Pathophysiology of Adipocyte Defects and Dyslipidemia in HIV Lipodystrophy: New Evidence from Metabolic and Molecular Studies

[1,6]Ashok Balasubramanyam, [1,6]Rajagopal V. Sekhar, [2,3]Farook Jahoor
[4]Henry J. Pownall and [5]Dorothy Lewis
[1]Translational Metabolism Unit, Division of Diabetes, Endocrinology and Metabolism,
[2]Department of Pediatrics, [3]Children's Nutrition Research Center
[4]Section of Atherosclerosis and Lipoprotein Metabolism, [5]Department of Immunology
Baylor College of Medicine; [6]Endocrine Service, Ben Taub General Hospital, Houston, Texas

Abstract: Despite a burgeoning mass of descriptive information regarding the epidemiology, clinical features, body composition changes, hormonal alterations and dyslipidemic patterns in patients with HIV lipodystrophy syndrome (HLS), the specific biochemical pathways that are dysregulated in the condition and the molecular mechanisms that lead to their dysfunction, remain relatively unexplored. In this paper, we review studies that detail the metabolic basis of the dyslipidemia - specifically, the hypertriglyceridemia - that is the serologic hallmark of HLS and present new data relevant to mechanisms of dyslipidemia in the postprandial state. We also describe preliminary experiments showing that in addition to the well-known effects of highly-active antiretroviral drugs, the functional disruption of adipocytes and preadipocytes by factors intrinsic to HIV-infected immunocytes may play a role in the pathogenesis of HLS.

Key words: Triglycerides, cholesterol, lipoprotein lipase, lipolysis, lymphocyte

INTRODUCTION

Characteristics of dyslipidemia and its relationship to lipodystrophy: A characteristic dyslipidemic pattern observed in the majority of patients with HLS is moderate to severe hypertriglyceridemia and reduced serum HDL cholesterol, with serum LDL cholesterol that is slightly elevated or altered in composition to small, dense particles[1-5]. This resembles the typical dyslipidemia of the Metabolic Syndrome, albeit in an accelerated and exaggerated form. Indeed, insulin resistance[6-9] and increased cardiovascular risk[10-16] have been associated with HLS in several studies. A subset of these patients also manifest generalized lipoatrophy or truncal fat accumulation associated with peripheral lipoatrophy. We have previously proposed, on the basis of whole body lipid kinetic data, a pathophysiologic scheme whereby fundamental defects in adipocyte function lead to a chain of metabolic consequences that explain hypertriglyceridemia, insulin resistance and increased cardiovascular risk in HLS[17, 18]. The mechanisms associated with depot-specific adipose alterations, i.e., atrophy in some regions and hypertrophy in others, are not easy to explain on the basis of whole-body metabolic measurements and they require investigations of lipid turnover in specific body regions, which are currently under way.

Metabolic basis of HLS - studies in the fasting and fed state: Whole body kinetic studies have demonstrated defects in specific lipid metabolic pathways in HLS patients in both the fasting[19-21] and fed (25) states. Studies in the fasted state uniformly reveal accelerated whole body lipolysis in patients with different phenotypic forms of HLS. Sekhar *et al*. studied patients with the "mixed" form (i.e., with peripheral atrophy and central obesity) of HLS using infusions of $^{13}C_1$-palmitate, $^2H_2$-glycerol and indirect calorimetry[19] and showed that, compared to age-, gender- and BMI-matched non-HIV healthy controls, these patients had markedly increased rates of both total and net lipolysis. Despite a modest concomitant increase in the rate of intra-adipocyte reesterification, there was a net excess release of free fatty acids (FFA's) into the plasma pool. The HLS patients lacked the ability to increase fat oxidation proportionately, resulting in a net increase in hepatic flux of FFA's resulting in increased availability of FFAs for hepatic reesterification and increased release of VLDL-triglyceride into the circulation. Reeds *et al*. confirmed the latter finding by direct measurement of the rate of VLDL-triglyceride synthesis in HLS patients[20].

