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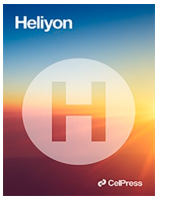

Research article

A non-targeted metabolomics comparative study on plasma of pfizer and sinopharm COVID-19 vaccinated individuals, assessed by (TIMS-QTOF) mass spectrometry

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ABSTRACT

COVID-19 is a highly contagious infectious disease that has posed a global threat, leading to a widespread pandemic characterized by multi-organ complications and failures. Aims: The present study was conducted to evaluate the impact of Pfizer and Sinopharm vaccines on metabolomic changes and their correlations with immune pathways. Main methods: The study used a cross-sectional design and implemented an untargeted metabolomics-based approach. Plasma samples were obtained from three groups: non-vaccinated participants, Sinopharm-vaccinated participants, and Pfizer-vaccinated participants. Comparative metabolomic analysis was conducted using TIMS-QTOF, and multiple t-tests with a 5 % false discovery rate (FDR) were performed using MetaboAnalyst software. Key findings: Out of the 105 metabolites detected, 72 showed statistically significant changes (p-*value* < 0.05) across the different groups. Notably, several metabolites such as neopterin, pyridoxal, and syringic acid were markedly altered in individuals vaccinated with Pfizer. Conversely, in the Sinopharm-vaccinated group, significant alterations were observed in sphinganine, neopterin, and sphingosine. These metabolites hold potential as biomarkers for evaluating vaccine efficacy. Additionally, both Pfizer and Sinopharm vaccinations were found to influence sphingolipid and histidine metabolisms compared to the control group.

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The Sinopharm group also displayed changes in lysine degradation relative to the control group. When comparing the enriched pathways between the Pfizer and Sinopharm-vaccinated groups, differences were observed in purine metabolism. Furthermore, alterations in tryptophan and vitamin B6 metabolism were noted when comparing the Pfizer-vaccinated group with both the control and Sinopharm-vaccinated groups. Significance: These findings highlight the importance of metabolomics in assessing vaccine effectiveness and identifying potential biomarkers for monitoring the efficacy of newly developed vaccines in a shorter timeframe.

1. Introduction

The financial repercussions of COVID-19 had a considerable impact on individuals and society. This urgency has led to a race among pharmaceutical companies to develop vaccines that can mitigate the effects of COVID-19. The COVID-19 pandemic expedited the development of the COVID-19 vaccine, compressing the timeline for Phase I completion from the typical 3–9 years to just 6–9 months. Global efforts to combat COVID-19 have relied heavily on vaccines manufactured by Sinopharm and Pfizer-BioNTech [1]. These vaccines can stimulate the immune system and provide protection against COVID-19 by utilizing distinct technologies. Inactivated vaccines, such as Sinopharm, contain weakened or inactivated SARS-CoV-2 virus that the body recognizes and defends itself from future infections once it is administered, triggering an immune response [2]. The Pfizer-BioNTech vaccine, messenger RNA (mRNA) vaccine, uses a different approach. A small piece of the virus's genetic material, specifically mRNA, instructs the cells to produce the spike protein, and then an immune response is triggered by this protein, training the body to recognize and respond to the virus if it is encountered [3].

Although the mecha nisms of action of these vaccines differ, both have proven effective against COVID-19 and have contributed to global vaccination campaigns. Clinical studies have shown varying levels of vaccine effectiveness among individuals who received different vaccines. The Pfizer-BioNTech vaccine's effectiveness in adults has been estimated to be 96 % [4]. On the other hand, studies have shown that adults who have been fully vaccinated with the Sinopharm vaccine have reported vaccine effectiveness of approximately 81 % [5]. These findings indicate that there are differences in the immunity levels and immune responses generated by the two vaccines.

Previous studies evaluated the immunogenicity and safety of COVID-19 vaccines, as well as the involvement of specific immune cell types and cytokines [6]. Vaccine-induced protection involved not only immune cells but also significant alterations in cellular metabolic pathways. To gain a comprehensive understanding of the immune response elicited by vaccines, researchers turned to integrative vaccinology, also known as vaccinomics [7]. This field utilizes high-throughput cellular and molecular omics technologies to explore and analyze various aspects of vaccine-induced immunity to unravel the complex mechanisms underlying vaccine efficacy and identify key molecular players and pathways involved in the immune response to vaccination. The rapid evaluation of vaccine effectiveness and the identification of specific metabolite alterations play a crucial role in mitigating epidemics and pandemics [8]. Recent studies have employed multiple omics analyses, such as single-cell RNA sequencing (scRNA-seq), proteomics, metabolomics, and lipidomics, to compare the immunological characteristics of COVID-19 patients with those of healthy individuals. By utilizing a multi-omics approach and conducting in vitro immune response analyses, it becomes possible to assess the protective effects of vaccines, aid in vaccine development, and identify the pathways involved in antibody production [9]. This study aims to identify metabolomic signatures associated with immune responses following the administration of Pfizer and Sinopharm in a Jordanian cohort. UHPLC-ESI-QTOF-MS technology was used to perform advanced monitoring of the efficacy of novel and newly developed vaccines.

2. Material and methods

2.1. Study design and samples collection

A total of 340 subjects, aged 10–85 years, were enrolled from Al-Quds Medical Labs in Al-Zarqa, Jordan. Blood samples were collected from three groups: 77 healthy individuals who had not received any COVID-19 vaccines, 107 individuals who received the

Table 1
Patient's demographics data.

	Patient's demographics			
	Total number of samples (n = 340)	Control (n = 77)	Pfizer vaccinated (n = 156)	Sinopharm vaccinated (n = 107)
Females	215 (63.23 %)	62 (80.51 %)	92 (58.97 %)	61 (57 %)
Males	125 (36.76 %)	15 (19.48 %)	64 (41.02 %)	46 (42.99 %)
Age (years)	8–90	8–90	14–85	19–85
Participants aged less than 30 years	178 (52.35 %)	42 (54.55 %)	81 (51.9 %)	55 (51.4 %)
Participants aged more than 30 years	162 (47.65 %)	35 (45.45 %)	75 (48.1 %)	52 (48.6 %)

Sinopharm vaccine, and 156 individuals who received the Pfizer vaccine. The blood samples were collected in heparinized tubes and centrifuged for 5 min to obtain plasma.

The study was approved by the Institutional Review Board at The University of Jordan, and all participants provided informed consent prior to sample collection. Exclusion criteria included individuals who tested positive for COVID-19, received booster doses, a third dose of any COVID-19 vaccine, or a single dose of either the Pfizer or Sinopharm vaccines.2.2. Preparation of samples for metabolites extraction.

After aliquoting the samples into 100 μL portions in Eppendorf tubes, 300 μL of methanol from Wunstorfer Strasse, Seelze, Germany, was added. The tubes were then vortexed and incubated at −20 °C for 2 h. Post-incubation, the samples were vortexed again and centrifuged for 15 min at 14,000 rpm. The resulting supernatant was evaporated at 35–40 °C using speed vacuum evaporation.

