Trends in Peptide and Protein Sciences
Volume 7 (2022): e5

DOI: http://dx.doi.org/10.22037/tpps.v7i0.38445

Original Article

Leginsulin Peptide from Legumes as a Ligand of Growth Hormone-Releasing Hormone Receptor for Lipodystrophy Management in Patients with AIDS

Mohsen Danaeifar

[a] *Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran.*

Article history:
Received: 19 May 2022
Accepted: 15 June 2022

Keywords:
AIDS
Leginsulin
Lipodystrophy
Peptides
Protein docking

HIGHLIGHTS

- Leginsulin is a plant secondary metabolite with 37 amino acids peptide structure.
- Binding of leginsulin and GHRH receptor was studied through in silico approaches.
- Results support the leginsulin as a potential management of lipodystrophy in AIDS patients.

ABSTRACT

Since the appearance of acquired immunodeficiency syndrome (AIDS) disease, millions of people got infected, thousands are died and many suffered from its complications. One of the chronic and late symptoms of AIDS is lipodystrophy that leads to losing of fat in some parts of the body while gaining it at other organs and sites. One of the main targets in drug development for management of lipodystrophy is growth hormone-releasing hormone (GHRH) receptor. As a secondary metabolite from plants, leginsulin is a peptide with 37 amino acids and considered as hormone-like peptide. In this study, through in silico approaches, binding of leginsulin and GHRH receptor was studied. The results showed strong binding of the two molecules with docking score of -324.16 and ligand RMSD of 47.26. The molecular dynamic investigation also revealed these two proteins remained bound for almost 104 ns. Evaluation of the peptide toxicity in the body had shown that it is not toxic to the human organs and also, it doesn't pass through the blood brain barrier. The results support the use of legumes as a source of leginsulin for potential management of lipodystrophy in the patients with AIDS.

Cite this article as: Danaeifar, M. (2022). Leginsulin peptide from legumes as a ligand of growth hormone-releasing hormone receptor for lipodystrophy management in patients with AIDS. *Trends Pept. Protein Sci.*,**7**: e5.

[*] *Corresponding Author:*
Email: mdanaeifar@alumni.ut.ac.ir (M. Danaeifar)
: https://orcid.org/0000-0002-1712-9922

Introduction

Human immunodeficiency virus (HIV) causes a spectrum of conditions that is called acquired

immunodeficiency syndrome (AIDS) (Schuman *et al*., 1987). According to the statistics from the World Health Organization (WHO), 37.7 million people were living with HIV at the end of 2020 and 0.7% of adults were infected with this virus worldwide (Peate, 2021). Common symptoms of AIDS are headache, muscle aches, joint pain, rash, fever, night sweating, cough, weight loss, swollen lymph glands, sore throat and painful mouth sores (Yi *et al*., 2006). In the chronic stages of the disease, some symptoms appear such as: chills, chronic diarrhea, sweating, swollen lymph glands, skin rashes, weight loss, weakness, persistent and unexplained fatigue, and persistent white spots (Scandlyn, 2000). One of the complications and late symptoms is lipodystrophy. This phenomenon consist of a group of syndromes, which lead to losing of fat in some parts of the body while gaining it in other organs and sites (Fiorenza *et al*., 2011). This symptom is first recognized in AIDS patients at 1999. Several parts of the patient's body are affected by lipodystrophy including the face, buttocks, limbs, breasts, dorso-cervical spine and abdomen (Finkelstein *et al*., 2015). Lipodystrophy may predispose the patients to the development of second phase complications, such as cardiovascular diseases and impairing their quality of life (Shevitz *et al*., 2001). The burden of the HIV-related lipodystrophy is considered high in low income countries (Finkelstein *et al*., 2015). Lipodystrophy develops within four to six months after the initiation of antiviral therapy for AIDS (Guaraldi *et al*., 2013). Lipodystrophy is considered as a complex metabolic disease and therefore, the changes in lifestyle is very important in its management. Some metabolic features are related to the lipodystrophy including hypercholesterolemia, hypertriglyceridemia, low levels of high-density lipoprotein cholesterol, type 2 diabetes mellitus, insulin resistance, elevated hepatic transaminases and lactic acidemia. The pathogenesis of lipodystrophy is still not fully understood, but some conditions are linked to this manifestation. For example, it was hypothesized that protease inhibitors target the catalytic region of HIV-1 protease. This region is homologous with low-density-lipoprotein-receptor-related protein and cytoplasmic retinoic-acid-binding protein 1 (CRABP-1) which regulate the lipid metabolism (Carr *et al*., 1998) and lipodystrophy can be secondary to the protease inhibitors that is due to inhibition of regulatory proteins with similar catalytic site of HIV-1 protease (Galescu *et al*., 2013). Some evidences showed diminished nocturnal secretion of growth hormone in patients suffered from AIDS (Grinspoon and Gelato, 2001, Rietschel et al., 2001). It was also showed low-dose amounts of growth hormone for 18 months in AIDS patients resulted in abdominal accumulation of fat go together with a reduction in visceral fat (Lo *et al*., 2008). Based on these observations, one of main targets for management of lipodystrophy in the body, determined to be receptors related to the release of the growth hormone (Zhou *et al*., 2020). Many drugs affected growth hormone axis have been used for the treatment of this metabolic disorder, but none of them have shown satisfactory results in the management of lipodystrophy.

