↑ T14 [f]	-	-	-	-	-	-
Dihydroxy-eicosatetraenoic acid (DHETs)	-	-	↑ T7; ↓ T14 [f]	-	-	-	-	-	-
5-Hydroxy-eicosatetraenoic acid (5-HETEs)	-	-	↑ T2; ↓ T7; ↑ T14 [f]	-	-	-	-	-	-

[>] Unsaturated Fatty Acids; * In low responders compared with high responders; [†] In young low responders; ^ In young high responders; [§] In infants vaccinated early compared with infants vaccinated later; [f] Return to the baseline level.

Author Proofs "For Personal Use Only"

lymphocytes, lymphocyte proliferation, and the synthesis of various cytokines, such as IL-1, IL-6, IFN-γ, and Tumor Necrosis Factor (TNF). Most PGE2 effects could be mediated by T_{reg} cells by inhibiting Th1 cells and $CD8^+$ T lymphocytes [46, 47]. In individuals vaccinated with Zostavax®, the linoleate pathway was ranked 12th among the top 14 differentially abundant metabolic pathways at 1/0 and 3/0 time points; in addition, the linoleate pathway significantly correlated with some modules of transcriptomics, especially with genes encoding glycoproteins presenting viral antigens (TLR7 and TLR8, MCH, B lymphocyte signature, myeloid DC activation *via* nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) [30] (Table **3**).

In individuals vaccinated with Hantavax®, among the top 20 discriminant pathways, the arachidonic acid pathway is the most significantly enriched pathway discriminating the metabolome before and after vaccination [29]. Arachidonic acid metabolism is associated with genes encoding the synthesis of leukotrienes and prostaglandin D2 (PGD2). The upregulation of arachidonic acid metabolism was dose-dependent, reaching a peak after the third dose and then stabilizing after the fourth one. FAs metabolism was the eleventh and linoleate the thirteen (Table **3**).

In CoronaVac (Sinovac®)-vaccinated subjects, the FAs synthesis was ranked second among the top 35 most significantly enriched pathways. It was significantly upregulated after the first dose and returned to baseline after the second. The linoleate pathway was the 22nd and behaved like the previous one after the first dose but fell below baseline after the second [31] (Table **3**). In subjects vaccinated against seasonal influenza with the trivalent inactivated influenza vaccine Fluzone®, the FAs biosynthesis was the second among the top 46 most significantly enriched pathways at 1/0 and 7/0 time points. The linoleate metabolism pathway was the 32nd after seven days from vaccination [27] (Table **3**).

Seven PUFAs, including arachidonic acid, α-linoleic acid, and γ-linolenic acid, were found to be significantly decreased in a group of "high responder" young adults immunized with the seasonal trivalent inactivated influenza vaccine (TIV, A/California/7/09 (H1N1)-like virus; A/Perth/16/2009 (H3N2); and B/Brisbane/60/2008) [48]; briefly, in young adults, PUFAs were significantly decreased after seven days from vac-

cination in low responders and after 28 days, in high responders.

In older adults, PUFAs did not vary significantly after vaccination, both in low- and high-responders. The immune response to influenza vaccination in high-responder young adults may originate PUFA depletion after 28 days (Table **3**).

A longitudinal lipidomic-based study conducted on 10 healthy adults vaccinated against *F. tularensis* identified 116 lipids [48]; after vaccination, two by-products of arachidonic acid and oleic acid, oleoylethanolamine and arachidonoylethanolamine, respectively, significantly decreased after 48 hours, and then increased after 7 and 14 days. These two bioactive lipid mediators are ligands of the peroxisome proliferator-activated receptor, isoform α; the activation of this transcription factor inhibits pro-inflammatory cytokines. In fact, their plasma levels positively correlated with anti-inflammatory cytokines and activated $CD8^+$ T cells [49] (Table **3**).

The neonatal lipid metabolism is strongly influenced by BCG vaccination administered either at birth (early) or after six weeks of life (delayed), as demonstrated in an *in vivo* and *in vitro* study conducted on three independent infant newborn cohorts [50]. By comparing early versus delayed BCG-vaccinated newborns, four free FAs (20:0, 22:2, 22:4, 24:0) were significantly increased in the early group. The *in vitro* BCG stimulation of cord blood samples from full-term neonates delivered *via* cesarean section induced a significant reduction in docosahexaenoic acid, a very long-chain PUFA, linoleic acid, and arachidonic acid, together with a significant increase in PGE2 (Table **3**).

2.5. Glicerophospholipids (Phospholipids) and LYSOPHOSPHOLIPIDS

Glicerophospholipids, commonly known as phospholipids (PLs), their by-product lysophospholipids (LysoPLs), and sphingolipids are key components of the cellular membrane-generating mediators involved in the metabolism of cell signaling. In the innate immune response, phospholipases hydrolyze membrane PLs, initiating various fundamental activities, including cell membrane remodeling and the synthesis of bioactive signaling mediators [51]. In particular, the enzymatic activity of the isoform phospholipase A2 (PLA2) generates FAs and LysoPLs, triggering the cascade of oxylipin synthesis. Oxylipins are a class of enzymatically oxidized PLs; they include eicosanoids and play a unique role in innate immune cells. In leukocytes, the cytosolic and calcium-independent PLA2 subclasses cPLA2 and iPLA2 generate lysophosphatidic acid and arachidonic acid, which serve as signaling molecules for monocyte migration toward chemoattractant protein 1 (MCP-1) [52]. Lysophosphatidylcholines (LPCs) are the most abundant class of LysoPLs in human blood; they include four main types, namely LPC16:0, LPC18:1, LPC20:4, and LPC22:6. Depending on various factors, including the biochemical structure and the type of immune cells, LPC could exert either pro- or anti-inflammatory effects. For example, saturated and monosaturated LPC species are pro-inflammatory mediators; conversely, polyunsaturated LPC species are anti-inflammatory mediators [53, 54]. The role of LPC on the adaptative immune system is to enhance the effect of T lymphocytes; moreover, LPC stimulates the production of INF-γ by $CD4^+$ and $CD8^+$ T cells and promotes the immunosuppression of T_{reg} through increased expression of transforming growth factor-β (TGF-β).

In individuals vaccinated with Zostavax®, PLs and phosphatidyl-inositol phosphate (PIP) pathways were found within the top 14 differentially abundant metabolic pathways at the 1/0 timepoint. Significant changes in the concentration of metabolites involved in the PIP pathway were observed after one day from vaccination compared to baseline (pre-vaccination, timepoint 0). After three days from vaccination, PLs metabolism was correlated with genes regulating B lymphocyte activity (B cell signature). A further investigation carried out through the multiscale multifactorial response network (MMRN) was aimed at correlating networks related to genes, metabolites, cytokines, and peripheral blood mononuclear cells. At 3/0 timepoint, the SN3 metabolite network containing the pathways of TCA, nucleotides, and PIP was linked at 7/0 with genes related to $CD4^+$ signature, nucleotide metabolism, T cell activation, enriched B cells, inositolphosphate (IP) metabolism, and phosphatidyl-inositol (PPI) signaling. IP results from the hydrolysis of PIP by phospholipase C and acts as a second messenger in several cellular processes by modulating calcium release from stores. After one day from vaccination, the PCR-based molecular test for SARS-CoV-2 was positive in eight subjects, demonstrating the presence of viral replication and negative in the remaining 27 vaccinated individuals. Interestingly, subjects with positive PCR showed higher gene expression for IP metabolism; by day 7/0, this group showed the activation of various cellular processes, *e.g.*, G-protein-mediated calcium signaling, cytoskeleton remodeling, and cell adhesion, suggesting the activated response to viral infection. However, by day 7/0, the same group demonstrated the decreased expression of genes associated with plasma cell activity [30] (Table **4**).

"Author Proofs, For Personal Use Only"

In the lipidomic-based study on adults vaccinated against *F. tularensis*, 116 lipids were identified; at days 1, 2, 7, and 14 post-vaccination, 14 lipids were differentially abundant, 50% PLs and 28.6% oxylipins [49]. Among the pro-inflammatory oxylipins, significant changes in plasma level of 5-Hydroxyeicosatetraenoic acid (5-HETE) and dihydroxyeicosatetraenoic acid (DHET) species (4 metabolites) were observed. In detail, seven days after vaccination, all the DHET species increased 2-fold over pre-vaccination; concomitantly, 5-HETE, the precursor of DHETs, significantly decreased over time, returning to the baseline level (before vaccination) at day 14. The decrease in 5-HETE was attributed to the increased synthesis of the less active DHET metabolite species, suggesting the resolution of the inflammatory response mediated by DHET. LPC decreased the day after vaccination and was negatively correlated with the activation of $CD4^+$ T cells (Table **3**). In CoronaVac (Sinovac®)-vaccinated subjects, the PL pathway was among the top 20 differentially abundant pathways; it was downregulated after the first dose and returned to baseline after the second [31] (Table **4**).

Two recent metabolomics-based studies conducted on healthy individuals vaccinated against SARS-CoV-2 with the mRNA vaccine BNT162b2 reported significant changes in lipoproteins plasma levels; changes were associated with the intensity of the immune response. The first study utilized both ^{1}H NMR spectroscopy and LC-MS [55]; changes in plasma levels of PUFAs, PLs, and triacylglycerols from lipoproteins reflected the immune response to the BNT162b2 vaccine.

A study demonstrated that the plasma metabolome predicts the immune response in terms of neutralizing antibodies level at three different time points, before the first vaccine dose, before the second dose (day 22), and after 90 days from the first dose. In particular, PLs and PUFAs were found to increase in low-responders before the first dose, discriminating low- from high-responders. Plasma metabolome deciphered by LC-MS identified the upregulation of 2 lysophospholipidethanolamines (LysoPE P-16:0/0:0 and lysoPE 0:0/24:6 or 24:6/0:0) in low responder individuals (Tables **3** and **4**). Using ^{1}H NMR spectroscopy, a small-size pilot study found that vaccination does not induce any significant change in the serum metabolic profile in healthcare workers who previously developed COVID-19 and those COVID-19 naïve [56]; rather, vaccination is associated with significant changes in lipoproteins concentration. In COVID-19 naïve individuals, there was an overall depletion in lipoproteins serum level with an increase after the second dose (at day 28), followed by a decrease one month after the second dose. Moreover, the number of altered lipoproteins increased as time increased from vaccination. In COVID-19-recovered subjects, lipoproteins variations were far milder and of the opposite sign, excluding free cholesterol and PLs associated with VLDL-5; these changes were negligible after the second dose (Table **4**).

