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RESEARCH ARTICLE

# The effect of postprandial in vivo and experimental *in vitro* hyperlipidemia on human peripheral blood monocytes

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**Objective**: In this study, we aimed to investigate the effect of transient postprandial in vivo and prolonged experimental in vitro hyperlipidemia on human peripheral blood monocytes. Methods: Peripheral blood was collected from seven healthy subjects after an overnight fast and three hours after a standardized high-fat meal. Both fasting and postprandial samples were stained for surface markers CD14/CD11b and intracellular lipids using BODIPY493/503. Postprandial samples only were used for isolation of peripheral blood mononuclear cells that were further incubated overnight with postprandial hyperlipidemic autologous plasma, then stained as described above. All samples were analyzed on a FACSAria III flow cytometer. **Results**: Flow cytometric analysis revealed two monocyte populations (CD14+): CD14low and CD14high. In fasting, these populations show similar morphology (FSC/SSC), but different expressions of CD14, CD11b, and BODIPY493/503. At three hours postprandially, a moment of maximum hyperlipidemia, neither population suffered significant changes. After the 24-hour incubation, cell activation was observed in both populations: similar fold change increase in SSC, increase in FSC for CD14high cells only, similar foldchange increase in CD14, slightly higher foldchange increase in CD11b for CD14low monocytes, and significantly higher foldchange increase in lipid content for CD14high monocytes. CD14high monocytes appear to undergo a more intense activation than CD14low monocytes.
**Conclusions**: We conclude that all monocytes react after prolonged in vitro exposure to plasma lipids, each subset having its own activation pattern. All monocyte types may play a role in inflammation and the development of plaques. Monocyte assays are a valuable tool for the investigation of atherosclerosis at the cellular level.

**Keywords**: CD14 CD11b monocyte, lipids, cell activation, postprandial hyperlipidemia, bodipy

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## Introduction

Human peripheral blood mononuclear cells (PBMC) are mainly comprised of lymphocytes and monocytes. PBMCs have many applications and are widely used in the medical and pharmaceutical fields of research. Monocytes (Mo) are the precursors of macrophages (Mϕ), effector cells of the innate immune system. For a long time, the role of macrophages was considered limited to phagocytosis. While it is true that macrophages are extremely proficient phagocytes, research over the last decades has shown that monocytes and macrophages exhibit remarkable heterogeneity, versatility and plasticity [1-4]. Beyond phagocytosis, cells of the mononuclear phagocyte system are able to shape the microenvironment by reacting to and secreting molecular mediators [5, 6]. These cells are involved in all stages of inflammation, phagocytosis, antigen presentation, tissue repair, cytokine-signaling, and maintenance of tissue homeostasis [4, 5, 7]. Throughout these processes, monocytes and macrophages can change their functional phenotype in response to stimuli from the microenvironment [5, 6]. As such, researchers have sought to classify these cells based on phenotype and function. In humans, circulating monocytes are classified based on relative expression levels of surface proteins cluster of differentiation (CD)14 and CD16 [3, 8].

Given their importance in innate immunity and role as a bridge between innate and adaptive immunity, Mo/Mϕ are involved in many diseases. Atherosclerosis is the leading cause of coronary artery disease, peripheral artery disease, and cerebrovascular disease. Traditionally thought of as a lipid storage disease caused by lipoprotein retention in the intimal space, atherosclerosis was shown to have an important immune component and is currently classified as a chronic inflammatory disease with an autoimmune component [9, 10]. Indeed, an essential step in atherogenesis is the recruitment of circulating monocytes to the intimal space where the inflammatory microenvironment triggers monocyte differentiation into macrophages [9]. The retained lipoproteins, mainly low-density lipoprotein (LDL), are modified and taken up by macrophages via scavenger receptors for phagocytosis [9]. This maladaptive process results in the formation of lipid-laden macrophages (also called “foam cells”) and continuous growth of the inflammatory fatty infiltrates that will appear as plaques [9]. Some studies suggest that intracellular fat may accumu-

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late in the circulation even before monocyte migration and differentiation, as "foamy monocytes" have been identified and shown to be implicated in atherogenesis [11, 12]. There is a well-documented association between clinical atherosclerosis and plasma lipids, especially total cholesterol, LDL cholesterol, and some apolipoproteins [13]. The deleterious effects of prolonged hyperlipidemia, as seen in dyslipidemic patients, have been well documented. However, less is known about the effects of transient hyperlipidemia, as seen postprandially, on circulating monocytes.

