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Immunometabolic profiling of human monocytes in response to lipid exposure

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ABSTRACT

Background: Monocytes are central in inflammation and atherogenesis. Postprandial lipemia is a known pro-atherogenic state, yet its impact on monocyte activation remains incompletely understood. This study aimed to investigate morphological and immunophenotypical changes in circulating monocytes following lipid exposure.

Methods: Monocytes from 19 healthy volunteers were analyzed at baseline (fasting/T0), three hours postprandially (T3), and after 24-hour incubation with autologous serum (T24). Flow cytometry was used to assess monocyte subsets (classical/CM, intermediate/IM, and nonclassical/NCM) based on CD14/CD16 expression, alongside markers of activation (CD11b, CD14), lipid uptake (BODIPY), and lipid handling (LDLR, CD36). Morphological parameters (FSC, SSC) were also evaluated.

Results: At T3, all monocyte subsets showed increased size, with CM and NCM also showing increased granularity (↑SSC). LDLR expression decreased in all subsets, while CD36 increased only in NCM. At T24, monocytes displayed further increase in size, as well as higher lipid accumulation and activation (CD11b, CD14, LDLR), particularly in CM and IM. NCMs uniquely increased in size while decreasing granularity and showed no rise in activation markers. Overall, IMs consistently exhibited the most proinflammatory phenotype, while NCMs retained an anti-inflammatory profile. A novel inverse correlation was found between BMI and monocyte LDLR expression.

Conclusions: These findings underscore the dynamic and subset-specific responses of monocytes to lipid exposure, highlighting intermediate monocytes as key proinflammatory players and suggesting a more quiescent role for non-classical monocytes. This phenotypic distinction may hold clinical relevance for early immune monitoring in cardiometabolic risk states, as well as for advancing novel therapeutic interventions targeting monocytes at the cellular level.

Keywords: lipemia, monocyte, postprandial, LDLR, CD36

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INTRODUCTION

Postprandial lipemia, defined as a transient elevation in plasma lipids—primarily triglycerides—has emerged as a significant and prevalent cardiovascular risk factor [1,2]. This is particularly relevant in Western populations, who spend most of their waking hours in a postprandial state. In such individuals, elevated postprandial triglyceride levels contribute independently to cardiovascular disease, especially in those with insulin resistance or diabetes [2]. The extent of postprandial lipemia is highly dependent on meal composition, particularly fat and caloric content [3]. Larger, high-fat meals lead to greater production of chylomicrons and VLDL, resulting in a more pronounced rise in plasma lipids. Saturated fats, in particular, delay chylomicron clearance and are associated with increased inflammation and vascular dysfunction, whereas unsaturated fats tend to provoke a milder lipemic and inflammatory response, offering more cardiovascular protection [3,4].

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The mechanisms by which postprandial lipemia contributes to atherogenesis are complex [5]. Nevertheless, several key processes have been elucidated. Elevated triglyceride-rich lipoproteins (TRLs) in the postprandial phase can directly activate monocytes, enhancing their adhesion to VCAM-1 and ICAM-1. This promotes the formation of "foamy" monocytes enriched in lipid droplets and displaying increased activation marker expression, such as CD11b/CD11c, particularly in individuals with metabolic syndrome, who typically exhibit higher triglyceride levels and delayed lipid clearance [6]. The presence of lipid droplets in monocytes during the postprandial period is linked to pro-inflammatory activation and heightened endothelial adhesion [6,7]. Oxidative stress also plays a critical role: the postprandial state is marked by increased reactive oxygen species (ROS) from TRL lipolysis and mitochondrial activity, reduced antioxidant defenses, and elevated markers of oxidative stress in blood and urine [5,8]. This oxidative environment impairs endothelial function by lowering nitric oxide bioavailability, reducing flow-mediated dilation, and elevating soluble VCAM-1 and ICAM-1 levels—a consistent response after high-fat meals [5,8]. Furthermore, oxidative stress elevates oxLDL levels, which activate monocytes via TLR4, TLR6, and CD36 pathways. Monocytes that internalize oxLDL adopt a foamy morphology and secrete pro-inflammatory cytokines and chemokines [9,10]. Prolonged exposure to oxLDL also "trains" monocytes through epigenetic reprogramming, resulting in long-term pro-inflammatory phenotypes and foam cell formation [11].

Monocytes are central to innate immunity, inflammation, and the development of atherosclerosis. They are functionally heterogeneous and classified into three subsets based on CD14 and CD16 expression: classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$), and non-classical ($CD14^{+}CD16^{++}$), each with distinct inflammatory and migratory profiles [12]. One effective way to study their function is by analyzing surface and intracellular markers, as well as their lipid handling capabilities. A deeper understanding of monocyte biology, particularly their response to lipid exposure, holds promise for identifying novel therapeutic targets in metabolic and cardiovascular disease. In the present study, we aimed to explore monocyte behavior in response to lipids by simulating two experimental conditions of lipid exposure, using the fasting state as a baseline for comparison.

