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Further investigation of the occurrence mechanism of lipodystrophy with HIV protease inhibitors in HIV-infected patients

Étude du mécanisme d'apparition de la lipodystrophie avec les inhibiteurs de la protéase du VIH chez les patients infectés par le VIH

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Article original

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Mots-Clés: Alpha glucosidase; Inhibiteurs de protéase du VIH ; Lipodystrophie ; Alpha amylase pancréatique ; Lipase pancréatique

ABSTRACT

Introduction: Lipodystrophy is a long-term metabolic complication in HIV infected patients on antiretroviral therapy. We aimed to determine the mechanism by which lipodystrophy occurs with HIV protease inhibitors.
Methods: We performed an *in-vitro* analytical study, by setting our sights on carbohydrate-lipid metabolism enzymes such as alpha glucosidase, alpha amylase and lipase, the activity of which most often undergoes disorders observed in cases of lipodystrophy. The principle of this methodology consisted in carrying out *in-vitro* enzyme inhibition assays by the spectrophotometric method on these enzymes, in order to determine a probable inhibition of their activity by the HIV protease inhibitors used in antiretroviral therapy. And then, to compare the inhibition obtained with the one of reference inhibitory molecules such as acarbose and orlistat.
Results: The results revealed that in the presence of HIV protease inhibitor like 59.98% with an IC50 of 3.76±0.03 mg/ml and similarly in the presence of Lopinavir 200 mg /Ritonavir 100 mg, this pancreatic lipase activity was reduced to 63.78% with an IC_{50} of 4.62±0.09 mg/ml. The two antiretroviral drugs presented IC_{50} which were statistically significant compared with the one of orlistat (p.value=0.01) with an inhibition percentage of 58.98% and IC_{50} of 3.62 ± 0.01 mg/ml.
Conclusion: HIV protease inhibitors, used in antiretroviral therapy exhibit an inhibitory effect on some enzymes involved in biological processes in the body, thus causing the lipodystrophy.

RESUME

Introduction: La lipodystrophie est une complication métabolique retrouvée chez les patients sous traitement antirétroviral. Le but de l'étude était de déterminer le mécanisme par lequel la lipodystrophie survient avec les inhibiteurs de protéase du VIH.
Méthodes : Il s'agissait d'une étude analytique in-vitro, s'intéressant aux enzymes telles que l'alpha glucosidase, l'alpha amylase et la lipase, dont l'activité est altérée en cas de lipodystrophie. Le principe consistait à réaliser des tests d'inhibition enzymatique in-vitro par la méthode spectrophotométrique, afin de déterminer une inhibition de leur activité par les inhibiteurs de protéase du VIH. Puis, de comparer l'inhibition obtenue avec celle de molécules inhibitrices de référence telles que l'acarbose et l'orlistat.
Résultats : Les résultats révélaient qu'en présence d'un inhibiteur de protéase du VIH comme l'Atazanavir 300 mg/Ritonavir 100 mg, l'activité de la lipase pancréatique était réduite à 59,98% avec un IC50 de 3,76±0,03 mg/ml et de même en présence de Lopinavir 200 mg/Ritonavir 100 mg, cette activité de la lipase pancréatique était réduite à 63,78% avec un IC50 de 4,62±0,09 mg/ml. Les deux médicaments présentaient des IC50 statistiquement significatives par rapport à celles de l'orlistat (valeur p=0,01) avec un pourcentage d'inhibition de 58,98% et une IC50 de 3,62 ± 0,01 mg/ml.
Conclusion : Les inhibiteurs de la protéase du VIH, présentent un effet inhibiteur sur certaines enzymes impliquées dans les processus biologiques provoquant la lipodystrophie.

