Computational Investigation of the Impact of the Ghrelin Hormone Gene Variation (R51q) on the Hormone-receptor Binding Pattern

Baraa Adil*, Saleha Shahar, Syazwani I. Amran, Mohd S. S. Omar, M. Abu Naser

Department of Bioscience, Faculty of Science, University Technology Malaysia (UTM)

ABSTRACT

Ghrelin is a peptide hormone made up of 28 amino acids. It is involved in various biological processes, including the stimulation of growth hormone release, control of food intake, and metabolic and cytoprotective effects. In 1999, this hormone was identified as a ligand for the GHSR1a growth hormone secretagogue receptor. Ghrelin hormone-receptor complexes have been modeled in a few previous *in-silico* studies, but none of them have investigated the effects of the gene variation rs34911341/(R51Q) on the full-length ghrelin model and on the hormone-receptor binding. It was established that the full-length ghrelin model's secondary structure was unaffected by the R to Q amino acid substitution. Additionally, the mutant hormone-receptor complex exhibited better outcomes and altered the molecular interactions between the mutant ligand and the receptor by creating novel interactions, according to the post-molecular dynamic simulation analysis.

Keywords: GHSR-1a, R51Q, Ghrelin, Molecular docking.

INTRODUCTION

Ghrelin is a 28 amino acid peptide generated and secreted largely by X/A-like cells in the oxyntic glands of the stomach mucosa.[1,2] This peptide was initially identified as a powerful stimulator of growth hormone (GH) release from the anterior pituitary,[3] but it has now become obvious that it serves many functions throughout the organs. Ghrelin regulates food intake and satiety, gastrointestinal motility,[4,5] lipid storage control, and glucose metabolism.[6] Moreover, it has cardiovascular effects, protects against ischemia/reperfusion injury in the heart, has anti-inflammatory properties, and reduces anti-oxidant stress in a variety of diseases.[7,8]

It appears that the structure and functions of ghrelin hormone are highly conserved in vertebrates. It is the endogenous ligand of the growth hormone secretagogue receptor (GHSR) , a class A, G protein-coupled receptor (GPCR) that induces calcium mobilization, identified in pigs, humans, teleosts, and birds.[8,9] This receptor has two alternative splice variants: GHS-R1a, the active receptor that mediates the biological actions of growth hormone secretagogues (GHS) and ghrelin, and GHS-R1b, an inactive receptor with the first 5 transmembrane helices that regulate the expression of GHSR1a at the cell surface.[10]

To activate its receptor, ghrelin requires the acylation of a fatty acid (octanoyl or decanoyl) to its serine 3 residue, a modification carried out by the enzyme ghrelin-o-acyltransferase (GOAT).[11] Recent research suggested that the ghrelin octanoyl moiety is essential for forming the hydrophobic core and facilitating ghrelin N-terminal access to the receptor binding pocket.[12]

Only one gene variation, rs34911341 had been identified as a missense mutation in the ghrelin hormone, and it caused an amino acid substitution at positions 28 of the mature ghrelin and 51 of the preproghrelin gene from arginine R to glutamine Q. This gene variation was found to be associated with a few diseases, such as T2DM, hypertension, and obesity. The arginine residue at position 28 of the mature ghrelin hormone was thought to be essential for endoprotease identification and action.[13] Endoprotease catalyzes the proteolytic cleavage during the production of ghrelin.[14,15] Yet, it is unclear if this genetic variation alters ghrelin's physiologic characteristics or action.

Ghrelin and ghrelin receptor agonists can effectively treat anorexia and cachexia in cancer[16,17] and chronic kidney disease patients.[18] Treatment with ghrelin has been shown to improve renal function and attenuate renal fibrosis and inflammation.[18,19] Ghrelin and ghrelin receptor agonists can also be useful in aquaculture, contributing to growth enhancement and fish

*Author for Correspondence: Baraa.adil@graduate.utm.my

health improvement. Due to its applications, multiple efforts have been directed toward finding new active molecules, prompting the discovery of new GHS.

