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# Lipid Profile and Prevalence of Dyslipidemia in Preand Postmenopausal Women in Yaounde, Cameroon

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## Authors' contributions

This work was carried out in collaboration among all authors. Authors NG and TTJJ designed the study, authors NG, TTJJ, BAT, SG, ACR and TCB performed the analysis, Author NG wrote the protocol and wrote the first draft of the manuscript. Author NG managed the analyses of the study. All authors read and approved the final manuscript.

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### **ABSTRACT**

**Aims:** Lipids serve several important physiologic functions like energy homeostasis or biosynthesis of hormone within the human body. The deterioration of lipid metabolism which is favoured by menopause is the source of cardiovascular diseases. This study aimed to determine the plasma lipid profile and dyslipidemia prevalence of some Cameroonian women.

Study Design: This was a cross-sectional study.

**Place and Duration of Study:** The enrollment took place at the Yaounde Military Hospital from November 2016 to July 2017.

Methodology: To achieve this goal, two sets of 105 postmenopausal and 127 premenopausal

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participants were enrolled in this study. 5ml of blood were collected on EDTA coated tubes from each participant. Enzymatic methods were used to evaluate total cholesterol, HDL cholesterol and triglyceride, while LDL cholesterol was determined using the Friedewald equation. These biological parameters were used to determine dyslipidemia. Thereafter, they were compared within age and year since menopause subgroups.

**Results:** There was no significant difference in the mean values of total cholesterol, HDL-C, LDL-C as well as triglyceride between premenopausal and postmenopausal women. The mean HDL-C concentration was stable in the age subgroups while the mean total cholesterol and LDL-C were slightly increasing as postmenopausal participants got older. LDL-C level was significantly higher in women who had been in menopause for more than 15 years. The overall dyslipidemia prevalence was 53%. Dyslipidemia was significantly higher in the subgroup of women who had freshly entered menopause.

**Conclusion:** Even though there was no change in the mean concentration of the plasma lipid parameters, of pre- and postmenopausal participants there was a general need to improve their lifestyle in order to reduce dyslipidemia prevalence.

Keywords: Lipid profile; dyslipidemia; premenopausal; postmenopausal; total cholesterol; LDL cholesterol; triglyceride.

## 1. INTRODUCTION

Lipids serve several important physiologic functions like enerav homeostasis biosynthesis of hormone within the human body. They can also lead to major health issues. Dyslipidemia is a primary risk factor for atherosclerotic cardiovascular disease (ASCVD) and may even be a prerequisite for ASCVD, occurring before other major risk factors come into play [1]. Menopause is defined as the permanent cessation of menstruation resulting from the loss of ovarian follicular activity [2]. During this period, there is a cessation of ovarian estrogen production.

The deterioration of the plasma lipid profile is one of the cardiovascular disease risk factors favoured by estrogen deficiency. Studies have shown relations between postmenopausal status and elevated concentrations of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) [3,4,5]. The difference in mean TC observed among postmenopausal women and premenopausal women may reach 0.8 mmol/l [6]. Women are at lesser risk of developing disease than their cardiovascular male counterparts before menopause. However, mortality from cardiovascular events is higher in women after menopause (53.8%) than in men (49%) [7]. Menopausal estrogen decline is associated with adverse metabolic changes such as insulin resistance and dyslipidemia which is characterized by high triglyceride (TG) levels, low high-density lipoprotein cholesterol (HDL-C) and an increased frequency of small dense lowdensity lipoprotein particles. The importance of estrogens in the development of metabolic diseases during menopause is disputed. Both menopause and lipids are highly correlated with age; it remains unclear whether menopausal lipid changes are independent of age effects [8,9].

Cross-sectional studies have been done in Europe [10,11,12], Asia [6] and America [13] on the lipid profile in premenopausal women versus postmenopausal. Women represent more than half of the Cameroonian population and they will be now spending more of their life in the postmenopausal state because of an increase in life expectancy. It is, therefore, necessary to evaluate all parameters leading to the promotion of knowledge of menopause-related metabolic diseases. Data on lipid metabolic changes and dyslipidemia among women in the Central Africa sub-region and in Cameroon particularly are nonexistent. Our study hypothesized that plasma lipid profile and dyslipidemia could be affected by age, menopausal status, as well as menopausal duration. Thus, this cross-sectional study aimed to determine the plasma lipid profile and dyslipidemia prevalence in preand postmenopausal women living in Yaounde, Cameroon.

