

Supplementary material for the article

**Immunometabolic profiling of human monocytes in response to lipid exposure**

Ion Bogdan Mănescu<sup>1,2</sup>, Sara Thomas<sup>3</sup>, Daniel Gîrlea<sup>4</sup>, Liliana Demian<sup>2,5</sup>, Ana Maria Fotache<sup>2\*</sup>, Minodora Dobreanu<sup>1,2,5</sup>

1. Department of Laboratory Medicine, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Romania
2. Clinical Laboratory, Emergency County Clinical Hospital of Targu Mures, Romania
3. Medical campus Targu Mures, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Romania
4. Medical campus Hamburg (UMCH), George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Hamburg, Germany
5. Immunology Laboratory, Center for Advanced Medical and Pharmaceutical Research (CCAMF), George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Romania

\* Corresponding author: Ana Maria Fotache, Clinical Laboratory, Emergency County Clinical Hospital of Targu Mures, Romania. E-mail: fotache.anamaria@yahoo.com

**Supplementary table 1.** Comparison of monocyte subsets – T0 (fasting)

|            | Classical               | Intermediate            | Nonclassical            | Classical vs Intermediate | Classical vs Nonclassical | Intermediate vs Nonclassical |
|------------|-------------------------|-------------------------|-------------------------|---------------------------|---------------------------|------------------------------|
| Subset (%) | 92.8<br>[88.6-94.2]     | 3.4*<br>[2.5-6.7]       | 3.5<br>[2.3-5.9]        | <0.0001                   | <0.0001                   | 0.22                         |
| FSC        | 5.030<br>[5.011-5.065]  | 5.053<br>[5.030-5.082]  | 5.041<br>[5.026-5.065]  | 0.0001                    | 0.08                      | 0.24                         |
| SSC        | 4.776<br>[4.753-4.810]  | 4.772<br>[4.747-4.805]  | 4.719<br>[4.701-4.782]  | 0.97                      | 0.0002                    | <0.0001                      |
| CD14       | 3.426*<br>[3.060-3.483] | 3.364*<br>[3.058-3.472] | 2.920*<br>[2.824-3.013] | 0.76                      | <0.0001                   | <0.0001                      |
| CD16       | -                       | 2.946*<br>[2.707-3.019] | 3.614<br>[3.452-3.665]  | -                         | -                         | <0.0001                      |
| CD11b      | 3.514<br>[3.423-3.625]  | 3.604<br>[3.476-3.716]  | 3.381<br>[3.268-3.502]  | 0.0010                    | 0.0001                    | <0.0001                      |
| CD36       | 2.937<br>[2.898-2.981]  | 2.947<br>[2.886-2.976]  | 2.529<br>[2.324-2.681]  | 0.64                      | <0.0001                   | <0.0001                      |
| LDLR       | 2.982*<br>[2.655-3.060] | 3.098<br>[2.956-3.333]  | 2.754*<br>[2.450-2.989] | <0.0001                   | 0.0304                    | <0.0001                      |
| BODIPY     | 3.548*<br>[3.441-3.651] | 3.596<br>[3.520-3.645]  | 3.605<br>[3.494-3.655]  | 0.22                      | 0.0071                    | 0.77                         |

The comparisons were performed using log<sub>10</sub> values of the raw mean fluorescence data (MFI). Results are expressed as median with [IQR – interquartile range], regardless of the statistical distribution. \*Data set with nonparametric distribution.