METHODS

This study was approved by the Ethics Committee of the George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș (Approval No. 3217/10.06.2024). All procedures and investigations were conducted at the University's Center for Advanced Medical and Pharmaceutical Research (CCAMF) in Târgu Mureș, Romania.

This small-scale study aimed to explore differences between monocyte subsets and their interactions with lipids in both ex vivo and in vitro settings. To establish a reliable baseline, only healthy individuals were included. Due to its exploratory scope and limited sample size, this investigation was considered a pilot study.

Study participants and sample collection

The study included 19 clinically healthy participants who provided written informed consent prior to any procedures. Each participant had two peripheral venous blood samples collected on two separate occasions: once in a fasting state (T0) and once three hours after consuming a standardized high-fat, high-calorie meal (postprandial, T3). Beyond the inclusion of only healthy participants, each individual acted as their own control, with measurements at T3 and T24 compared to their respective baseline values at T0. The blood samples were collected in three types of tubes: K_2EDTA tubes for complete blood count (BD Vacutainer, product no. 367841), SST tubes for biochemical analyses (BD Vacutainer, product no. 367986), and lithium heparin tubes (BD Vacutainer, product no. 367526) for peripheral blood mononuclear cell (PBMC) isolation. The standardized meal, containing both animal and vegetable fats, provided approximately one gram of lipid per kilogram of body weight and accounted for 70% of the total caloric intake. Participants were instructed to refrain from eating, drinking (except water), smoking, or engaging in physical activity during the three-hour postprandial period.

Complete blood count and plasma lipid analysis

Blood samples from both timepoints were processed individually within one hour of collection under identical conditions. A complete blood count (CBC) with differential was first performed using a SYSMEX XS-800i hematology analyzer. Serum separator tubes (SSTs) were allowed to clot at room temperature for 30 minutes and then centrifuged at 3500 rpm for 10 minutes. The resulting serum was transferred into 2.0 mL safe-lock tubes (Eppendorf, product no. EP022363344) and stored at –80 °C until further analysis. Subsequently, serum samples were thawed and analyzed in batch for a standard lipid profile, which included triglycerides (TG), total cholesterol (TC), HDL-cholesterol (HDLC), and directly measured LDL-cholesterol (LDLC). Non-HDL cholesterol (non-HDLC) was calculated as TC minus HDLC, and remnant cholesterol (RemC) as TC minus HDLC minus LDLC. The atherogenic index of plasma (AIP) was calculated as $\log_{10}$(TG/HDLC).

PBMC isolation

Peripheral blood mononuclear cells (PBMCs) were isolated from the lithium heparin tubes within two hours from collection. The isolation was performed using the gradient centrifugation method using Histopaque-1077 (Sigma-Aldrich, product no. 10771). PBMCs were then washed two times with Dulbecco's Phosphate-Buffered Saline without calcium or magnesium (PBS; Biowest, product no. L0615) and the supernatant was discarded after centrifugation for five minutes at 300×g. Cell pellets were resuspended in complete medium RPMI-1640 (cRPMI) medium – Sigma-Aldrich, product no. R8758 supplemented with 20% fetal bovine serum (FBS, product no. F7524) and 1% antibiotic antimycotic solution (Penicillin/ Streptomycin/ Amphotericin B, Sigma-Aldrich, product no. A5955). The cRPMI was added 10% dimethyl sulfoxide (DMSO, Sigma-Aldrich, product no. D2650) for cryopreservation purposes, all at a cell density of 1×106 PBMC/mL. Cell suspensions were sampled in 1.5 cryotubes and stored at-150°C until further processing.

Cell staining protocol

From each K_2EDTA tube (T0 and T3), 100 µL of whole blood was transferred into two Falcon flow cytometry tubes (BD, product no. 352063), labeled "Unstained" and "Stained," respectively. To the "Stained" tube, 4 µL of each of the following anti-human antibodies was added: APC-Cy7 anti-CD14 (BD, product no. 557831), Alexa Fluor 647 anti-CD16 (BD, product no. 557710), PE-Cy7 anti-CD11b (BD, product no. 552850), BV510 anti-CD36 (BD, product no. 744986), and PE anti-LDLR (BD, product no. 565653). Tubes were incubated at room temperature in the dark for 15 minutes.

Following incubation, 1 mL of 1× BD FACS Lysing Solution (BD, product no. 349202) was added to each tube, and the samples were further incubated for 10 minutes under the same conditions. The lysing solution serves a dual purpose: lysing red blood cells and fixing white blood cells. After lysis, 2 mL of Dulbecco's PBS was added to each tube, followed by brief vortexing. Tubes were centrifuged at 800×g for 5 minutes. The supernatant was discarded, and the cell pellet was washed twice more by resuspension in 2 mL of PBS followed by centrifugation under the same conditions.

Subsequently, 0.5 µL of BODIPY 493/503 (1 µg/µL; Invitrogen, product no. D3922) was added to the pellet, and the tube was mixed thoroughly. The sample was incubated at room temperature in the dark for 20 minutes, followed by three PBS washes as described above. Finally, the cell pellet was resuspended in 300 µL of BD FACSFlow sheath fluid (BD, product no. 342003), and samples were analyzed within 4 hours on a BD FACSAria III flow cytometer. The cytometer was properly configured with compensation settings specific to the experiment, and quality control was performed daily using BD FACSDiva CS&T Research Beads (BD, product no. 655050).