## 2. MATERIALS AND METHODS

#### 2.1 Study Area and Study Population

Participants were premenopausal and postmenopausal women, apparently normal. The menopausal status was considered here as the permanent cessation of menstruation resulting

from loss of ovarian follicular activity for at least 12 months. The enrollment took place at the Yaounde Military Hospital from November 2016 to July 2017. The aim of the study was first explained to prospective participants. This process was done in markets, schools, offices, churches, gatherings and the hospital. Volunteers were invited to the Yaounde Military Hospital for enrollment. The enrolled participants were grouped into postmenopausal (105) and premenopausal (127) apparently normal women. All women under any medication interfering with lipid metabolism were excluded. Participants with known pathology of lipid metabolism, diabetes or major heart, liver or kidney diseases or malignancies were also excluded. Premenopausal participants were divided into 4 age range subgroups: 18-27, 28-37, 38-47 and more than 47 years while postmenopausal participants were instead divided into 3 subgroups: 45-54, 55-64 and more than 64 years. Postmenopausal women were further shared in 4 sets according to the length of their menopausal state. These sets were respectively: 1-5, 6-10, 11-15, and more than 15 years since menopause (YSM).

#### 2.2 Data and Samples Collection

Anthropometric parameters (height, weight, waist and hip circumferences) were taken and the body mass index (BMI) and waist to hip ratio (WHR) was calculated as weight (Kg) / height (m)² and waist/hip respectively. The participants attended the laboratory in the morning after 12 hours overnight fast. A volume of 5ml of venous blood sample was then collected in EDTA coated tubes. Plasma was separated by centrifugation at 3000 rpm for 10 minutes and aliquots were stored at 70°C until analysis. EDTA tubes give Homogeneous mixing. Used for whole blood hematology testings, Immunohematology testing & Blood donor screening.

# 2.3 Biochemical Analysis

Biochemical analysis was performed on the plasma sample of each enrolled participant in order to determine the concentrations of total cholesterol, HDL-C, and triglyceride. The kits and reagents used were from BIOLABO. Each of these biochemical parameters was measured with a semi-automated analyzer (Kenza Max Biochemistry from BIOLABO) following the manufacturer instructions.

Total cholesterol level was determined as described by Allain et al. [14]. The enzymatic CHOD PAP method is a succession of three reactions in which quinoneimine, (a pink complex) is formed from cholesterol esters.

Plasma samples were treated by phosphotungstic acid (PTA) and magnesium chloride. After centrifugation, HDL cholesterol obtained in the supernatant was measured by the total cholesterol reagent.

Triglyceride concentration was evaluated by the Fossati and Prencipe [15] associated with Trinder [16] reaction. This is a succession of reactions in which quinoneimine is formed from triglycerides.

LDL cholesterol plasma concentration was obtained by Friedewald et al. [17] equation: [LDL-chol] = [Total chol] - [HDL-chol] - ([TG]/5). None of the participants had plasma TG > 400 mg/dL thus the calculation of LDL-C by Friedwald equation was appropriate.

# 2.4 Dyslipidemia

Dyslipidemia was defined according to the criteria of the National Cholesterol Education Program Adult Treatment Panel III [18]. Hypercholesterolemia was declared participants with either a total cholesterol > 200 mg/dL or an LDL cholesterol > 130 mg/dL while hypocholesterolemia was at HDL cholesterol < 40 mg/dL. Hypertriglyceridemia was declared for triglyceride plasma level > 150 mg/dL. A combination of hypercholesterolemia and hypertriglyceridemia was classified as mixed hyperlipidemia while atherogenic dyslipidemia was a combination of HDL hypocholesterolemia. hypertrialyceridemia and LDL hypercholesterolemia.

# 2.5 Data Analysis

Data were cleaned, coded and entered using Microsoft Excel and exported to EPI info 7 for statistical analysis. Data were presented as mean ± SD for continuous variables and percentages and frequencies for categorical variables. Variables were analyzed using bivariate and multivariate regression analysis, one-way analysis of variance, and Student's t-test. P < .05 was considered statistically significant.

#### 3. RESULTS AND DISCUSSION

# 3.1 Subject Characteristics

In this study, a total of 232 participants were enrolled and divided into two sets. The first set was 105 (45.3%) postmenopausal women between 45 and 81 years with a mean age of  $57.8 \pm 7.4$  years, a mean waist circumference of  $100 \pm 10$  cm and a mean BMI of  $30.4 \pm 6.4$  Kg/m². The second set was 127 (54.7%) premenopausal women between 18 and 61 years. Their mean age was  $31.9 \pm 10.0$  years while their mean waist circumference and BMI were  $80 \pm 20$  cm, and  $27.3 \pm 6.2$  Kg/m²

respectively. Table 1 shows more details on the descriptive characteristics of the participants. Waist circumference, as well as BMI, was significantly higher in postmenopausal participants when compared to premenopausal.