Although atherosclerosis is one of the most investigated diseases, little progress has been made so far regarding the identification of clinically useful biomarkers and potential molecular/ cellular therapeutic targets [14]. Therefore, given their central role, the involvement of monocytes and macrophages in atherosclerosis remains a topic of interest. There are several methods currently used for investigating the role of Mo/Mϕ in atherosclerosis including, but not limited to, cytokine assays, reactive oxygen species assays, adhesion tests, surface and/or intracellular markers detection, lipid uptake assays, and genomic and/or proteomic approaches. In this study, we aimed to investigate the effect of postprandial *in vivo* and experimental *in vitro* hyperlipidemia on human peripheral blood monocytes from healthy subjects. For this purpose, we combined two of the previously mentioned methods: surface marker detection and a lipid uptake assay.

## Methods

This study was approved by the Ethics Committee of the George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Romania (UMFST, approval no. 1558/13.01.2022). Subject sampling, sample processing and data acquisition were performed at the Center for Advanced Medical and Pharmaceutical Research of the UMFST (CCAMF, Targu Mures, Romania). All subjects participated voluntarily after signing an informed consent form. Peripheral blood samples were collected from 7 healthy young adults aged 23 to 28 years (3 male and 4 female, average age 25 years) using 9-mL sodium heparin vacutainers (Greiner Bio-One, cat. No. 455051). Throughout the study, preliminary blood tests were performed and complete blood count (CBC, Sysmex XS-800i) abnormalities and high-sensitivity C-reactive protein (hsCRP, Siemens BN ProSpec) levels above 3 mg/L were considered exclusion criteria.

In order to establish the moment of maximum postprandial hyperlipidemia, 3 subjects were inserted a peripheral venous catheter after fasting overnight and 3 mL of fasting blood was collected (T0). Subjects were then offered a standardized high-fat meal: approximately 1 g of lipid per kg body weight, accounting for 70% of calories, both animal and vegetal fats. For the following 6 hours, subjects restrained from any physical exercise, food, or drinks except for water, and 3 mL of blood was collected every hour (T1 to T6). Blood samples were centrifuged for 10 minutes at 300×g and an extended lipid profile was performed from plasma samples: triglycerides, total cholesterol, LDL cholesterol, high density lipoprotein (HDL) cholesterol (on a Cobas Integra 400 Plus instrument, Roche, Switzerland), lipoprotein (a), and apolipoproteins (Apo) A-I, A-II, B, and E (on Siemens BN ProSpec).

All 7 subjects were called on a different occasion and fasting blood samples were collected (T0) before they were offered a standardized high-fat meal as described above. According to the moment of maximum postprandial hyperlipidemia deduced from the initial experiment, postprandial blood samples were collected after 3 hours (T3). The following blood tests were initially performed on T0 and T3 samples: CBC, hsCRP, triglycerides, total cholesterol, LDL cholesterol, and HDL cholesterol. T0 and T3 whole blood samples were then stained for surface markers using anti-human BV421-anti-CD14 (BD Biosciences, cat. No. 563743) and APC-Cy7-anti-CD11b (Biolegend, cat. No. 101226) fluorochrome-conjugated monoclonal antibodies (3 µL / 100 µL whole blood). Corresponding unstained samples were also prepared. All samples were then treated with BD FACS Lysing Solution (BD Biosciences, cat. No. 349202) for removal of red blood cells and washed twice with Phosphate-Buffered Saline (PBS – Sigma, cat. No. D1283). A final staining was performed with the neutral lipid dye BODIPY 493/503 (Thermo Fisher, cat. No. D3922), followed by incubation in the dark for 30 minutes and 2 washes with PBS. Pellets were resuspended in PBS and analyzed within 1 hour on a BD FACSAria III flow cytometer using FACSDiva software version 8.0 (both BD Biosciences).

Additional processing was performed for T3 samples collected at the moment of maximum postprandial hyperlipidemia. PBMCs were isolated using a density gradient centrifugation protocol established in our laboratory [15], and cultured for 24 hours in a medium consisting of 50% PBS and 50% autologous hyperlipidemic plasma (T3) at a density of $2\times10^6$ cells/mL, in a $CO_2$ incubator (37°C, 5% $CO_2$, 100% humidity). After the incubation, non-adherent cells were recuperated, washed with PBS, and stained for surface markers and intracellular lipids as described above, followed by flow cytometry analysis (T24).