**Supplementary table 2.** Comparison of monocyte subsets – T3 (three hours postprandial)

|            | Classical               | Intermediate            | Nonclassical            | Classical vs Intermediate | Classical vs Nonclassical | Intermediate vs Nonclassical |
|------------|-------------------------|-------------------------|-------------------------|---------------------------|---------------------------|------------------------------|
| Subset (%) | 93.7*<br>[89.5-96.1]    | 3.2*<br>[1.9-5.0]       | 3.1<br>[1.5-4.7]        | <0.0001                   | <0.0001                   | 0.51                         |
| FSC        | 5.080<br>[5.019-5.103]  | 5.091<br>[5.048-5.115]  | 5.086<br>[5.062-5.094]  | <0.0001                   | 0.0009                    | 0.82                         |
| SSC        | 4.791<br>[4.767-4.834]  | 4.784<br>[4.766-4.805]  | 4.751<br>[4.730-4.777]  | 0.0123                    | <0.0001                   | 0.0047                       |
| CD14       | 3.032*<br>[2.826-3.502] | 2.986*<br>[2.772-3.451] | 2.839<br>[2.541-3.004]  | 0.07                      | <0.0001                   | <0.0001                      |
| CD16       | -                       | 2.808*<br>[2.640-3.010] | 3.498<br>[3.350-3.623]  | -                         | -                         | <0.0001                      |
| CD11b      | 3.486<br>[3.420-3.568]  | 3.518*<br>[3.456-3.700] | 3.292<br>[3.218-3.488]  | 0.09                      | <0.0001                   | <0.0001                      |
| CD36       | 2.944<br>[2.814-3.030]  | 2.966<br>[2.825-3.020]  | 2.693<br>[2.450-2.878]  | 0.63                      | 0.0004                    | 0.0004                       |
| LDLR       | 2.583*<br>[1.887-2.724] | 2.695<br>[2.491-3.004]  | 2.199*<br>[0.462-2.618] | 0.0005                    | 0.0290                    | 0.0002                       |
| BODIPY     | 3.560<br>[3.515-3.620]  | 3.579<br>[3.526-3.595]  | 3.588<br>[3.547-3.635]  | 0.85                      | 0.0397                    | 0.13                         |

The comparisons were performed using log<sub>10</sub> values of the raw mean fluorescence data (MFI). Results are expressed as median with [IQR – interquartile range], regardless of the statistical distribution. \*Data set with nonparametric distribution.

**Supplementary table 3.** Comparison of monocyte subsets – T24-PRE (24h incubation with T0 serum)

|            | Classical               | Intermediate            | Nonclassical            | Classical vs Intermediate | Classical vs Nonclassical | Intermediate vs Nonclassical |
|------------|-------------------------|-------------------------|-------------------------|---------------------------|---------------------------|------------------------------|
| Subset (%) | 80.2<br>[75.5-88.1]     | 9.7<br>[7.8-14.6]       | 7.8<br>[3.1-11.3]       | <0.0001                   | <0.0001                   | 0.07                         |
| FSC        | 5.143<br>[5.104-5.183]  | 5.215*<br>[5.194-5.275] | 5.155<br>[5.125-5.216]  | <0.0001                   | 0.0372                    | <0.0001                      |
| SSC        | 4.841*<br>[4.819-4.917] | 4.913<br>[4.869-4.957]  | 4.669<br>[4.645-4.722]  | <0.0001                   | <0.001                    | <0.0001                      |
| CD14       | 3.574<br>[3.515-3.630]  | 3.712<br>[3.646-3.773]  | 2.818<br>[2.759-2.992]  | <0.0001                   | <0.0001                   | <0.0001                      |
| CD16       | -                       | 2.845<br>[2.805-2.886]  | 3.581<br>[3.491-3.677]  | -                         | -                         | <0.0001                      |
| CD11b      | 3.860<br>[3.797-3.917]  | 3.946<br>[3.903-4.121]  | 3.249<br>[3.036-3.455]  | <0.0001                   | <0.0001                   | <0.0001                      |
| CD36       | 2.814*<br>[2.708-2.867] | 2.932<br>[2.791-2.995]  | 2.360<br>[2.181-2.551]  | 0.0001                    | 0.0012                    | <0.0001                      |
| LDLR       | 3.753<br>[3.556-3.815]  | 3.802<br>[3.567-4.077]  | 2.995<br>[2.600-3.343]  | 0.13                      | <0.0001                   | <0.0001                      |
| BODIPY     | 3.902*<br>[3.780-3.994] | 3.892*<br>[3.771-4.010] | 3.853*<br>[3.742-3.882] | 0.92                      | 0.14                      | 0.07                         |

The comparisons were performed using log<sub>10</sub> values of the raw mean fluorescence data (MFI). Results are expressed as median with [IQR – interquartile range], regardless of the statistical distribution. \*Data set with nonparametric distribution.