The impact of vaccination against the H1N1 influenza virus on lipid metabolism (and many other metabolisms) differs significantly between individuals treated with antibiotics and those untreated [27]. After one day from vaccination, in the group of antibiotic-free vaccinated subjects, PLs and PIP metabolisms were the eleventh and fifteenth most significantly enriched pathways, respectively. After three days, these pathways were not discriminant for the untreated subjects. On the other hand, in the group of antibiotic-treated subjects, the same results were found after three days from vaccination; in addition, glycosphingolipids biosynthesis was the sixteenth most significantly enriched pathway after three days. As previously mentioned, antibiotics reduce gut bacterial load, and dysbiosis significantly impacts the metabolic response to flu vaccination (Table **4**).

In subjects vaccinated with the trivalent influenza vaccine, phospholipids belonging to various classes, such as phoshatidylcholines (PCs), phosphatidylethanolamines (PEs), and LPCs, discriminated high-responder older adults from low-responder older adults, being significantly increased in high responders at different time points (Table **4**) [48].

In the *in vivo* and *in vitro* study on BCG vaccination in three cohorts of infant newborns, results largely converged towards alterations in lysophospholipids; notably, changes in LPCs were closely associated with innate and adaptative immunity [50]. In the group of early BCG vaccination, lipid metabolite levels were higher than that in delayed vaccination, with the increased abundance in monoacylglycerol, steroid, lysophospholipid metabolites, and glycophosphocholines associated with the decrease in LPCs. In addition, glycerolipid pathways shared common nodes with the pathway of bile acid biosynthesis and the metabolism of glycosphingolipids, glycophosphocholine, and linoleic acid. By using complex panel infusion-MS and MRM-based lipidomics, it was found that the early BCG immunization induces the reduction of most lysophosphocholines (excluded LPCs 22:2 and 16:0), four lysophosphatidylethanolamines, two phosphocholines, three phosphoethanolamines, nine monoglicerols, and three triacylglycerols. The *in vitro*

"Author Proofs
For Personal Use Only"

Table 4. Effects of vaccination on the metabolism of phospholipids and cholesterol at various time points.

	Zostavax® [30]	Hantavax® [29]	DVC-LVS® [28, 49]	CoronaVac (Sinovac®) [31]	BNT162b2 COVID-19* [55]	BNT162b2 COVID-19 [56]	Fluzone®(Trivalent Flu) [27]	Seasonal Trivalent Flu [48]	BCG-SSI (DK) [50]
Phospholipids (PLs)	↑ T1	-	↓ T1, T7	↓ T1	↑ T0 *	↓ T7, T14, T21, T28, T51	↑ T1	↑ T2, T7, T28 ^	-
Phosphatidylcholines (PCs)	-	-	-	-	↑ T0 >	-	↑ T2, T7, T14	↑ T2, T7, T28 ^	-
Phosphatidyl-ethanolamines (PEs)	-	-	-	-	-	-	-	↑ T2, T7, T28 ^	-
Phosphatidyl-inositol phosphate (PIP) and inositolphosphate (IP) metabolisms	↑ T1	-	-	-	-	-	↑ T1	↑ T28 †: ↑ T28 ▼	-
Lysophosphatidyl-cholines (LPCs)	-	-	↓ T1, T2, T7	-	↑ T0 >	-	-	↑ T2, T7 ^	-
Lysophosphatidyl-ethanolamines (LPEs)	-	-	-	-	↑ T0 >	-	-	-	-
Sphingolipids	↑ T1	-	-	↑ T1; ↓ T21	↑ T0 >	-	↑ T1, T3	-	↑ T0 §
Ceramides	-	-	↓ T7	-	↓ T0, T22, T90 >	-	-	-	-
Cholesterol synthesis and metabolism	↑ T1	↑ T1, T2, T3, T4	↑ T2	-	↑ T0 >	↓ T7, T14, T21, T28, T51	↑ T3, T7	↑ T7 ‡, T14 ‡, T28 ‡,^	-

* In low responders compared with high responders; > In high responders compared with low responders; ^ In old high responders compared with young high responders; † In young responders compared with old responders; ‡ In old responders compared with young responders; § In high responders compared with low responders; ▼ In young and old low responders

Author Proofs "For Personal Use Only"

BCG stimulation of cord blood samples from full-term neonates delivered *via* cesarean section induced a significant decrease in choline phosphate, glycophosphocholine, and phosphoethanolamine (PE) (Table **4**).

2.6. Sphingolipids

Sphingolipids are the primary lipid component of lipid rafts, membrane domains enriched in cholesterol and sphingolipids in contiguity with the outer leaflet of the plasma membrane. Sphingolipids include ceramides, sphingomyelins, and hundreds of glycosphingolipids [57, 58]. Sphingolipid metabolites, particularly ceramide, ceramide 1-phosphate (C1P), and sphingosine-1-phosphate (S1P), are signaling molecules that regulate a wide range of relevant immune cellular processes. Infections, oxidative stress, and cytokines affect sphingolipid metabolism by regulating key enzymes and leading to the limited production of ceramide, C1P, and S1P. The latter plays several roles in innate and adaptive immunity, including immunosurveillance, immune cell trafficking and differentiation, immune response, and endothelial barrier integrity [58]. The considerable difference in SP1 concentration between plasma (high level) and tissues (low level) promotes the migration and trafficking of most immune cells from the thymus, lymph nodes, and spleen into circulation and *vice versa* [59, 60]. S1P is enzymatically released from sphingosine by the sphingosine kinase 1 (SPHK1); during sepsis, the SPHK1 upregulation in phagocytes and macrophages inhibits the activation of NF-κB by TLRs and consequently, the production of pro-inflammatory cytokines [61]. Ceramides consist of a sphingolipid linked to a fatty acid *via* an amide bond. Ceramides are essential intermediates in the biosynthesis and metabolism of all sphingolipids and are involved in cellular inflammation and apoptosis; experimental studies demonstrated that ceramides are upregulated in macrophages stimulated with LPS [62]. C1P is engaged in eicosanoid production through stimulation of PLSA2; furthermore, C1P inhibits metalloproteinase 17 (ADAM 17), the most important metalloprotease responsible for cleaving pro-TNF with the release of the active form [57].

The day after vaccination, glycosphingolipid metabolism was the fourth most enriched pathway (comparison between day 0 and day 1) in subjects vaccinated with Zostavax®. After three days, glycosphingolipid metabolism was associated with gene expression for B cell signatures, DC activation *via* NF-κB, and MHC-TLR7-TLR8 cluster [30] (Table **4**).

The longitudinal lipidomic-based study conducted on subjects vaccinated against *F. tularensis* showed

that compared with pre-vaccination, ceramide 22:0 was among the top seven differentially abundant metabolites seven days after vaccination [49]. The significant reduction in ceramide 22:0 seven days after vaccination may be associated with the mitigation of inflammation due to the vaccine dose (Table 4).

In subjects vaccinated with CoronaVac (Sinovac®), the sphingolipid metabolism was found among the top 35 differentially abundant pathways, being over-expressed at 1/0; after the second dose, it dropped below baseline [31] (Table **4**).

Before the first dose of the mRNA vaccine BNT162b2 against SARS-CoV-2, the plasma level of four ceramides (D18:0/22:0, D18:1/23:0, D18:0/20:0, and D18:1/25:0) was found significantly lower in high-responders compared with that in low-responders [55]. Low levels persisted in high-responders 22 days after the first dose, whereas, in low-responders, ceramides plasma level was significantly decreased compared with that before vaccination (Table **4**). After three months, no statistically significant difference in ceramides plasma level was found between groups.

In the group of untreated individuals immunized with the trivalent influenza vaccine Fluzone®, glycosphingolipids metabolism was the fourth most significantly enriched pathway one day after vaccination and persisted after three days [27] (Table **4**). However, after three days, the same result was observed in vaccinated individuals treated with antibiotics.

2.7. Cholesterol and Steroids

Cholesterol originates from acetyl-CoA through the mevalonate pathway; synthesis and accumulation are regulated by the SREBP-1 and 2. On the other hand, cellular cholesterol efflux depends on the liver receptors LXRα (nuclear receptor 1H3) and LXRβ (nuclear receptor 1H2), transcription factors belonging to the nuclear receptor superfamily. Both transcription factors are coordinated with each other and are regulated by two feedback mechanisms, the cholesterol- and the oxysterol-based feedback [63]. Cholesterol excess can cause endoplasmic reticulum stress, thereby decreasing T lymphocyte function [64, 65]. Cholesterol synthesis is up-regulated in T_{reg} cells and promotes proliferation and suppressive action, likely through the mechanistic target of mTORC1. Conversely, cholesterol accumulation inhibits these cells [64]. SREBP-1 and 2 control the growth and proliferation of CD8 lymphocytes by a direct mechanism [66, 67]. Cholesterol and especially oxysterols stimulate the differentiation and maturation of DCs. The lipid raft and the MCH system enable the DCs in their main function of antigen presentation to T lymphocytes and promote their activation, initiating the specific, adaptive response and concomitantly reinforcing the innate response [64]. Cholesterol promotes the macrophage pro-inflammatory phenotype by producing antibacterial cytokines and ROS. Several cholesterol precursor metabolites have important functions in the immune response. For example, in cultured human monocytes treated with β glucan, the accumulation of mevalonate was one of the mechanisms underlying trained immunity; moreover, the incubation of healthy monocytes with mevalonate alone produced trained macrophages epigenetically similar to those induced by β-glucan [68]. These findings provide evidence that mevalonate is an essential metabolite in the interplay of metabolic-epigenetic mechanisms mediating trained immunity.

Beyond cholesterol, oxysterols are a large family of bioactive signaling metabolites involved in a wide range of biological properties; they are early intermediates in the metabolism of cholesterol to bile acids and can be derived from the diet or can be generated by enzyme-mediated mechanisms or by free radical oxidation [69]. Oxysterols can bind nuclear receptors, cell membrane receptors, and transport proteins [70]. The activation of macrophages or DCs through TLR4 or TLR3 up-regulates the *Ch25h* gene expression; this gene encodes cholesterol 25-hydroxylase (CH25H), the enzyme catalyzing the conversion of cholesterol to 25-Hydroxy cholesterol (25-HC), a potent immunoregulatory oxysterol [71]. 25-HC suppresses the biosynthesis of immunoglobulin A (IgA) in B lymphocytes and curbs IL-1β and inflammasome activation [72, 73]. 25-HC exerts an antiviral action against SARS-CoV-2 through spike protein blockade and spike protein-catalyzed membrane fusion [74]; interestingly, *Ch25h* is up-regulated in macrophages and lung epithelial cell lines of bronchoalveolar lavage fluid (BAL) from patients with COVID-19 [75].