The following flow cytometry gating strategy was applied (in this order): setting fluorescence thresholds using unstained samples, identifying singlets (Forward Scatter height vs Forward Scatter area, FSC-H vs FSC-A), and gating singlets into $CD14^{low}$ and $CD14^{high}$ populations. The following parameters were then compared between these populations: % of parent population (CD14+ cells), FSC, Side Scatter (SSC), and mean fluorescence intensities (MFI) of CD14, CD11b, and BODIPY expressions. Flow cytometric data on monocytes (CD14+ cells) was considered representative for the *in vivo* status of these cells during fasting (T0) and for the *in vivo* effect of postprandial transient hyperlipidemia (T3). Flow cytometric data on monocytes recovered after 24 hours of incubation with li-

pid-rich autologous plasma, was considered representative for the *in vitro* effect of prolonged hyperlipidemia.

All data was stored in Microsoft Excel files (Microsoft Corporation) and statistical processing was performed in MedCalc statistical software version 20.104 (MedCalc Software Ltd, Ostend, Belgium). Data sets were tested for normality using the Shapiro-Wilk test. Since the end-goal of our studies is to establish and implement protocols for precision medicine research, outliers were not excluded. All data sets were paired, therefore T student and Wilcoxon tests were used to compare either the same cell population at various moments, or different cell populations at the same moment. A two-tailed alpha was set to 0.05 and significant *p* values were marked as follows: *p≤0.05, **p≤0.01, ***p≤0.001, and ****p≤0.0001. All images were generated in MedCalc software.

## Results

The initial experiment revealed a similar evolution of plasma lipid levels in all subjects (N=3, data not shown). Triglyceride levels showed the highest variation, increasing postprandially with a peak at T3 (+69.3%, +106%, +219%) before returning to fasting-like levels at T6. Apo-E and Lp(a) levels were also slightly increased at T3, but with no statistical or clinical significance. Contrarily, the levels of cholesterol, LDL cholesterol, HDL cholesterol, and Apo A-I, A-II, and B, were slightly decreased (data not shown). T3 was considered the moment of maximum postprandial hyperlipidemia and used as a sampling moment for postprandial samples in the second experiment.

The variations in plasma lipid levels seen at T3 vs T0 in the second experiment (N=7) confirmed the observations made in the initial experiment (data not shown). For flow cytometry data on monocyte parameters (FSC, SSC, CD14 MFI, CD11b MFI, and BODIPY MFI), a fold-change approach was employed for data normalization, using MFI values recorded at T0 or T3 as baseline (fold change = 1). Cell parameter variations (T3 vs T0 and T24 vs T0, T0 as baseline) are shown in Figures 1A and 1C for $CD14^{low}$ and $CD14^{high}$ monocytes, respectively. Neither population showed significant cell parameter variations at T3 compared to T0. Therefore, T0 and T3 moments were considered highly similar in terms of recorded parameters of $CD14^{low}$ and $CD14^{high}$ monocytes. More importantly, cell parameters were compared between T24 and T3 (T3 as baseline) in order to assess the different effects of *in vivo* postprandial (T3) and *in vitro* prolonged (T24) hyperlipidemia. Results are shown in Figures 1B and 1D for $CD14^{low}$ and $CD14^{high}$ monocytes, respectively. A comparison of the two monocyte populations was performed at each of the three moments T0, T3, and T24, and results are shown in Table I.

## Discussion

### Fasting vs non-fasting plasma lipids and atherosclerosis

Around the world, laboratories, professional societies, guidelines, and scientific consensuses are starting to recommend non-fasting lipid testing. Postprandial elevation of triglyceride levels has been reported in many studies on healthy subjects [16, 17], but apart from triglycerides, plasma lipids do not vary significantly between fasting and non-fasting samples [18]. Moreover, the non-fasting approach holds multiple advantages over the traditional fasting lipid profile: more time efficient, doesn't require fasting nor sampling in the morning, reflects a more accurate overview of the patient's lipid metabolism and homeostasis, reduces the risk of hypoglycemia in diabetic patients, and increases overall patient compliance [18]. The non-fasting lipid profile was shown not to be inferior to the fasting profile regarding the assessment of cardiovascular risk and response to hypolipemic treatment [19]. Other studies have found non-fasting lipid testing to be superior regarding the assessment of cardiovascular risk [19-21]. Several studies suggest that postprandial hyperlipidemia is associated with atherosclerosis, as elevated non-fasting triglyceride levels may cause lipid accumulation over time possibly due to delayed clearance [16]. A prospective study on 14000 subjects has shown that elevated non-fasting triglyceride levels are associated with increased risk of coronary heart disease, myocardial infarction, and death [20]. Some studies have shown that prolonged hyperlipidemia generates and maintains a highly atherogenic triade: elevated triglycerides, elevated LDL cholesterol, and reduced HDL cholesterol [22, 23]. Thus, postprandial hyperlipidemia is currently considered an independent risk factor for cardiovascular disease [20].