Another fate of un-oxidized plasma FFA's derived from excessive lipolysis would be increased deposition of fatty acids in myocytes and hepatocytes. Indeed, excessive intramyocellular fat has been noted in patients with HLS[7] and this is strongly associated with

Corresponding Author: Ashok Balasubramanyam, M.D., Baylor College of Medicine, Room 700B, One Baylor Plaza, Houston, Texas 77030, Tel: 713-798-8654, Fax: 713-798-3810

the development of insulin resistance in skeletal muscle[7, 22]. An indirect correlate of this effect is that the FFA's that form the substrate for fat oxidation in HLS patients are derived mainly from non-plasma sources[19] such as intramyocellular lipid depots. Finally, elevated plasma FFA's in HLS patients would also exacerbate an atherogenic plasma lipid profile by increasing the activity of cholesteryl ester transfer protein, which catalyzes transfer of triglycerides of VLDL to HDL and LDL[23,24]. The resulting TG-rich HDL and LDL would be good substrates for hepatic lipase, generating smaller, more atherogenic (small, dense LDL) and less atheroprotective (HDL3) lipoprotein particles. We have proposed the term "systemic steatosis" for the whole body disruption of lipid metabolism that results from a primary adipocyte defect in HLS, to emphasize that this unusual pathogenic cascade causes not only dyslipidemia but also excess lipid deposition in other tissues (muscle, liver, beta cells), leading to lipotoxic insulin resistance[17].

These findings in HLS patients in the fasted state do not completely account for the degree of hypertriglyceridemia in HLS patients. Recently, Sekhar *et al*. have demonstrated profound defects in adipocyte function and lipase action in the postprandial state that result in significant hypertriglyceridemia in the plasma chylomicron fraction[25]. HLS patients were fed a meal mixed with two grams of a triglyceride labeled in the terminal carbon position on all three fatty acids ($^{13}C_3$-tripalmitin). At baseline, in the fasted state, chylomicron-TG concentrations were markedly higher in the HLS patients than in normal non-HIV controls. Blood and breath sampling for 8 hours following the meal showed that the ability of HLS patients to dispose of the labeled triglyceride from the CM pool was impaired five-fold compared to that of non-HIV healthy persons. Furthermore, tracking the labeled free palmitic acid revealed that the HLS patients also had a marked inability to store diet-derived fatty acids within adipocytes in the postprandial period (10% of the fatty acid label within the chylomicron pool was stored 8 hours after the meal in HLS patients, compared to 90% in the controls). These data imply that there is a profound defect in the postprandial function or regulation of adipocyte lipoprotein lipase in HLS patients (Fig. 2). Since the elevated lipolytic rates in the fasted state point to dysregulation of hormone sensitive lipase (HSL), the lipid kinetic data *in toto* indicate that two key insulin-regulated lipases - HSL and LPL - are severely dysfunctional in HLS and that these defects can account for most of the cascade of metabolic abnormalities that form the basis of the dyslipidemia in HLS. The extent to which these defects arise from specific inhibition of the enzymes as opposed to a general disruption of adipocyte function, remains an open issue, as is the question of the underlying

mechanisms (HAART drugs, viral factors, immune responses).