Natural products are a group of molecules that are produced by living organisms. Secondary metabolites are a group of natural products that are not essential for the life of the organism, but they give the producer, properties that make it more adaptable to the peripheral environment over a long period of time. Secondary metabolites of plants generally fall into following categories: polyketides, non-ribosomal peptides (NRP) and plant secondary metabolites (Danaeifar and Mazlomi, 2022). As a secondary metabolite from plants, leginsulin is a peptide with 37 amino acids (Kim *et al*., 2012). This peptide belongs to the cysteine-knot family. The aim of this study is to evaluate the potency of leginsulin peptide in controlling the AIDS-related lipodystrophy and to predict its pharmacokinetics and pharmacodynamics features through *in silico* tools and functional simulations.

Materials and Methods

Structural homology search

The sequence and structure of the leginsulin peptide were retrieved from the PDB database (PDB ID: 1JU8). In order to find drugs with a similar structure to leginsulin, it was structurally aligned with all drug structures in the GoDrugBank database (Wishart *et al*., 2018). The similarity search was set against the whole structure and the threshold ST was set at 0.7.

ADME tests

To predict the toxicity of proteins in different body organs and tissues, eMolTox online web-server was used (Ji *et al*., 2018). Acute toxicity was calculated using the web-based tool Toxicity Profiler (AbdulHameed *et al*., 2021). ADME@NCATS web server was also used to predict rat liver microsomal (RLM) stability, parallel artificial membrane permeability assay (PAMPA), solubility, human liver cytosolic (HLC) stability and CYP450 toxicity tests of leginsulin (Gonzalez *et al*., 2021). The metabolism and membrane transport of leginsulin was predicted and evaluated using the online web server vNN-ADMET (Schyman *et al*., 2017).

Protein-protein docking

The structurally similar drug was evaluated for its function and receptors in GoDrugBank and the drug receptor sequence was retrieved from UniProt databank. Molecular docking was performed between the leginsulin and the target receptor to study their interaction and the binding strength using the HDOCK web server (Yan *et al.*, 2020).

Molecular dynamic

Classical molecular dynamics simulations with leginsulin peptide bound to the growth hormone-releasing hormone receptor (GHRHR) were performed using GROMACS 2018 software. The initial native structure of the leginsulin peptide was retrieved from the RCSB protein data bank (PDB ID: 1JU8). The native structure of the GHRHR was also obtained from UniProt database (Q02643). The water solvated forms of the both proteins were obtained using TIP4P, as the water model and within rhombic dodecahedron box, and the minimum distance was set on 3.0 nm between protein atoms and the box. To monitor the stability of the leginsulin in their native motion, the root mean square deviation (RMSD) was estimated. Contact analysis and the root mean square fluctuation (RMSF) were determined for each complex. Constants for the pressure and temperature were set at 1.01325 atm and 300° K, respectively.