**Supplementary table 4.** Comparison of monocyte subsets – T24-POST (24h incubation with T3 serum)

|            | Classical               | Intermediate            | Nonclassical            | Classical vs Intermediate | Classical vs Nonclassical | Intermediate vs Nonclassical |
|------------|-------------------------|-------------------------|-------------------------|---------------------------|---------------------------|------------------------------|
| Subset (%) | 79.1<br>[75.9-87.4]     | 11.9<br>[8.8-17.5]      | 6.1*<br>[3.1-8.5]       | <0.0001                   | <0.0001                   | 0.0124                       |
| FSC        | 5.115<br>[5.058-5.141]  | 5.195*<br>[5.180-5.232] | 5.144*<br>[5.125-5.172] | 0.0001                    | 0.0204                    | 0.0039                       |
| SSC        | 4.843<br>[4.797-4.882]  | 4.890<br>[4.855-4.987]  | 4.685<br>[4.626-4.767]  | 0.0003                    | <0.0001                   | <0.0001                      |
| CD14       | 3.519<br>[3.478-3.605]  | 3.660<br>[3.619-3.712]  | 2.960<br>[2.761-3.015]  | <0.0001                   | <0.0001                   | <0.0001                      |
| CD16       | -                       | 2.860<br>[2.831-2.917]  | 3.521<br>[3.395-3.674]  | -                         | -                         | <0.0001                      |
| CD11b      | 3.802<br>[3.721-3.915]  | 3.976<br>[3.897-4.155]  | 3.276<br>[2.996-3.459]  | <0.0001                   | <0.0001                   | <0.0001                      |
| CD36       | 2.698*<br>[2.657-2.804] | 2.911<br>[2.824-2.976]  | 2.274<br>[2.146-2.446]  | <0.0001                   | 0.0001                    | <0.0001                      |
| LDLR       | 3.728<br>[3.581-3.884]  | 3.803<br>[3.643-4.078]  | 2.813<br>[2.618-3.236]  | 0.09                      | <0.0001                   | <0.0001                      |
| BODIPY     | 3.744*<br>[3.575-3.908] | 3.885*<br>[3.758-3.981] | 3.799<br>[3.707-3.853]  | 0.12                      | 0.55                      | 0.06                         |

The comparisons were performed using log<sub>10</sub> values of the raw mean fluorescence data (MFI). Results are expressed as median with [IQR – interquartile range], regardless of the statistical distribution. \*Data set with nonparametric distribution.