"Rational" treatments aimed at the metabolic defects: Treatment of the dyslipidemia of HLS has proven to be difficult and in some patients, an intractable problem. There is inadequate response to many standard lipid lowering agents, a high frequency of adverse effects and interference by some agents of the metabolism of antiretroviral drugs. Metformin, a drug that enhances insulin sensitivity, has produced modest benefit, suggesting an interconnection between the mechanisms of dyslipidemia and insulin resistance[26,27]. There is also evidence that the dietary habits and physical activity of patients with HLS tend to be quite suboptimal[28]. In this context, the specific metabolic defects uncovered by the lipid kinetic studies offer specific targets at which to direct rational treatment to improve dyslipidemia in HLS (Fig 2). Agents directed at inhibiting lipolysis (such as niacin or acipimox), increasing fat oxidation (such as fibrates and other PPAR-alpha agonists, or leptin), attenuating the redistribution of fat (such as growth hormone) or enhancing adipocyte development and function (such as thiazolidinediones or other PPAR-gamma agonists) would appear to be attractive therapeutic candidates, alone or in combination. Lifestyle interventions would also be a key aspect of rational therapy: a low fat diet (rather than the usual low-carbohydrate diet recommended for hypertriglyceridemia) might help attenuate post-prandial hypertriglyceridemia and regular exercise would diminish plasma and tissue free fatty acids by enhancing their oxidative disposal. Each of these interventions is currently under study in different clinical trials. One multiple