Natural Product Likeness test

To examine the potential of leginsulin as a natural drug product, the PDB file format of this peptide was converted to Mol file type and the resulting data uploaded in Natural Product Likeness Score calculator (NaPLeS) (Sorokina and Steinbeck, 2019).

Results

Structural similarity search

The general structure of the leginsulin is shown in Fig. 1. Leginsulin was structurally aligned with GoDrugBank deposits, and the most similar chemical structure was identified that was tesamorelin. This drug is a peptide composed of 44 amino acids (Diao and Meibohm, 2013). The drug is a GHRH receptor agonist used to treat abdominal lipodystrophy (Wang and Tomlinson, 2009). The GHRH receptor is a receptor protein and a Gs protein that leads to cascade of cAMP by adenylate cyclase. GHRHR has 423 amino-acid residues (Zhou *et al.*, 2020). The tertiary structure of leginsulin consists of 4 helixes, 3 beta strands (Fig. 1).

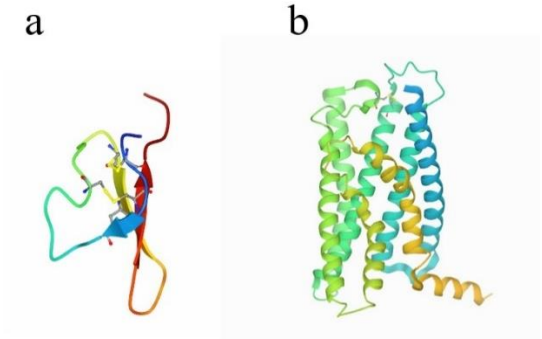

Figure 1. The tertiary structure of ligand and receptor, a) leginsulin and b) growth hormone-releasing hormone receptor respectively. The simulation was carried on solvated form of the protein. The NMR ensemble structures were retrieved from the protein data bank.

Protein-protein docking

Protein-protein docking was performed using HDOCK web server. This server uses a hybrid algorithm for the prediction and affinity of the binding, based on both template-based and template-free docking that leads to the accuracy of the prediction (Yan *et al.*, 2020). The best molecular docking energy score for these two proteins was -324.16 and the ligand RMSD (Å) calculated to be 47.26, which was designated as model 1. The binding site for the leginsulin was located in a region between amino acid 12 to 27 and for the GHRH receptor between amino acids 78 to 93. This region is the binding site of the receptor, which can targeted by various medications. The most prominent amino acid in the binding region of leginsulin were arginine and cysteine, respectively. The binding schematic of model 1 and other models of ligand-receptor interaction are presented in Fig. 2 and their affinity are mentioned in Table 1.

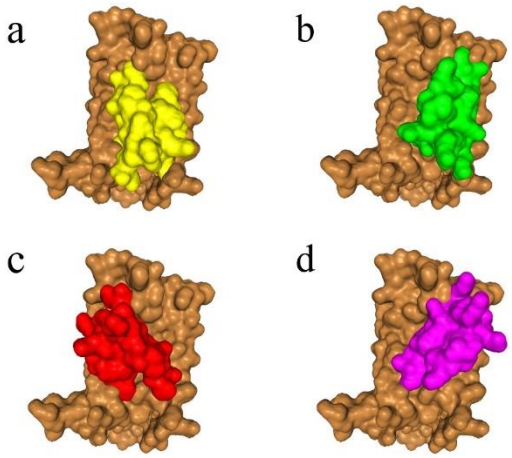

Figure 2. Four models of leginsulin peptide and growth hormone-releasing hormone receptor interactions. α) Model 1 of interaction that had the best docking score and b, c and d) model 2, 3 and 4 of ligand receptor interactions. The strongest binding of the ligand and receptor observed in model 1 (a) with docking score of -324.16, and the weakest one was seen in model 4 (d) with docking score of -288.14. The images were generated by HDOCK web server.