To evaluate the repeatability of the analysis, a quality control (QC) sample was prepared by pooling equal volumes (10 μL) from each sample. The extracted samples were resuspended in 100 μL of Honeywell's LC-MS CHROMASOLV 0.1 % formic acid in deionized water (Wunstorfer Strasse, Seelze, Germany). Following resuspension, 100 μL of the prepared sample was filtered through a 0.45 μm hydrophilic nylon syringe filter and collected in inserts within LC glass vials for LC-MS/MS analysis.2.3. Ultra-high-performance liquid chromatography coupled with electrospray ionization and quadrupole time of flight mass spectrometry (UHPLC-ESI-QTOF-MS)

For the untargeted analysis, we employed an ultra-high-performance liquid chromatography (UHPLC) system from Bruker Daltonic GmbH, Bremen, Germany, coupled with a quadrupole time-of-flight mass spectrometer (QTOF). The system included an electrospray ionization (ESI) source, a solvent delivery pump (HPG 1300), an autosampler, and a thermostated column compartment. Data management was handled using Bruker Compass HyStar 5.0 SR1 Patch1 (5.0.37.1) and Compass 4.1 for otofSeries, Control Version 6.2 software. The mobile phases used were A (water with 0.1 % formic acid) and B (acetonitrile with 0.1 % formic acid). The gradient program was as follows: from 0 to 2 min, 99 % A and 1 % B; from 2 to 17 min, 99 %–1 % A and 1 %–99 % B; from 17 to 20 min, 99 % B and 1 % A. The flow rate was maintained at 0.25 mL/min. Then, from 20 to 20.1 min, the composition was adjusted to 99 % B and 1 % A, with the flow rate increased to 0.35 mL/min. From 28.5 to 30 min, the composition was reverted to 1 % B and 99 % A, with the flow rate reduced back to 0.25 mL/min. A 10 μL sample was injected into a Hamilton® Intensity Solo 2C18 column (100 mm × 2.1 mm × 1.8 μm) maintained at a column oven temperature of 35 °C. The separation method, approved by Bruker, initially utilized 99 % water as the first solvent, allowing more polar metabolites to elute first, while the less polar metabolites were retained longer [10].To enhance separation based on polarity, we implemented an acetonitrile (ACN) gradient that gradually increased to elute less polar metabolites [11]. We maintained a micro flow rate of 0.25 mL/min, with an intermediate flow rate of 0.35 mL/min for column washing. The electrospray ionization (ESI) source conditions were kept consistent for each injection, including a drying gas flow rate of 10.0 L/min at 220 °C, a capillary voltage of 4500 V, and a nebulizer pressure of 2.2 bar. For MS2 acquisition, we used collision energy stepping between 100 % and 250 % set at 20 eV and an End Plate offset of 500 V. Sodium formate served as an external calibrant during acquisition, which included an auto MS scan and an auto MS/MS segment. Both segments were performed in positive ionization mode at 12 Hz, with an automatic in-run mass scan range of 20–1300 *m/z*. The active exclusion was configured to exclude after 3 spectra and reintroduce after 0.2 min. This method followed a protocol approved by Bruker, with system stability confirmed by testing various reconstitution solvents.

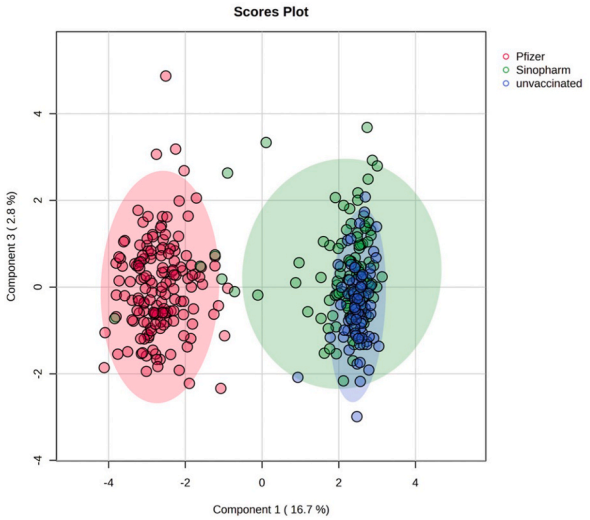

Fig. 1. A well-defined alteration in the metabolomic profile across all the groups was noticed using sparse partial least squares-discriminant analysis (sPLS-Da). Red color denotes Pfizer-vaccinated individuals, the green color denotes Sinopharm-vaccinated individuals, while the blue color denotes unvaccinated individuals.

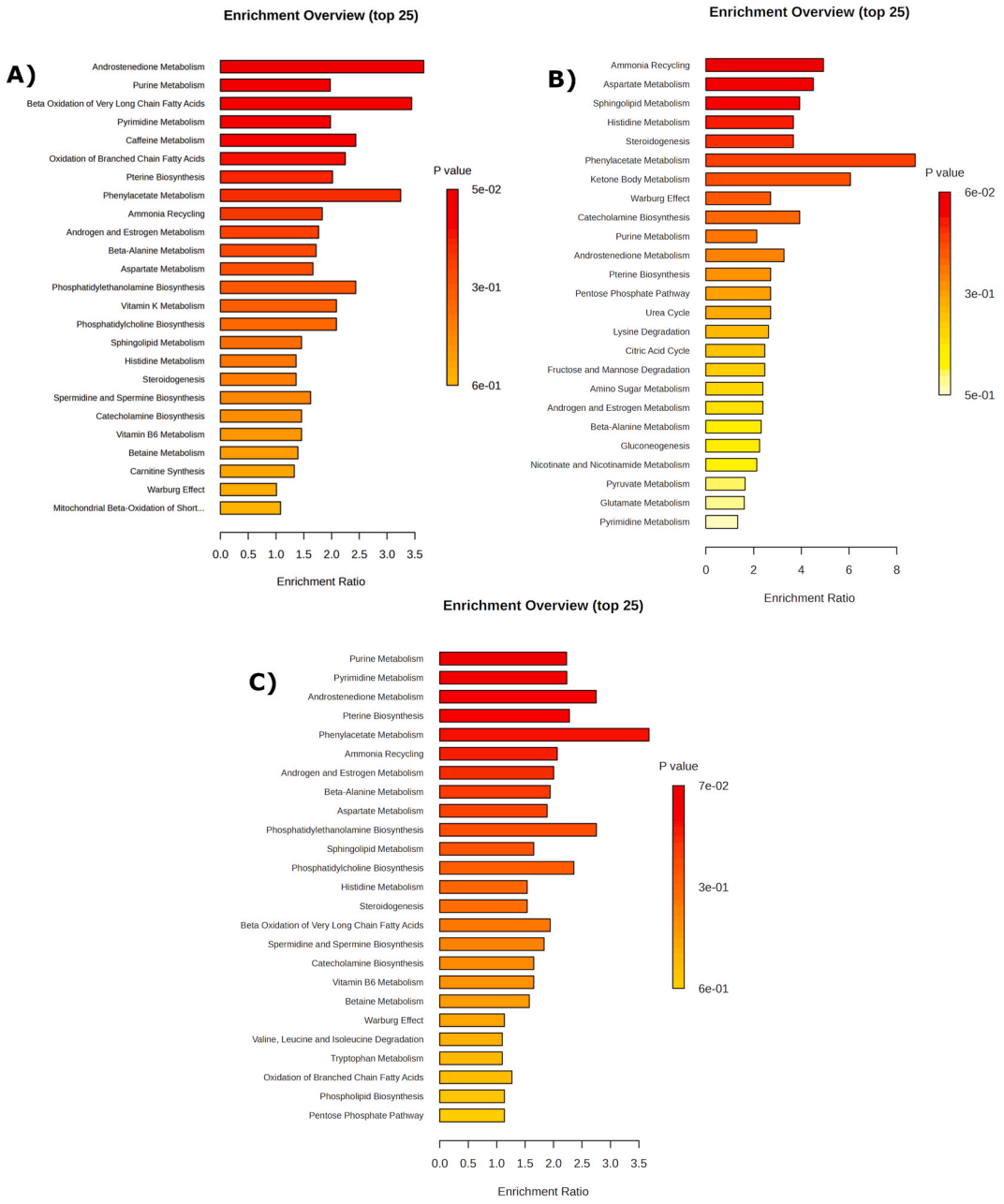

Fig. 2. Metabolomic functional enrichment analysis of significantly altered metabolites in **A)** Pfizer-vaccinated individuals vs control. **B)** Sinopharm-vaccinated individuals vs control. **C)** Pfizer-vaccinated individuals and Sinopharm-vaccinated individuals. Colour intensity indicates significance, the darker the colour, represents a higher significance.