arm, blinded, placebo-controlled trial sponsored by the NIH, termed "Heart Positive", is investigating the effects of stepwise addition of sustained-release niacin, fenofibrate, or both agents against the background of a carefully supervised program of aerobic and resistance exercise together with a regulated diet designed for treatment of the Metabolic Syndrome (the National Cholesterol Education Program Adult Treatment Panel-III diet). The effects of these interventions on the primary endpoint (plasma triglyceride lowering) will not be available for three years; however, it is interesting to note that some of the most significant improvements in hypertriglyceridemia in HLS patients have been observed in small trials of fibrates or niacin[4,29-31].

We have utilized a mechanistically-driven approach that complements standard clinical trials for assessing rational therapy for dyslipidemia in patients with HLS. Because the stable isotope protocols have been optimized to define the specific lipid kinetic defects in a quantitative manner in HLS patients, targeted interventions can be applied for brief periods of time to assess their effects on the defective kinetic
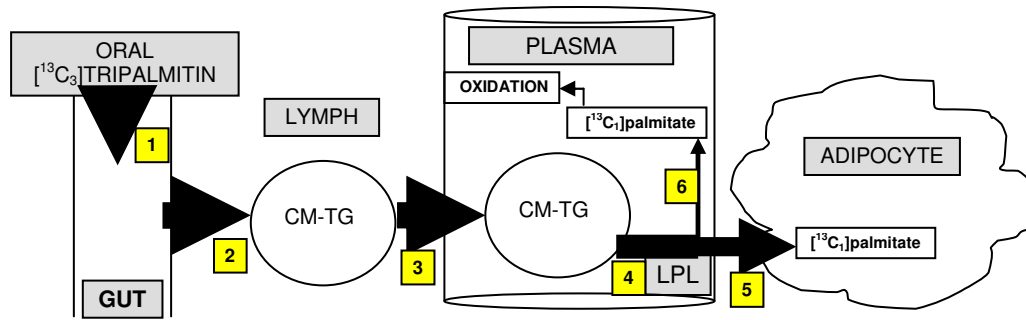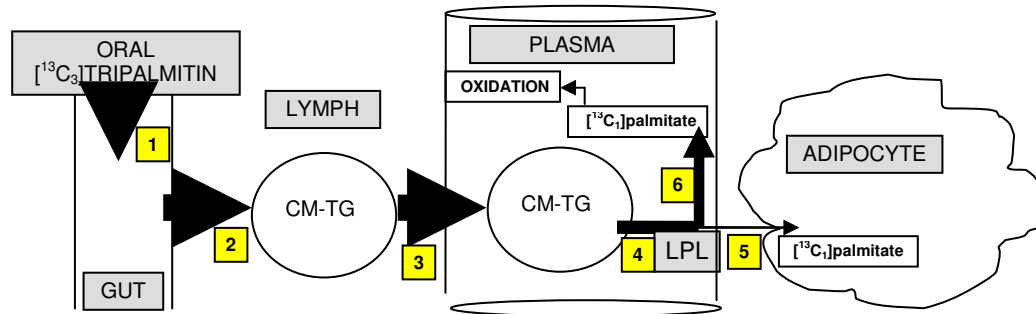
Fig. 1: Molecular defects in the disposal of dietary triglycerides in HIV lipodystrophy. 1 = entry of labeled triglyceride into the gut, 2 = absorption into chylomicrons, 3 = entry of chylomicrons into plasma, 4 = triglyceride hydrolysis, 5 = fatty acid "entrapment" in adipocyte, 6 = free fatty acid release into plasma. The major defect seems to occur at step 5.
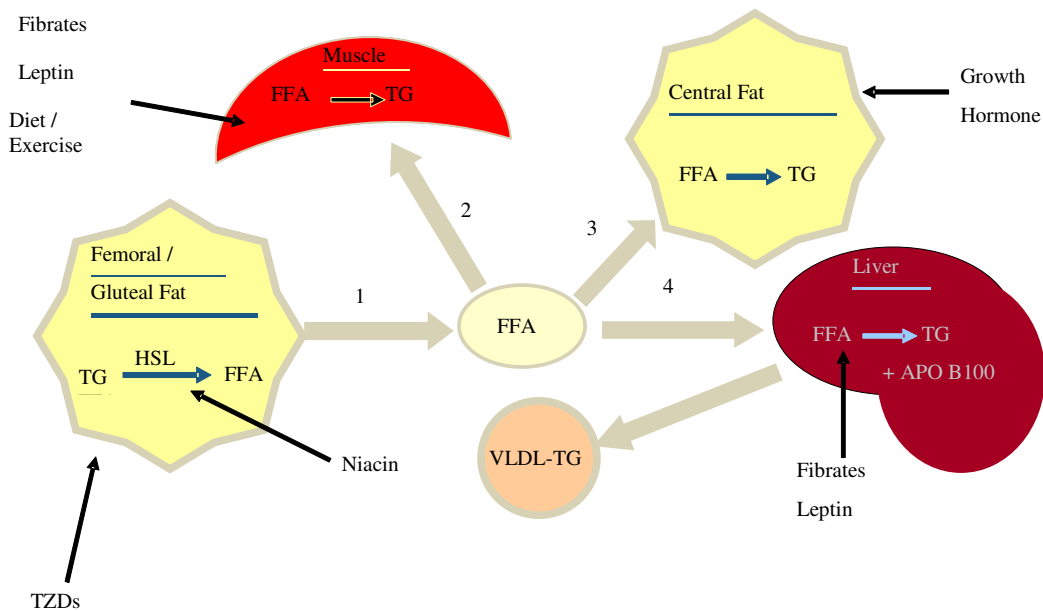