PBMC cultures

PBMC samples isolated from the T0 (fasting) samples were thawed rapidly in a 37°C water bath. Cells were resuspended in room temperature cRPMI and left for one hour to accommodate. Then, PBMCs were seeded at 5×105 cells/well in a 24-well tissue culture plate (VWR, product no. 10062-896). For each study participant, two wells were used, both with fasting PBMC, but one with T0 (fasting) autologous serum (T24-PRE) and one with T3 (postprandial) autologous serum (T24-POST). Cells were then incubated for 24 hours in a 100% humidity incubator at 37°C with 5% CO2 atmosphere. After the 24h incubation, cells were detached from the well bottoms by vigorous shaking and under pippetting aspiration-rejection force. Cells were washed with Dulbecco's PBS and then stained as described above.

Flow cytometry gating strategy

For all samples analyzed by flow cytometry (T0, T3, T24-PRE, and T24-POST), the flow cytometry gating strategy focused on identifying singlet monocytes and classifying them into three subsets: classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$), and nonclassical ($CD14^{+}CD16^{++}$). For each subset, mean fluorescence intensity (MFI) data were exported and included in the statistical analysis.

In total, eight parameters were analyzed. Two were morphological (non-fluorescent): forward scatter (FSC), indicating cell size, and side scatter (SSC), indicating cell complexity or granularity. The remaining six were fluorescent markers of functional surface proteins (CD14, CD16, CD11b, CD36, and LDLR) and intracellular lipid content (BODIPY).

Statistical analysis

Statistical analysis was conducted on serum lipid profile and data collected in all experimental conditions (T0, T3, T24-PRE, and T24-POST). Pearson or Spearman correlations were used to assess correlation between datasets. Differences in each cellular parameter between the timepoints were assessed using paired tests – either the parametric T-test or the non-parametric Wilcoxon signed-rank test. Statistical significance was set at α=0.05, though marginally significant *p*-values (up to 0.10) were also noted. All statistical analyses were performed using MedCalc Statistical Software, version 20.104, and IBM SPSS Statistics, version 20.

RESULTS

Clinical, demographic, and biochemical test results are presented in Table 1. The study group showed a predominance of female participants, with all individuals being young adults. Regarding body mass index (BMI), the group included some outliers at both ends of the spectrum: underweight and obese individuals. Routine biochemical analyses were within normal ranges, consistent with values expected in young, apparently healthy individuals. Concerning the lipid profile, the most notable dyslipidemic feature observed in some participants was a low HDLC level, which contributed to elevated values of the AIP.

Given the established relationship between body mass and circulating lipids, we examined the correlations between BMI and plasma lipid parameters at baseline (T0). Our analysis revealed moderate, statistically significant or near-significant positive correlations between BMI and several atherogenic lipid markers, including TC (ρ=0.438, *p*=0.06), LDLC (r=0.430, *p*=0.06), non-HDLC (r=0.468, *p*=0.04), RemC (ρ=0.549, *p*=0.014), TG (ρ=0.548, *p*=0.01), and the AIP (r=0.463, *p*=0.04). A weak negative correlation was also observed with HDLC (ρ=-0.308, *p*=0.19).

In terms of monocyte subtypes, BMI was negatively correlated with the proportion of classical monocytes (CM; r=-0.444, *p*=0.056) and positively correlated with nonclassical monocytes (NCM; r=0.498, *p*=0.030) in the fasting state. A similar trend was observed with intermediate monocytes (IM) at T24 (ρ=0.440, *p*=0.059). In addition, fasting BODIPY fluorescence, indicating intracellular lipid content, showed a positive association with BMI in IM (ρ=0.422, *p*=0.071). Notably, BMI was strongly and consistently negatively correlated with LDLR expression across all monocyte subsets: CM (r=-0.758, *p*=0.0002), IM (r=-0.660, *p*=0.0021), and NCM (r=-0.695, *p*=0.0010).

For the analysis of cellular marker expression, raw flow cytometry data were log-transformed using base 10 to ensure appropriate data normalization. A detailed comparison of the three monocyte subsets (classical, intermediate, and nonclassical) under each experimental condition (T0, T3, T24-PRE, and T24-POST) is provided in Supplementary Tables 1–4, respectively. To facilitate interpretation of these differences, between-subset comparisons were summarized in Figure 1 as a heatmap, highlighting relative expression patterns across experimental conditions.