# 3.2 Lipid Profile

There was no significant difference in the mean values of total cholesterol, HDL-C, LDL-C as well as triglyceride between premenopausal and postmenopausal participants (Table 1). The mean HDL-C and LDL-C plasma concentrations were stable among the various age sub groups in premenopausal participants (Fig. 1). In

Table 1. Descriptive and biochemical characteristics

Variables	Premenopausal	Postmenopausal	P value	
Age (year)	31.9 ± 10.0	57.8 ± 7.4	.00*	
Height (m)	1.6 ± 0.7	1.6 ± 0.6	.35	
Weight (kg)	72.5 ± 16.5	79.5 ± 16.9	.34	
Waist (m)	$0.8 \pm 0.2$	1.0 ± 0.1	.00*	
Hip (m)	1.0 ± 0.2	1.1 ± 0.1	.03*	
BMI (Kg/m <sup>2</sup> )	27.3 ± 6.2	$30.4 \pm 6.4$	.00*	
WHR	$0.9 \pm 0.6$	$0.9 \pm 0.9$	.45	
Total Cholesterol (g/l)	1.8 ± 0.4	$1.9 \pm 0.4$	.37	
HDL Cholesterol (g/l)	0.5 ± 0.1	$0.5 \pm 0.2$	.64	
LDL Cholesterol (g/l)	1.1 ± 0.4	$1.2 \pm 0.4$	.43	
Triglyceride (g/l)	$0.7 \pm 0.3$	$0.9 \pm 0.4$	.45	

WHR: waist to hip ratio, BMI: Body mass Index, \* Statistically significant

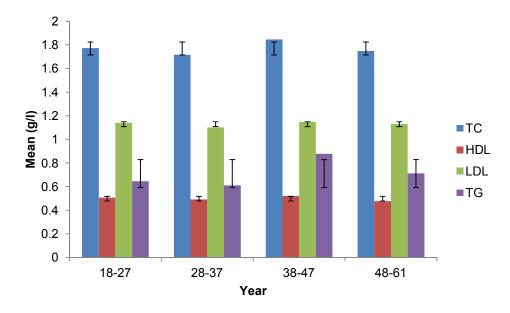


Fig. 1. Lipid profile of premenopausal participants according to age group TC: total cholesterol, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, TG: Triglyceride

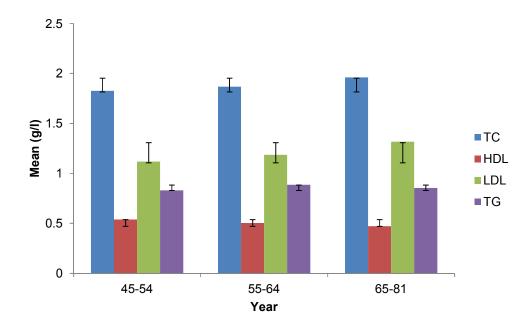


Fig. 2. Lipid profile of postmenopausal participants according to age group TC: total cholesterol, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, TG: Triglyceride

Table 2. Lipid profile according to the duration of menopause

	Total cholesterol (g/l)	HDL cholesterol (g/l)	LDL cholesterol (g/l)	Triglyceride (g/l)
YSM (year)				
1-5	1.8 ± 0.4	0.6 ± 0.2	1.1 ± 0.3	$0.8 \pm 0.4$
6-10	1.8 ± 0.4	$0.5 \pm 0.1$	$1.2 \pm 0.3$	$0.8 \pm 0.3$
11-15	1.8 ± 0.2	$0.5 \pm 0.1$	1.1 ± 0.2	$0.9 \pm 0.4$
>15	$2.1 \pm 0.4$	$0.5 \pm 0.2$	$1.4 \pm 0.2$	$1.0 \pm 0.4$
P value	.05	.15	.02*	.41

Data are presented as mean±SD, YSM: year since menopause, \* statistically significant