2.2. Data processing and analysis

The MetaboScape® 4.0 program was used to evaluate the data (Bruker Daltonics). The following data bucketing parameters were used by the T-ReX 2D/3D workflow: The feature's magnitude is determined by examining the area of its peak, with a minimum measured intensity of 1000 and a minimum peak length of 7. It took 0–0.3 min for the mass spectral calibration to complete and contained characteristics from 100 to 365 samples. On the other hand, auto MS/MS scanning was carried out in a fairly standard manner. The mass range for the scan was 20–1300 *m/z*, while the retention time range was 0.3–25 min. 340 samples from the three groups under examination were pooled to form a data collection with 9281 features by utilizing LC-QTOF to evaluate each sample twice.

The MS/MS spectra and retention times were mapped to the human metabolite database (HMDB) 4.0.

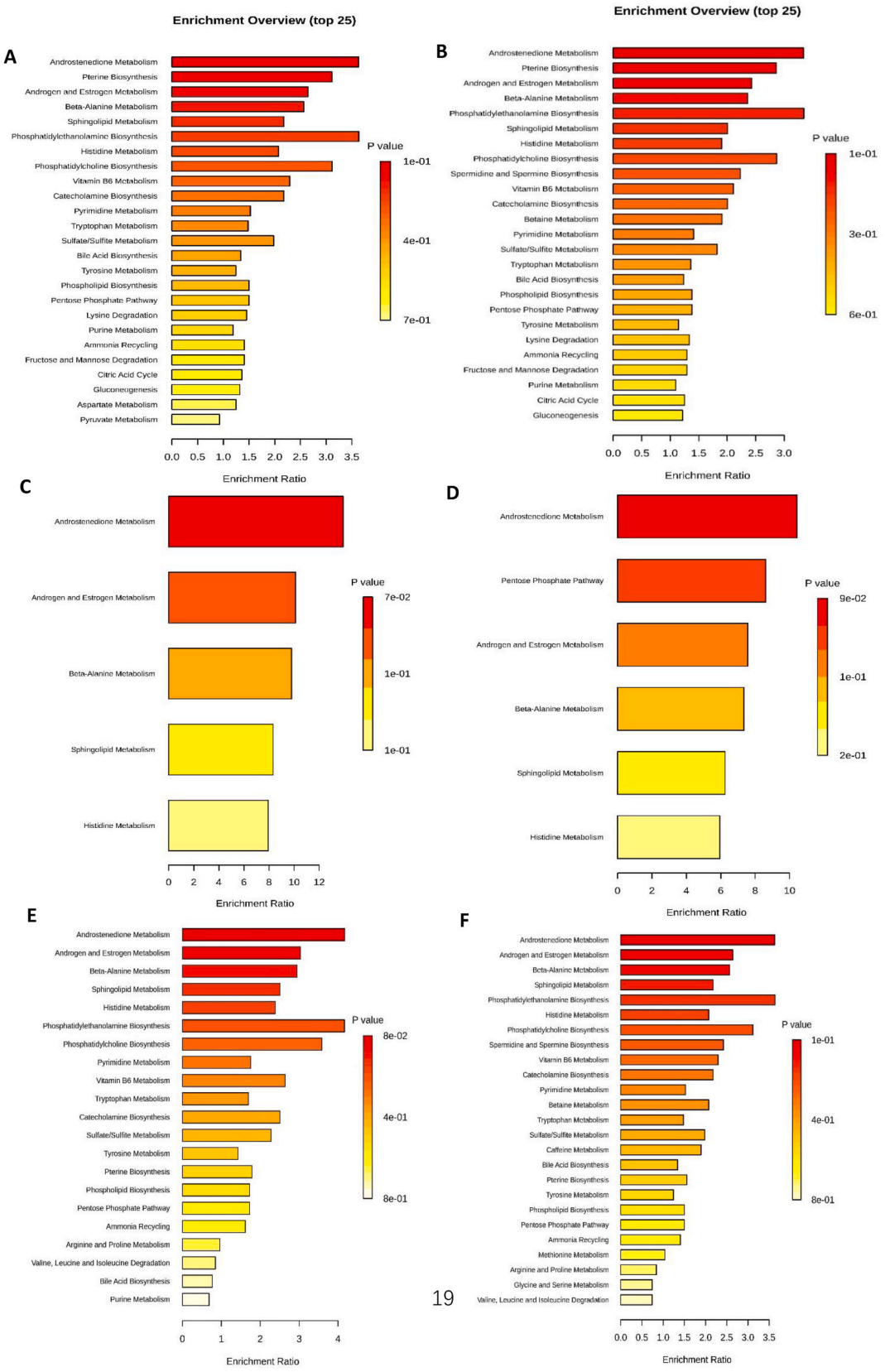

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Fig. 3. Metabolomic functional enrichment analysis of significantly altered metabolites across all groups age. **A and B** represent Pfizer-vaccinated individuals aged less than 30 years and more than 30 years respectively. **C and D** represent Sinopharm-vaccinated individuals aged less than 30 years and more than 30 years respectively. **E and F** represent Pfizer-vaccinated individuals compared to Sinopharm-vaccinated individuals aged less than 30 years and more than 30 years respectively. Colour intensity indicates significance, the darker the colour, represents a higher significance.

While annotating the compounds, the spectrum library was compared to identify the compounds with MS/MS. Following that, a group of chosen metabolites was filtered using parameters including the AQ score, retention time values, MS/MS score, *m/z* values, mSigma, and analyte list spectral library. There were still 105 distinct metabolites after this filtering. The peak intensity of each metabolite was used to quantify the data matrix. Only important substances that were reported in HMDB 4.0, which has 850 metabolites overall, have been incorporated into the metabolite databases. The metabolites files were generated as a CSV file and integrated into the comprehensive method for metabolomics data analysis known as MetaboAnalyst 5.0 software.

The sPLS-DA was carried out to determine the most distinctive characteristics in the analyzed group for labeled samples. Multiple hypothesis testing was corrected by applying 5 % FDR.

2.3. Metabolic pathway and statistical analysis

To process the enrichment metabolite sets and pathway analysis, MetaboAnalyst version 5.0 software was used [12]. Multiple unpaired t-tests were conducted for pairwise comparisons between the groups (Unvaccinated and Sinopharm vaccinated group; Unvaccinated and Pfizer vaccinated group; Sinopharm and Pfizer vaccinated group). A *p-value* threshold of <0.05 was used to determine statistical significance and it was corrected by applying 5 % FDR.

Table 2
The statistically significant metabolites among Pfizer vaccinated individuals and controls.