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Introduction

The advent of highly active antiretroviral therapy (HAART) has significantly reduced the morbidity and mortality associated with the Human Immunodeficiency Virus (HIV) [1], but, as life expectancy has increased, the incidence of chronic diseases has also increased [2]. This change in the type of morbidity associated with HIV highlights the importance of understanding the effects of HIV and Highly Active Antiretroviral Therapy (HAART) on the biological processes in the body. Lipodystrophy is one of the long-term metabolic complications in people with HIV infection undergoing the Highly Active Antiretroviral Therapy (HAART). It is a very remarkable metabolic complication associated with glucose-lipid disorders such as the insulin resistance and dyslipidemia which can also affect the cardiovascular system.

Lipodystrophy is characterized by an abnormal distribution of fat in the body, manifested by atrophy of the cheeks, face, arms, legs and buttocks (lipoatrophy). And sometimes there is also an accumulation of fat in the trunk, chest or neck (lipohypertrophy). Sometimes there are areas of the body that are quite enlarged, especially the chest, neck (sometimes with a buffalo hump) and abdomen due to an increase in visceral adipose tissue. The combination of the two (lipohypertrophy and lipoatrophy) gives a mixed syndrome.

Metabolic disorders occurring in cases of lipodystrophy, depend on the therapeutic molecules used. While the Stavudine and especially some Protease Inhibitors (PI) such as the Ritonavir and the Lopinavir give dyslipidemia, the Indinavir will primarily affect the glucose metabolism and give a resistance to insulin which can progress to diabetes [3-4]. T

The very presence of lipodystrophy with peripheral lipoatrophy and especially inflammation of the visceral adipose tissue, insulin-resistant, will also alter metabolic parameters. The lipid profile frequently found in these patients is an atherogenic profile with an increase of triglycerides and total cholesterol, a drop of HDL-Cholesterol, an increase of LDL-Cholesterol in the body. Therefore, these patients are at high cardiovascular risk.

The prevalence of lipodystrophy is estimated at 3.07 cases per million inhabitants worldwide [5]. The prevalence of the lipodystrophy depends on the type of therapy used. Estimates of 20% to 70% of affected patients have been advanced [6]. However, new molecules available on the market made this metabolic complication less common in patients on antiretroviral therapy [6]. A recent study done on the lipoatrophy seen by double absorbsiometry-X scanner (DEXA-scan) reported a reduction of 20% of the peripheral fat [7]. In this study, 32% of patients were on two Reverse Transcriptase Inhibitors (RTI) and Efavirenz, 18% of patients were on two Reverse Transcriptase Inhibitors (RTI) and Lopinavir boosted by the Ritonavir, but only 8% of patients treated without Reverse Transcriptase Inhibitors (RTI) developed a lipoatrophy. In the Aquitaine cohort in France, 38% of patients had signs of lipodystrophy, including 16% for lipoatrophy, 12% for lipohypertrophy and 10% for mixed syndrome [8]. In sub-Saharan Africa, a Rwandan study has estimated the prevalence of lipodystrophy at 34% [9]. Another study carried out on patients infected with HIV undergoing antiretroviral therapy at the regional hospital of Bertoua in Cameroon, reported a prevalence of 27.64% for lipoatrophy cases, 20.95% for lipohypertrophy cases and 1,93% for mixed syndrome cases [10].

The mechanism by which Nucleotide Reverse Transcriptase Inhibitors (NRTI) cause lipodystrophy is less obvious. It has been hypothesized that it is through the inhibition of gamma DNA polymerase, the enzyme responsible for mitochondrial DNA replication [11]. The subsequent depletion of mitochondrial DNA leads to a decrease of the transcription of mitochondrial enzymes and ultimately, mitochondrial dysfunction. This is at origin of an alteration of the oxidation of fatty acids and disturbs the balance between production and energy reserves, leading to lipodystrophy and insulin resistance. On the other hand, the occurrence mechanism of lipodystrophy with Protease Inhibitors (PI) merits further investigation.