In subjects vaccinated with Zostavax®, cholesterol metabolism was the 7th pathway included in the top fourteen differentially abundant metabolic pathways at 1/0 timepoint [30] (Table **4**). At the 1/0 time point, MMRN highlighted the presence of an extensive metabolite network (TN1.1) containing multiple steroid hormone-associated metabolic pathways (squalene and cholesterol, androgens and estrogens, and C21 steroids). TN1.1 metabolic network was associated with the TN3.76 gene network at the 3/0 timepoint. TN3.76 gene network included gene modules controlling B lymphocytes, plasma cells, and Sterol Element Binding Transcription Factor-1 (SREBF-1) modu-

Author Proofs
"For Personal Use Only"

lating SREBP-1. In turn, the SREBF-1 gene module was strongly correlated with several gene modules related to B and T lymphocytes and metabolites belonging to the sterol class. MMRN analysis thus revealed a large number of genes and metabolites that were integrated through SREBF-1 activities, which may be the cornerstone in B and T cell development.

In subjects vaccinated against *F. tularensis*, nine metabolites belonging to the steroid biosynthesis pathway were significantly increased from pre-vaccination to two days after and correlated with transcriptomic modules related to T cell activation and differentiation [28]. A study performed on the same subjects reported that among the 40 differentially abundant lipids, two were cholesterol esters, namely 16:1 and 20:2 [49]. The former significantly decreased at 2/0 and 7/0 time points (p = 0.004 and p = 0.037, respectively). The latter was decreased at 2/0 (p=0.02) (Table **4**). The ABCA1 gene encoding the main protein responsible for cholesterol efflux from cells was downregulated at all post-vaccination time points unless 2/0. These data may be related to the activation of TLR signalling, inducing the reduction of cholesterol efflux. As a result, cholesterol accumulates within immune cells, and the inflammatory response is amplificated.

In subjects vaccinated with Hantavax®, the steroid hormones synthesis was the second most enriched pathway differentiating pre-vaccination from all post-vaccination time points [29] (Table **4**).

By comparing high-responder with low-responder individuals to the mRNA vaccine BNT162b2 against SARS-CoV-2, cholesterol (free and cholesterol esters) was significantly increased in high-responders before vaccination [55]. In healthcare workers vaccinated with the mRNA vaccine BNT162b2, free cholesterol, LDL-cholesterol, and apolipoprotein B100 were considerably downregulated in COVID-19 naïve; conversely, in healthcare workers who previously developed COVID-19, these changes were almost negligible and limited to a few lipoproteins [56] (Table **4**). It is reasonable to assume that differences in the inflammatory response to vaccination between recovered and naïve healthcare workers promote differences in cholesterol biosynthesis, considering that cholesterol is a crucial mediator of inflammation.

In the group of individuals immunized with the trivalent influenza vaccine Fluzone® and treated with antibiotics, squalene and cholesterol biosynthesis were among the most significantly enriched pathways seven days after vaccination [27]. In untreated immunized individuals, steroid hormone biosynthesis and metabolism were significantly enriched three days after vaccination and persisted seven days after (Table **4**).

In older high responders to the seasonal trivalent inactivated influenza vaccine, researchers found the accumulation of cholesteryl esters; on the other hand, in younger high responders, cholesteryl esters were found to be significantly decreased seven days after vaccination [48] (Table **3**). It is reasonable to assume that differences in fatty acids and sterols levels between younger and older individuals immunized with the influenza vaccine may reflect a different balance between biosynthesis and catabolism of these lipids.

2.8. Amino Acids

The immune response requires a considerable utilization of amino acids to support the activity of immune cells. When the cellular demand for amino acids exceeds the capacity of their synthesis, the non-essential amino acids become conditionally essential. Amino acids strongly impact most immune cellular functions, supporting metabolic reprogramming by multiple mechanisms [76]. On the one hand, amino acids are involved in important signaling pathways, *e.g.*, mTORC1, that sense their presence and drive cellular anabolic processes [77]. On the other hand, amino acids modulate the differential utilization of glycolysis, TCA cycle, OXPHOS, and complex interplay with each other [78]. Besides the primary role of glutamine in supporting the increased biosynthetic requirements of activated immune cells, branched-chain amino acids (BCAA) play a crucial role by increasing the translocation of the glucose transporters GLUT1 and GLUT4, promoting glycolysis and providing acetyl-CoA intermediates to the TCA cycle. Serine supports mitochondrial metabolism by activating pyruvate kinase, the key glycolytic enzyme in macrophages; in T cells, serine promotes the translation of mitochondrial proteins, including those of the electron transport chain [76]. Several amino acids control and maintain the redox balance, especially after the increase in ROS levels following cell activation; in particular, BCAA, cysteine, glutamine, and glycine are needed for the synthesis of glutathione [76, 79]. Even histidine exerts antioxidant actions by forming complexes with metal ions. Ultimately, the accumulation and storage of amino acids within lysosomes are crucial to driving autophagy as a protective mechanism during cellular stress.

Amino acids facilitate specific gene expression programs in immune cells by supplying methyl and acetyl groups for DNA and histones, originating intermediates that drive metabolic rewiring upon immune cell

Author Proofs For Personal Use Only

Table 5. Effects of vaccination on the metabolism of amino acids at various timepoints.

	Zostavax® [30]	Hantavax® [29]	DVC-LVS® [28, 49]	CoronaVac (Sinovac®) [31]	CoronaVac (Sinovac®) [32]	BNT162b2 COVID-19 [55]	Seasonal Trivalent Flu [48]
Amino acids synthesis	-	-	↑ T2	-	-	-	-
Aminoacyl tRNA	-	↑ TNR	↑ T2	↑ T2	-	-	-
Arginine	-	↑ T1, T2, T3, T4	-	↓ T1, T21	-	-	-
Arginine-Proline metabolism	-	-	↑ T2	↓ T1, ↑ T21	-	-	-
Phenylalanine	-	↑ T1, T2, T3, T4	-	↓ T1, T21	-	-	-
Phenylalanine metabolism	-	↑ TNR	-	↑ T1, ↓ T21	-	↑ T0, T1, T2, T22; ↑ T1, T2, T22, T90 ^	-
Tyrosine	-	↑ T4 *	-	↑ T1, T21	-	-	↑ T28 †
Tyrosine metabolism	-	↑ TNR	-	↑ T1, T21	-	-	-
Tryptophan	↑ T1	↑ T1, T2, T3, T4	-	↑ T1, T21	-	-	↑ T28 †
Kynurenine	-	-	-	↓ T21	-	-	-
Tryptophan metabolism	-	↑ TNR	-	↑ T1, ↓ T21	-	-	↑ T28 †
Histidine	-	-	↓ T2	-	-	↓ T22; ↓ T90 ^	-
Histidine metabolism	-	-	↓ T2	↓ T2	-	-	-
Glutamine	-	-	-	-	-	↑ T0, T1, T22; ↑ T0, ↓ T90 ^	-
Glutamine metabolism	-	-	-	↓ T1, T21	-	-	-
Taurine	-	-	-	-	↑ T30	-	-
Asparagine	-	-	↑ T2	-	-	-	-

* In high responders; ^ In high responders compared with low responders; In young high responders

activation. Moreover, amino acids promote nucleotide biosynthesis and drive cellular translation. Arginine is a key amino acid for macrophage differentiation and polyamine metabolism. In M1 macrophages, arginine is converted into NO and citrulline by the enzyme NO-synthetase, whereas in M2 macrophages, the enzyme arginase hydrolyzes arginine into ornithine and urea. Ornithine may fuel the critical downstream pathways leading to the synthesis of polyamine and proline, which are strongly involved in cell proliferation and tissue repair [80]. In immune cells, several inflammatory mediators, such as IFN-γ and LPS, activate the tryptophan metabolism leading to a progressive depletion in tryptophan counterbalanced by the increase in kynurenine due to the activity of indoleamine 2,3-dioxygenase (IDO). The latter is expressed mainly in peripheral lymphatic organs, including Peyer's plaques [81]. Kynurenine binds to the aryl hydrocarbon receptor (AhR), a vital component of the immune response [82]. During inflammation, AhR is overexpressed, being involved in both the generation of T_{reg} cells and a particular type of DC with immunomodulatory phenotype [83, 84].

Vaccinations impact amino acid metabolism; in particular, three studies observed that amino acid biosynthesis or aminoacyl-tRNA biosynthesis were among the most enriched pathways after vaccination [28, 29, 31] (Table **5**). In individuals vaccinated with Hantavax®, arginine, histidine, valine, tryptophan, leucine, isoleucine, homocysteine, methionine, threonine, glycine, cystathionine, glutamine, and citrulline positively correlated with the antibody response, as demonstrated by differences in their levels among non-responders, poor responders, and regular responders to vaccination.

In individuals vaccinated with DVC-LVS®, asparagine blood level increased by 23% the day after vaccination (1/0) compared to the baseline sample collected before vaccination; after two days, histidine decreased by 9% [28]. It is well known that asparagine inhibits T cell activation; thus, the increase in asparagine

Author Proofs
"For Personal Use Only"

the day after vaccination mirrors the vaccine efficacy and could be used in the future to assess the optimal dose for the best immune activation. On the other hand, the decrease in histidine could be associated with the conversion of histidine into histamine. Pathway enrichment analysis showed that the arginine metabolism was enriched in differentially abundant metabolites at day 2/0 (Table **5**).

Both studies on individuals vaccinated with CoronaVac (Sinovac®) found significant changes in amino acid metabolism [31, 32]. In the study by Wang *et al.* [31], valine, tryptophan, and betaine blood levels were upregulated after vaccination, both after the first and after the second dose, whereas leucine and tyrosine were upregulated only after the second dose. Methionine increased after the first dose and decreased after the second dose; phenylalanine was significantly downregulated after the first and the second dose. Vaccination significantly impacted the metabolism of arginine, proline, phenylalanine, glycine, serine, and threonine; for example, the arginine-proline pathway was significantly under-expressed after the first dose and then progressively increased above the baseline level after the second dose. This response was confirmed by the upregulation of arginine biosynthesis after the second dose. Conversely, phenylalanine and tryptophan metabolisms were upregulated after the first dose, returning near and below the baseline level after the second dose, respectively. Glutamine metabolism was under-expressed after the first and second doses, whereas histidine metabolism was under-expressed after the second dose only [31]. Differences were observed between immunized subjects and subjects infected with SARS-CoV-2. In particular, valine, tryptophan, leucine, and betaine were significantly upregulated in immunized individuals compared with infected individuals. In the study by He *et al.* [32], leucine and taurine were significantly upregulated after the second dose; the correlation between taurine and immunized antibodies unveiled the strong and positive relationship between this amino acid and the immune response. Indeed, taurine is crucial for modulating the immune system and attenuating oxidative stress and inflammation, with a direct suppression of cytokine synthesis. It is reasonable to assume that vaccination promotes a reciprocal control between taurine and neutralizing antibodies (Table **5**).