Table 1. Docking score and RMSD quantities of various leginsulin and GHRHR interactions

Model	1	2	3	4
Docking Score	-324.16	-315.79	-293.5	-288.14
Ligand RMSD (A)	47.26	48.75	55.49	56.32

Stability of the leginsulin and GHRHR binding

Molecular dynamics technique was used to assess the stability of the leginsulin and GHRHR binding in triplicate for 150 ns. The results showed that the leginsulin - GHRHR remained bound for more than 104 ns. By analyzing the root mean square deviation (RMSD) of the protein backbone, it was observed that equilibrium was reached after 56 ns. Root mean square fluctuation (RMSF) analysis was performed to study the protein backbone flexibility. The most significant flexibility was seen at residues 24-37 of leginsulin.

Functional simulations

The blood-brain barrier (BBB) test showed that leginsulin cannot be transferred across the BBB and is therefore, safe for brain cells. The leginsulin showed not to be a P-gp (glycoprotein) inhibitor or P-gp substrate. Rat liver microsomal stability module showed that leginsulin is stable with the predicted class (probability) of 0.93. Acute toxicity for oral use was predicted based on the most similar compound in toxicity database and it was 0.57 (mmol/kg [RMSE]). Human liver cytosolic stability test showed this protein is very stable in the cytosol with a predicted class of 0.55 as the confidence score. CYP2C9-inhibitor and substrate prediction confidence scores were 0.51 and 0.53, respectively. CYP2D6 inhibitor and substrate activity were calculated to be 0.51 and 0.52 and for CYP3A4 inhibitor and substrate, they were 0.50 and 0.51. The parallel artificial membrane permeability assay (PAMPA) test measure was performed and it was predicted to be high at pH 5 and it was low at pH 7.4 with the confidence score of 0.98. The results also showed that it was highly soluble in the body fluids (predicted score of 0.97). Leginsulin has been shown to be neither a matrix metalloproteinase inhibitor, nor a mutagenic chemical, as its AMES (*Salmonella*/microsome bacterial mutagenicity test) was negative. It was also showed that this natural peptide was not a hERG blocker. QSAR estimation of the maximum recommended therapeutic dose (MRTD) calculated to be 1956 mg/d. Probable toxicity of the leginsulin for different organs of the body is presented in Table 2. The natural product likeness scorer was developed based on the sum of frequency of compounds fragments among natural products and synthetic molecules. This score is calculated for each atom in the molecular structure. The natural product score is used to predict the applicability of a compound or putative structure from a library as a natural product. The mode score of all natural products is 2.5 and for synthetic products is -0.8. The score for leginsulin was calculated 0.5.

Discussion

Since the onset of AIDS in the early 1980s and its outburst in 1990s, millions of people have been infected, thousands have died and many suffered from its complications and impacts on their lives (Granich *et al*., 2015). Lipodystrophy, as a manifestation related to AIDS and its viral therapy, has a serious impact on the quality of life and survival rate of the patients (Fiorenza *et al*., 2011). The main medication for lipodystrophy management is tesamorelin. This drug is a synthetic form of GHRH that can mimic the natural peptide action (Sivakumar *et al.*, 2011). This drug has 44 amino acids compared to leginsulin with 37 amino acids. Leginsulin is a natural product that has found in some plant genera, including legumes and soybeans, making them as both a food source and an anti-lipodystrophy drug for AIDS patients (Yoshizawa *et al.*, 2011). *In silico* toxicity evaluations are also support the use of leginsulin containing plants and crops in AIDS patients.

Table 1. The possible toxicity of the leginsulin peptide for various tissues of the body

Target	Action	Organ	Confidence
Hepatotoxicity	Activators of the heat shock response signaling pathway	Liver	0.982
Nephrotoxicity	Cytotoxicity in HEK293 cells - 8 hour	Kidney	0.992
Neurotoxicity	Agonist of the androgen receptor (AR) signaling pathway	Central nervous system	0.99
Reproduction toxicity	Agonist of the androgen receptor (AR) signaling pathway	Endocrine	0.97
Cell toxicity	Agonist of H2AX	DNA Damage	0.992