Hence the research question related to the problem of our study which revolved around the cessation of antiretroviral therapy by the HIV infected patient following the physical and psychological discomfort caused by the constant occurrence of lipodystrophy despite the repeated modification therapeutic

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protocols. Here, we aimed to determine the mechanism by which lipodystrophy occurs.

Materials and Methods

Site, period and type of study

We performed an *in-vitro* analytical study from 10th February 2021 to 30th September 2022 at the laboratory of pharmacology and drug discovery from the Institute of Medical Research and Medicinal Plants Studies (IMPM) in Yaounde Cameroon.

General procedure of enzyme inhibition assay

In the general procedure of our enzyme inhibition assays, the inhibitor solution consisted of the antiretroviral molecule (HIV Protease inhibitor). Acarbose was used as the reference inhibitor for the enzymes alpha glucosidase and pancreatic alpha amylase. The orlistat was the reference inhibitor for pancreatic lipase. Enzyme solutions included either pancreatic alpha amylase, alpha glucosidase, pancreatic lipase. The phosphate buffer solution was added to the mixture to make the pH of the solution constant. The substrate solution consisted of either starch for the pancreatic alpha amylase inhibition assay, the 4-nitrophenyl α-D-glucopyranoside for the alpha glucosidase inhibition assay, and the Olive oil for the pancreatic lipase inhibition assay. After adding the substrate solution and incubation, we proceeded to reading the absorbance on the spectrophotometer to then determine the concentration of the solution.

The percentage inhibition was determined through the formula: **%= Abs (control) - Abs (sample) / Abs (control) x 100 (Abs= Absorbance or the Optical Density).**
The minimum inhibitory concentration of inhibitor was determined graphically from the logarithm of the solution concentration: IC_{50} = f [log (concentration)].

Statistical analysis

The student test with a significant threshold value fixed at 5% was used to compare the means of minimum inhibitory concentration (IC_{50}) through the R software version 2.13. The Graphpad software allowed us to plot the graphs and more easily calculate the minimum inhibitory concentrations (IC_{50}) of the enzymatic activities.

The inhibitory effects of the antiretroviral drugs used on the enzymes were expressed as a inhibition percentages and the IC_{50} data as means ± standard error (SE) for at least three determinations (n=3).

We worked in this study only with HIV protease inhibitors more used in Cameroon (Atazanavir, Lopinavir and Ritonavir) to evaluate their inhibitory effects on enzymes from the carbohydrate-lipid metabolism with the spectrophotometry method. The enzymes used were the alpha glucosidase (EC 3.2.1.20) extracted from the intestinal mucosa of the wistar rat fasted for 24 hours, the pancreatic alpha amylase (E.C.3.2.1.1) extracted from pig pancreas in freeze-dried form, and the pancreatic lipase (EC 3.1.1.3) extracted from mouse pancreas.