Table 3. Prevalence of dyslipidemia in pre- and postmenopausal participants

	Overall n=232 n (%)	Premenopausal n=127 n (%)	Postmenopausal n=105 n (%)	P value
Dyslipidemia	123 (53.0)	58 (45.6)	64 (61.0)	.03*
Hypercholesterolemia	88 (37.9)	46 (36.2)	42 (40.0)	.55
HDL hypocholesterolemia	52 (22.4)	24 (18.9)	28 (26.7)	.08
LDL hypercholesterolemia	78 (33.6)	42 (33.1)	36 (34.3)	.42
Hypertriglyceridemia	11 (4.7)	3 (2.4)	8 (7.6)	.44
Atherogenic dyslipidemia	2 (0.9)	1 (0.8)	1 (1.0)	.89
Mixed hyperlipidemia	4 (1.7)	2 (1.6)	2 (1.9)	.84

\* statistically significant

contrary, as highlighted in Fig. 2, only the mean HDL-C was stable in postmenopausal women while the mean total cholesterol and LDL-C plasma concentrations were slightly increasing as the postmenopausal participants got older. Detail analysis showed that there was no

triglyceride variation pattern observed in both pre- and postmenopausal women according to the age subgroups.

As shown in Table 2, there was a significantly higher (P = .02) mean plasma LDL-C level

Table 4. Dyslipidemia related to age category in premenopausal participants

	Age group (year)				
	18-27 (n=62)	28-37 (n=25)	38-47 (n=29)	≥48 (n=11)	P value
	n (%)	n (%)	n (%)	n (%)	
Dyslipidemia	30 (48.4)	11 (44.0)	14 (48.3)	4 (36.4)	.33
Hypercholesterolemia	25 (40.3)	7 (28.0)	11 (37.9)	3 (27.3)	.38
HDL hypocholesterolemia	11 (17.7)	7 (28.0)	5 (17.2)	1 (9.1)	.54
LDL hypercholesterolemia	23 (37.1)	7 (28.0)	9 (31.0)	3 (27.3)	.80
Hypertriglyceridemia	0 (0.0)	0 (0.0)	3 (10.3)	0 (0.0)	.02*
Atherogenic dyslipidemia	0 (0.0)	0 (0.0)	1 (1.3)	0 (0.0)	.22
Mixed hyperlipidemia	0 (0.0)	0 (0.0)	2 (6.9)	0 (0.0)	.08

\* statistically significant

Table 5. Dyslipidemia related to age category in postmenopausal participants

	Age group (year)				
	45-54 (n=43)	55-64 (n=45)	≥65 (n=16)	P value	
	n (%)	n (%)	n (%)		
Dyslipidemia	24 (55.8)	27 (60.0)	13 (81.3)	.02*	
Hypercholesterolemia	20 (46.5)	17 (37.8)	5 (31.3)	.72	
HDL hypocholesterolemia	8 (18.6)	14 (31.1)	6 (37.5)	.24	
LDL hypercholesterolemia	17 (39.5)	14 (31.1)	5 (31.3)	.67	
Hypertriglyceridemia	1 (2.3)	4 (8.9)	3 (18.8)	.07	
Atherogenic dyslipidemia	0 (0.0)	1 (2.2)	0 (0.0)	.49	
Mixed hyperlipidemia	0 (0.0)	2 (4.4)	0 (0.0)	.33	

\* statistically significant

Table 6. Dyslipidemia related to menopause duration

	Dyslipidemia	Hyper-C	HDL hypo-C	LDL hyper-C	Hyper TG	Atherogenic dyslipidemia	Mixed hyperlipidemia
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
YSM (ye	ar)						
1-5	28 (43.8)	21 (50.0)	13 (46.4)	19 (52.8)	3 (37.5)	1 (100)	1 (50.0)
6-10	14 (21.9)	8 (19.0)	7 (25.0)	6 (16.6)	0 (0.0)	0 (0.0)	0 (0.0)
11-15	2 (3.0)	0 (0.0)	1 (3.6)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)
>15	20 (31.3)	13 (31.0)	7 (25.0)	11 (30.6)	4 (50.0)	0 (0.0)	1 (50.0)
P value	.03*	.10	.89	.16	.09	.81	.73

YSM: year since menopause, Hyper-C: hypercholesterolemia, HDL hypo-C: HDL hypocholesterolemia, LDL hyper-C: LDL hypercholesterolemia, HyperTG: hypertriglyceridemia, \* statistically significant

 $(1.4 \pm 0.2 \text{ g/I})$  in participants who have been in the postmenopausal state since more than 15 years. The mean total cholesterol concentration was also higher in women with YSM > 15 (2.1  $\pm$  0.4 g/I) than the other subgroups. However, there was no significant difference between them (P = .05). HDL-C and triglyceride did not differ between the YSM subgroups.