Fig. 2: Potential targets for "rational" therapy of dyslipidemia and lipotoxic insulin resistance in HIV lipodystrophy. 1 = increased release of free fatty acids from adipocytes, 2 = intramyocellular deposition of lipids, 3 = selective deposition of free fatty acids in central visceral fat depots, 4 = intrahepatocellular deposition of lipids.

rates and metabolic endpoints. In this manner, the effects of growth hormone replacement therapy on lipid kinetics in HLS have been assessed and those of treatment with leptin and rosiglitazone are under way. The rationale for GH treatment is that patients with HLS are frequently partially GH-deficient (in relative proportion to the degree of visceral adiposity)[32] and that a putative metabolic benefit of GH treatment is to enhance fat oxidation. GH replacement in HLS patients with unequivocal GH deficiency appears to have the salutary though somewhat paradoxical effect of decreasing the rates of lipolysis without increasing fat oxidation (33). These preliminary results suggest a mechanistic basis for the recently reported clinical effects of restoring physiologic plasma levels of GH on improving body composition and fat redistribution in patients with HLS[33].

Molecular basis of HLS - role of immunocyte-adipocyte interactions: What is the molecular basis of the adipocyte defects that lead to the metabolic disorders described above? A great deal of *in vitro* investigation has been focused on the role of antiretroviral drugs on adipocyte function[34, 35] and on the ability of preadipocytes to differentiate into mature adipocytes[36]. As in most metabolic illnesses, the pathophysiology is likely to be multifactorial and complex and there is clearly evidence that hypertriglyceridemia was observed in HIV-infected patients even prior to the era of treatment with effective antiretroviral drugs. We are currently investigating several novel hypotheses regarding the molecular pathogenesis of HLS. One hypothesis is that interactions between specific, HIV-infected or activated immune cells and adipocytes or preadipocytes lead to defects in function or differentiation of the adipocytes. It is based on the observation that adipocyte depots are anatomically associated with specific lymph node aggregates - hence it is possible that there are distinct functional interactions between activated lymphocytes and the surrounding adipocytes and pre-adipocytes(37]. We have investigated these potential interactions in the context of *in vitro* HIV-1 infection, using an assay system in which primary human pre-adipocytes (stromal vascular cells) or 3T3-L1 mouse pre-adipocytes are incubated with uninfected or HIV-infected lymphocytes for varying periods of time, with or without direct contact between the two cell types and each cell type is assayed for cell cycle distributions, apoptosis and biochemical function. Exposure of acutely HIV-infected T lymphocytes to human pre-adipocytes leads to a marked increase in HIV-1 production in the lymphocytes. In chronically HIV-infected lymphocytes, exposure to the pre-adipocytes leads to a cell cycle block and increased apoptosis in the lymphocytes. These effects are clearly mediated by a soluble factor secreted by the preadipocytes in response to the cell-cell interaction. Conversely, the pre-adipocytes undergo a differentiation block following exposure to the lymphocytes, as

demonstrated by a marked reduction in both lipid staining by Oil Red O and quantitative gene expression of key adipocyte developmental transcription factors. These effects are observed in both human and murine pre-adipocytes, suggesting that they are not due to direct HIV-1 infection of the pre-adipocytes. The specificity of these interactive effects is underscored by the fact that these results can be replicated using three different human T lymphocyte lines, but not using two different human macrophage cell lines.

These data indicate that there are significant interactions between pre-adipocytes and HIV-infected lymphocytes, which result on the side of the lymphocytes in increased HIV-1 production, cell cycle block and apoptosis and on the side of the pre-adipocytes in a marked block to differentiation. The interactions are mediated by soluble factors secreted by the pre-adipocytes (? adipokines), lymphocytes (? cytokines) or secreted components of HIV-1 itself. Identifying these factors and specifying their mechanism could be important for understanding the complex pathophysiology of HIV lipodystrophy.

ACKNOWLEDGEMENTS

REFERENCES

1. Carr, A., K. Samaras and S. Burton *et al*., 1998. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. AIDS, 12: F51-8.

2. Badiou, S., C. Merle De Boever, A.M. Dupuy, V. Baillat, J.P. Cristol and J. Reynes, 2003 Decrease in LDL size in HIV-positive adults before and after lopinavir/ritonavir-containing regimen: an index of atherogenicity? Atherosclerosis, 168:107-13

3. Sposito, A.C., B. Caramelli and A.M. Sartori, 1997. The Lipoprotein Profile in HIV Infected Patients. Braz. J. Infect. Dis., 1: 275-283

4. Fessel, W.J., S.E. Follansbee and J. Rego, 2002 High-density lipoprotein cholesterol is low in HIV-infected patients with lipodystrophic fat expansions: implications for pathogenesis of fat redistribution. AIDS, 16: 1785-9.

5. Tsiodras, S., C. Mantzoros, S. Hammer and M. Samore, 2000. Effects of protease inhibitors on hyperglycemia, hyperlipidemia and lipodystrophy: a 5-year cohort study. Arch. Intern. Med., 160: 2050-6.

6. Murata, H., P.W. Hruz and M. Mueckler, 2002. Indinavir inhibits the glucose transporter isoform Glut4 at physiologic concentrations. AIDS, 16: 859-63.

7. Gan, S.K., K. Samaras and C.H. Thompson *et al.*, 2002. Altered Myocellular and Abdominal Fat Partitioning Predict Disturbance in Insulin Action in HIV Protease Inhibitor-Related Lipodystrophy. Diabetes, 51: 3163-3169.