Despite testing a small group of subjects, our results concur with the literature. A meta-analysis of 113 studies has shown that plasma triglyceride levels peak 4 hours postprandially [21], while other studies report a plasma triglyceride concentration plateau at 3-4 hours postprandially [11]. In our study, the maximum concentration of triglycerides was recorded 3 hours postprandially. Regarding the other parameters of the lipid profile, our results align with other large populational studies that have reported decreased levels of total, LDL, and HDL cholesterol together with decreased levels of various apolipoproteins [18].

**Table I. Statistical differences between flow cytometric data of $CD14^{high}$ and $CD14^{low}$ monocyte populations**

| | FSC | SSC | CD14 | CD11b | BODIPY |
|---|---|---|---|---|---|
| Fasting (T0) | 0.37 | 0.22 | < 0.0001 | 0.013 | 0.0048 |
| Postprandial (T3) | 0.39 | 0.81 | 0.0002 | 0.0012 | 0.0025 |
| After incubation (T24) | 0.0001 | 0.09 | 0.015 | 0.47 | 0.001 |

Raw flow cytometric data (MFI – mean fluorescence intensity) of the two populations were statistically compared at each of the three moments (T0, T3, T24) and p values are presented in the table. Every time a difference is significant (p<0.05), the higher MFI value was recorded for the $CD14^{high}$ population. FSC – forward scatter (flow cytometric indicator of cell size), SSC – side scatter (flow cytometric indicator of cell internal complexity).