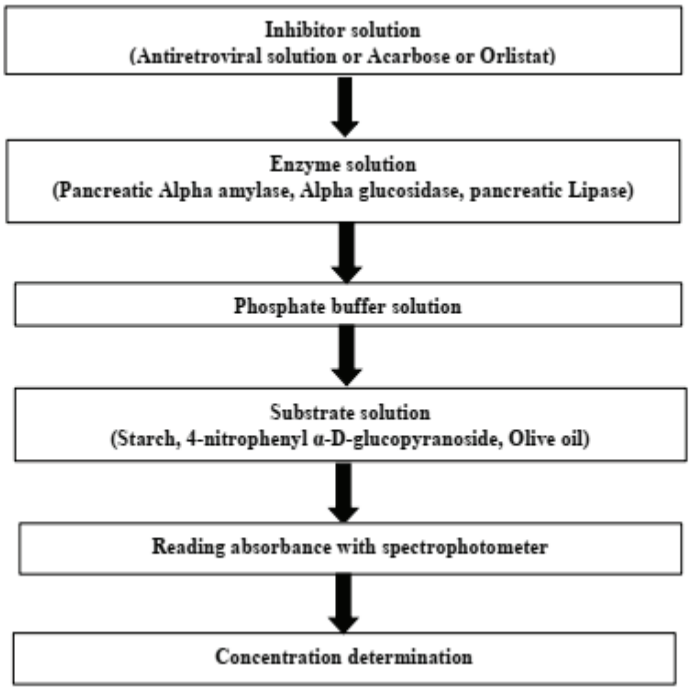

Figure 1: Graphical representation of the enzyme inhibition assay

The enzyme activities of pancreatic alpha-amylase and alpha-glucosidase were measured *in vitro* with their respective substrates such as the starch and the ρ-nitrophenyl glucopyranoside (ρNP-glucose). We have nevertheless chosen these substrates essentially for their convenience in carrying out the assays by the spectrophotometric method.

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The inhibitions assays of activities enzymes pancreatic alpha-amylase and alpha-glucosidase were carried out using respectively the pig pancreas alpha-amylase and the alpha-glucosidase extracted from the intestinal mucosa of the wistar rats, all close structurally and kinetically from those encountered in humans [17]. Acarbose was used as a positive control and reference inhibitor [18] with an IC_{50} of 0.07 ± 0.002 mg/ml for the pancreatic alpha-amylase and 0.05 ± 0.001 mg/ml for alpha-glucosidase with respective inhibition percentages of 59.98% and 63.95% (**Table 1**).

Table 1: Distribution of IC_{50} values and inhibition percentages of enzymes activities

Enzymes Inhibitory molecules	Pancreatic Alpha amylase		Alpha glucosidase		Pancreatic lipase		OR (CI to 95%)	P.value
	IC50 (mg/ml)	%	IC50 (mg/ml)	%	IC50 (mg/ml)	%		
Atazanavir 300 mg / Ritonavir 100 mg	33.92 ± 1.64	58.97	24.04 ± 1.54	47.96	3.76 ± 0.03	59.98	3.66 (2.11-5.36)	0.01
Lopinavir 200 mg / Ritonavir 100 mg	45.78 ±1.77	60.97	38.59 ± 1.83	65.98	4.02 ± 0.09	63.78	4.56 (2.36-6.89)	0.01
Ascarbose	0.07± 0.002	59.98	0.05 ± 0.001	63.95	/	/	1.58 (0.23-3.29)	0.76
Orlistat	/	/	/	/	3.62 ± 0.001	58.98	1.04 (0.99-3.56)	0.51

C_{50}= Minimum inhibitory concentration; %= Inhibition Percentage; OR= Odd-Ratio; CI= Confidence Interval

The results obtained concerning the *in vitro* assessment of the inhibitory effect from the respective activities of pancreatic alpha-amylase and alpha-glucosidase with the antiretroviral drugs used (Atazanavir 300 mg / Ritonavir 100 mg and Lopinavir 200 mg / Ritonavir 100 mg) compared with the inhibitory effect of acarbose considered as a reference inhibitor of the same enzymes, presented non-statistically significant values, respectively IC_{50} of 33.92±1.64 mg/ml (P.value = 0.87) and 45.78±1.77 mg/ml (P.value= 0.96) for the alpha-amylase (**Figure 1a, 1b, 1c**) and IC_{50} of 24.04±1.54 mg/ml (P.value = 0.76) and 38.59±1.83 mg/ml (P.value = 0.91) for the alpha-glucosidase (**Figure 2a, 2b, 2c)**.

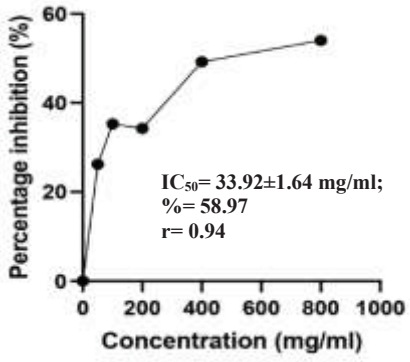

Figure 1a: Representative curve of the inhibitory effect of Atazanavir 300mg/Ritonavir 100 mg on pancreatic alpha amylase activity.