In individuals vaccinated with the mRNA vaccine BNT162b2, phenylalanine, histidine, 3-methylhistidine, and glutamine were the most significant amino acids discriminating low-responder individuals from high responders before vaccination [55]. Notably, their basal levels closely correlated with antibody levels 22 days after the first dose. Both high- and low-responder individuals showed an increase in phenylalanine and glutamine serum levels from pre-vaccination to 22 days after the first dose; phenylalanine was significantly higher in high responders at all time points whereas glutamine only before the first dose. After three months from the first dose, phenylalanine serum levels became again closely comparable to those before vaccination in both groups, whereas glutamine levels decreased in high responders and remained almost equal to those found after 22 days from vaccination. Histidine showed a different trend between high-responders and low-responders; compared to baseline levels before vaccination, histidine serum levels dropped 22 days after vaccination ($p < 0.0001$) and then increased three months later, reaching approximately half of the baseline level in high responders. Conversely, no significant variation in histidine serum levels was observed in low-responders over all time points. Histidine serum levels significantly differed between high- and low-responders only before vaccination (Table **5**).

Author Proofs
"For Personal Use Only"

The metabolic footprint of the older adults after vaccination with the seasonal trivalent inactivated influenza vaccine was marked by the increase in several amino acids, including arginine, serine glycine, threonine, cysteine, alanine, aspartate, and glutamate [48]. In particular, the accumulation of serine suggests that in older adults, the immune response to flu vaccination does not depend upon T-cell proliferation; in fact, the latter requires serine for activation, and in these individuals, serine remains unused. An increase in tryptophan and its intermediates 5-Hydroxy-L-Tryptophan and indoleacetic acid discriminated high responders from low responders in young adults (Table **5**). Finally, older adults exhibited high levels of C-glycosyl tryptophan, an intermediate of the tryptophan pathway closely correlated with age [85].

3. DISCUSSION

Vaccinology is progressing into the 21st century by applying the systems biology approach to decipher molecular networks driving the protective immune responses. This emerging approach, termed system vaccinology, integrates immunogenomics, proteomics, metabolomics, and bioinformatics with each other, aimed to develop safe vaccines and induce an optimal immunological response based on genetic, epigenetic, environmental, and lifestyle factors for each person [86]. Since metabolites produced by immune cells are powerful immune signaling molecules involved in innate and adaptative immunity, metabolomics is strategic for the immune response readout (Fig. **2**).

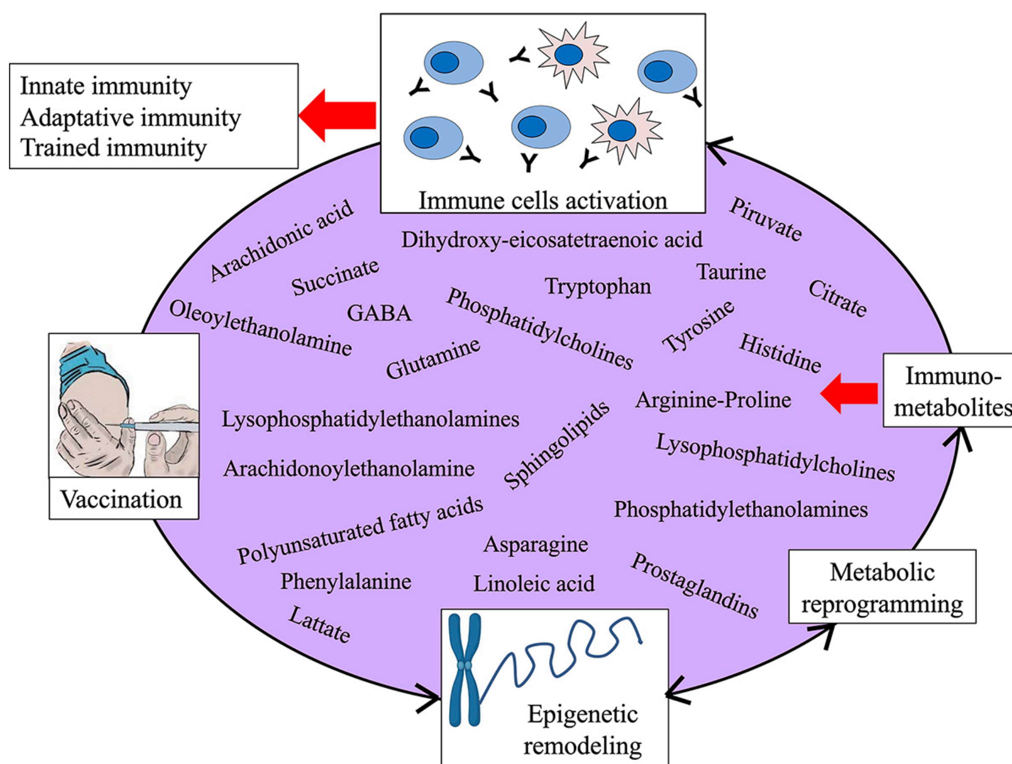


Fig. (2). Metabolites involved in the response to vaccination reported as significant in the current literature. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Author Proof - For Personal Use

Data reported in studies investigating the immunometabolic response to vaccinations indicate a chain of events orchestrated by the interplay of pro-inflammatory mediators and inflammation-resolving factors. In particular, vaccines promote an early activation of the innate immune system by a pro-inflammatory response; this step is followed by the activation of T lymphocytes, leading to the adaptive response that contributes to reinforcing innate immunity (Table **6**).

A system vaccinology-based study demonstrated that vaccination with BNT162b2 induces a mild-to-moderate innate immune response after the first dose; however, a broader innate response is activated by the booster immunization, consisting of the proliferation of considerable amounts of $CD14^{+}$ $CD16^{+}$ inflammatory monocytes, high IFN-γ plasma levels, and a transcriptional signature of innate immunity against SARS-CoV-2 [87].

Studies included in this review are highly heterogeneous because they tested different vaccines with different technological approaches, patient cohorts, and objectives. The analysis of the metabolic changes induced by vaccinations is limited by the small number of metabolomics-based studies currently available in the literature and the small number of vaccinated individuals enrolled in these studies. Seven studies explored longitudinal changes in the metabolomic profile [27-32] and lipidomic profile [49] pre- and post-vaccination. The remaining studies investigated differences between early and late neonatal vaccination [50], high- and low-responders to vaccination [55], young and old-vaccinated individuals [48], and individuals who developed COVID-19 prior to vaccination and naïve individuals [56]. Nevertheless, we could extrapolate some conclusions. Firstly, vaccination does not induce glycolysis as the main metabolic pathway for ATP synthesis; instead, the TCA cycle predominates over glycolysis in five studies [28-32]. It is reasonable to assume that the immune cell response to vaccination does not require a rapid energy availability as that necessary during a viral or bacterial infection. The increase in citrate, observed in three studies [28, 30, 31], may be associated with the activation of the trained immunity in activated macrophages, DCs, and NKs. Second, lipids play a primary role in response to vaccines and infections. Vaccines strongly impact either saturated or unsaturated FAs metabolism; in particular, linoleic acid and arachidonic acid pathways are upregulated

Table 6. Main mechanisms involved in the vaccination-induced immunometabolism.

Metabolic Pathway and/or Metabolite	Innate Immunity	Adaptive Immunity
Prevalence of TCA *vs.* glycolysis and accumulation of citrate	1. ROS production 2. Macrophages M1, DCs and NK activation 3. Acetyl-CoA production (Histone acetylation) 4. Precursor of FA, PG, cholesterol 5. M1→M2 macrophages	1. Macrophages, DCs and NK activation □ CD8 T memory phenotype 2. Activation of T cells and adaptive response
Fatty acids synthesis	Macrophages and DCs activation	Effector T cell activation
Fatty acids oxidation	M2 macrophages phenotype	T_{reg} phenotype and CD8 T memory
Prostaglandin synthesis from arachidonic acid	1. Inhibition of DCs, NK, CD8 2. Synthesis of IL-1, IL-6, IFN-γ, TNFα	-
Leukotrienes (5-HETEs, DHETs)	Pro-inflammatory effects	-
AEA, OEA	1. Activation of PPARγ in macrophages and DCs 2. Inflammation resolution	-
Lysophosphatidylcholines (PCs)	1. Correlation with TLR-induced CKs 2. Pro-inflammatory action 16:0. 18:0, 18:1 by stimulation of monocyte migration, chemotaxis, and phagocytosis 3. ROS production 4. Activation of the M1 phenotype 5. Anti-inflammatory effects 6. Inhibition of IL-6	1. T cell activation 2. INF-γ release by CD4 and CD8 T immune cells 3. T_{reg} promotion
Sphingolipids	1. NK migration from lymph nodes 2. Monocyte chemotaxis 3. Pro-inflammatory cytokines 4. TNF signaling modulation	1. Regulators of T cell systemic circulation and B cell movements 2. B cell activation
Cholesterol	1. Innate immunity cell proliferation 2. M1 phenotype promoter 3. Trained immunity mediator 4. Regulation of TLR and MCH signalling	1. B and T cell proliferation, upregulated in T_{reg} cells, 2. Regulation of BCR and TCR signalling 3. Control of CD8 growth 4. Th17 differentiation
Arginine and proline	1. Innate immunity cell proliferation 2. M1 phenotype promotion through No-synthetase 3. M2 phenotype promotion through arginase	1. B and T cell proliferation 2. TCR integrity through the arginine-NO pathway 3. Inflammatory response of effector T cells inhibition through arginase
Phenylalanine	M1 phenotype promotion through No-synthetase	Promotion of TCR integrity through arginine-NO pathway
Tryptophan	Immunomodulatory DCs phenotype (tryptophan-IDO-kynurenine pathway)	T_{reg} phenotype promotion through the tryptophan-IDO-kynurenine pathway
Histidine	1. M1 phenotype promotion through NO-synthetase 2. Chemotaxis pathways 3. Neutrophil degranulation 4. NK activity 5. Histamine precursor	-
Glutamine	M1 phenotype promotion through Arg-NO pathway TCA entry in activated macrophages Trained immunity induction	Supporting the BCR and TCR antigen response
Asparagine	-	TCR signalling and CD8 T-cell activity

Author Proofs "For Personal Use Only"

after the first and the second dose [27, 29, 31]. However, two days after the Coronavac (Sinovac®) vaccination, the depletion of linoleic acid was observed [31]. A reasonable explanation might be the rapid conversion of linoleic acid to arachidonic acid, a precursor of bioactive lipid mediators. Vaccines also impact the

metabolism of IP and cholesterol; the former is overexpressed in subjects with a viral infection and closely correlated with poor IgG response. The latter, together with its precursors, intermediates, and derivatives, was found to be significantly involved in the immune response to vaccines in seven studies [27-30, 48, 49, 55, 56]. Data emerging from these studies confirmed that cholesterol is strategic for the synthesis of 25-HC in M1 macrophages and DCs. In addition, 25-HC stimulates the conversion of macrophages and T cells in the M2 macrophage phenotype and T_{reg}, respectively. Beyond PLs and sphingolipids, further lipids and their precursors may be theoretically involved in response to vaccinations; this represents a challenge for future studies on the role of lipids in vaccine immunity. For example, the role of β-hydroxybutyrate, a ketone body, in supporting mitochondrial function is well-defined in infections but not in the immune response to vaccination.