**Supplementary table 5.** Changes in mean fluorescence intensity (MFI) at T3 vs T0

| <b>Classical monocytes</b>    |                      |                      |                |
|-------------------------------|----------------------|----------------------|----------------|
|                               | <b>T0</b>            | <b>T3</b>            | <b>p value</b> |
| Subset%                       | 92.8 [88.6-94.2]     | 93.7* [89.5-96.1]    | 0.29           |
| FSC                           | 5.030 [5.011-5.065]  | 5.080 [5.019-5.103]  | 0.0061         |
| SSC                           | 4.776 [4.753-4.810]  | 4.791 [4.767-4.834]  | 0.0222         |
| CD14                          | 3.426* [3.060-3.483] | 3.032* [2.826-3.502] | 0.51           |
| CD11b                         | 3.514 [3.423-3.625]  | 3.486 [3.420-3.568]  | 0.67           |
| CD36                          | 2.937 [2.898-2.981]  | 2.944 [2.814-3.030]  | 0.93           |
| LDLR                          | 2.982* [2.655-3.060] | 2.583* [1.887-2.724] | 0.0046         |
| BODIPY                        | 3.548* [3.441-3.651] | 3.560 [3.515-3.620]  | 0.82           |
| <b>Intermediate monocytes</b> |                      |                      |                |
|                               | <b>T0</b>            | <b>T3</b>            | <b>p value</b> |
| Subset%                       | 3.4* [2.5-6.7]       | 3.2* [1.9-5.0]       | 0.65           |
| FSC                           | 5.053 [5.030-5.082]  | 5.091 [5.048-5.115]  | 0.0055         |
| SSC                           | 4.772 [4.747-4.805]  | 4.784 [4.766-4.805]  | 0.76           |
| CD14                          | 3.364* [3.058-3.472] | 2.986* [2.772-3.451] | 0.13           |
| CD16                          | 2.946* [2.707-3.019] | 2.808* [2.640-3.010] | 0.31           |
| CD11b                         | 3.604 [3.476-3.716]  | 3.518* [3.456-3.700] | 0.73           |
| CD36                          | 2.947 [2.886-2.976]  | 2.966 [2.825-3.020]  | 0.95           |
| LDLR                          | 3.098 [2.956-3.333]  | 2.695 [2.491-3.004]  | 0.0049         |
| BODIPY                        | 3.596 [3.520-3.645]  | 3.579 [3.526-3.595]  | 0.84           |
| <b>Nonclassical monocytes</b> |                      |                      |                |
|                               | <b>T0</b>            | <b>T3</b>            | <b>p value</b> |
| Subset%                       | 3.5 [2.3-5.9]        | 3.1 [1.5-4.7]        | 0.18           |
| FSC                           | 5.041 [5.026-5.065]  | 5.086 [5.062-5.094]  | 0.0003         |
| SSC                           | 4.719 [4.701-4.782]  | 4.751 [4.730-4.777]  | 0.0688         |
| CD14                          | 2.920* [2.824-3.013] | 2.839 [2.541-3.004]  | 0.79           |
| CD16                          | 3.614 [3.452-3.665]  | 3.498 [3.350-3.623]  | 0.23           |
| CD11b                         | 3.381 [3.268-3.502]  | 3.292 [3.218-3.488]  | 0.39           |
| CD36                          | 2.529 [2.324-2.681]  | 2.693 [2.450-2.878]  | 0.0086         |
| LDLR                          | 2.754* [2.450-2.989] | 2.199* [0.462-2.618] | 0.0028         |
| BODIPY                        | 3.605 [3.494-3.655]  | 3.588 [3.547-3.635]  | 0.69           |

The comparisons were performed using log<sub>10</sub> values of the raw mean fluorescence data (MFI). Results are expressed as median with [IQR – interquartile range], regardless of the statistical distribution. \*Data set with nonparametric distribution.