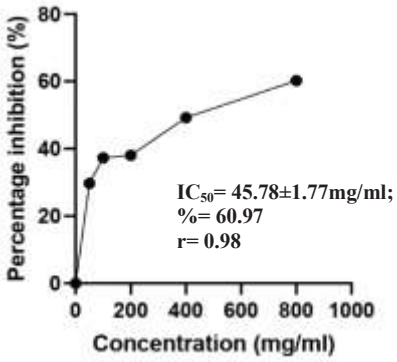

Figure 1b: Representative curve of the inhibitory effect of Lopinavir 200mg/Ritonavir 100 mg on pancreatic alpha amylase activity.

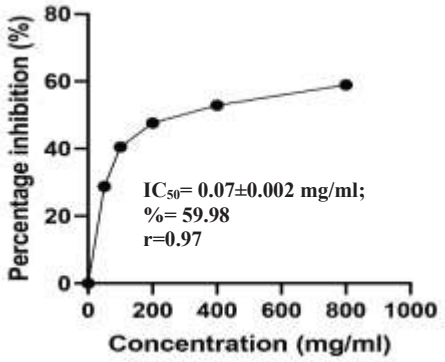

Figure 1c: Representative curve of the inhibitory effect of Acarbose on pancreatic alpha amylase activity.

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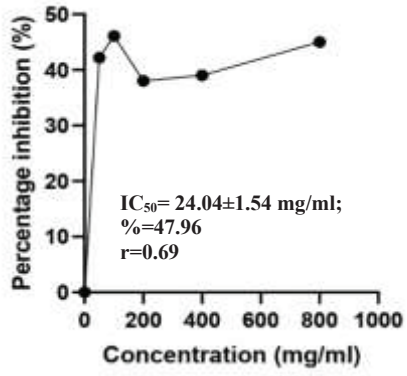

Figure 2a: Representative curve of the inhibitory effect of Atazanavir 300 mg/Ritonavir 100 mg on alpha glucose

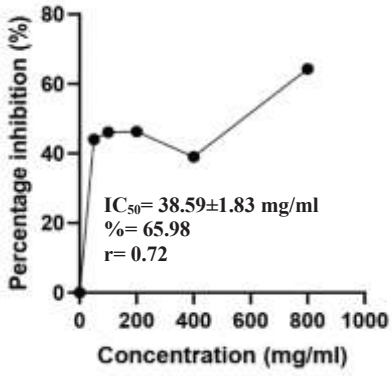

Figure 2b: Representative curve of the inhibitory effect of Lopinavir 200 mg/Ritonavir 100 mg on alpha glucosidase activity.

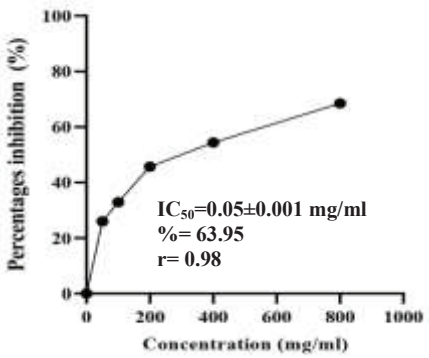

Figure 2c: Representative curve of the inhibitory effect of Acarbose on alpha glucosidase activity.

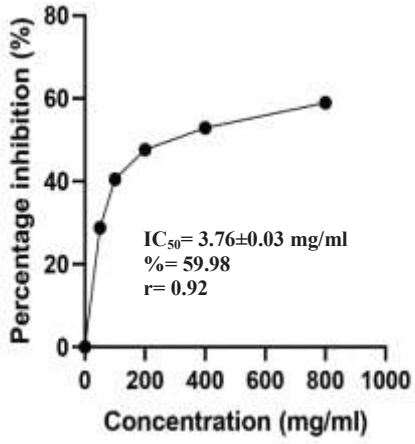

Figure 3a: Representative curve of the inhibitory effect of Atazanavir 300 mg/Ritonavir 100 mg on pancreatic lipase activity.