Flow cytometry data were also analyzed across timepoints, specifically comparing T3 vs T0 and T24 vs T0. The full results are reported in Supplementary Tables 5 and 6, respectively. Due to the minimal differences in mean fluorescence intensity (MFI) between T24-PRE and T24-POST (Supplementary Table 6), the T24-POST

Table 1. Clinical, demographic, and laboratory data (n=19)

	Range	Mean ± SD	Median [IQR]
Sex (n, % female)		13 (68.4%)	
Age (years)	21.0 – 33.0	24.6 ± 2.9	25.0 [22.0 – 25.0]
BMI (kg/m2)	17.7 – 38.3	23.4 ± 4.6	22.7 [20.2 – 24.9]
Uric acid (mg/dL)	2.89 – 7.57	4.38 ± 1.27	3.96 [3.54 – 4.60]
Albumin (g/dL)	4.54 – 5.36	4.93 ± 0.21	4.95 [4.80 – 5.00]
Amylase (U/L)	13.0 – 102.0	58.3 ± 24.2	50.7 [39.1 – 77.0]
ALT (U/L)	4.5 – 46.2	14.4 ± 11.4	10.8 [7.1 – 16.5]
AST (U/L)	12.6 – 31.1	18.9 ± 5.3	17.2 [14.9 – 22.8]
Total bilirubin (mg/dL)	0.20 – 0.83	0.41 ± 0.18	0.39 [0.24 – 0.49]
Creatinine (mg/dL)	0.61 – 0.92	0.76 ± 0.09	0.77 [0.69 – 0.83]
Creatinkinase (U/L)	50.9 – 345.5	108.3 ± 85.1	81.6 [57.8 – 97.3]
Phosphate (mg/dL)	3.13 – 5.16	3.94 ± 0.49	3.86 [3.54 – 4.25]
GGT (U/L)	8.8 – 128.5	21.5 ± 26.6	13.5 [10.3 – 22.8]
Glucose (mg/dL)	81.5 – 103.7	90.9 ± 5.9	89.7 [87.1 – 94.6]
LDH (U/L)	112.1 – 275.5	177.1 ± 43.8	169.7 [146.1 – 201.8]
Magnesium (mg/dL)	1.83 – 2.29	2.02 ± 0.11	2.00 [1.93 – 2.10]
Iron (µg/dL)	9.37 – 180.8	60.9 ± 39.6	58.0 [31.3 – 80.1]
Urea (mg/dL)	14.3 – 46.7	29.4 ± 7.7	28.2 [23.9 – 34.4]
Total cholesterol (mg/dL)	118.6 – 223.2	167.9 ± 29.4	166.3 [141.7 – 187.7]
LDLC (mg/dL)	65.1 – 151.7	98.0 ± 27.5	94.7 [74.9 – 114.3]
HDLC (mg/dL)	24.8 – 91.4	59.1 ± 15.1	56.7 [50.3 – 71.5]
Non-HDLC (mg/dL)	74.9 – 173.9	108.8 ± 31.0	95.5 [83.0 – 133.3]
RemC (mg/dL)	0.76 – 28.3	10.8 ± 6.7	8.9 [7.5 – 13.5]
Triglycerides (mg/dL)	44.9 – 150.3	79.2 ± 30.4	69.2 [57.5 – 103.7]
AIP	-0.174 – 0.680	0.114 ± 0.246	0.063 [-0.103 – 0.325]

Abbreviations: AIP – atherogenic index of plasma, ALT – alanine transaminase, AST – aspartate aminotransferase, GGT – gamma-glutamyl transferase, IQR – interquartile range, LDH – lactate dehydrogenase, RemC – remnant cholesterol, SD – standard deviation.