8. Sutinen, J., A.M. Hakkinen and J. Westerbacka *et al.*, 2002. Increased fat accumulation in the liver in HIV-infected patients with antiretroviral therapy-associated lipodystrophy. AIDS, 16: 2183-93

9. Behrens, G.M., A.R. Boerner and K. Weber *et al.*, 2002 Impaired glucose phosphorylation and transport in skeletal muscle cause insulin resistance in HIV-1-infected patients with lipodystrophy. J. Clin. Invest., 110: 1319-1327.

10. Depairon, M., S. Chessex and P. Sudre *et al.*, 2001. Premature atherosclerosis in HIV-infected individuals--focus on protease inhibitor therapy. AIDS, 15: 329-34.

11. Friis-Moller, N., R. Weber and P. Reiss *et al.*, 2003 Cardiovascular disease risk factors in HIV patients--association with antiretroviral therapy. Results from the DAD study. AIDS, 17: 1179-93.

12. Law, M., N. Friis-Moller and R. Weber *et al.*, 2003 Modelling the 3-year risk of myocardial infarction among participants in the Data Collection on Adverse Events of Anti-HIV Drugs (DAD) study. HIV Med., 4: 1-10.

13. Mary-Krause, M., L. Cotte, A. Simon, M. Partisani and D. Costagliola, 2003. Increased risk of myocardial infarction with duration of protease inhibitor therapy in HIV-infected men. AIDS, 17: 2479-86.

14. Lai, S., H. Lai and D.D. Celentano *et al.*, 2003 Factors associated with accelerated atherosclerosis in HIV-1-infected persons treated with protease inhibitors. AIDS Patient Care STDS, 17: 211-9.

15. Holmberg, S.D., A.C. Moorman and J.M. Williamson *et al.*, 2002 Protease inhibitors and cardiovascular outcomes in patients with HIV-1. Lancet, 360: 1747-8.

16. Varriale, P., G. Saravi, E. Hernandez and F. Carbon, 2004. Acute myocardial infarction in patients infected with human immunodeficiency virus. Am. Heart J., 147: 55-9.

17. Balasubramanyam, A., R.V. Sekhar, F. Jahoor, P.H. Jones and H.J. Pownall, 2004. Pathophysiology of dyslipidemia and increased cardiovascular risk in HIV lipodystrophy: a model of 'systemic steatosis'. Curr. Opin. Lipidol., 15: 59-67.

18. Sekhar, R.V., F. Jahoor, H.J. Pownall, C.M. Ballantyne and A. Balasubramanyam, 2004. Cardiovascular implications of HIV-associated dyslipidemic lipodystrophy. Curr. Atheroscler. Rep., 6: 173-9.

19. Sekhar, R.V., F. Jahoor and A.C. White *et al.*, 2002. Metabolic basis of HIV-lipodystrophy syndrome. Am. J. Physiol. Endocrinol. Metab., 283: E332-7.

20. Reeds, D.N., B. Mittendorfer, B.W. Patterson, W.G. Powderly, K.E. Yarasheski and S. Klein, 2003 Alterations in lipid kinetics in men with HIV-dyslipidemia. Am. J. Physiol. Endocrinol. Metab., 285: E490-E497.

21. Hadigan, C., S. Borgonha, J. Rabe, V. Young and S. Grinspoon, 2002 Increased rates of lipolysis among human immunodeficiency virus-infected men receiving highly active antiretroviral therapy. Metabolism, 51: 1143-7.

22. Dobbins, R.L., L.S. Szczepaniak, B. Bentley, V. Esser, J. Myhill and J.D. McGarry, 2001 Prolonged inhibition of muscle carnitine palmitoyltransferase-1 promotes intramyocellular lipid accumulation and insulin resistance in rats. Diabetes, 50: 123-30.

23. Barter, P.J., L.B. Chang, H.H. Newnham, K.A. Rye and O.V. Rajaram, 1990. The interaction of cholesteryl ester transfer protein and unesterified fatty acids promotes a reduction in the particle size of high-density lipoproteins. Biochim. Biophys. Acta, 1045: 81-9.