β-hydroxybutyrate fuels OXPHOS, acting as an alternative carbon source for intermediates in the TCA cycle and promoting the synthesis of bioenergetic amino acids and glutathione. Some acute viral infections of the respiratory tract promote a metabolic switch towards ketogenesis, leading to the increased bioavailability of ketone bodies, which support the energy demand of T cells, strongly involved in the clearance of viruses. Thus, ketogenesis supports T-cell immunity. However, infection-induced ketogenesis is compromised in patients with acute respiratory distress syndrome due to moderate and severe COVID-19 and bacterial pneumonia [88]; as a result, the antiviral immunity of the T cells, including cytokines production, is severely impaired in these patients. Whether or not a similar mechanism is present after vaccination against flu or COVID-19 remains to be deciphered. In particular, the next challenge may be to assess whether ketogenesis is involved in the efficacy of vaccines, including those against flu and SARS-CoV-2.

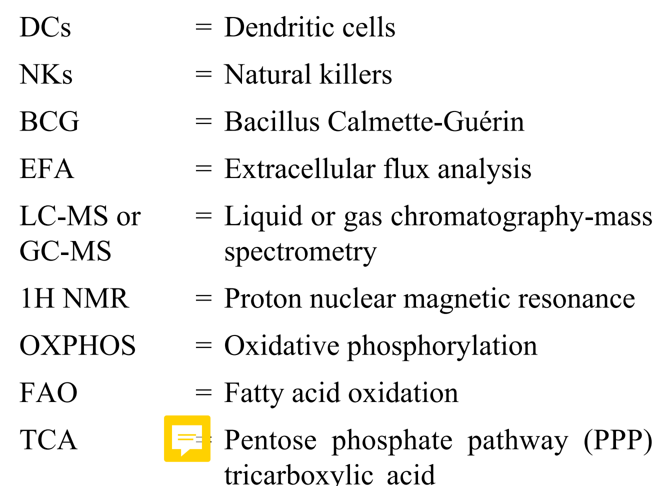
Given the importance of the metabolic reprogramming induced by vaccines, future studies are required to decipher the relationship between changes in the individual metabotype and vaccine effectiveness, harmful side effects, safety, and duration of the immunity following a complete immunization schedule. Indeed, metabolomics could predict the individual predisposition to develop an adverse event following immunization. A pilot study found that the pre- and post-vaccination metabotypes of individuals who developed an adverse event (clinically verified myocarditis or asymptomatic elevation of troponins) following immunization with the smallpox vaccine were significantly different from the metabotype of individuals with no adverse events or clinical symptoms [89]. In a mouse model, changes in the urine metabolome were closely correlated to the vaccine-induced toxicity; if confirmed, this result may aid the development of innovative and more effective vaccine safety test methods [90]. A further primary need is to assess the interindividual variability in vaccine response by identifying specific metabolomic signatures and consequently, biomarkers able to predict with high sensitivity and specificity the individual tolerability to a given vaccine, allowing a personalized approach to the vaccination strategy. A recent study has demonstrated that the individual metabotype predicts the innate and adaptive immune response after BCG vaccination, especially trained immunity [91]. The study evidenced that the concentration of taurine, an anti-inflammatory and anti-oxidant metabolite, positively correlates with trained immunity induced by BCG vaccination. This finding confirmed the role of specific metabolites, especially those involved in the TCA cycle (*e.g.*, fumarate), on epigenetic remodeling, which is the molecular basis for trained immunity.

Author Proofs
"For Personal Use Only"

CONCLUSION

In conclusion, the large-scale application of metabolomics and the close cooperation and interaction between "omics" could lead to the identification of candidate predictive biomarkers of vaccine efficacy/-tolerability with positive effects for patients and public health.

LIST OF ABBREVIATIONS



DCs	= Dendritic cells
NKs	= Natural killers
BCG	= Bacillus Calmette-Guérin
EFA	= Extracellular flux analysis
LC-MS or GC-MS	= Liquid or gas chromatography-mass spectrometry
1H NMR	= Proton nuclear magnetic resonance
OXPHOS	= Oxidative phosphorylation
FAO	= Fatty acid oxidation
TCA	= Pentose phosphate pathway (PPP) tricarboxylic acid

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Domínguez-Andrés, J.; van Crevel, R.; Divangahi, M.; Netea, M.G. Designing the next generation of vaccines: Relevance for lics. *MBio,* **2020**, *11*(6), e02616-20. http://dx.doi.org/10.1128/mBio.02616-20 PMID: 33443120

[2] Mayer, A.; Balasubramanian, V.; Walczak, A.M.; Mora, T. How a well-adapting immune system remembers. *Proc Natl Acad Sci.,* **2019**, *116*(18), 8815-23. http://dx.doi.org/10.1073/pnas.1812810116

[3] Netea, M.G.; Domínguez-Andrés, J.; Barreiro, L.B.; Chavakis, T.; Divangahi, M.; Fuchs, E.; Joosten, L.A.B.; van der Meer, J.W.M.; Mhlanga, M.M.; Mulder, W.J.M.; Riksen, N.P.; Schlitzer, A.; Schultze, J.L.; Stabell Benn, C.; Sun, J.C.; Xavier, R.J.; Latz, E. Defining trained immunity and its role in health and disease. *Nat. Rev. Immunol.,* **2020**, *20*(6), 375-388. http://dx.doi.org/10.1038/s41577-020-0285-6 PMID: 32132681

[4] Dominguez-Andres, J.; Netea, M.G. Long-term reprogramming of the innate immune system. *J. Leukoc. Biol.,* **2019**, *105*(2), 329-338. http://dx.doi.org/10.1002/JLB.MR0318-104R PMID: 29999546

[5] Sánchez-Ramón, S.; Conejero, L.; Netea, M.G.; Sancho, D.; Palomares, Ó.; Subiza, J.L. Trained immunity-based vaccines: A new paradigm for the development of broad--spectrum anti-infectious formulations. *Front. Immunol.,* **2018**, *9*, 2936. http://dx.doi.org/10.3389/fimmu.2018.02936 PMID: 30619296

[6] Netea, M.G.; Giamarellos-Bourboulis, E.J.; Domínguez-Andrés, J.; Curtis, N.; van Crevel, R.; van de Veerdonk, F.L.; Bonten, M. Trained immunity: A tool for reducing susceptibility to and the severity of SARS-CoV-2 infection *Cell,* **2020**, *181*(5), 969-77. http://dx.doi.org/10.1016/j.cell.2020.04.042

[7] Pinti, M.; Appay, V.; Campisi, J.; Frasca, D.; Fülöp, T.; Sauce, D.; Larbi, A.; Weinberger, B.; Cossarizza, A. Aging of the immune system: Focus on inflammation and vaccination. *Eur. J. Immunol.,* **2016**, *46*(10), 2286-2301. http://dx.doi.org/10.1002/eji.201546178 PMID: 27595500

[8] Diray-Arce, J.; Conti, M.G.; Petrova, B.; Kanarek, N.; Angelidou, A.; Levy, O. Integrative metabolomics to identify molecular signatures of responses to vaccines and infections. *Metabolites,* **2020**, *10*(12), 492. http://dx.doi.org/10.3390/metabo10120492 PMID: 33266347

[9] Voss, K.; Hong, H.S.; Bader, J.E.; Sugiura, A.; Lyssiotis, C.A.; Rathmell, J.C. A guide to interrogating immunometabolism. *Nat. Rev. Immunol.,* **2021**, *21*(10), 637-652. http://dx.doi.org/10.1038/s41577-021-00529-8 PMID: 33859379

[10] Mussap, M.; Noto, A.; Piras, C.; Atzori, L.; Fanos, V. Slotting metabolomics into routine precision medicine. *Expert Rev. Precis. Med. Drug Dev.,* **2021**, *6*(3), 173-187. http://dx.doi.org/10.1080/23808993.2021.1911639

[11] Poland, G.A.; Ovsyannikova, I.G.; Kennedy, R.B. Personalized vaccinology: A review. *Vaccine,* **2018**, *36*(36), 5350-5357. http://dx.doi.org/10.1016/j.vaccine.2017.07.062 PMID: 28774561

[12] O'Neill, L.A.J.; Kishton, R.J.; Rathmell, J. A guide to immunometabolism for immunologists. *Nat. Rev. Immunol.,* **2016**, *16*(9), 553-565. http://dx.doi.org/10.1038/nri.2016.70 PMID: 27396447

[13] Sun, L.; Yang, X.; Yuan, Z.; Wang, H. Metabolic reprogramming in immune response and tissue inflammation. *Arterioscler. Thromb. Vasc. Biol.,* **2020**, *40*(9), 1990-2001. http://dx.doi.org/10.1161/ATVBAHA.120.314037 PMID: 32698683

[14] Shen, W.; Gao, C.; Cueto, R.; Liu, L.; Fu, H.; Shao, Y.; Yang, W.Y.; Fang, P.; Choi, E.T.; Wu, Q.; Yang, X.; Wang, H. Homocysteine-methionine cycle is a metabolic sensor system controlling methylation-regulated pathological signaling. *Redox Biol.,* **2020**, *28*, 101322. http://dx.doi.org/10.1016/j.redox.2019.101322 PMID: 31605963

[15] Cameron, A.M.; Lawless, S.J.; Pearce, E.J. Metabolism and acetylation in innate immune cell function and fate. *Semin. Immunol.,* **2016**, *28*(5), 408-416. http://dx.doi.org/10.1016/j.smim.2016.10.003 PMID: 28340958

[16] Rodríguez-Prados, J.C.; Través, P.G.; Cuenca, J.; Rico, D.; Aragonés, J.; Martín-Sanz, P.; Cascante, M.; Boscá, L. Substrate fate in activated macrophages: A comparison between innate, classic, and alternative activation. *J. Immunol.,* **2010**, *185*(1), 605-614. http://dx.doi.org/10.4049/jimmunol.0901698 PMID: 20498354

[17] Galván-Peña, S.; O'Neill, L.A. Metabolic reprograming in macrophage polarization. *Front. Immunol.,* **2014**, *5*, 420. http://dx.doi.org/10.3389/fimmu.2014.00420 PMID: 25228902

[18] Arts, R.J.W.; Novakovic, B.; ter Horst, R.; Carvalho, A.; Bekkering, S.; Lachmandas, E.; Rodrigues, F.; Silvestre, R.; Cheng, S.C.; Wang, S.Y.; Habibi, E.; Gonçalves, L.G.; Mesquita, I.; Cunha, C.; van Laarhoven, A.; van de Veerdonk, F.L.; Williams, D.L.; van der Meer, J.W.M.; Logie, C.; O'Neill, L.A.; Dinarello, C.A.; Riksen, N.P.; van Crevel, R.; Clish, C.; Notebaart, R.A.; Joosten, L.A.B.; Stunnenberg, H.G.; Xavier, R.J.; Netea, M.G. Glutaminolysis and fumarate accumulation integrate immunometabolic and epigenetic programs in trained immunity. *Cell Metab.,* **2016**, *24*(6), 807-819. http://dx.doi.org/10.1016/j.cmet.2016.10.008 PMID: 27866838

[19] Netea, M.G.; Joosten, L.A.B.; Latz, E.; Mills, K.H.G.; Natoli, G.; Stunnenberg, H.G.; O'Neill, L.A.J.; Xavier, R.J. Trained immunity: A program of innate immune memory in health and disease. *Science,* **2016**, *352*(6284), aaf1098. http://dx.doi.org/10.1126/science.aaf1098 PMID: 27102489

[20] Riksen, N.P.; Netea, M.G. Immunometabolic control of trained immunity. *Mol. Aspects Med.,* **2021**, *77*, 100897.