Metabolite name	t.Stat	p.Value	FDR	Fold Change
Neopterin	10.892	1.45E-22	7.31E-22	140.01
3,4-Dihydroxymandelic acid	28.388	2.84E-77	7.53E-76	22.581
Urocanic acid	8.4983	2.40E-15	7.47E-15	19.907
Pyridoxal	15.574	7.19E-38	6.36E-37	13.359
Syringic acid	24.62	1.60E-66	3.39E-65	11.707
Succinylacetone	23.497	3.59E-63	4.75E-62	7.8155
Aspartyl-lysine	33.978	7.93E-92	4.20E-90	7.3405
Phosphocreatine	19.871	6.66E-52	7.84E-51	6.8864
Canavanine	16.298	2.88E-40	3.05E-39	5.99
1,3-Dimethyluracil	33.105	1.17E-89	4.15E-88	5.3327
5-Methoxytryptophol	9.3251	9.33E-18	3.00E-17	5.3009
Nutriacholic acid	48.347	8.33E-123	8.83E-121	5.0389
Dihydrothymine	23.936	1.73E-64	2.61E-63	4.8264
Saccharopine	15.076	3.21E-36	2.62E-35	3.6133
Ribose	12.909	4.61E-29	2.87E-28	3.3863
L-Dopa	16.228	4.90E-40	4.72E-39	2.7839
3-Methylxanthine	3.9673	9.70E-05	2.10E-04	2.0153
6-(Methylamino) purine	−9.6734	8.42E-19	2.98E-18	0.48233
Cortisol	−11.782	2.11E-25	1.17E-24	0.47762
p-Octopamine	−7.0634	1.90E-11	5.29E-11	0.44396
Guanosine 5′-triphosphate	−10.316	9.12E-21	3.62E-20	0.43342
Sphingosine	−9.4445	4.11E-18	1.40E-17	0.36942
Coumarin	−10.389	5.40E-21	2.29E-20	0.3122
Sphinganine	−10.268	1.28E-20	4.84E-20	0.28339
Xanthine	−9.4369	4.33E-18	1.43E-17	0.26045
m-Coumaric acid	−11.51	1.58E-24	8.38E-24	0.25515
Uracil	−10.882	1.55E-22	7.49E-22	0.24758
2,4-Diaminobutyric acid	−24.065	7.09E-65	1.25E-63	0.21628
Butanone	−8.2979	8.85E-15	2.68E-14	0.17935
Testosterone	−10.581	1.37E-21	6.03E-21	0.16644
Glycocholic acid	−5.1723	5.01E-07	1.33E-06	0.1522
Quinaldic acid	−14.025	9.68E-33	6.41E-32	0.13314
L-Kynurenine	−10.314	9.23E-21	3.62E-20	0.13301
Ethanolamine	−14.5	2.60E-34	1.97E-33	0.10855
Androstenedione	−7.5321	1.12E-12	3.21E-12	0.075751
Serotonin	−14.282	1.37E-33	9.71E-33	0.033592
Deoxycholic acid glycine conjugate	−12.415	1.88E-27	1.11E-26	0.018838

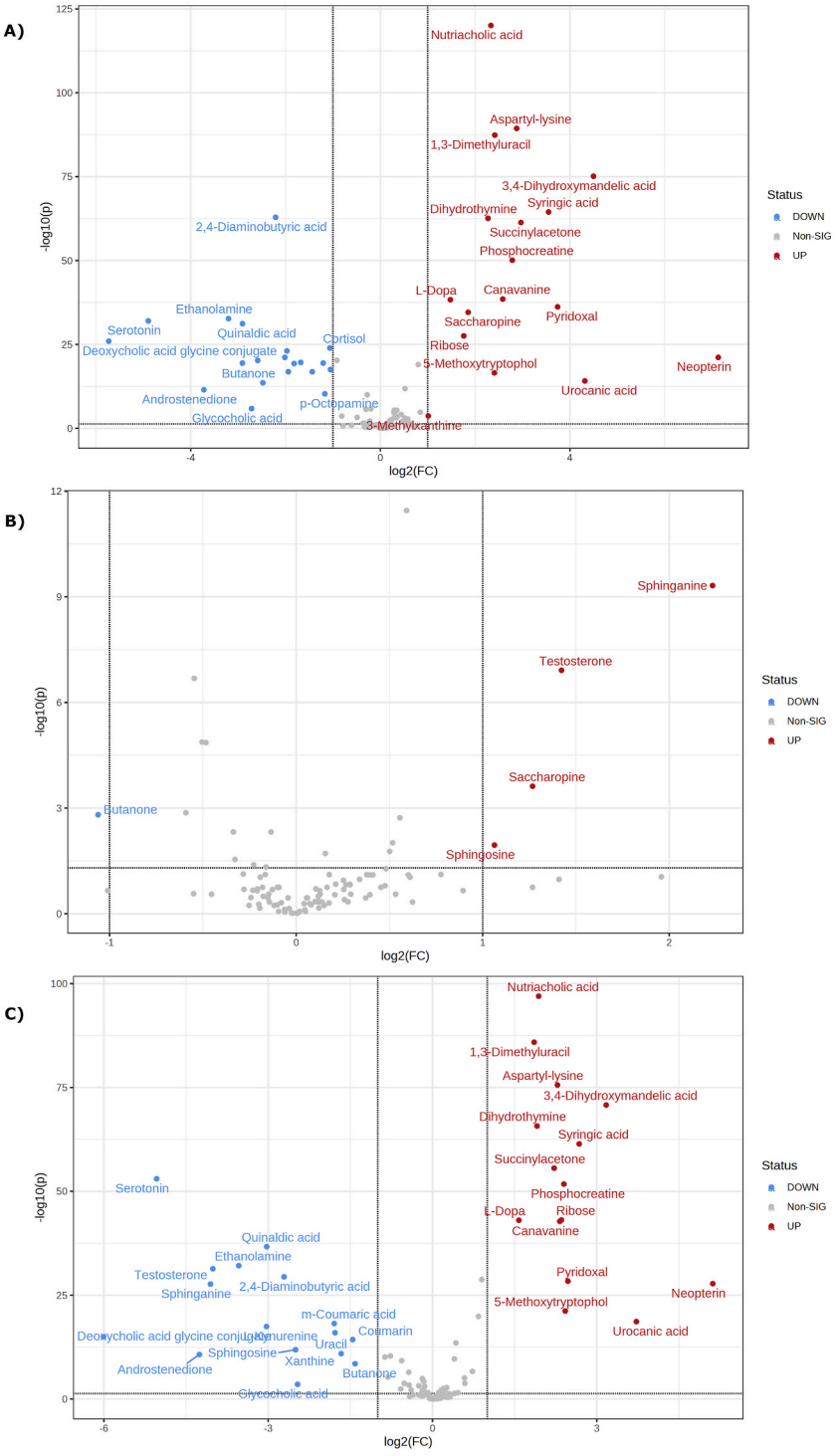

Fig. 4. Volcano plots showing the metabolites' abundance across all groups. X-axis indicates $\log_2$(fold-change) plotted against the -$\log_{10}$(*q-value*) in the y-axis. **A)** Pfizer-vaccinated individuals vs control. **B)** Sinopharm-vaccinated individuals vs control. **C)** Pfizer-vaccinated individuals and Sinopharm-vaccinated individuals.

Table 3
The statistically significant metabolites among Sinopharm vaccinated individuals and controls.

Metabolite name	t.Stat	p.Value	FDR	Fold change
Sphinganine	8.5199	7.31E-14	2.58E-12	4.6994
Neopterin	2.6556	0.0090738	0.039253	3.8861
Testosterone	7.05	7.27E-11	1.93E-09	2.681
Saccharopine	5.2087	8.99E-07	1.59E-05	2.4078
Sphingosine	3.7084	0.00030885	0.0029762	2.0897
Succinylacetone	2.6688	0.0083806	0.038623	1.713
Aspartyl-lysine	2.7168	0.0076081	0.036657	1.5171
Urocanic acid	9.0914	3.04E-16	3.22E-14	1.5074
Ribose	−4.1291	5.56E-05	0.00061767	0.66379
Butanone	−3.5133	0.00066624	0.0049322	0.47902

FDR: false discovery rate.

Table 4
The statistically significant metabolites among Pfizer vaccinated individuals and Sinopharm vaccinated individuals.