Another beneficial of using leginsulin is that unlike tesamorelin, it is not a synthetic compound, thus expected not to elicit considerable allergic or immunologic reactions in the body. Previous studies on leginsulin demonstrated this peptide had high ligand activity and was also stable to heat and many digestive enzymes, possibly due to its three intramolecular disulfide bonds (Komatsu *et al.,* 1994). Some studies demonstrated insulin-like function of leginsulin not only in plants, but also in animal bodies (Yamazaki *et al.,* 2003). Dun *et al.* evaluated the insulin-like action of leginsulin and demonstrated its involvement in the regulation of blood glucose concentration in the mice model. In the previous studies, the ability of leginsulin in eliciting accelerated cell growth and cell differentiation was also showed, supporting its role as a hormone-like peptide (Kim *et al.,* 2012). In recent studies, it has been clarified that leginsulin interacts with soybean seed basic 7S globulin, as a storage protein, which has a similar structure to the insulin receptor (de Medeiros *et al.,* 2022). Some patients with diabetes who receives insulin develops lipodystrophy due to some types of allergy resulting from impurities in the insulin mixture. These reactions are thought to be the result of an immune-mediated inflammatory process (Jedlowski *et al.,* 2019). Considering this phenomenon, leginsulin, taking advantage of its insulin-like functions, can decrease the need for insulin and therefore, decrease the possibility of lipodystrophy development in patients. Soybean as the main source of leginsulin peptide was evaluated for its insulin-like effects as a diet in patients with AIDS and the results showed the 60% increase of insulin sensitivity following consumption of soybean rich diet regime (Marcel *et al.,* 2011). The leginsulin is present in legumes and commonly consumed by humans as a dietary source (Kim *et al.,* 2012). When it comes to the market as a therapeutic agent, all functional aspects of it should be clarified. The results showed leginsulin did not inhibit the p-glycoproteins. The p-glycoprotein, also known as multidrug resistance protein 1 (MDR1), is an important protein of cell membrane that pumps many foreign compounds and substances out of the cell and also, a drug transporter which determines the uptake and efflux of a broad range of drugs (Husain *et al.,* 2022). Based on the p-glycoprotein inhibition test, leginsulin did not affect the function of this protein and would not alter the effects of related drugs.

The blood brain barrier is a semi-permeable membrane, which separates circulating blood from the central nervous system, thus prevents the entry of foreign substances or microorganisms into the brain cells (Abbott *et al.,* 2010). The results showed that leginsulin is unable to move across the BBB, which is in favor of its use as a therapeutic agent.

Leginsulin may affect the liver cells through activation of heat shock proteins (Hsp). These proteins play a role in many metabolic pathways and interactions including G protein-coupled receptor kinases, heterotrimeric G proteins, small GTPases and signaling kinases (Reinle *et al.,* 2022). Based on the *in silico* prediction, leginsulin may have cytotoxic effects on HEK 293 cells. This cell line exhibit epithelial morphology and it was isolated from the kidney of a human embryo (Thomas and Smart, 2005).
In the cytoplasm of human cells, heat shock proteins and the androgen receptor (AR), form a complex, causing of AR mediated activation of signaling pathway. The leginsulin showed AR agonist activity. In the previous studies, it was also showed that, AR signaling pathway is involved in neuroprotective actions (Pike *et al.,* 2008).

Conclusion

Based on our result of the protein docking between leginsulin and GHRH receptor, and also, its low possible toxicity to human body, it is suggested to consider leginsulin containing plants, like legumes, as a source of anti-lipodystrophy and glucose-regulating peptides.

Ethical Statement

This article does not contain any studies with human and animal subjects performed by any of the authors.

Competing Interests

The author declares that there is no potential conflict of interest related to this research and publication.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

Abbott, N.J., Patabendige, A.A., Dolman, D.E., Yusof, S.R. and D.J. Begley, (2010). ″Structure and function of the blood–brain barrier.″ *Neurobiology of Disease*, **37**(1): 13-25. DOI: https://doi.org/10.1016/j.nbd.2009.07.030.