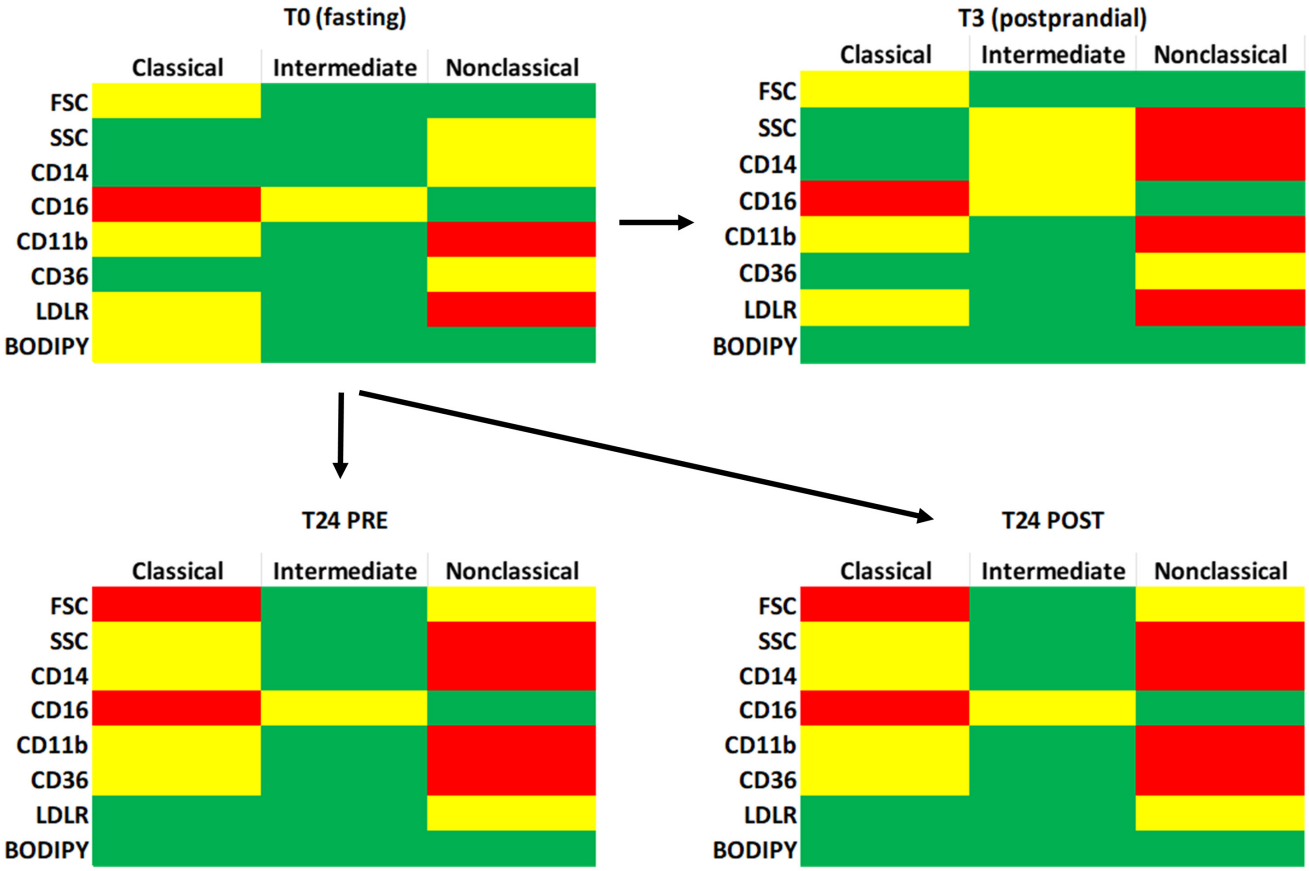

Figure 1. Relative expression of monocyte subsets markers at different experimental timepoints

dataset was excluded from further analyses involving the 24-hour timepoint. Accordingly, comparative analyses of T24 vs T3 are based on the T24-PRE values and are detailed in Supplementary Table 7. To aid visualization, the between-timepoint comparisons were compiled in Figure 2, allowing clearer insight into temporal changes within each monocyte subset.

DISCUSSION

BMI and monocyte immunophenotype

Our group consisted of clinically healthy young individuals. In this study, we found BMI to be positively correlated with all atherogenic lipid profile parameters (TC, LDLC, non-HDLC, RemC, AIP, and TG), while negatively correlated with HDLC. The marginally significant p-values seen in the analysis can be attributed to the small study group. However, it is well established that elevated BMI is linked to an adverse immunometabolic profile, marked by higher levels of atherogenic lipids, reduced HDL cholesterol, increased inflammatory biomarkers, and notable shifts in peripheral immune cell phenotypes [13–21].

Regarding the association of BMI with monocyte subsets and immunophenotype, obesity has been linked to increased proportions of CMs and IMs [22], as well as IMs and NCMs [23–25], along with enhanced migratory capacity due to increased expression of CXCR1 (all subsets), and CCR2 and CCR5 in IMs and NCMs [23]. Monocytes from obese patients have also been shown to contain more lipid droplets upon BODIPY staining [26]. Nevertheless, the effect of body mass on monocyte surface markers appears to be dynamic, with positive changes observed in obese patients following weight loss, including significant decreases in the proportion of IMs and NCMs [25], as well as reductions in CD14 and CD36 expression in CMs and IMs, and CD16 in IMs and NCMs [27].

Our findings also align with current literature, as we found a positive association between BMI and the proportion of NCMs (T0), and IMs (T24). In IMs, lipid content at T0 was also associated with BMI. Nevertheless, the literature is scarce regarding the relation between BMI and monocyte LDLR expression. A substudy of the Multi-Ethnic Study of Atherosclerosis (MESA) revealed increased LDLR mRNA in the circulating monocytes of obese patients [28]. At the same time, genes involved in cholesterol efflux (ABCA1, ABCG1) were downregulated, suggesting increased LDL uptake by monocytes in obese individuals [28]. Given the inverse relationship between cellular uptake of LDL and LDLR expression (due to internalization of LDLR), these findings seem to align with our