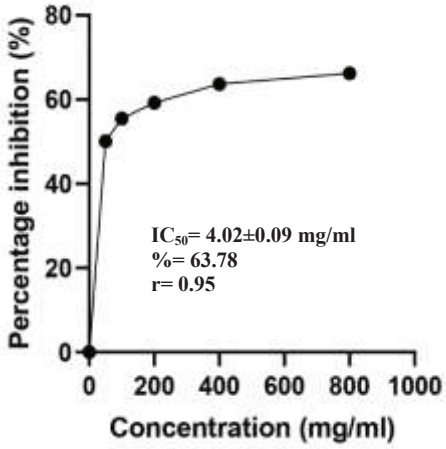

Figure 3b: Representative curve of the inhibitory effect of Lopinavir 200 mg/Ritonavir 100 mg on pancreatic lipase activity.

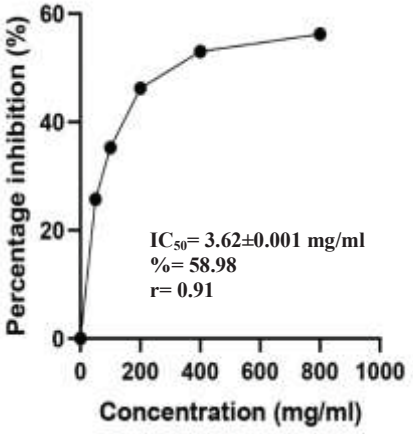

Figure 3c: Representative curve of the inhibitory effect of Orlistat on pancreatic lipase activity.

The non-statistically significant value found in this study could be justified by the difference in the inhibition mechanism of the inhibitors used. These inhibitory effects obtained with antiretroviral drugs used on the pancreatic alpha-amylase and alpha-glucosidase also had non-statistically significant values compared to the inhibitory effects of thirteen plants used in the study carried out in Algeria by BECHIRI-ABBES on the same enzymes, namely: P. angustifolia (0.61 mg/ml), M. inodora (0.66 mg/ml), O. europaea (0.99 mg/ml), J. oxydrus (1.20 mg/ml), O. sylvestris (1.23 mg/ ml), S. officinalis (1.30 mg/ml), A. iva (1.51 mg/ml), A. halimus (1,56 mg/ml), B. dioica (2.09 mg/ml), U. dioica (2.25 mg/ml), Z. album (2.52 mg/ml), P. persica (2.86 mg/ml), N. oleander (3.02 mg/ml) for the pancreatic alpha-amylase (P.value = 0.59) [19] And S. officinalis (10.14 µg / ml), P. angustifolia (35.48 µg/ ml), J. oxydrus (66.06), O. europaea (75.96 µg/ ml) for the alpha-glucosidase (P.value = 0.66) [19].

The non-significant inhibitory effects observed with the antiretroviral drugs (Atazanavir 300 mg/Ritonavir 100 mg and Lopinavir 200 mg/Ritonavir 100 mg) used in our study on pancreatic alpha-amylase and alpha-glucosidase compared with the one of acarbose could be justified by the difference in the inhibition mechanism of the inhibitors used and this

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took us away from the hypothesis of a probable inhibition of these enzymes in the occurrence mechanism of the lipodystrophy in HIV-infected patients undergoing antiretroviral therapy.