Abdulhameed, M.D.M., Liu, R., Schyman, P., Sachs, D., Xu, Z., Desai, V. and A. Wallqvist, (2021). ″ToxProfiler: Toxicity-target profiler based on chemical similarity.″ *Computational Toxicology*, **18**: 100162. DOI: https://doi.org/10.1016/j.comtox.2021.100162.

Carr, A., Samaras, K., Burton, S., Law, M., Freund, J., Chisholm, D.J. and D.A. Cooper, (1998). ″A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors.″ *AIDS*, **12**(7): F51-58. DOI: https://doi.org/10.1097/00002030-199807000-00003.

Danaeifar, M. and M.A. Mazlomi, (2022). ″Combinatorial biosynthesis: playing chess with the metabolism.″ *Journal of Asian Natural Products Research*, 1-20. DOI: https://doi.org/10.1080/10286020.2022.2065265.

De Medeiros, A.F., De Queiroz, J.L.C., Maciel, B.L.L. and A.H. De Araújo Morais, (2022). ″Hydrolyzed proteins and vegetable peptides: anti-inflammatory mechanisms in obesity and potential therapeutic targets.″ *Nutrients*, **14**(3): 690. DOI: https://doi.org/10.3390/nu14030690.

Diao, L. and B. Meibohm, (2013). ″Pharmacokinetics and pharmacokinetic–pharmacodynamic correlations of therapeutic peptides.″ *Clinical Pharmacokinetics*, **52**: 855-868. DOI: https://doi.org/10.1007/s40262-013-0079-0.

Finkelstein, J.L., Gala, P., Rochford, R., Glesby, M.J. and S. Mehta, (2015). ″HIV/AIDS and lipodystrophy: implications for clinical management in resource-limited settings.″ *Journal of the International AIDS Society*, **18**(1): 19033. DOI: https://doi.org/10.7448/IAS.18.1.19033.

Fiorenza, C.G., Chou, S.H. and C.S. Mantzoros, (2011). ″Lipodystrophy: pathophysiology and advances in treatment.″ *Nature Reviews Endocrinology*, **7**: 137-150. DOI: https://doi.org/10.1038/nrendo.2010.199.

Galescu, O., Bhangoo, A. and S. Ten, (2013). ″Insulin resistance, lipodystrophy and cardiometabolic syndrome in HIV/AIDS.″ *Reviews in Endocrine and Metabolic Disorders*, **14**: 133-140. DOI: https://doi.org/10.1007/s11154-013-9247-7.

Gonzalez, E., Jain, S., Shah, P., Torimoto-Katori, N., Zakharov, A., Nguyễn, Đ.-T., Sakamuru, S., Huang, R., Xia, M. and R.S. Obach, (2021). ″Development of robust quantitative structure-activity relationship models for CYP2C9, CYP2D6, and CYP3A4 Catalysis and Inhibition.″ *Drug Metabolism and Disposition*, **49**(9): 822-832. DOI: https://doi.org/10.1124/dmd.120.000320.

Granich, R., Gupta, S., Hersh, B., Williams, B., Montaner, J., Young, B. and J.M. Zuniga, (2015). ″Trends in AIDS deaths, new infections and ART coverage in the top 30 countries with the highest AIDS mortality burden; 1990–2013.″ *PLoS One*, **10**(7): e0131353. DOI: https://doi.org/10.1371/journal.pone.0131353.

GrinspooN, S. and M. Gelato, (2001). ″The rational use of growth hormone in HIV-infected patients.″ *The Journal of Clinical Endocrinology and Metabolism*, **86**(8): 3478-3479. DOI: https://doi.org/10.1210/jcem.86.8.7879.

Guaraldi, G., Stentarelli, C., Zona, S. and A. Santoro, (2013). ″HIV-associated lipodystrophy: impact of antiretroviral therapy.″ *Drugs*, **73**: 1431-1450. DOI: https://doi.org/10.1007/s40265-013-0108-1.

Husain, A., Makadia, V., Valicherla, G.R., Riyazuddin, M. and J.R. Gayen, (2022). ″Approaches to minimize the effects of P-glycoprotein in drug transport: A review.″ *Drug Development Research*, 1-17. DOI: https://doi.org/10.1002/ddr.21918.