Metabolite name	t.Stat	P.Value	FDR	Fold change
Neopterin	12.525	1.69E-28	9.41E-28	34.738
Urocanic acid	9.7521	2.32E-19	1.07E-18	13.206
3,4-Dihydroxymandelic acid	25.086	1.68E-71	4.45E-70	9.0116
Syringic acid	22.226	3.83E-62	6.77E-61	6.4044
Pyridoxal	12.699	4.29E-29	2.53E-28	5.5535
5-Methoxytryptophol	10.554	6.45E-22	3.26E-21	5.3675
Phosphocreatine	19.387	1.79E-52	2.11E-51	5.2768
Ribose	16.91	8.00E-44	8.48E-43	5.1143
Canavanine	16.818	1.68E-43	1.48E-42	5.0095
Aspartyl-lysine	26.607	2.64E-76	9.34E-75	4.8652
Succinylacetone	20.502	2.60E-56	3.94E-55	4.6633
Nutriacholic acid	33.939	1.05E-97	1.11E-95	3.8332
Dihydrothymine	23.525	1.91E-66	4.05E-65	3.7573
1,3-Dimethyluracil	30.018	1.27E-86	6.75E-85	3.622
L-Dopa	16.892	9.25E-44	8.91E-43	2.9858
Butanone	−6.1316	3.20E-09	8.93E-09	0.37546
Coumarin	−8.3209	4.91E-15	1.79E-14	0.36434
Xanthine	−7.0986	1.19E-11	3.95E-11	0.31478
Uracil	−8.8777	1.12E-16	4.40E-16	0.29131
m-Coumaric acid	−9.6034	6.78E-19	2.99E-18	0.28837
Glycocholic acid	−3.6715	0.00029246	0.0006596	0.18131
Sphingosine	−7.4503	1.37E-12	4.67E-12	0.17677
2,4-Diaminobutyric acid	−13.002	3.90E-30	2.58E-29	0.15275
Quinaldic acid	−15.083	2.16E-37	1.76E-36	0.12266
L-Kynurenine	−9.3714	3.54E-18	1.45E-17	0.12235
Ethanolamine	−13.778	7.95E-33	6.02E-32	0.086121
Testosterone	−13.564	4.43E-32	3.13E-31	0.062066
Sphinganine	−12.492	2.20E-28	1.17E-27	0.060201
Androstenedione	−7.0162	1.96E-11	6.29E-11	0.052329
Serotonin	−19.753	9.69E-54	1.28E-52	0.030449
Deoxycholic acid glycine conjugate	−8.5477	1.07E-15	4.05E-15	0.01555

3. Results

3.1. Participants and blood sample characteristics

All study participants were Jordanians, with 215 females (63.23 %) and 125 males (36.76 %). The participant's age varied from 8 to 90 years old between the three groups (Table 1).

3.2. Metabolic changes

The sparse partial least squares-discriminant analysis (sPLS-DA) showed minimal overlap between the Pfizer vaccinated group compared to the overlapped groups of the Sinopharm vaccinated group and the unvaccinated control group, indicating statistically significant differences among these groups (as depicted in Fig. 1).

Fig. 2 presents the functional enrichment analysis of significantly altered metabolites. In Fig. 2A, the altered metabolic pathways are shown for the comparison between the Pfizer-vaccinated group and the unvaccinated group, highlighting specific pathways that exhibit significant changes due to the Pfizer vaccine. Fig. 2B illustrates the altered pathways for the comparison between the

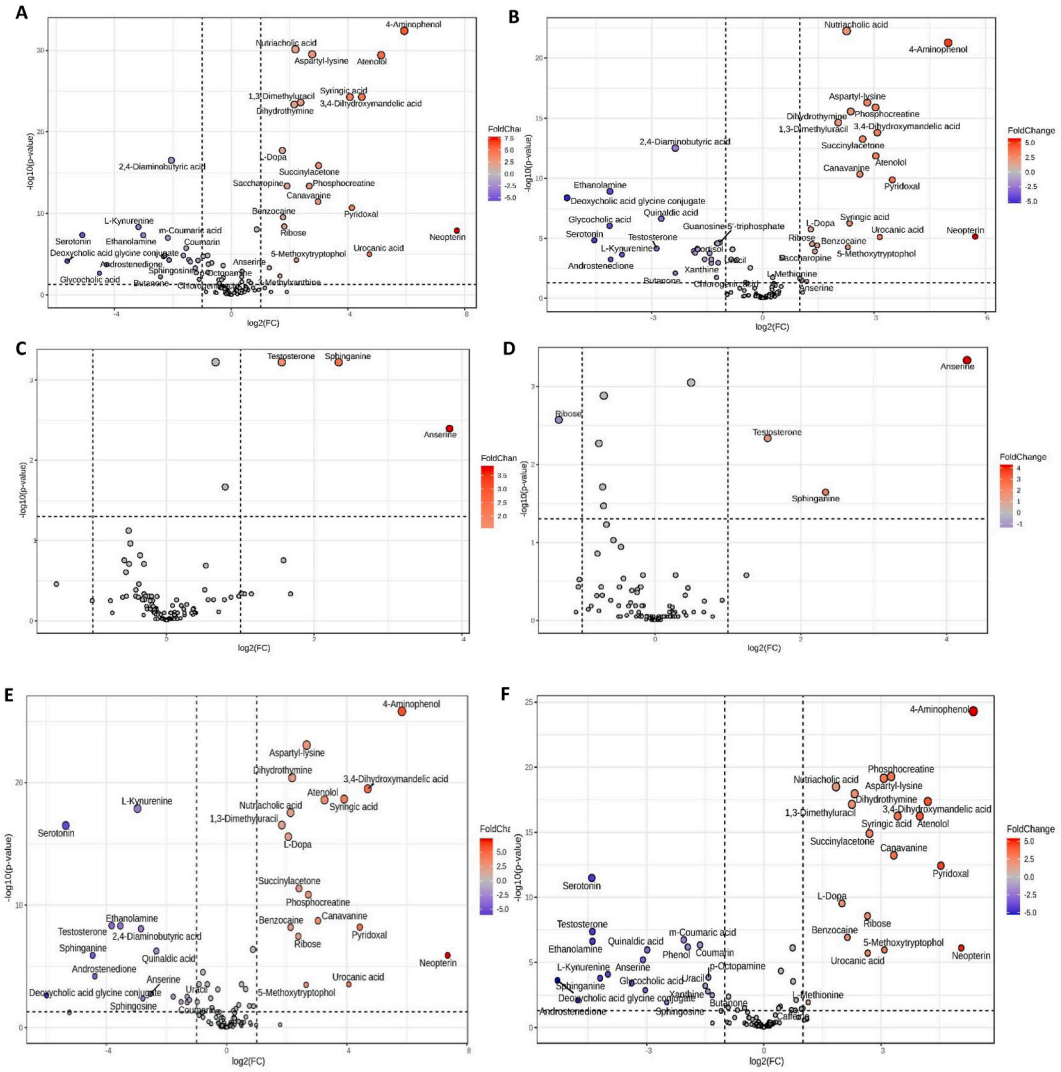

Fig. 5. Volcano plots showing the metabolites' abundance across all groups age. X-axis indicates $\log_2$(fold-change) plotted against the -$\log_{10}$(q-value) in the y-axis. **A and B** represent Pfizer-vaccinated individuals compared to control in patients aged less than 30 years and more than 30 years respectively. **C and D** represent Sinopharm-vaccinated individuals compared to control in patients aged less than 30 years and more than 30 years respectively. **E and F** represent Pfizer-vaccinated individuals compared to Sinopharm-vaccinated individuals in patients aged less than 30 years and more than 30 years respectively.