Author Proofs
"For Personal Use Only"

http://dx.doi.org/10.1016/j.mam.2020.100897 PMID: 32891423

[21] Haschemi, A.; Kosma, P.; Gille, L.; Evans, C.R.; Burant, C.F.; Starkl, P.; Knapp, B.; Haas, R.; Schmid, J.A.; Jandl, C.; Amir, S.; Lubec, G.; Park, J.; Esterbauer, H.; Bilban, M.; Brizuela, L.; Pospisilik, J.A.; Otterbein, L.E.; Wagner, O. The sedoheptulose kinase CARKL directs macrophage polarization through control of glucose metabolism. *Cell Metab.,* **2012**, *15*(6), 813-826. http://dx.doi.org/10.1016/j.cmet.2012.04.023 PMID: 22682222

[22] O'Sullivan, D.; van der Windt, G.J.W.; Huang, S.C.C.; Curtis, J.D.; Chang, C.H.; Buck, M.D.; Qiu, J.; Smith, A.M.; Lam, W.Y.; DiPlato, L.M.; Hsu, F.F.; Birnbaum, M.J.; Pearce, E.J.; Pearce, E.L. Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. *Immunity,* **2014**, *41*(1), 75-88. http://dx.doi.org/10.1016/j.immuni.2014.06.005 PMID: 25001241

[23] Hertz, L.; Hertz, E. Cataplerotic TCA cycle flux determined as glutamate-sustained oxygen consumption in primary cultures of astrocytes. *Neurochem. Int.,* **2003**, *43*(4-5), 355-361. http://dx.doi.org/10.1016/S0197-0186(03)00022-6 PMID: 12742079

[24] Ferreira, A.V.; Domiguéz-Andrés, J.; Netea, M.G. The Role of Cell Metabolism in Innate Immune Memory. *J. Innate Immun.,* **2022**, *14*(1), 42-50. http://dx.doi.org/10.1159/000512280 PMID: 33378755

[25] Schebb, N.H.; Kühn, H.; Kahnt, A.S.; Rund, K.M.; O'Donnell, V.B.; Flamand, N.; Peters-Golden, M.; Jakobsson, P.J.; Weylandt, K.H.; Rohwer, N.; Murphy, R.C.; Geisslinger, G.; FitzGerald, G.A.; Hanson, J.; Dahlgren, C.; Alnouri, M.W.; Offermanns, S.; Steinhilber, D. Formation, signaling and occurrence of specialized pro-resolving lipid mediators—what is the evidence so far? *Front. Pharmacol.,* **2022**, *13*, 838782. http://dx.doi.org/10.3389/fphar.2022.838782 PMID: 35308198

[26] Bosch, M.; Sánchez-Álvarez, M.; Fajardo, A.; Kapetanovic, R.; Steiner, B.; Dutra, F.; Moreira, L.; López, J.A.; Campo, R.; Marí, M.; Morales-Paytuví, F.; Tort, O.; Gubern, A.; Templin, R.M.; Curson, J.E.B.; Martel, N.; Català, C.; Lozano, F.; Tebar, F.; Enrich, C.; Vázquez, J.; Del Pozo, M.A.; Sweet, M.J.; Bozza, P.T.; Gross, S.P.; Parton, R.G.; Pol, A. Mammalian lipid droplets are innate immune hubs integrating cell metabolism and host defense. *Science,* **2020**, *370*(6514), eaay8085. http://dx.doi.org/10.1126/science.aay8085 PMID: 33060333

[27] Hagan, T.; Cortese, M.; Rouphael, N.; Boudreau, C.; Linde, C.; Maddur, M.S.; Das, J.; Wang, H.; Guthmiller, J.; Zheng, N.Y.; Huang, M.; Uphadhyay, A.A.; Gardinassi, L.; Petitdemange, C.; McCullough, M.P.; Johnson, S.J.; Gill, K.; Cervasi, B.; Zou, J.; Bretin, A.; Hahn, M.; Gewirtz, A.T.; Bosinger, S.E.; Wilson, P.C.; Li, S.; Alter, G.; Khurana, S.; Golding, H.; Pulendran, B. Antibiotics--Driven gut microbiome perturbation alters immunity to vaccines in humans. *Cell,* **2019**, *178*(6), 1313-1328.e13. http://dx.doi.org/10.1016/j.cell.2019.08.010 PMID: 31491384

[28] Goll, J.B.; Li, S.; Edwards, J.L.; Bosinger, S.E.; Jensen, T.L.; Wang, Y.; Hooper, W.F.; Gelber, C.E.; Sanders, K.L.; Anderson, E.J.; Rouphael, N.; Natrajan, M.S.; Johnson, R.A.; Sanz, P.; Hoft, D.; Mulligan, M.J. Transcriptomic and metabolic responses to a live-attenuated *Francisella tularensis* vaccine. *Vaccines,* **2020**, *8*(3), 412. http://dx.doi.org/10.3390/vaccines8030412 PMID: 32722194

[29] Khan, A.; Shin, O.S.; Na, J.; Kim, J.K.; Seong, R.K.; Park, M.S.; Noh, J.Y.; Song, J.Y.; Cheong, H.J.; Park, Y.H.; Kim, W.J. A systems vaccinology approach reveals the mechanisms of immunogenic responses to hantavax vaccination in humans. *Sci. Rep.,* **2019**, *9*(1), 4760. http://dx.doi.org/10.1038/s41598-019-41205-1 PMID: 30886186

[30] Li, S.; Sullivan, N.L.; Rouphael, N.; Yu, T.; Banton, S.; Maddur, M.S.; McCausland, M.; Chiu, C.; Canniff, J.; Dubey, S.; Liu, K.; Tran, V.; Hagan, T.; Duraisingham, S.; Wieland, A.; Mehta, A.K.; Whitaker, J.A.; Subramaniam, S.; Jones, D.P.; Sette, A.; Vora, K.; Weinberg, A.; Mulligan, M.J.; Nakaya, H.I.; Levin, M.; Ahmed, R.; Pulendran, B. Metabolic phenotypes of response to vaccination in humans. *Cell,* **2017**, *169*(5), 862-877.e17. http://dx.doi.org/10.1016/j.cell.2017.04.026 PMID: 28502771

[31] Wang, Y.; Wang, X.; Luu, L.D.W.; Chen, S.; Jin, F.; Wang, S.; Huang, X.; Wang, L.; Zhou, X.; Chen, X.; Cui, X.; Li, J.; Tai, J.; Zhu, X. Proteomic and metabolomic signatures associated with the immune response in healthy individuals immunized with an inactivated SARS-CoV-2 vaccine. *Front. Immunol.,* **2022**, *13*, 848961. http://dx.doi.org/10.3389/fimmu.2022.848961 PMID: 35686122

[32] He, M.; Huang, Y.; Wang, Y.; Liu, J.; Han, M.; Xiao, Y.; Zhang, N.; Gui, H.; Qiu, H.; Cao, L.; Jia, W.; Huang, S. Metabolomics-based investigation of SARS-CoV-2 vaccination (Sinovac) reveals an immune-dependent metabolite biomarker. *Front. Immunol.,* **2022**, *13*, 954801. http://dx.doi.org/10.3389/fimmu.2022.954801 PMID: 36248825

[33] Choi, I.; Son, H.; Baek, J.H. Tricarboxylic Acid (TCA) cycle intermediates: Regulators of immune responses. *Life,* **2021**, *11*(1), 69. http://dx.doi.org/10.3390/life11010069 PMID: 33477822

[34] Williams, N.C.; O'Neill, L.A.J. A role for the krebs cycle intermediate citrate in metabolic reprogramming in innate immunity and inflammation. *Front. Immunol.,* **2018**, *9*, 141. http://dx.doi.org/10.3389/fimmu.2018.00141 PMID: 29459863

[35] Langston, P.K.; Shibata, M.; Horng, T. Metabolism supports macrophage activation. *Front. Immunol.,* **2017**, *8*, 61. http://dx.doi.org/10.3389/fimmu.2017.00061 PMID: 28197151

[36] Hooftman, A.; O'Neill, L.A.J. The immunomodulatory potential of the metabolite itaconate. *Trends Immunol.,* **2019**, *40*(8), 687-698. http://dx.doi.org/10.1016/j.it.2019.05.007 PMID: 31178405

[37] Hooftman, A.; Angiari, S.; Hester, S.; Corcoran, S.E.; Runtsch, M.C.; Ling, C.; Ruzek, M.C.; Slivka, P.F.; McGettrick, A.F.; Banahan, K.; Hughes, M.M.; Irvine, A.D.; Fischer, R.; O'Neill, L.A.J. The immunomodulatory metabolite itaconate modifies nlrp3 and inhibits inflammasome activation. *Cell Metab.,* **2020**, *32*(3), 468-478.e7. http://dx.doi.org/10.1016/j.cmet.2020.07.016 PMID: 32791101

[38] Tannahill, G.M.; Curtis, A.M.; Adamik, J.; Palsson-McDer-

"Author Proofs "For Personal Use Only"

mott, E.M.; McGettrick, A.F.; Goel, G.; Frezza, C.; Bernard, N.J.; Kelly, B.; Foley, N.H.; Zheng, L.; Gardet, A.; Tong, Z.; Jany, S.S.; Corr, S.C.; Haneklaus, M.; Caffrey, B.E.; Pierce, K.; Walmsley, S.; Beasley, F.C.; Cummins, E.; Nizet, V.; Whyte, M.; Taylor, C.T.; Lin, H.; Masters, S.L.; Gottlieb, E.; Kelly, V.P.; Clish, C.; Auron, P.E.; Xavier, R.J.; O'Neill, L.A.J. Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature,* **2013**, *496*(7444), 238-242. http://dx.doi.org/10.1038/nature11986 PMID: 23535595