# 3.3 Dyslipidemia

Out of the 232 pre- and postmenopausal participants of this study, 123 (53%) had

dyslipidemia. Its prevalence was higher in postmenopausal women 64 (61%) than premenopausal 58 (45.6%) with a statistical significant difference (P = .03). As shown in Table 3, the overall pattern of dyslipidemia in both groups was 88 (37.9%)hypercholesterolemia, followed by 78 (33.6%) LDL hypercholesterolemia, 52 (22.4%) HDL hypocholesterolemia and 11 (4.7%)hypertriglyceridemia. HDL hypocholesterolemia prevalence was higher in postmenopausal participants 28 (26.7%)compare premenopausal 24 (18.9%) though it was not statistically significant (P = .08). The prevalence of hypercholesterolemia, LDL hypercholesterolemia and hypertriglyceridemia statistically did not differ between the two groups (Table 3).

Less than half of premenopausal participants in each age subgroup had dyslipidemia with the highest prevalence of hypercholesterolemia and LDL hypercholesterolemia among the 18-27 years while the highest prevalence for HDL hypocholesterolemia was among the 28-37 years (Table 4).

As highlighted in Table 5, more than half of postmenopausal in each age subgroup had dyslipidemia. Dyslipidemia was significantly (P = .02) higher in women  $\ge 65$  years with more than 4 out of 5 (81.3%) women. The highest prevalence of hypercholesterolemia and LDL hypercholesterolemia was among the 45-54 years while the highest prevalence of HDL hypocholesterolemia was among the  $\ge 65$  years.

Regarding menopause duration, dyslipidemia prevalence 28 (43.8%) was significantly higher (P = .03) in the subgroup of women who have freshly entered the postmenopausal state. This 1-5 year since menopause (YSM) subgroup also had the highest hypercholesterolemia 21 (50.0%), HDL hypocholesterolemia 13 (46.4%) and LDL hypercholesterolemia 19 (52.8%) prevalences though they were not statistically significant (Table 6).

#### 3.4 Discussion

Estrogen deficiency or decline in estrogen levels after menopause often leads to dysregulation of metabolism [19]. These changes are in part due to the age-related metabolic impairment, but may also be associated with postmenopausal hypoestrogenism [20]. During menopause, there is a shift towards a more atherogenic lipid profile with an increase in plasma total cholesterol, LDL cholesterol and triglycerides and a decrease of HDL cholesterol [21,22,23,24]. Results of our study showed no significant change in the mean plasma concentration of total cholesterol, HDL, LDL cholesterols as well as triglyceride between pre- and postmenopausal participants. In other African countries such as Nigeria, Manafa et al. [25] reported also no significant variation in the mean values of total cholesterol and HDL cholesterol in pre- and postmenopausal subjects. However, the LDL cholesterol and triglyceride concentrations were significantly higher in these

postmenopausal Nigerian women compared to premenopausal subjects.

Changes in the concentration of HDL-C after menopause have been subjecting of controversy. In our study, the mean HDL-C concentrations were identical between premenopausal (0.5  $\pm$  0.1 g/l) and postmenopausal (0.5  $\pm$  0.2 g/l) subjects. Similar observations were reported by Zhou et al. [26] in pre- and postmenopausal Chinese women. In addition, stable mean HDL-C concentrations were also observed among different age subgroups of either pre- or postmenopausal participants. Unchanged HDL-C concentrations may be explained by unaltered visceral adipose tissue after menopause [27]. Low HDL-C in postmenopausal women in most previous reports may not be due to menopause, but due to weight gain, lack of activity reduced alcohol intake and associated metabolic diseases in old women [28]. HDL particles not only mediate reverse cholesterol transport but also exhibit anti-oxidant, anti-inflammatory, antithrombotic and vasodilatory activities [29,30]. With this high mean HDL cholesterol concentration, postmenopausal participants of this study may less likely have cardiovascular disease.

The mean total cholesterol and LDL-C concentrations were stable among pre- and postmenopausal participants. Age has been said to contribute to elevated lipid parameters. However, in this study, it was only in postmenopausal women that a marginal and continuous increase of both parameters from one age subgroup to another was found. Polotsky et al. [21] reported that these changes seem to be independent of age and more related to the increased abdominal fat mass. In our study, the total and LDL cholesterol concentrations were higher in subjects who have been experiencing menopause for more than 15 years. Similar observations have been reported by Manafa et al. [25] who found not only a progressive increase of total and LDL cholesterol concentrations but also the same pattern on triglyceride concentrations. However, Quadri and Dhundsi, [31] found no relation between lipid profile and time since menopause. This inconsistency may be explained by the fact that lifestyle and genetic variability between the populations could studied he determining factors affecting lipid profile in postmenopausal women rather than menopause duration.