Jedlowski, P.M., Te, C.H., Segal, R.J. and M.T. Fazel, (2019). ″Cutaneous adverse effects of diabetes mellitus medications and medical devices: a review.″ *American Journal of Clinical Dermatology*, **20**: 97-114. DOI: https://doi.org/10.1007/s40257-018-0400-7.

Ji, C., Svensson, F., Zoufir, A. and A. Bender, (2018). ″eMolTox: prediction of molecular toxicity with confidence.″ *Bioinformatics*, **34**(14): 2508-2509. DOI: https://doi.org/10.1093/bioinformatics/bty135.

Kim, W.S., Jang, S. and H.B. Krishnan, (2012). ″Accumulation of leginsulin, a hormone-like bioactive peptide, is drastically higher in asian than in north american soybean accessions.″ *Crop Science*, 52: 262-271. DOI: https://doi.org/10.2135/cropsci2011.08.0454.

Komatsu, S., Koshio, O. and H. Hirano, (1994). ″Protein kinase activity and insulin-binding activity in plant basic 7S globulin.″ *Bioscience, Biotechnology, and Biochemistry*, **58**(9): 1705-1706. DOI: https://doi.org/10.1271/bbb.58.1705.

Lo, J., You, S.M., Canavan, B., Liebau, J., Beltrani, G., Koutkia, P., Hemphill, L., Lee, H. and S. Grinspoon, (2008). ″Low-dose physiological growth hormone in patients with HIV and abdominal fat accumulation: a randomized controlled trial.″ *JAMA*, **300**(5):509-519. DOI: https://doi.org/10.1001/jama.300.5.509.

Marcel, A.-K., Ekali, L.G., Eugene, S., Arnold, O.E., Sandrine, E.D., Von Der Weid, D., Gbaguidi, E., Ngogang, J. and J.C. Mbanya, (2011). ″The effect of Spirulina platensis versus soybean on insulin resistance in HIV-infected patients: a randomized pilot study.″ *Nutrients*, **3**(7): 712-724. DOI: https://doi.org/10.3390/nu3070712.

Peate, I. (2021). ″World AIDS Day 2021: a comparison of HIV and COVID-19.″ *British Journal of Healthcare Assistants*, **15**: 506-509. DOI: https://doi.org/10.12968/bjha.2021.15.10.506.

Pike, C.J., Nguyen, T.-V.V., Ramsden, M., Yao, M., Murphy, M.P. and E.R. Rosario, (2008). ″Androgen cell signaling pathways involved in neuroprotective actions.″ *Hormones and Behavior*, **53**(5): 693-705. DOI: https://doi.org/10.1016/j.yhbeh.2007.11.006.

Reinle, K., Mogk, A. and B. Bukau, (2022). ″The diverse functions of small heat shock proteins in the proteostasis network.″ *Journal of Molecular Biology*, **434**(1): 167157. DOI: https://doi.org/10.1016/j.jmb.2021.167157.

Rietschel, P., Hadigan, C., Corcoran, C., Stanley, T., Neubauer, G., Gertner, J. and S. Grinspoon, (2001). ″Assessment of growth hormone dynamics in human immunodeficiency virus-related lipodystrophy.″ *The Journal of Clinical Endocrinology and Metabolism*, **86**(2): 504-510. DOI: https://doi.org/10.1210/jcem.86.2.7175.

Scandlyn, J. (2000). ″When AIDS became a chronic disease.″ *Western Journal of Medicine*, **172**(2): 130-133. DOI: https://doi.org/10.1136/ewjm.172.2.130.

Schuman, J.S., Orellana, J., Friedman, A.H. and S.A. Teich, (1987). Acquired immunodeficiency syndrome (AIDS). *Survey of Ophthalmology*, **31**(6): 384-410. DOI: https://doi.org/10.1016/0039-6257(87)90031-2.