[39] Liu, P.S.; Wang, H.; Li, X.; Chao, T.; Teav, T.; Christen, S.; Di Conza, G.; Cheng, W.C.; Chou, C.H.; Vavakova, M.; Muret, C.; Debackere, K.; Mazzone, M.; Huang, H.D.; Fendt, S.M.; Ivanisevic, J.; Ho, P.C. α-ketoglutarate orchestrates macrophage activation through metabolic and epigenetic reprogramming. *Nat. Immunol.,* **2017**, *18*(9), 985-994. http://dx.doi.org/10.1038/ni.3796 PMID: 28714978

[40] Jha, A.K.; Huang, S.C.C.; Sergushichev, A.; Lampropoulou, V.; Ivanova, Y.; Loginicheva, E.; Chmielewski, K.; Stewart, K.M.; Ashall, J.; Everts, B.; Pearce, E.J.; Driggers, E.M.; Artyomov, M.N. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity,* **2015**, *42*(3), 419-430. http://dx.doi.org/10.1016/j.immuni.2015.02.005 PMID: 25786174

[41] Mills, E.; O'Neill, L.A.J. Succinate: A metabolic signal in inflammation. *Trends Cell Biol.,* **2014**, *24*(5), 313-320. http://dx.doi.org/10.1016/j.tcb.2013.11.008 PMID: 24361092

[42] Domínguez-Andrés, J.; Joosten, L.A.B.; Netea, M.G. Induction of innate immune memory: The role of cellular metabolism. *Curr. Opin. Immunol.,* **2019**, *56*, 10-16. http://dx.doi.org/10.1016/j.coi.2018.09.001 PMID: 30240964

[43] Wang, R.; Green, D.R. Metabolic reprogramming and metabolic dependency in T cells. *Immunol. Rev.,* **2012**, *249*(1), 14-26. http://dx.doi.org/10.1111/j.1600-065X.2012.01155.x PMID: 22889212

[44] Wang, R.; Dillon, C.P.; Shi, L.Z.; Milasta, S.; Carter, R.; Finkelstein, D.; McCormick, L.L.; Fitzgerald, P.; Chi, H.; Munger, J.; Green, D.R. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity,* **2011**, *35*(6), 871-882. http://dx.doi.org/10.1016/j.immuni.2011.09.021 PMID: 22195744

[45] Lochner, M.; Berod, L.; Sparwasser, T. Fatty acid metabolism in the regulation of T cell function. *Trends Immunol.,* **2015**, *36*(2), 81-91. http://dx.doi.org/10.1016/j.it.2014.12.005 PMID: 25592731

[46] Miles, E.A.; Childs, C.E.; Calder, P.C. Long-Chain Polyunsaturated Fatty Acids (LCPUFAs) and the developing immune system: A narrative review. *Nutrients,* **2021**, *13*(1), 247. http://dx.doi.org/10.3390/nu13010247 PMID: 33467123

[47] Kalinski, P. Regulation of immune responses by prostaglandin E2. *J. Immunol.,* **2012**, *188*(1), 21-28. http://dx.doi.org/10.4049/jimmunol.1101029 PMID: 22187483

[48] Chou, C.H.; Mohanty, S.; Kang, H.A.; Kong, L.; Avila--Pacheco, J.; Joshi, S.R.; Ueda, I.; Devine, L.; Raddassi, K.; Pierce, K.; Jeanfavre, S.; Bullock, K.; Meng, H.; Clish, C.; Santori, F.R.; Shaw, A.C.; Xavier, R.J. Metabolomic and transcriptomic signatures of influenza vaccine response in healthy young and older adults. *Aging Cell,* **2022**, *21*(9), e13682. http://dx.doi.org/10.1111/acel.13682 PMID: 35996998

[49] Maner-Smith, K.M.; Goll, J.B.; Khadka, M.; Jensen, T.L.; Colucci, J.K.; Gelber, C.E.; Albert, C.J.; Bosinger, S.E.; Franke, J.D.; Natrajan, M.; Rouphael, N.; Johnson, R.A.; Sanz, P.; Anderson, E.J.; Hoft, D.F.; Mulligan, M.J.; Ford, D.A.; Ortlund, E.A. Alterations in the human plasma lipidome in response to tularemia vaccination. *vaccines,* **2020**, *8*(3), 414. http://dx.doi.org/10.3390/vaccines8030414 PMID: 32722213

[50] Diray-Arce, J.; Angelidou, A.; Jensen, K.J.; Conti, M.G.; Kelly, R.S.; Pettengill, M.A.; Liu, M.; van Haren, S.D.; McCulloch, S.D.; Michelloti, G.; Idoko, O.; Kollmann, T.R.; Kampmann, B.; Steen, H.; Ozonoff, A.; Lasky-Su, J.; Benn, C.S.; Levy, O. Bacille Calmette-Guérin vaccine reprograms human neonatal lipid metabolism *in vivo* and *in vitro*. *Cell Rep.,* **2022**, *39*(5), 110772. http://dx.doi.org/10.1016/j.celrep.2022.110772 PMID: 35508141

[51] O'Donnell, V.B.; Rossjohn, J.; Wakelam, M.J.O. Phospholipid signaling in innate immune cells. *J. Clin. Invest.,* **2018**, *128*(7), 2670-2679. http://dx.doi.org/10.1172/JCI97944 PMID: 29683435

[52] Cathcart, M.K. Signal-activated phospholipase regulation of leukocyte chemotaxis. *J. Lipid Res.,* **2009**, *50*(Suppl)(-Suppl.), S231-S236. http://dx.doi.org/10.1194/jlr.R800096-JLR200 PMID: 19109234

[53] Tan, S.T.; Ramesh, T.; Toh, X.R.; Nguyen, L.N. Emerging roles of lysophospholipids in health and disease. *Prog. Lipid Res.,* **2020**, *80*, 101068. http://dx.doi.org/10.1016/j.plipres.2020.101068 PMID: 33068601

[54] Knuplez, E.; Marsche, G. An updated review of pro- and anti-inflammatory properties of plasma lysophosphatidylcholines in the vascular system. *Int. J. Mol. Sci.,* **2020**, *21*(12), 4501. http://dx.doi.org/10.3390/ijms21124501 PMID: 32599910

[55] Dagla, I.; Iliou, A.; Benaki, D.; Gikas, E.; Mikros, E.; Bagratuni, T.; Kastritis, E.; Dimopoulos, M.A.; Terpos, E.; Tsarbopoulos, A. Plasma metabolomic alterations induced by COVID-19 vaccination reveal putative biomarkers reflecting the immune response. *Cells,* **2022**, *11*(7), 1241. http://dx.doi.org/10.3390/cells11071241 PMID: 35406806

[56] Ghini, V.; Maggi, L.; Mazzoni, A.; Spinicci, M.; Zammarchi, L.; Bartoloni, A.; Annunziato, F.; Turano, P. Serum NMR profiling reveals differential alterations in the lipoproteome induced by Pfizer-BioNTech Vaccine in COVID-19 recovered subjects and naïve subjects. *Front. Mol. Biosci.,* **2022**, *9*, 839809. http://dx.doi.org/10.3389/fmolb.2022.839809 PMID: 35480886

[57] Maceyka, M.; Spiegel, S. Sphingolipid metabolites in inflammatory disease. *Nature,* **2014**, *510*(7503), 58-67. http://dx.doi.org/10.1038/nature13475 PMID: 24899305

[58] Spiegel, S.; Milstien, S. The outs and the ins of sphingosine-1-phosphate in immunity. *Nat. Rev. Immunol.,* **2011**, *11*(6), 403-415. http://dx.doi.org/10.1038/nri2974 PMID: 21546914

[59] Hannun, Y.A.; Obeid, L.M. Sphingolipids and their metabolism in physiology and disease. *Nat. Rev. Mol. Cell*

Author Proofs
"For Personal Use Only"

Biol., **2018**, *19*(3), 175-191.
http://dx.doi.org/10.1038/nrm.2017.107 PMID: 29165427

[60] Arnon, T.I.; Horton, R.M.; Grigorova, I.L.; Cyster, J.G. Visualization of splenic marginal zone B-cell shuttling and follicular B-cell egress. *Nature,* **2013**, *493*(7434), 684-688. http://dx.doi.org/10.1038/nature11738 PMID: 23263181

[61] Walzer, T.; Chiossone, L.; Chaix, J.; Calver, A.; Carozzo, C.; Garrigue-Antar, L.; Jacques, Y.; Baratin, M.; Tomasello, E.; Vivier, E. Natural killer cell trafficking *in vivo* requires a dedicated sphingosine 1-phosphate receptor. *Nat. Immunol.,* **2007**, *8*(12), 1337-1344. http://dx.doi.org/10.1038/ni1523 PMID: 17965716

[62] Gaggini, M.; Pingitore, A.; Vassalle, C. Plasma ceramides pathophysiology, measurements, challenges, and opportunities. *Metabolites,* **2021**, *11*(11), 719. http://dx.doi.org/10.3390/metabo11110719 PMID: 34822377

[63] Reboldi, A.; Dang, E. Cholesterol metabolism in innate and adaptive response. *F1000Res,* **2018**, *7*, 1647. http://dx.doi.org/10.12688/f1000research.15500.1

[64] Fessler, M.B. Regulation of adaptive immunity in health and disease by cholesterol metabolism. *Curr. Allergy Asthma Rep.,* **2015**, *15*(8), 48. http://dx.doi.org/10.1007/s11882-015-0548-7 PMID: 26149587

[65] Aguilar-Ballester, M.; Herrero-Cervera, A.; Vinué, Á.; Martínez-Hervás, S.; González-Navarro, H. Impact of cholesterol metabolism in immune cell function and atherosclerosis. *Nutrients,* **2020**, *12*(7), 2021. http://dx.doi.org/10.3390/nu12072021 PMID: 32645995

[66] Kidani, Y.; Elsaesser, H.; Hock, M.B.; Vergnes, L.; Williams, K.J.; Argus, J.P.; Marbois, B.N.; Komisopoulou, E.; Wilson, E.B.; Osborne, T.F.; Graeber, T.G.; Reue, K.; Brooks, D.G.; Bensinger, S.J. Sterol regulatory element–binding proteins are essential for the metabolic programming of effector T cells and adaptive immunity. *Nat. Immunol.,* **2013**, *14*(5), 489-499. http://dx.doi.org/10.1038/ni.2570 PMID: 23563690