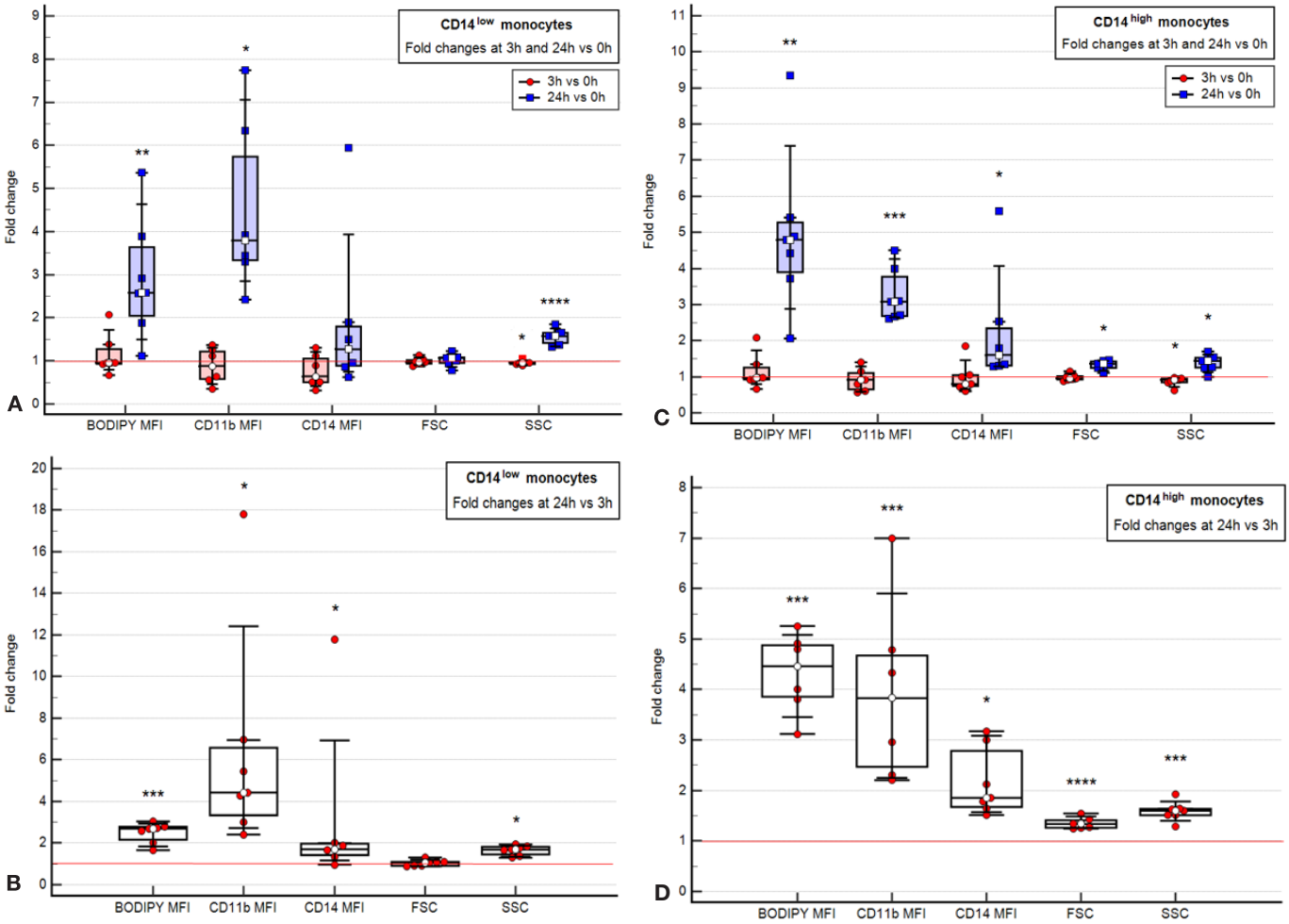


**Fig. 1. Flow cytometric cell parameter variations at different moments (T0 – fasting, T3 – three hours postprandially, T24 – after 24 hours of incubation with autologous hyperlipidemic plasma). Variations of monocyte parameters at T3 and T24 vs baseline (T0, red horizontal line) are shown in subfigures A for CD14low monocytes and C for CD14high monocytes. Variations of monocyte parameters at T24 (prolonged in vitro hyperlipidemia) vs a T3 baseline (transient in vivo hyperlipidemia, red horizontal line) are shown in subfigures B for CD14low monocytes and D for CD14high monocytes. All parameter variations are shown as fold changes in mean fluorescence intensity (MFI) compared to the specified baseline. FSC – forward scatter (flow cytometric indicator of cell size), SSC – side scatter (flow cytometric indicator of cell internal complexity). Statistical significance levels: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.**

### The effect of *in vivo* transient hyperlipidemia

Human monocytes are classified in three populations based on the relative expressions of CD14 and CD16 surface markers: classical ($CD14^{high}CD16^{neg}$), intermediate ($CD14^{high}CD16^{low}$), and non-classical ($CD14^{low}CD16^{high}$) [3, 8]. Due to lack of surface CD16 staining in our study, $CD14^{high}$ monocytes represent both classical and intermediate monocytes, while the $CD14^{low}$ population represents the non-classical monocytes. The study of monocytes in atherosclerosis is of great interest because an increase in monocyte activation leads to polarization and differentiation into M1/M2 macrophages, thus having direct implications on chronic inflammation and plaque formation [24]. Recent research has shown that CD14 is not just a lipopolysaccharide (LPS) receptor as it can interact with other molecules such as endogenous lipids [25]. Moreover, increased CD14 expression was observed in the acute phase of myocardial infarction [26]. Thus, CD14 is currently regarded as a marker of monocyte activation both in physiological and pathological conditions such as ischemic heart disease. Other studies have shown that postprandial products of VLDL lipolysis increase monocyte secretion of cytokines (TNF-α, IL-1β, IL-8) and adhesion molecules (Mac-1/ CD11b, ICAM-1, VCAM-1), thus possibly playing a role in plaque initiation and growth by enhancing monocyte adhesion and transmigration across the vessel wall [27]. Mac-1 (CD11b) is a marker of cell adhesion that plays an important role in inflammation. Current evidence indicate that CD11b is a potent marker of monocyte activation [28]. Not only surface markers undergo postprandial modifications in monocytes. Following lipid uptake, cytosolic lipid droplets occur along with a pro-inflammatory status of the monocyte [11, 29]. Postprandial triglycerides and free fatty acids released through lipolysis can activate monocytes by dynamizing and stimulating inflammatory pathways. Thus, there is a positive correlation between postprandial plasma triglyceride levels and the occurence of monocyte cytosolic lipid droplets [11, 29].