Schyman, P., Liu, R., Desai, V. and A. Wallqvist, (2017). ″vNN web server for ADMET predictions.″ *Frontiers in Pharmacology*, **8:** 889. DOI: https://doi.org/10.3389/fphar.2017.00889

Shevitz, A., Wanke, C.A., Falutz, J. and D.P. Kotler, (2001). ″Clinical perspectives on HIV-associated lipodystrophy syndrome: an update.″ *AIDS*, **15**(15): 1917-1930. DOI: https://doi.org/10.1097/00002030-200110190-00003

Sivakumar, T., Mechanic, O., Fehmie, D. and B. Paul, (2011). ″Growth hormone axis treatments for HIV-associated lipodystrophy: a systematic review of placebo-controlled trials.″ *HIV Medicine*, **12**: 453-462. DOI: https://doi.org/10.1111/j.1468-1293.2010.00906.x.

Sorokina, M. and C. Steinbeck, (2019). ″NaPLeS: a natural products likeness scorer—web application and database.″ *Journal of Cheminformatics*, **11**: 55. https://doi.org/10.1186/s13321-019-0378-z.

Thomas, P. and T.G. Smart, (2005). ″HEK293 cell line: a vehicle for the expression of recombinant proteins.″ *Journal of Pharmacological and Toxicological Methods*, **51**(3): 187-200. DOI: https://doi.org/10.1016/j.vascn.2004.08.014.

Wang, Y. and B. Tomlinson, (2009). ″Tesamorelin, a human growth hormone releasing factor analogue.″ *Expert Opinion on Investigational Drugs*, **18**(3): 303-310. DOI: https://doi.org/10.1517/13543780802707658

Wishart, D.S., Feunang, Y.D., Guo, A.C., Lo, E.J., Marcu, A., Grant, J.R., Sajed, T., Johnson, D., LI, C. and Z. Sayeeda, (2018). ″DrugBank 5.0: a major update to the DrugBank database for 2018.″ *Nucleic Acids Research*, **46**(D1): D1074-D1082. DOI: https://doi.org/10.1093/nar/gkx1037.

Yamazaki, T., Takaoka, M., Katoh, E., Hanada, K., Sakita, M., Sakata, K., Nishiuchi, Y. and H. Hirano, (2003). ″A possible physiological function and the tertiary structure of a 4-kDa peptide in legumes.″ *European Journal of Biochemistry*, **270**(6): 1269-1276. DOI: https://doi.org/10.1046/j.1432-1033.2003.03489.x.

Yan, Y., Tao, H., He, J. and S.-Y. HuaNG, (2020). ″The HDOCK server for integrated protein–protein docking.″ *Nature Protocols*, **15:** 1829-1852. DOI: https://doi.org/10.1038/s41596-020-0312-x.

Yi, M.S., Mrus, J.M., Wade, T.J., Ho, M.L., Hornung, R.W., Cotton, S., Peterman, A.H., Puchalski, C.M. and J. Tsevat, (2006). ″Religion, spirituality, and depressive symptoms in patients with HIV/AIDS.″ *Journal of General Internal Medicine*, **21**(S5): S21-S27. DOI: https://doi.org/10.1111/j.1525-1497.2006.00643.x.

Yoshizawa, T., Shimizu, T., Yamabe, M., Taichi, M., Nishiuchi, Y., Shichijo, N., Unzai, S., Hirano, H., Sato, M. and H. Hashimoto, (2011). ″Crystal structure of basic 7S globulin, a xyloglucan-specific endo-β-1, 4-glucanase inhibitor protein-like protein from soybean lacking inhibitory activity against endo-β-glucanase.″ *The FEBS Journal*, **278**(11): 1944-1954. DOI: https://doi.org/10.1111/j.1742-4658.2011.08111.x.

Zhou, F., Zhang, H., Cong, Z., Zhao, L.-H., Zhou, Q., Mao, C., Cheng, X., Shen, D.-D., Cai, X. and C. Ma, (2020). ″Structural basis for activation of the growth hormone-releasing hormone receptor.″ *Nature Communications*, **11**: 5205 (1-10). DOI: https://doi.org/10.1038/s41467-020-18945-0.