Sinopharm-vaccinated group and the unvaccinated group, identifying the metabolic shifts associated with the Sinopharm vaccine. Lastly, Fig. 2C highlights the significantly altered metabolic pathways when comparing individuals vaccinated with Sinopharm to those who received the Pfizer vaccine, providing insights into the differential metabolic responses elicited by the two vaccines.Fig. 3 illustrates the enriched pathways of significantly altered metabolites in patients aged above and below 30 years across different groups. Some differences were observed in the enriched metabolic pathways between participants above and below 30 years who received the Pfizer vaccine. For instance, spermidine and spermine biosynthesis was enriched in patients above 30 years but not in those below 30 years (Fig. 3A and B). Additionally, the pentose phosphate pathway was enriched in participants above 30 years who received the Sinopharm vaccine, a pattern not observed in participants below 30 years (Fig. 3C and D). Furthermore, when comparing Sinopharm-vaccinated individuals to those who received the Pfizer vaccine, differences were observed in enriched metabolic pathways, such as betaine metabolism, which was enriched in participants above 30 years but not in those below 30 years (Fig. 3E and F). Three binary comparisons were made. In the comparison between the control group and the Pfizer vaccinated individuals, a total of 38 metabolites exhibited significant differences (Table 2). Among these metabolites, 18 showed increased levels, while 20 displayed decreased levels. The five most notable metabolites with increased level in Pfizer vaccinated group were neopterin, 3,4-dihydroxymandelic acid, urocanic acid, pyridoxal, and syringic acid. Conversely, deoxycholic acid glycine conjugate, serotonin, androstenedione, ethanolamine, and cortisol exhibited considerably decreased levels in the Pfizer vaccinated group (as depicted in Fig. 4A).

Based on the *t*-test analysis, a comparison between the Sinopharm vaccinated group and the control group revealed significant

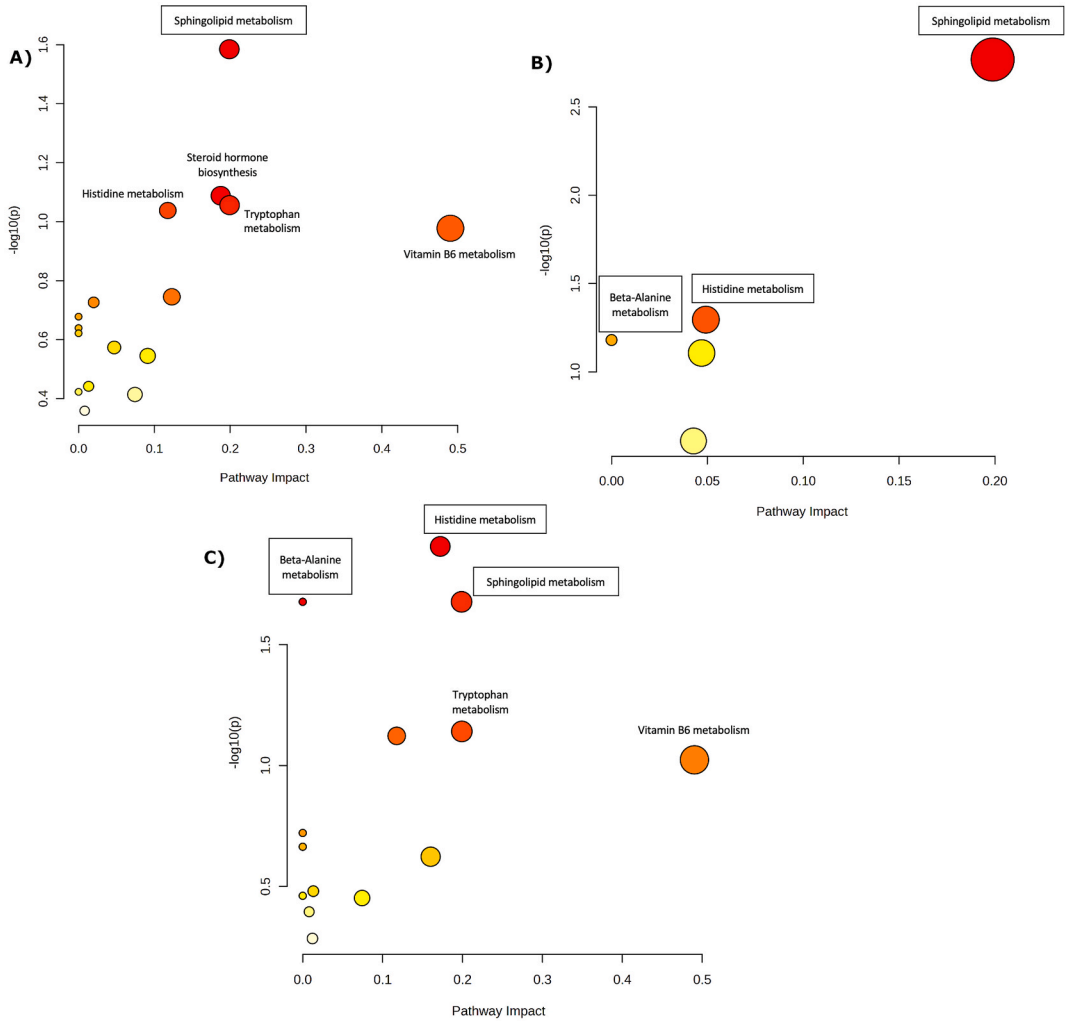

Fig. 6. Pathway analysis of the three groups **A)** Pfizer-vaccinated individuals vs control. **B)** Sinopharm-vaccinated individuals vs control. **C)** Pfizer-vaccinated individuals and Sinopharm-vaccinated individuals. Colour intensity indicates significance, the darker the colour, represents a higher significance.

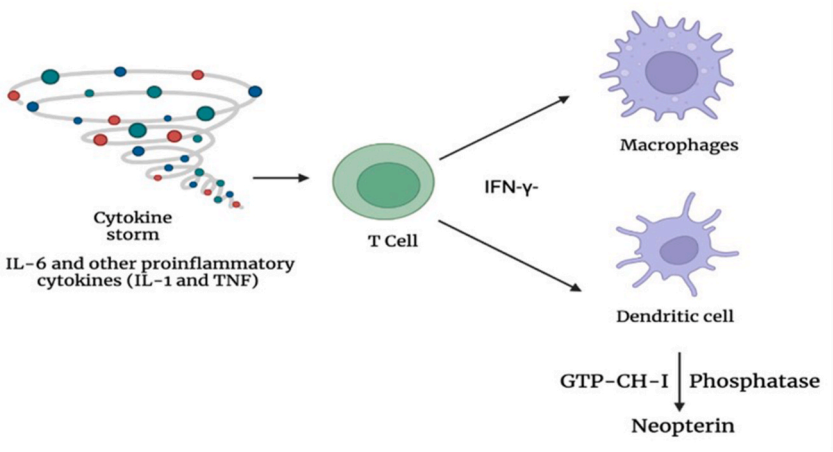

Fig. 7. The effect of the metabolite Neopterin on the immune system.

differences in 10 metabolites (Table 3). Among these metabolites, 8 exhibited increased levels in the Sinopharm vaccinated group, while 2 displayed decreased levels. Notably, the Sinopharm vaccinated group showed significantly higher levels of sphinganine and neopterin compared to the control group. Conversely, the control group had higher levels of butanone and ribose compared to the Sinopharm vaccinated group (as shown in Fig. 4B).