In this study, we aimed to quantify monocyte activation by measuring the expression of surface markers CD14 and CD11b, along with the intracellular accumulation of lipids as shown by expression of BODIPY. Early activation of monocytes in response to postprandial hyperlipidemia has been reported, some authors considering triglycerides as potent monocyte activators [27, 30]. Contrarily, our study does not provide any evidence of such early activation of monocytes triggered by postprandial hyperlipidemia. For both $CD14^{low}$ and $CD14^{high}$ populations, the *in vivo* transient hyperlipidemia (T3 vs T0) showed no apparent effect on neither of the markers used (CD14, CD11b, BODIPY). Given that our subjects were young, healthy, had normal body weight and normal lipid profiles, it is possible that the transient hyperlipidemia caused by a single high-fat meal was not enough to activate circulating monocytes, at least not to the same extent as a prolonged hyperlipidemia. Another explanation may be a delayed activation of monocytes after the moment of postprandial sampling (T3). However, our findings agree with another study that reported no change in CD14 and CD11b expressions at 3 hours after a high-fat meal [31]. There are multiple possible causes for the contradicting reports in the literature. Monocyte activation can be induced by *ex vivo* and *in vitro* manipulation, as these cells are well known for their fragility in such experiments. As such, high activations reported by some studies may be caused, at least partially, by this phenomenon. Moreover, there is a lack of standardization regarding the composition of high-fat meals and the investigation of monocyte activation for these purposes. Last, but not least, the activation markers used in this study cover only a fraction of the monocyte activation pathways.

#### The effect of *in vitro* prolonged hyperlipidemia

After a 24-hour incubation with autologous lipid-rich postprandial plasma, several changes were observed for both $CD14^{low}$ and $CD14^{high}$ populations. The flow cytometric parameter SSC increased similarly for both populations due to lipid uptake and formation of intracellular lipid droplets. Consequently, monocytes transformed into lipophages, also known as "foamy monocytes" [11, 12, 29]. Interestingly enough, FSC also increased after 24 hours, but only for the $CD14^{high}$ monocytes. This may be explained by both lipid accumulation and cellular activation.

Regarding the surface markers, we recorded a similar median increase of about 1.7-fold in CD14 expression for both monocyte populations at T24 compared to T3. However, given that polarized monocytes can oscillate between phenotypes, together with the fact that only non-adherent cells were investigated in this study, we could not establish whether *in vitro* prolonged hyperlipidemia caused any phenotype shifts. Thus, in this regard, we can only conclude that, after the 24-hour incubation, the two populations still coexist in culture and are both activated. A similar median increase of about 4-fold in CD11b expression was also observed for both monocyte populations at T24 compared to T3. Other studies have also reported increased monocyte CD11b expression after prolonged exposure to lipids [30]. The slightly higher median fold change in CD11b expression observed for $CD14^{low}$ vs $CD14^{high}$ monocytes is also confirmed by other studies reporting that intermediary and non-classical monocytes express more CD11b in a hyperlipidemic environment [32, 33].

Regarding the intracellular accumulation of lipids, data analysis revealed that $CD14^{high}$ monocytes have a higher expression of BODIPY than $CD14^{low}$ monocytes at all three moments (T0, T3, T24). Although both populations increased their BODIPY expression at T24, the median fold change increase was higher for $CD14^{high}$ vs $CD14^{low}$ monocytes (approximate fold change values 4.5 vs 2.8). Increased accumulation of intracellular lipids correlates well with increased SSC (both seen at T24) and is regarded by many as an activator of monocytes after prolonged exposure to a hyperlipidemic environment [34]. It remains to be seen whether intracellular accumulation of lipids leads exclusively to a pro-inflammatory phenotype. It is known, however, that internalized lipids alter monocyte behavior, particularly the classical subset. Studies have shown that *in vitro* incubation of these cells with VLDL particles results in lipid uptake and alteration of cellular biological processes [29, 34].