24. Lagrost, L., E. Florentin and V. Guyard-Dangremont *et al.*, 1995 Evidence for nonesterified fatty acids as modulators of neutral lipid transfers in normolipidemic human plasma. Arterioscler. Thromb. Vasc. Biol., 15: 1388-96.

25. Sekhar, R.V., F. Jahoor and H.J. Pownall *et al.*, 2005 Severely dysregulated disposal of postprandial triacylglycerols exacerbates hypertriacylglycerolemia in HIV-lipodystrophy syndrome. Am. J. Clin. Nutr. 81:1405-10.

26. Hadigan, C., C. Corcoran, N. Basgoz, B. Davis, P. Sax and S. Grinspoon, 2000. Metformin in the treatment of HIV lipodystrophy syndrome: A randomized controlled trial. JAMA, 284: 472-7.

27. Hadigan, C., J. Rabe and S. Grinspoon, 2002. Sustained benefits of metformin therapy on markers of cardiovascular risk in human immunodeficiency virus-infected patients with fat redistribution and insulin resistance. J. Clin. Endocrinol. Metab., 87: 4611-5.

28. Hadigan, C., S. Jeste, E.J. Anderson, R. Tsay, H. Cyr and S. Grinspoon, 2001 Modifiable dietary habits and their relation to metabolic abnormalities in men and women with human immunodeficiency virus infection and fat redistribution. Clin. Infect. Dis., 33: 710-7.

29. Rao, A., S. D'Amico, A. Balasubramanyam and M. Maldonado, 2004. Fenofibrate is effective in treating hypertriglyceridemia associated with HIV lipodystrophy. Am. J. Med. Sci., 327: 315-8.

30. Bonnet, F., E. Balestre and R. Thiebaut *et al.*, 2004 Fibrates or statins and lipid plasma levels in 245 patients treated with highly active antiretroviral therapy. Aquitaine Cohort, France, 1999-2001. HIV Med., 5: 133-9.

31. Badiou, S., C. Merle De Boever, A.M. Dupuy, V. Baillat, J.P. Cristol and J. Reynes, 2004. Fenofibrate improves the atherogenic lipid profile and enhances LDL resistance to oxidation in HIV-positive adults. Atherosclerosis, 172: 273-9.

32. Rietschel, P., C. Hadigan and C. Corcoran *et al.*, 2001. Assessment of growth hormone dynamics in human immunodeficiency virus-related lipodystrophy. J. Clin. Endocrinol. Metab., 86: 504-10.

33. D'Amico, S., J. Shi, R.V. Sekhar, F. Jahoor F, K.J. Ellis, K. Rehman, J. Willis J, M. Maldonado and A. Balasubramanyam. 2006 Physiologic growth hormone replacement improves fasting lipid kineticsin patients with HIV lipodystrophy syndrome. Am. J. Clin. Nutr., 84: 204-11.

34. Koutkia, P., B. Canavan, J. Breu, M. Torriani, J. Kissko and S. Grinspoon, 2004 Growth hormone-releasing hormone in HIV-infected men with lipodystrophy: a randomized controlled trial. JAMA, 292: 210-8.

35. Murata, H., P.W. Hruz and M. Mueckler, 2002 Indinavir inhibits the glucose transporter isoform Glut4 at physiologic concentrations. AIDS, 16: 859-63.

36. Gougeon, M.L., L. Penicaud, B. Fromenty, P. Leclercq, J.P. Viard and J. Capeau, 2004 Adipocytes targets and actors in the pathogenesis of HIV-associated lipodystrophy and metabolic alterations. Antivir. Ther., 9: 161-77.

37. Dowell, P., C. Flexner, P.O. Kwiterovich and M.D. Lane, 2000 Suppression of preadipocyte differentiation and promotion of adipocyte death by HIV protease inhibitors. J. Biol. Chem., 275: 41325-32.