Triglyceride concentration did not differ between pre- and postmenopausal women. Meanwhile, Guo et al. [32] have reported significantly higher levels of triglyceride in postmenopausal women compared to premenopausal. It seems like the variation in the plasma triglyceride concentration cannot be linked directly to pre- or postmenopausal status. As already reported by previous authors [33,8,9], increase levels of plasmatic triglyceride concentration were explained by age effects.

53% of these participants About had dyslipidemia. This percentage is lower than what Doupa et al. [34] reported in Senegal and very high than the value reported in Chinese women [35]. Dyslipidemia was higher in postmenopausal (61%)than premenopausal participants. Gomina et al. [36] have reported 86.98% dyslipidemia in postmenopausal Beninese women. The overall dyslipidemia pattern [88 (37.9%) hypercho-lesterolemia, followed by 78 (33.6%) LDL hypercholesterolemia, 52 (22.4%) HDL hypocho-lesterolemia and 11 (4.7%) hypertriglyceridemia] is lower than what is reported by Doupa et al. [34]. Cultural differences, race, diet which vary from one region to another as well as ethnic differences can affect dyslipidemia. In the Multi-Ethnic Study of Atherosclerosis done in America, Goff et al. [37] observed that ethnic differences in the prevalence of dyslipidemia were Chinese observed only American for subjects.

In premenopausal participants, the highest LDL hypercholesterolemia prevalence (37.1%) was among the 18-27 years subgroup. These results are in line with previous observation reporting that LDL cholesterol levels tend to increase after the age of 20 years [38]. The highest level of dyslipidemia (81.3%) in postmenopausal women ≥ 65 years corroborates results reported in Black American women [37]. In general, dyslipidemia prevalence is greater in the older age groups [37]. The highest prevalence of hypercho-lesterolemia (46.5%)and LDL hypercho-lesterolemia (39.5%) was among the 45-54 years subgroup in postmenopausal participants. In Benin, Gomina et al. [36] had instead reported the highest prevalence in women of 56 years and over. These differences could be due to genetic variability or cultural dissimilarities.

Participants with menopause duration between 1-5 years presented the highest overall dyslipidemia prevalence as well as the worse cholesterolemia patterns. The rate of dysregulation of lipid metabolism might be higher in the early postmenopausal period.

The primary limitation of this study was the characterization of participants at one point in adulthood without knowledge of the sequence of previous cardiovascular health risk factors. This cross-sectional study might not provide accurate estimates of relationships between menopausal status and these biochemical variables that could change with time. Another issue was the nonrepresentative nature of the sample that could reduce the generalizability of results, the potential for residual confounding and the potential for errors in or bias self-reported data. Furthermore, other metabolites metabolism were not analyzed. Despite these limitations, these data generated here have provided a descriptive picture on lipid profile and dyslipidemia of Cameroonian women. They could, therefore, be used as a baseline for subsequent studies. Additional studies with more parameters and lipid metabolites will enable to establish a good relationship between lipid metabolism and menopausal status in central Africa.

## 4. CONCLUSION

This study concluded that there was no significant difference in the mean values of total cholesterol, HDL-C, LDL-C as well as triglyceride between premenopausal and postmenopausal women. The mean HDL-C concentration was stable in postmenopausal participants thought their total cholesterol and LDL-C levels were slightly increased as they got older. LDL-C level was significantly higher in women who have been experiencing menopause for more than 15 years. Overall 53% of the participants had dyslipidemia with a higher prevalence in postmenopausal women. Dyslipidemia was significantly higher in the subgroup of women who have freshly entered menopause. To maintain a good lipid profile, women should be encouraged to have a good lifestyle with moderate physical activity and lower dietary fat intake.

# **CONSENT**

All participants provided informed consent. Each informed consent was written because each participant enrolled in this study gave its approval by signing an informed consent form.

#### ETHICAL APPROVAL

This study was approved by the National Committee Ethics for Human Health Research (ethic clearance N° 2016/08/803/CE/CNERSH/SP). Participants of cross-sectional study were healthy volunteers living in Yaounde to whom the research aim was explained and their questions properly answered. They were informed of their complete right to withdraw from the study before the publication of the results without any explanation. During analysis, data collected from each subject were anonymous and kept confidential.

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#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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