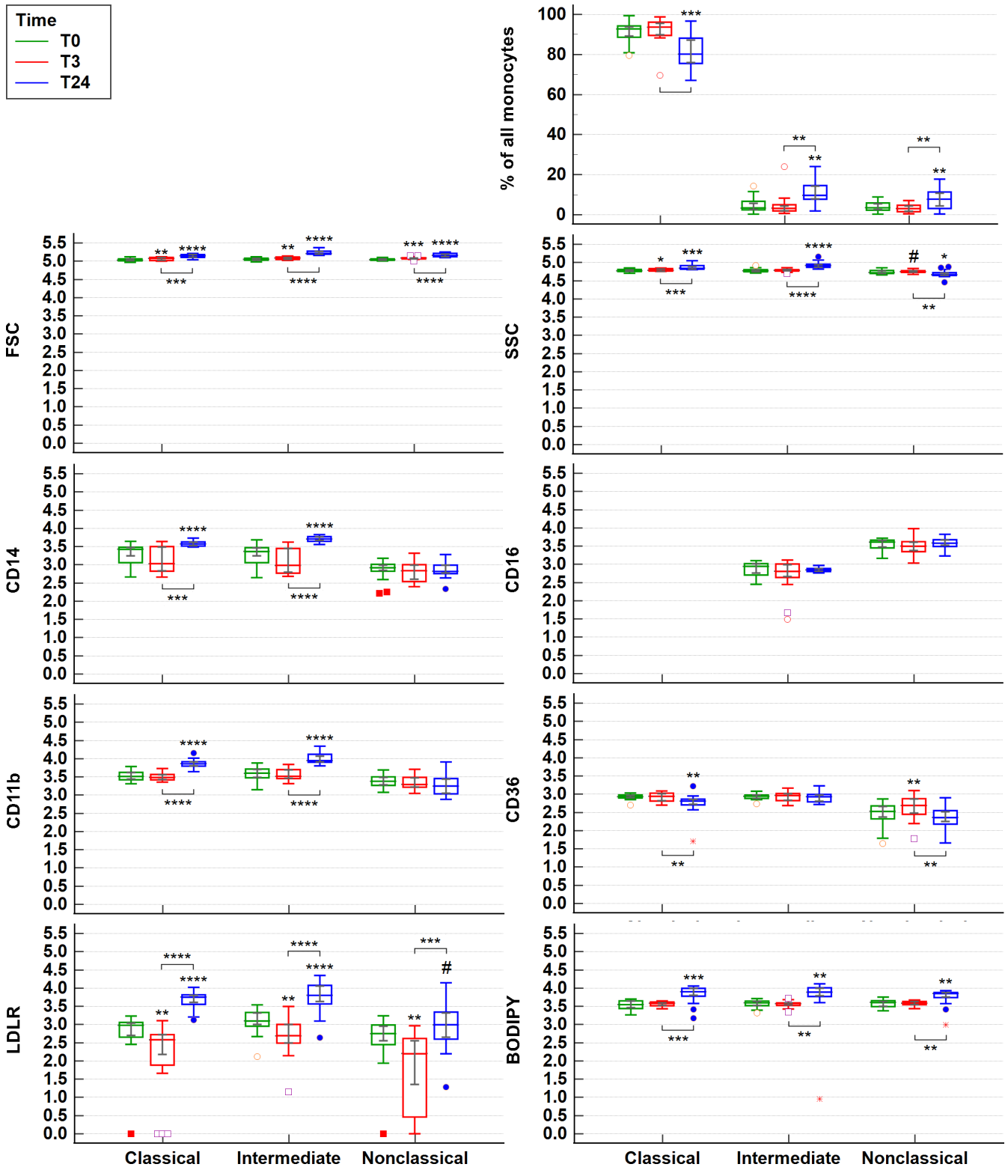

Figure 2. Monocyte subsets evolution across different experimental conditions
Significance: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, $^{\#}p$ value 0.05-0.10 (marginally significant).

observation of a strong negative correlation between BMI and LDLR expression in all monocyte subsets, as obese individuals appear to have fewer LDLRs on monocytes due to their internalization triggered by increased LDL uptake.

Overall, these findings further support the established hypothesis that metabolic dysfunction, expressed globally as elevated BMI among other indicators, affects monocyte subset distribution and immunophenotype. The novelty in this case lies in the observation of a global negative relationship between BMI and monocyte LDLR surface protein expression, which, to our knowledge, is first reported here.

Exclusion of T24-POST monocytes

In the present study, we aimed to explore monocyte behavior in response to lipids by simulating two experimental conditions of lipid exposure, using the fasting state as a baseline for comparison. First, the T3 approach simulated the in vivo short-term, transient postprandial exposure of circulating monocytes to blood lipids. Although the analysis was conducted ex vivo, this condition closely reflected in vivo effects, as monocytes were processed and fixed soon after blood collection. Second, the T24 approach simulated prolonged lipid exposure by incubating monocytes in vitro with autologous serum for 24 hours. For T24, we intended to compare the effects of fasting (PRE) versus postprandial (POST) serum. However, as shown in Supplementary Table 6 and Figure 1, there were minimal differences between the T24-PRE and T24-POST conditions. Therefore, subsequent analyses involving the 24h timepoint were based solely on the T24-PRE dataset, as presented in Supplementary Table 7 and Figure 2.

A "heatmap" overview of monocyte phenotypes

Figure 1 illustrates in a simplified manner the changes undergone by the three monocyte populations from the fasting state to the T3 and T24 timepoints. Since all flow cytometry parameters in this study are linked to inflammation [6,29–33], it is evident in Figure 1 that, in the fasting state, the monocyte populations exhibit distinct morphological and immunophenotypical features: IMs are the largest and most granular, with the highest expression of CD14, CD11b, CD36, LDLR, and intracellular lipids. Some of these features are also shared by CMs. In contrast, NCMs appear to be the "least inflammatory" subset. At T3, and more so at T24, IMs further establish themselves as the most proinflammatory monocyte population, while the minimal inflammatory profile of NCMs becomes even more pronounced. Notably, after both short (T3) and prolonged (T24) lipid exposure, all monocyte subsets display comparable levels of lipid accumulation.