#### Classical and intermediate monocytes ($CD14^{high}$)

Overall, the two monocyte populations in our study showed both similar and distinct patterns of activation. Numerous studies have shown that monocyte subsets have different functional properties, which explains our findings. In humans, the majority of circulating monocytes are classical type, which are excellent phagocytes, rich in scavenger receptors capable of internalizing plasma lipids [8, 35]. This may explain the intense activation of $CD14^{high}$ monocytes after prolonged exposure to lipids, as well as the efficient internalization of lipids observed in our study. Classical monocytes are also the major population present in atherosclerotic plaques [35], suggesting their central role in the pathogenesis of this disease. Elevated triglyceride levels are known to cause receptor hyper-regulation (CD14, CD11b), increased secretion of inflammatory molecules, and local oxidative stress in intermediate monocytes [3, 33, 35]. Although CD16 surface staining was not used in this study, it is known that intermediate monocytes account for a small fraction of the monocytes (2-10%). Thus, our observations on $CD14^{high}$ monocytes are based mainly on changes that occurred in classical monocytes. Both classical and intermediate monocytes are pro-inflammatory cells that express high levels of scavenger receptors, are capable of lipid internalization, and are recruited early on in the inflammatory process. Some studies have shown that intermediate [36] and classical monocytes [26] are highly predictive of cardiovascular events. All these data correlate well with the intense activation of $CD14^{high}$ monocytes observed in our study.

### Non-classical monocytes (CD14$^{low}$)

Non-classical monocytes are known for actively patrolling the luminal side of the vascular endothelium. However, they are not limited to this role as they can be recruited in later stages of inflammation [35, 37]. The late mobilization, together with the mostly anti-inflammatory phenotype of these cells, may explain the lesser *in vitro* activation of CD14$^{low}$ monocytes observed in this study. Non-classical monocytes also require CD11b for patrolling and attachment to vascular endothelial cells [37]. Moreover, this monocyte subset was shown to express high levels of CD11b in response to triglyceride exposure [33]. Our findings correlate with these data given the slightly higher median fold change increase in CD11b observed for CD14$^{low}$ vs CD14$^{high}$ monocytes in this study. It is important to emphasize that intermediate and non-classical monocytes are in fact derived from classical monocytes released from the bone marrow. Data from the literature show that there is a continuous spectrum of CD14/CD16 expression in monocytes and the three subsets are not separated by well-defined criteria [38, 39]. In inflammation, a phenotype shift was observed from classical to intermediate and non-classical monocytes [38]. In cardiovascular diseases, a shift from classical and non-classical monocytes to the intermediate type was observed [39]. Due to lack of CD16 surface staining, we could not conclude whether the *in vitro* prolonged exposure to a hyperlipidemic environment was able to generate an inflammatory stimulus strong enough to trigger phenotype shifts between monocyte populations. This study had two other limitations: a small group of subjects and the investigation of non-adherent monocytes only after the 24-hour incubation.

## Conclusion

In this study, we investigated the effect of *in vivo* transient and *in vitro* prolonged hyperlipidemia on CD14$^{low}$ and CD14$^{high}$ monocytes by looking at the size (FSC), internal complexity (SSC), expressions of surface CD14 and CD11b, and intracellular lipid content. In fasting, these two populations show similar morphology (FSC, SSC), but different expressions of surface CD14 and CD11b, as well as lipid content. At three hours postprandially, a moment of maximum hyperlipidemia, neither population suffered significant changes. After a 24-hour *in vitro* exposure to autologous hyperlipidemic plasma, cell activation was observed in both populations: similar fold change increase in SSC, increase in FSC for CD14$^{high}$ cells only, slightly higher foldchange increase in CD11b for CD14$^{low}$ monocytes, and significantly higher foldchange increase in lipid content for CD14$^{high}$ monocytes. CD14$^{high}$ monocytes appear to undergo a more intense activation than CD14$^{low}$ monocytes. We conclude that all monocytes react after prolonged *in vitro* exposure to plasma lipids, each subset having its own activation pattern. Thus, all monocyte types may play a role in inflammation and the development of plaques. In a puzzling research field still riddled with unknowns, monocyte assays are a valuable tool for the investigation of atherosclerosis at the cellular level.

## Authors' contributions

IBM, MM, and MD designed the study. IBM, MM, ECP, and DRM collected the samples and performed experiments, data acquisition, analysis, and interpretation. All authors participated in drafting the work and revising it critically. All authors agreed to be accountable for all aspects of the work and have read and approved of the final manuscript. IBM and MM have contributed equally to this work and share first authorship.

## Conflict of interest

The authors declare no conflicts of interest.

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