**Supplementary table 6.** Changes in mean fluorescence intensity (MFI) at T24 vs T0

| Classical monocytes    |                      |                      |                      |               |                |                     |
|------------------------|----------------------|----------------------|----------------------|---------------|----------------|---------------------|
|                        | T0                   | T24 (PRE)            | T24 (POST)           | T0 vs T24 PRE | T0 vs T24 POST | T24 PRE vs T24 POST |
| Subset%                | 92.8 [88.6-94.2]     | 80.2 [75.5-88.1]     | 79.1 [75.9-87.4]     | 0.0007        | 0.0001         | 0.57                |
| FSC                    | 5.030 [5.011-5.065]  | 5.143 [5.104-5.183]  | 5.115 [5.058-5.141]  | <0.0001       | 0.0099         | 0.0119              |
| SSC                    | 4.776 [4.753-4.810]  | 4.841* [4.819-4.917] | 4.843 [4.797-4.882]  | 0.0001        | 0.0046         | 0.19                |
| CD14                   | 3.426* [3.060-3.483] | 3.574 [3.515-3.630]  | 3.519 [3.478-3.605]  | <0.0001       | 0.0024         | 0.0177              |
| CD11b                  | 3.514 [3.423-3.625]  | 3.860 [3.797-3.917]  | 3.802 [3.721-3.915]  | <0.0001       | <0.0001        | 0.36                |
| CD36                   | 2.937 [2.898-2.981]  | 2.814* [2.708-2.867] | 2.698* [2.657-2.804] | 0.0053        | 0.0010         | 0.15                |
| LDLR                   | 2.982* [2.655-3.060] | 3.753 [3.556-3.815]  | 3.728 [3.581-3.884]  | <0.0001       | <0.0001        | 0.12                |
| BODIPY                 | 3.548* [3.441-3.651] | 3.902* [3.780-3.994] | 3.744* [3.575-3.908] | 0.0001        | 0.13           | 0.06                |
| Intermediate monocytes |                      |                      |                      |               |                |                     |
|                        | T0                   | T24 (PRE)            | T24 (POST)           | T0 vs T24 PRE | T0 vs T24 POST | T24 PRE vs T24 POST |
| Subset%                | 3.4* [2.5-6.7]       | 9.7 [7.8-14.6]       | 11.9 [8.8-17.5]      | 0.0082        | 0.0004         | 0.18                |
| FSC                    | 5.053 [5.030-5.082]  | 5.215* [5.194-5.275] | 5.195* [5.180-5.232] | <0.0001       | <0.0001        | 0.14                |
| SSC                    | 4.772 [4.747-4.805]  | 4.913 [4.869-4.957]  | 4.890 [4.855-4.987]  | <0.0001       | 0.0006         | 0.70                |
| CD14                   | 3.364* [3.058-3.472] | 3.712 [3.646-3.773]  | 3.660 [3.619-3.712]  | <0.0001       | <0.0001        | 0.05                |
| CD16                   | 2.946* [2.707-3.019] | 2.845 [2.805-2.886]  | 2.860 [2.831-2.917]  | 0.86          | 0.95           | 0.0095              |
| CD11b                  | 3.604 [3.476-3.716]  | 3.946 [3.903-4.121]  | 3.976 [3.897-4.155]  | <0.0001       | <0.0001        | 0.66                |
| CD36                   | 2.947 [2.886-2.976]  | 2.932 [2.791-2.995]  | 2.911 [2.824-2.976]  | 0.57          | 0.73           | 0.63                |
| LDLR                   | 3.098 [2.956-3.333]  | 3.802 [3.567-4.077]  | 3.803 [3.643-4.078]  | <0.0001       | <0.0001        | 0.26                |
| BODIPY                 | 3.596 [3.520-3.645]  | 3.892* [3.771-4.010] | 3.885* [3.758-3.981] | 0.0017        | 0.0033         | 0.82                |
| Nonclassical monocytes |                      |                      |                      |               |                |                     |
|                        | T0                   | T24 (PRE)            | T24 (POST)           | T0 vs T24 PRE | T0 vs T24 POST | T24 PRE vs T24 POST |
| Subset%                | 3.5 [2.3-5.9]        | 7.8 [3.1-11.3]       | 6.1* [3.1-8.5]       | 0.0072        | 0.0323         | 0.36                |
| FSC                    | 5.041 [5.026-5.065]  | 5.155 [5.125-5.216]  | 5.144* [5.125-5.172] | <0.0001       | 0.0012         | 0.13                |
| SSC                    | 4.719 [4.701-4.782]  | 4.669 [4.645-4.722]  | 4.685 [4.626-4.767]  | 0.0212        | 0.16           | 0.66                |
| CD14                   | 2.920* [2.824-3.013] | 2.818 [2.759-2.992]  | 2.960 [2.761-3.015]  | 0.65          | 0.56           | 0.80                |
| CD16                   | 3.614 [3.452-3.665]  | 3.581 [3.491-3.677]  | 3.521 [3.395-3.674]  | 0.69          | 0.88           | 0.11                |
| CD11b                  | 3.381 [3.268-3.502]  | 3.249 [3.036-3.455]  | 3.276 [2.996-3.459]  | 0.10          | 0.26           | 0.77                |
| CD36                   | 2.529 [2.324-2.681]  | 2.360 [2.181-2.551]  | 2.274 [2.146-2.446]  | 0.35          | 0.10           | 0.45                |
| LDLR                   | 2.754* [2.450-2.989] | 2.995 [2.600-3.343]  | 2.813 [2.618-3.236]  | 0.07          | 0.54           | 0.60                |
| BODIPY                 | 3.605 [3.494-3.655]  | 3.853* [3.742-3.882] | 3.799 [3.707-3.853]  | 0.0046        | 0.0001         | 0.12                |

The comparisons were performed using log<sub>10</sub> values of the raw mean fluorescence data (MFI). Results are expressed as median with [IQR – interquartile range], regardless of the statistical distribution. \*Data set with nonparametric distribution.