These findings align with existing literature, which shows that despite their heterogeneity, IMs express the highest levels of antigen presentation-related molecules and secrete elevated amounts of proinflammatory cytokines [12,34]. In contrast, NCMs have a less pronounced secretory profile, can counteract CM activity, and are associated with wound healing [12,34]. In the context of cardiovascular disease, the predominant proinflammatory nature of IMs is reflected in their increased counts being independently associated with the prevalence of coronary artery disease [35], restenosis after angioplasty [36], coronary plaque vulnerability [37], and adverse cardiovascular events [38].

Short-term postprandial phenotype changes (T3)

Moving to a more detailed analysis, Figure 2 illustrates the evolution of each monocyte subset and the differences between them. At 3 hours postprandial, there were no changes in the proportion of monocyte subsets, nor in the expression levels of CD14, CD16, CD11b, or BODIPY. However, all subsets showed a slight but significant increase in cell size (FSC), while increased granularity (SSC) was observed only in the CM and NCM subsets. LDLR expression significantly decreased across all subsets, yet only NCMs displayed a change in CD36 expression, which increased. Based on the known roles of monocyte subsets and associated markers, these findings suggest that postprandially, all monocytes engaged in lipid uptake, triggering morphological changes (↑FSC, ↑/no change in SSC) and reduced cholesterol receptor expression (↓LDLR). Notably, NCMs appeared to respond distinctively, exhibiting the sharpest LDLR reduction while being the only subset to upregulate CD36. This aligns with their established patrolling sentinel function, where the scavenger receptor CD36 plays a central role [39]. Still, the unchanged BODIPY expression is intriguing if these alterations resulted from lipid uptake. It is possible that lipid internalization was incomplete – lipid droplets may not have yet formed or lipids were still being trafficked, and thus not stained by BODIPY. Alternatively, the observed morphological changes might not stem only from lipid uptake, but also from monocyte activation in response to sensing the postprandial hyperlipemic environment.

Long-term in vitro phenotype changes (T24)

The extended incubation of monocytes with autologous serum allowed them sufficient time to fully react and adapt to their environment. Given the minimal differences observed between cells incubated with fasting versus postprandial serum, it can be hypothesized that the activation observed at T24, relative to T0 or T3, results not only from prolonged lipid exposure but also from the stimulatory nature of the in vitro environment. As a result of this prolonged exposure, several significant changes occurred.

First, there was a clear shift from the CM phenotype toward IMs and NCMs. Since the in vitro setup prevented monocytes from migrating into tissues and there was no replenishment of the less mature CM subset from the bone marrow, this phenotypic shift became apparent. This is supported by the current theory that monocyte subsets represent a maturation spectrum, progressing from $CD16^-$ CMs to $CD16^+$ IMs and NCMs [31,34].

Second, there was a marked increase in intracellular lipid content (↑BODIPY), which in the more inflamma-

tory CM and IM subsets, led to increased cell size (↑FSC) and granularity (↑SSC). These subsets also exhibited significant increases in immune activation markers (↑CD14, ↑CD11b) and in the LDL receptor (↑LDLR), all consistent with immunometabolic activation. In contrast, the NCM subset displayed a distinct behavior. While it too showed increased cell size (↑FSC) and lipid content (↑BODIPY), it paradoxically exhibited decreased granularity (↓SSC). Although its LDL receptor expression increased (↑LDLR), there were no changes in CD14 and CD11b expression. The precise mechanism underlying this behavior remains unclear, but it may be that, consistent with their generally anti-inflammatory nature, NCMs – despite lipid uptake – do not undergo activation, possibly due to different metabolic processing or lipid storage mechanisms, as suggested by the decreased SSC. Regardless, this distinctive behavior of NCMs is clearly highlighted in this experiment.

Lastly, the findings at T3 and T24 are consistent with those from a previously published smaller pilot study conducted by the same team [40]. Although the earlier experiment used a similar design, it did not include CD16, which is essential for full identification of monocyte subsets, and was limited to the analysis of CD14, CD11b, and BODIPY. Despite these limitations, the results concerning these markers are similar to those observed in the present study [40].

Translational potential

We have entered the era of precision medicine, where understanding individual variability at the cellular and molecular level is essential for tailoring prevention and treatment strategies [41]. Although conventional biomarkers are well established (e.g., LDL-cholesterol), there remains an ongoing need to identify novel indicators capable of predicting disease onset or outcomes, particularly in the context of cardiovascular health and related conditions [42,43]. In this regard, investigating monocyte surface markers and their interactions with lipids offers significant translational potential in clinical medicine. Detailed phenotyping of monocyte subsets and their metabolic responses allows for early detection of inflammatory changes that precede overt cardiometabolic disease.