The *t*-test analysis identified 32 metabolites that exhibited significant differences (Table 4) between the Pfizer vaccinated group and the Sinopharm vaccinated group. Among these metabolites, 15 showed increased levels in the Pfizer vaccinated group, while 17 displayed decreased levels. Notably, neopterin, urocanic acid, 3,4-dihydroxymandelic acid, and syringic acid were significantly higher in the Pfizer vaccinated group compared to the Sinopharm vaccinated group. Conversely, sphinganine, androstenedione, serotonin, and deoxycholic acid glycine conjugate were significantly decreased in the Pfizer vaccinated group compared to the Sinopharm vaccinated group (as depicted in Fig. 4C).

While comparing participants aged less than 30 years across the groups ($q\text{-value} < 0.05$, fold change threshold = 2), 41 significantly altered metabolites were observed in the Pfizer-vaccinated group in comparison to the control group, with 21 metabolites increased and 20 metabolites decreased in the Pfizer-vaccinated individuals (Fig. 5A). For participants aged 30 years and older, the Pfizer-vaccinated group showed alterations in 43 metabolites compared to the control group, with 21 metabolites increased and 22 metabolites decreased (Fig. 5B).

The Sinopharm-vaccinated group, when compared to the control group, showed significant differences in 3 metabolites, all of which were increased in participants aged less than 30 years (Fig. 5C). For participants aged 30 years and older, the Sinopharm-vaccinated group revealed significant differences in 4 metabolites, with 3 metabolites increased and 1 metabolite decreased compared to the control group (Fig. 5D).

Additionally, when comparing the Pfizer-vaccinated group to the Sinopharm-vaccinated group for participants aged less than 30 years, 34 significantly altered metabolites were identified, with 16 metabolites increased in the Pfizer-vaccinated group and 18 metabolites increased in the Sinopharm-vaccinated group (Fig. 5E). When comparing the Pfizer-vaccinated group to the Sinopharm-vaccinated group for participants aged 30 years and older, 39 significantly altered metabolites were identified, with 19 metabolites increased in the Sinopharm-vaccinated group and 20 metabolites increased in the Pfizer-vaccinated group (Fig. 5F and refer to Table S1). Furthermore, we conducted a metabolic comparison between females and males who received the Pfizer or Sinopharm vaccine. This comparison is crucial as males and females often exhibit differences in metabolic pathways due to variations in hormones, body composition, and genetic factors, which can help tailor vaccine strategies. In the Pfizer-vaccinated group, there was no significant difference in the metabolic profile between males and females ($q\text{-value} > 0.05$). However, males who received the Sinopharm vaccine showed a significant increase in indolelactic acid and isovalerylcarnitine levels compared to females who received the same vaccine (See Table S2).

3.3. Functional analysis pathway changes

The first pathway analysis module focused on highly altered metabolites between the unvaccinated and Pfizer-vaccinated groups. Among the four metabolic pathways analyzed sphingolipid metabolism showed the most significant dysregulation ($p\text{-value} = 0.026$) (as shown in Fig. 6A).

Shingolipid metabolism ($p\text{-value} = 0.001706$) and histidine metabolism ($p\text{-value} = 0.050622$) were identified as affected metabolic pathways between the control group and the Sinopharm vaccinated group (as depicted in Fig. 6B).

Finally, a pathway analysis module was designed for the comparison between the Pfizer and Sinopharm-vaccinated groups. The metabolism of histidine ($p\text{-value} = 0.01241$), beta-alanine ($p\text{-value} = 0.02104$), and sphingolipids ($p\text{-value} = 0.02104$) was found to be significantly altered in both the Pfizer and Sinopharm vaccinated groups (as shown in Fig. 6C).

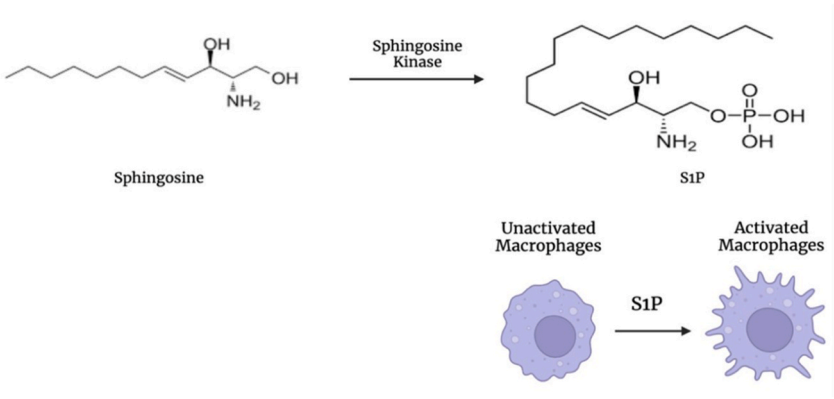

Fig. 8. The effect of the metabolite Sphingosine on the immune system.

4. Discussion

In this cross-sectional study, we compared the metabolome of individuals who received the Pfizer or Sinopharm COVID-19 vaccines with un-vaccinated controls using LC-MS/MS analysis.

Notably, individuals who received the Pfizer vaccine showed a significant increase in neopterin levels compared to both the Sinopharm-vaccinated group and unvaccinated controls. Neopterin is a critical biomarker for immune system activation. It is primarily produced by macrophages and dendritic cells in response to stimulation by interferon-γ (IFN-γ) (Fig. 7). This production is a hallmark of the Th1 immune response, which is essential for combating intracellular pathogens such as viruses and certain bacteria. IFN-γ stimulation leads to increased neopterin production by macrophages and dendritic cells, correlating with their enhanced activation and crucial roles in antigen presentation and the initiation of adaptive immune responses [13]. Furthermore, neopterin plays a role in regulating oxidative stress by generating reactive oxygen species (ROS), which are vital for pathogen clearance. This regulation ensures a balance between effective pathogen eradication and minimizing host tissue damage [14]. In our study, the significantly elevated neopterin levels observed in Pfizer-vaccinated individuals 35-fold higher than in the Sinopharm-vaccinated group and 140-fold higher than in unvaccinated controls indicate a robust immune activation. This aligns with previous findings on vaccines such as the MMR vaccine [15,16]. This finding highlights neopterin as a potential indicator of vaccination success and emphasizes its significance in assessing the efficacy of COVID-19 vaccines in activating the immune system against SARS-CoV-2 [16]. Monitoring neopterin levels could provide valuable insights into the immune response triggered by COVID-19 vaccination.

Moreover, our study found that the Pfizer-vaccinated group exhibited a roughly 5-fold increase in 5-methoxytryptophol compared to the Sinopharm-vaccinated group. 5-methoxytryptophol and melatonin, both released by the pineal hormone, possess immunomodulatory and antioxidant properties. Elevated 5-methoxytryptophol levels suggest immune protection resulting from mRNA vaccination [17]. Additionally, our study identified higher levels of syringic acid in the Pfizer-vaccinated group compared to both the control and Sinopharm-vaccinated groups. Syringic acid, known for its anti-inflammatory properties, is a major component in *Pistacia Lentiscus* leaf extract [18]. Syringic acid influences immune pathways through several mechanisms, making it a potential biomarker for vaccine-induced immune responses. Its strong antioxidant properties help scavenge free radicals and reduce oxidative stress, protecting immune cells and ensuring efficient operation during an immune response [19]. Additionally, syringic acid exhibits anti-inflammatory effects by inhibiting pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, thereby maintaining a balanced immune reaction and preventing tissue damage. It also modulates signaling pathways like NF-κB, which are pivotal in immune regulation, inflammation, and cell survival [20]. The increased levels of syringic acid observed in the Pfizer-vaccinated group approximately 11.7-fold higher than the control group and 6-fold higher than the Sinopharm-vaccinated group highlight its significant role in the immune response induced by mRNA vaccines. These findings suggest that syringic acid could serve as a biomarker for the immune response elicited by mRNA vaccines.