The inhibitory effect of antiretroviral drugs used was tested using the mouse pancreatic lipase extracted using the protocol of the study made by Shahani 1975 on the isolation, homogeneity and characterization of bovine pancreatic lipase [15], with appropriate modifications while also using olive oil as a substrate. The assessment showed that the activity of pancreatic lipase was affected by the antiretroviral drugs used. A maximum enzyme activity was observed during the incubation without the presence of antiretroviral drugs in the reaction environment, which confirmed that our source of enzyme was functional. As soon as the different concentrations of antiretroviral drugs were added in increasing order (50; 100; 200; 400 and 800 mg/ml), a decrease of the enzyme activity was observed up to a concentration of 800 mg/ml. Indeed, in the presence of the Atazanavir 300 mg/ Ritonavir 100 mg, the lipase activity was reduced to 59.98 % with an IC_{50} of 3.76 ± 0.03 mg/ml (**Figure 3a**) and similarly in the presence of the Lopinavir 200 mg/ Ritonavir 100 mg, this lipase activity was reduced to 63.78% with an IC_{50} of 4.62 ± 0.09 mg/ml (**Figure 3b**). The inhibitory effects of these two antiretroviral drugs had statistically significant values compared to the inhibitory effect of Orlistat used as a positive control and reference inhibitor [18] with an inhibition percentage of 58.98 % and an IC_{50} of 3.62 ± 0.01 mg/ml (**Figure 3c**).

These inhibitory effects also had statistically significant values (P.value= 0.03) compared to those of extracts from the plants Fraxinus angustifolia and Clematis flammula used in the study made in 2018 in Algeria by Azzem Celia & Benhellal Samia on the same enzyme, which had respective IC_{50} of 2.5 mg/ml and 5 mg/ml [20].

The significant inhibitory effects observed with antiretroviral drugs (Atazanavir 300 mg / Ritonavir 100 mg and Lopinavir 200 mg / Ritonavir 100 mg) used in our study on the lipase, joined the probable hypothesis of inhibition of this enzyme activity in the occurrence mechanism of lipodystrophy and its carbohydrate-lipid disorders observed in HIV-infected patients under antiretroviral therapy [21].

In fact, HIV Protease Inhibitors (PI) seem to bind to the LRP protein (LDL-Receptor-Related Protein), thus resulting an inhibition of lipase activity and consequently an alteration of the cellular uptake of chylomicrons [18]. This alteration of the chylomicrons uptake responsible for the transport of food-borne lipids in the liver is believed to cause steatorrhea, dyslipidemia and also steatosis in the liver and pancreas which will disturb the production of insulin by the pancreatic beta cells of the Langerhans islets with a harmful repercussion observed on the glucose metabolism in HIV-infected patients undergoing antiretroviral therapy.

The limitation of our study was noted in the assessment of the inhibitory effect of antiretroviral drugs used (Atazanavir 300 mg / Ritonavir 100 mg and Lopinavir 200 mg / Ritonavir 100 mg) on other enzymes of carbohydrate-lipid metabolism such as invertase, cholesterol esterase etc. and also on other metabolic pathways from the body which could allow us to better understand the impact of these antiretroviral drugs in the occurrence mechanism of lipodystrophy and its carbohydrate-lipid disorders in HIV-infected patients undergoing antiretroviral therapy.

Conclusion

Our study reported the presence of a partial inhibition of pancreatic lipase activity upon contact with HIV protease inhibitors such as the Atazanavir, Ritonavir and Lopinavir. Which amounts to saying that, inhibitors directed against HIV and used in antiretroviral therapy also have an inhibitory effect on some enzymes involved in biological processes in the body, like DNA gamma polymerase for nucleotide inhibitors of reverse transcriptase (NIRT) and pancreatic lipase for protease inhibitors (PI), thereby causing long-term metabolic complications like lipodystrophy in HIV-infected patients undergoing antiretroviral therapy. Given that the follow-up of lipodystrophy is not yet officially recognized and poses several problems affecting its diagnosis and therapeutic follow-up, the most effective option remains the prevention of its occurrence in perspectives of research relating to this pathological entity.

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Competing interest:
The authors have reported no competing interests.

Author's contributions: All the authors contributed to the design and execution of this study. All the authors have read and approved the final version of manuscript.

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