[67] Hu, X.; Wang, Y.; Hao, L.Y.; Liu, X.; Lesch, C.A.; Sanchez, B.M.; Wendling, J.M.; Morgan, R.W.; Aicher, T.D.; Carter, L.L.; Toogood, P.L.; Glick, G.D. Sterol metabolism controls TH17 differentiation by generating endogenous RORγ agonists. *Nat. Chem. Biol.,* **2015**, *11*(2), 141-147. http://dx.doi.org/10.1038/nchembio.1714 PMID: 25558972

[68] Bekkering, S.; Arts, R.J.W.; Novakovic, B.; Kourtzelis, I.; van der Heijden, C.D.C.C.; Li, Y.; Popa, C.D.; ter Horst, R.; van Tuijl, J.; Netea-Maier, R.T.; van de Veerdonk, F.L.; Chavakis, T.; Joosten, L.A.B.; van der Meer, J.W.M.; Stunnenberg, H.; Riksen, N.P.; Netea, M.G. Metabolic induction of trained immunity through the mevalonate pathway. *Cell,* **2018**, *172*(1-2), 135-146.e9. http://dx.doi.org/10.1016/j.cell.2017.11.025 PMID: 29328908

[69] Griffiths, W.J.; Wang, Y. Oxysterols as lipid mediators: Their biosynthetic genes, enzymes and metabolites. *Prostaglandins Other Lipid Mediat.,* **2020**, *147*, 106381. http://dx.doi.org/10.1016/j.prostaglandins.2019.106381 PMID: 31698146

[70] Mutemberezi, V.; Guillemot-Legris, O.; Muccioli, G.G. Oxysterols: From cholesterol metabolites to key mediators. *Prog. Lipid Res.,* **2016**, *64*, 152-169. http://dx.doi.org/10.1016/j.plipres.2016.09.002 PMID: 27687912

[71] Reinmuth, L.; Hsiao, C.C.; Hamann, J.; Rosenkilde, M.; Mackrill, J. Multiple targets for oxysterols in their regulation of the immune system. *Cells,* **2021**, *10*(8), 2078. http://dx.doi.org/10.3390/cells10082078 PMID: 34440846

[72] Spann, N.J.; Glass, C.K. Sterols and oxysterols in immune cell function. *Nat. Immunol.,* **2013**, *14*(9), 893-900. http://dx.doi.org/10.1038/ni.2681 PMID: 23959186

[73] Dang, E.V.; McDonald, J.G.; Russell, D.W.; Cyster, J.G. Oxysterol restraint of cholesterol synthesis prevents AIM2 inflammasome activation. *Cell,* **2017**, *171*(5), 1057-1071.e11. http://dx.doi.org/10.1016/j.cell.2017.09.029 PMID: 29033131

[74] Zang, R.; Case, J.B.; Yutuc, E.; Ma, X.; Shen, S.; Gomez Castro, M.F.; Liu, Z.; Zeng, Q.; Zhao, H.; Son, J. Cholesterol 25-hydroxylase suppresses SARS-CoV-2 replication by blocking membrane fusion. *Proc Natl Acad Sci.,* **2020**, *117*(50), 32105-32113. http://dx.doi.org/10.1073/pnas.2012197117

[75] Wang, S.; Li, W.; Hui, H.; Tiwari, S.K.; Zhang, Q.; Croker, B.A.; Rawlings, S.; Smith, D.; Carlin, A.F.; Rana, T.M. Cholesterol 25□Hydroxylase inhibits SARS □CoV□2 and other coronaviruses by depleting membrane cholesterol. *EMBO J.,* **2020**, *39*(21), e106057. http://dx.doi.org/10.15252/embj.2020106057 PMID: 32944968

[76] Kelly, B.; Pearce, E.L. Amino assets: How amino acids support immunity. *Cell Metab.,* **2020**, *32*(2), 154-175. http://dx.doi.org/10.1016/j.cmet.2020.06.010 PMID: 32649859

[77] Takahara, T.; Amemiya, Y.; Sugiyama, R.; Maki, M.; Shibata, H. Amino acid-dependent control of mTORC1 signaling: A variety of regulatory modes. *J. Biomed. Sci.,* **2020**, *27*(1), 87. http://dx.doi.org/10.1186/s12929-020-00679-2 PMID: 32799865

[78] Li, P.; Wu, G. Important roles of amino acids in immune responses. *Br. J. Nutr.,* **2022**, *127*(3), 398-402. http://dx.doi.org/10.1017/S0007114521004566 PMID: 34776020

[79] Holeček, M. Histidine in health and disease: Metabolism, physiological importance, and use as a supplement. *Nutrients,* **2020**, *12*(3), 848. http://dx.doi.org/10.3390/nu12030848 PMID: 32235743

[80] Rath, M.; Müller, I.; Kropf, P.; Closs, E.I.; Munder, M. Metabolism *via* Arginase or Nitric Oxide synthase: Two competing arginine pathways in macrophages. *Front. Immunol.,* **2014**, *5*, 532. http://dx.doi.org/10.3389/fimmu.2014.00532 PMID: 25386178

[81] Sorgdrager, F.J.H.; Naudé, P.J.W.; Kema, I.P.; Nollen, E.A.; Deyn, P.P.D. Tryptophan metabolism in inflammaging: From biomarker to therapeutic target. *Front. Immunol.,* **2019**, *10*(10), 2565. http://dx.doi.org/10.3389/fimmu.2019.02565 PMID: 31736978

[82] Lamas, B.; Natividad, J.M.; Sokol, H. Aryl hydrocarbon receptor and intestinal immunity. *Mucosal Immunol.,* **2018**, *11*(4), 1024-1038. http://dx.doi.org/10.1038/s41385-018-0019-2 PMID: 29626198

[83] Mezrich, J.D.; Fechner, J.H.; Zhang, X.; Johnson, B.P.; Burlingham, W.J.; Bradfield, C.A. An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *J. Immunol.,* **2010**, *185*(6), 3190-3198.

Author Proofs
'For Personal Use Only'

http://dx.doi.org/10.4049/jimmunol.0903670 PMID: 20720200

[84] Nguyen, N.T.; Kimura, A.; Nakahama, T.; Chinen, I.; Masuda, K.; Nohara, K.; Fujii-Kuriyama, Y.; Kishimoto, T. Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity *via* a kynurenine-dependent mechanism. *Proc Natl Acad Sci.,* **2010**, *107*(46), 19961-6. http://dx.doi.org/10.1073/pnas.1014465107

[85] Menni, C.; Kastenmüller, G.; Petersen, A.K.; Bell, J.T.; Psatha, M.; Tsai, P.C.; Gieger, C.; Schulz, H.; Erte, I.; John, S.; Brosnan, M.J.; Wilson, S.G.; Tsaprouni, L.; Lim, E.M.; Stuckey, B.; Deloukas, P.; Mohney, R.; Suhre, K.; Spector, T.D.; Valdes, A.M. Metabolomic markers reveal novel pathways of ageing and early development in human populations. *Int. J. Epidemiol.,* **2013**, *42*(4), 1111-1119. http://dx.doi.org/10.1093/ije/dyt094 PMID: 23838602

[86] Fanos, V.; Puddu, M.; Mussap, M. OMICS technologies and personalized vaccination in the COVID-19 era. *Journal of Pediatric and Neonatal Individualized Medicine,* **2022**, *11*(1), e110114. http://dx.doi.org/10.7363/110114

[87] Arunachalam, P.S.; Scott, M.K.D.; Hagan, T.; Li, C.; Feng, Y.; Wimmers, F.; Grigoryan, L.; Trisal, M.; Edara, V.V.; Lai, L.; Chang, S.E.; Feng, A.; Dhingra, S.; Shah, M.; Lee, A.S.; Chinthrajah, S.; Sindher, S.B.; Mallajosyula, V.; Gao, F.; Sigal, N.; Kowli, S.; Gupta, S.; Pellegrini, K.; Tharp, G.; Maysel-Auslender, S.; Hamilton, S.; Aoued, H.; Hrusovsky, K.; Roskey, M.; Bosinger, S.E.; Maecker, H.T.; Boyd, S.D.; Davis, M.M.; Utz, P.J.; Suthar, M.S.; Khatri, P.; Nadeau, K.C.; Pulendran, B. Systems vaccinology of the BNT162b2 mRNA vaccine in humans. *Nature,* **2021**, *596*(7872), 410-416. http://dx.doi.org/10.1038/s41586-021-03791-x PMID: 34252919

[88] Karagiannis, F.; Peukert, K.; Surace, L.; Michla, M.; Nikolka, F.; Fox, M.; Weiss, P.; Feuerborn, C.; Maier, P.; Schulz, S.; Al, B.; Seeliger, B.; Welte, T.; David, S.; Grondman, I.; de Nooijer, A.H.; Pickkers, P.; Kleiner, J.L.; Berger, M.M.; Brenner, T.; Putensen, C.; Abdullah, Z.; Latz, E.; Schmidt, S.; Hartmann, G.; Streeck, H.; Kümmerer, B.M.; Kato, H.; Garbi, N.; Netea, M.G.; Hiller, K.; Placek, K.; Bode, C.; Wilhelm, C. Impaired ketogenesis ties metabolism to T cell dysfunction in COVID-19. *Nature,* **2022**, *609*(7928), 801-807. http://dx.doi.org/10.1038/s41586-022-05128-8 PMID: 35901960

[89] McClenathan, B.M.; Stewart, D.A.; Spooner, C.E.; Pathmasiri, W.W.; Burgess, J.P.; McRitchie, S.L.; Choi, Y.S.; Sumner, S.C.J. Metabolites as biomarkers of adverse reactions following vaccination: A pilot study using nuclear magnetic resonance metabolomics. *Vaccine,* **2017**, *35*(9), 1238-1245. http://dx.doi.org/10.1016/j.vaccine.2017.01.056 PMID: 28169076

[90] Sasaki, E.; Kusunoki, H.; Momose, H.; Furuhata, K.; Hosoda, K.; Wakamatsu, K.; Mizukami, T.; Hamaguchi, I. Changes of urine metabolite profiles are induced by inactivated influenza vaccine inoculations in mice. *Sci. Rep.,* **2019**, *9*(1), 16249. http://dx.doi.org/10.1038/s41598-019-52686-5 PMID: 31700085

[91] Koeken, V.A.C.M.; Qi, C.; Mourits, V.P.; de Bree, L.C.J.; Moorlag, S.J.C.F.M.; Sonawane, V.; Lemmers, H.; Dijkstra, H.; Joosten, L.A.B.; van Laarhoven, A.; Xu, C.J.; van Crevel, R.; Netea, M.G.; Li, Y. Plasma metabolome predicts trained immunity responses after antituberculosis BCG vaccination. *PLoS Biol.,* **2022**, *20*(9), e3001765. http://dx.doi.org/10.1371/journal.pbio.3001765 PMID: 36094960

Author Proofs
"For Personal Use Only"