Furthermore, our data revealed a substantial increase approximately 7-fold in aspartyl-lysine levels in individuals who received the Pfizer vaccine compared to the control group. Lysine, a constituent amino acid, exhibits immune regulatory activity, and its deficiency can hinder protein production, including cytokines, impacting lymphocyte growth [21]. While aspartate contributes to immune regulation by aiding in the recycling of citrulline into arginine in activated macrophages [21,22]. This process, facilitated by nitric oxide synthase (iNOS), is vital for maintaining a high rate of nitric oxide (NO) generation in response to immunological challenges [23]. Activated macrophages, crucial in the immune response, release NO as a potent antimicrobial agent, inhibiting pathogen growth. Various immune cells, including neutrophils, dendritic cells, natural killer cells, and T cells, can produce and respond to NO, contributing to their respective immune functions [24]. The coordinated release of NO and other effector molecules by these cells represents a crucial mechanism for pathogen control and immune defense. In accordance with our results, increased aspartyl-lysine could be a potential biomarker for assessing the effectiveness of the Pfizer vaccine in stimulating immune responses.

Moreover, multiple pathways found to be dysregulated in in individuals who received the Pfizer or Sinopharm vaccine compared to the control group, including.

4.1. Sphingolipid metabolism pathway

Our results revealed significant alteration in the sphingolipid metabolism when comparing the Pfizer-vaccinated group, the Sinopharm-vaccinated group, and the control group. Additionally, there were notable differences between the Pfizer and Sinopharm-vaccinated groups (Fig. 6). Sphingolipids play a crucial role in innate immunity during infections, as demonstrated by several studies [25]. The metabolism of sphingolipids is involved in the regulation of inflammation and immune responses. One key aspect is the conversion of sphingosine into sphingosine-1-phosphate (S1P), which contributes to lymphocyte migration from lymphoid organs into the bloodstream due to the S1P gradient [25] (Fig. 8). Conversely, sphingosine inhibits the release of pro-inflammatory cytokines, mainly through the dephosphorylation of AKT and the p65 subunit of NFκB [26].

4.2. Histidine metabolism pathway

Vaccination response was positively associated with baseline amino acid levels, mostly according to our data on histidine, tryptophan, and lysine [27]. As most amino acids are required for the synthesis of antibodies and cytokines, amino acids and the immune response are closely connected. Through stimulating innate, adaptive, and regulatory immune responses [28]. Using the enrichment and pathway analysis, histidine metabolism was significantly impacted when comparing each Pfizer-vaccinated group and Sinopharm-vaccinated group with the control. Additionally, it was impacted in the Pfizer-vaccinated group vs. Sinopharm-vaccinated

group. (Figs. 2 and 6). Previously, immune reaction to acute inflammation has been linked to L-histidine [29]. This is thought to be caused by the imidazole functional group, which is involved in scavenging reactive oxygen species generated in cells during acute inflammatory responses.

4.3. Tryptophan metabolism pathway

Altered tryptophan metabolism was observed when comparing the Pfizer-vaccinated group with unvaccinated controls and the Sinopharm-vaccinated group (Fig. 6A and C). Tryptophan metabolism produces various immune-active metabolites called kynurenines, which have anti-inflammatory properties and influence energy homeostasis and behavior. Tryptophan metabolism, specifically the conversion of tryptophan to kynurenines by the enzyme indoleamine 2,3 dioxygenase 1 (IDO1), is an important immunomodulatory pathway that opposes hyperinflammation [30]. However, IL-6 can impair the ability of IDO1 to regulate immune homeostasis. Blocking IL-6 has been shown to counter COVID-19-associated cytokine release syndrome (CRS) [31].

4.4. Lysine dysregulation pathway

The degradation of lysine was found to be altered when comparing the Sinopharm vaccinated group with the unvaccinated control group (Fig. 6B). Lysin plays a crucial role in supporting various aspects of health, including bone health, skin integrity, and immune function [32]. In COVID-19, lysine has shown potential benefits. Studies have indicated that lysine supplementation can lead to an immediate elimination or significant reduction in fever in COVID-19 patients. These improvements in symptoms were observed within a short duration of 4–18 h, suggesting that lysine may have a rapid effect in improving symptoms [33,34].

4.5. Purine metabolism pathway

The analysis of enriched pathways revealed that purine metabolism was impacted when comparing the Pfizer-vaccinated group vs. Sinopharm-vaccinated group (Fig. 2). Purine nucleotides have long been recognized as important regulators of immune responses [35]. They provide energy and cofactors that support cell viability and growth. The purinergic signaling network, mediated by both pro- and anti-inflammatory effects, plays a role in immune modulation [35,36].

4.6. Vitamin B metabolism pathway

Our analysis revealed that vitamin B6 metabolism was perturbed when comparing the Pfizer-vaccinated group to the control and the Sinopharm-vaccinated group (Fig. 6A and C). Vitamin B6, also known as pyridoxine, is an essential compound that plays a crucial role in maintaining the functioning of the immune system [37]. Vitamin B6 is involved in various immune-related processes, including the production of T cells and interleukins. Adequate levels of vitamin B6 are necessary for optimal immune function, and a deficiency in vitamin B6 can lead to reduced immunity, decreased serum antibody levels, and imbalances in cytokine production, such as a decrease in IL-2 and an increase in IL-4 [38].

5. Conclusion, limitations and future work

In our study, we observed significant differences in metabolomic profile among the Sinopharm, Pfizer, and unvaccinated control groups within a Jordanian cohort. Specifically, neopterin, pyridoxal, 5-methoxytryptophol, and syringic acid showed pronounced alterations in Pfizer-vaccinated individuals compared to both Sinopharm-vaccinated and unvaccinated groups. These metabolites are closely associated with immune activation and modulation, suggesting their potential as biomarkers for monitoring the efficacy and effectiveness of newly developed vaccines in a shorter timeframe.

The study has certain constraints, notably it focused on a single cohort, which might restrict the applicability of the results to a wider population. In future research, we are aiming to integrate genomics and proteomics studies for a comprehensive understanding of immune responses and vaccine effectiveness. Moreover, validating the highly altered metabolites identified in our study, which can enhance precision by specifically measuring and quantifying identified metabolites.

Institutional Review Board statement

The study was conducted in accordance with the Declaration of Helsinki and approved by University of Jordan's Ethics Committee Ref. No.: UHS-HERC-094-21032022, March 21, 2022.

Informed consent statement

Informed consent has been obtained from all participants.

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Declaration of interest statement: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The metabolomics data of this study have been deposited in the Metabolomics Workbench under the study ID ST003039 (DOI: https://doi.org/10.21228/M88X46).

CRediT authorship contribution statement

Haneen I. Abufares: Writing – review & editing, Writing – original draft, Methodology. **Ruba A. Zenati:** Writing – review & editing, Writing – original draft, Methodology. **Nelson C. Soares:** Writing – review & editing, Methodology, Conceptualization. **Waseem El-Huneidi:** Writing – review & editing. **Lina A. Dahabiyeh:** Writing – review & editing. **Hamza M. Al-Hroub:** Writing – review & editing. **Mohammad A.Y. Alqudah:** Writing – review & editing. **Ahmad Y. Abuhelwa:** Writing – review & editing. **Karem H. Alzoubi:** Writing – review & editing. **Eman Abu-Gharbieh:** Writing – review & editing. **Wafa' Jehad Haza:** Writing – review & editing. **Mohammad A. Fararjeh:** Writing – review & editing. **Bashaer Abu-Irmaileh:** Writing – review & editing. **Yasser Bustanji:** Writing – review & editing, Conceptualization. **Mohammad H. Semreen:** Writing – review & editing, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35443.

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