**Supplementary table 7.** Changes in mean fluorescence intensity (MFI) at T24 vs T3

| <b>Classical monocytes</b>    |                      |                      |                |
|-------------------------------|----------------------|----------------------|----------------|
|                               | <b>T3</b>            | <b>T24-PRE</b>       | <b>p value</b> |
| Subset%                       | 93.7* [89.5-96.1]    | 80.2 [75.5-88.1]     | 0.0033         |
| FSC                           | 5.080 [5.019-5.103]  | 5.143 [5.104-5.183]  | 0.0006         |
| SSC                           | 4.791 [4.767-4.834]  | 4.841* [4.819-4.917] | 0.0008         |
| CD14                          | 3.032* [2.826-3.502] | 3.574 [3.515-3.630]  | 0.0002         |
| CD11b                         | 3.486 [3.420-3.568]  | 3.860 [3.797-3.917]  | <0.0001        |
| CD36                          | 2.944 [2.814-3.030]  | 2.814* [2.708-2.867] | 0.0095         |
| LDLR                          | 2.583* [1.887-2.724] | 3.753 [3.556-3.815]  | <0.0001        |
| BODIPY                        | 3.560 [3.515-3.620]  | 3.902* [3.780-3.994] | 0.0004         |
| <b>Intermediate monocytes</b> |                      |                      |                |
|                               | <b>T3</b>            | <b>T24-PRE</b>       | <b>p value</b> |
| Subset%                       | 3.2* [1.9-5.0]       | 9.7 [7.8-14.6]       | 0.0019         |
| FSC                           | 5.091 [5.048-5.115]  | 5.215* [5.194-5.275] | <0.0001        |
| SSC                           | 4.784 [4.766-4.805]  | 4.913 [4.869-4.957]  | <0.0001        |
| CD14                          | 2.986* [2.772-3.451] | 3.712 [3.646-3.773]  | <0.0001        |
| CD16                          | 2.808* [2.640-3.010] | 2.845 [2.805-2.886]  | 0.41           |
| CD11b                         | 3.518* [3.456-3.700] | 3.946 [3.903-4.121]  | <0.0001        |
| CD36                          | 2.966 [2.825-3.020]  | 2.932 [2.791-2.995]  | 0.61           |
| LDLR                          | 2.695 [2.491-3.004]  | 3.802 [3.567-4.077]  | <0.0001        |
| BODIPY                        | 3.579 [3.526-3.595]  | 3.892* [3.771-4.010] | 0.0012         |
| <b>Nonclassical monocytes</b> |                      |                      |                |
|                               | <b>T3</b>            | <b>T24-PRE</b>       | <b>p value</b> |
| Subset%                       | 3.1 [1.5-4.7]        | 7.8 [3.1-11.3]       | 0.0047         |
| FSC                           | 5.086 [5.062-5.094]  | 5.155 [5.125-5.216]  | <0.0001        |
| SSC                           | 4.751 [4.730-4.777]  | 4.669 [4.645-4.722]  | 0.0049         |
| CD14                          | 2.839 [2.541-3.004]  | 2.818 [2.759-2.992]  | 0.37           |
| CD16                          | 3.498 [3.350-3.623]  | 3.581 [3.491-3.677]  | 0.29           |
| CD11b                         | 3.292 [3.218-3.488]  | 3.249 [3.036-3.455]  | 0.32           |
| CD36                          | 2.693 [2.450-2.878]  | 2.360 [2.181-2.551]  | 0.0067         |
| LDLR                          | 2.199* [0.462-2.618] | 2.995 [2.600-3.343]  | 0.0008         |
| BODIPY                        | 3.588 [3.547-3.635]  | 3.853* [3.742-3.882] | 0.0020         |

The comparisons were performed using  $\log_{10}$  values of the raw mean fluorescence data (MFI). Results are expressed as median with [IQR – interquartile range], regardless of the statistical distribution. \*Data set with nonparametric distribution.