As discussed above, the distribution of monocyte subsets is altered in obesity and cardiovascular disease, with intermediate monocytes being most commonly associated with worse clinical outcomes. These changes may occur early in disease progression, and a simple, cost-effective peripheral blood monocyte “profiling” could provide cellular-level evidence of a shift toward inflammatory phenotypes and altered lipid metabolism. Such an approach could feasibly become part of routine clinical practice due to its affordability. While in vitro experiments demand dedicated infrastructure and greater investment of time and resources, a straightforward ex vivo 6- or 8-color monocyte panel would be sufficient, provided that the optimal surface markers are clearly defined.

Given the interindividual variability and analytical variability introduced by factors such as instrument recalibration, heatmaps offer a valuable tool by providing a visual representation that allows findings to be interpreted rapidly and intuitively. In this context, absolute numbers become less relevant, while the relationships between surface markers take precedence. Further research involving larger cohorts of healthy individuals as well as those with various conditions—such as obesity, metabolic syndrome, diabetes, and coronary heart disease—could enable the evidence-based development of “normal" (“healthy”) fasting or postprandial heatmaps against which patients with these pathologies could be compared. Such a visual tool could be readily integrated into clinical practice as a user-friendly pocket application, offering visual cues and automatically generated interpretations based on a continuously expanding and regularly reviewed database. These concepts illustrate the significant potential for incorporating cellular-level data into routine clinical practice. Nonetheless, further research and standardization are needed, as there is currently no consensus on which markers should be included, how the panel should be interpreted, or how its results should be integrated with clinical assessments.

There is, however, promising potential for translating these monocyte findings into more advanced research and, ultimately, into clinical practice. For instance, foundational studies on CD14 and CD16 expression led to the classification of monocytes into classical, intermediate, and non-classical subsets, a discovery that reshaped the understanding of their distinct roles in inflammation and atherogenesis [12]. Another key development was the identification of lipid-laden "foamy monocytes" using lipid staining and surface marker analysis, which revealed early proatherogenic changes in circulating immune cells [44]. Such cellular insights supported the development of targeted therapies such as canakinumab, which reduced cardiovascular events in the CANTOS trial by modulating innate immune pathways without affecting lipid levels [45]. Canakinumab was shown to reduce the number of circulating moncytes and their ability to accumulate in atherosclerotic plaques, a process that contributes to cardiovascular disease. These examples highlight how monocyte-based research can drive the emergence of novel diagnostic and therapeutic strategies, reinforcing its value in translational science.

Novelty and study limitations

This study offers several novel contributions to the understanding of monocyte behavior under lipid exposure. A key finding is the negative association between BMI and LDLR expression across all monocyte subsets, a relationship not previously described in the literature. Additionally, the analysis included intracellular lipid accumulation using BODIPY staining and morphological parameters such as FSC and side scatter SSC. These features, though rarely reported, are valuable early indicators of monocyte activation and provide important context for interpreting functional changes.

The study also has some limitations. The relatively small sample size limited the statistical power and generalizability of the results. Furthermore, the flow cytometry panel was restricted to a limited number of surface and intracellular markers, which may not capture the full spectrum of monocyte functional states. Future studies with larger cohorts, including both healthy individuals and patients with metabolic pathologies, as well as broader marker panels are needed to validate and expand upon these observations.

CONCLUSIONS

This study provides new insights into how circulating monocytes respond to lipid exposure under different experimental conditions, simulating both acute postprandial and prolonged serum contact. Through phenotypic profiling, lipid accumulation analysis, and morphological assessment, the results highlight the dynamic behavior of monocyte subsets, particularly the proinflammatory profile of intermediate monocytes and the distinct, less reactive nature of non-classical monocytes. In addition to the novel negative association between BMI and monocyte LDLR expression, we also report relevant changes in several other key markers (CD14, CD16, CD11b, CD36) as well as intracellular lipids (BODIPY), cell size (FSC), and granularity (SSC), parameters which are rarely discussed in the context of monocyte activation. These findings reinforce the relevance of detailed monocyte characterization in cardiometabolic research and suggest potential translational pathways for identifying early immunometabolic changes in disease development.

ABBREVIATIONS

AIP- atherogenic index of plasma

BMI – body mass index

CD- cluster of differentiation

CM – classical monocytes

FSC – forward scattered light

IM – intermediate monocytes

LDLR- LDL receptor

MFI- mean fluorescence intensity

NCM – nonclassical monocytes

oxLDL- oxidized LDL

PBMC- peripheral blood mononuclear cells

RemC – remnant cholesterol

ROS- reactive oxygen species

SSC – side scattered light

TC – total cholesterol

TG – triglycerides

TLR- toll-like receptor

VCAM- vascular cell adhesion molecule

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AUTHORS' CONTRIBUTION

IBM – conceptualization, investigation, formal analysis, project administration, resources, writing (original draft)

ST – investigation, formal analysis

DG – investigation, formal analysis

LD – investigation, formal analysis

AMF – investigation, formal analysis, writing (original draft)

MD – conceptualization, supervision, validation, writing (review & editing)

CONFLICT OF INTEREST

None to declare.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is available exclusively in electronic form on the journal's website at the following LINK.

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