Previous computational approach studies about ghrelin hormone and its receptor GHSR-1a have focused on their binding pattern, but none have investigated their gene mutation impacts and how these gene variations could alter the binding pattern and their final structures.[3,8,12,20,21] Thus, the current study focused on the impact of R51Q variation on the ghrelin hormone and how this amino acid substitution could alter the hormone-receptor binding pattern.

METHODS

Models of Wild Type and Mutant Ghrelin (R51Q) Hormone and GHSR-1a

According to the protein data bank server, crystal models of the ghrelin hormone and its receptor are available with the given codes 6h3e and 6ko5, respectively. Two distinct differences were discernible between the exact amino acid sequence of the ghrelin hormone and the 6h3e model. The first difference is the amino acid sequence length; ghrelin hormone consists of 28 residues, whereas the crystal model consists of 18 aa; the missing residues belong to the C-terminal region, which is inactive in hormone-receptor binding. During the modeling of the 6h3e model, it was discovered that aspartate (D) was modeled at position 3 instead of serine (S) due to stability concerns. In order to obtain the full-length homone, two structural modifications were made to the 6h3e model. First, the construction of the missing residues of its C-terminal region, and second, replacement of the D amino acid with the S amino acid at position 3. Both of these structural alterations were accomplished with the PyMol tool. In addition, the mutant model R51Q was generated via PyMol software as well. Finally, wildtype and mutant ghrelin hormone models were submitted to the YASARA server to minimize their energy.

In contrast, 6ko5 did not need any modifications due to it did not exhibit any missing residues in its 3D crystal structure. Furthermore, the 6ko5 model was submitted to PyMol in order to remove its stability ligands and prepare it for molecular docking with the modified wildtype and mutant ghrelin hormone models.

Molecular Docking

As previously mentioned, ligands and receptors were prepared via PyMol software. Moreover, both of the ligands models were docked to the wildtype receptor using the online docking server ClusPro 2.0.[21] The ClusPro server (https://cluspro.org) is a widely usedprotein–protein docking tool. The active site residues/ binding residues were highlighted and submitted to the docking server based on previously published studies.[3,8,12,20,21] According to the cited studies, the binding residues for the receptor included: D99, R102, Y106, Q120, S123, E124, E197, R199, F279, F286, F290, Q302, N305, F309, F312, and Y313. On the other hand, the active part of the ligand was the N-terminal rejoin only due to the C-terminal part is functionally inactive.[12]

Molecular dynamic simulations MDs

Hormone receptor complex MD simulations were performed using GORMACS, a freely available and open-source software tool for high-performance molecular dynamics and output analysis.[22,23] In addition, CHARMM GUI was applied to accomplish two objectives.[24] The ghrelin hormone receptor, a G-coupled protein with seven transmembranes domains, was submitted to the CHARMM GUI server for 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) membrane assembly.[25] Soluble builder was another CHARMM GUI server feature that helped to generate the initial phases of MD simulation by automating tasks including topology generation, periodic solvent box formation, ions addition, and force field configuration. This feature was implemented once the complex was inserted into a POPC lipid membrane and the pdb files were obtained. The MD simulations were applied with the following parameters: a force field of charmm36, a solvent box size of 10*10*10 nm, 0.15 M of Na^+ and Cl^- ions, 310 K of temperature, and a time constant of 100 ns due to a recently published study.[8] Moreover, the rest of the MD simulation steps, including minimization, equilibration, and production steps have been done manually via GROMACS command lines.

MDs Trajectory and Structural Analysis

The MDs trajectory analysis included root mean standard deviation (RMSD), root mean squared fluctuation (RMSF), the radius of gyration (Rg), solvent accessible surface area (SASA), and molecular mechanics with generalized Born and surface area solvation (mmgbsa). With the exception of mmgbsa, which was evaluated via prime mmgbsa software, the other parameters were calculated via QtGrace tool. Moreover, the final ligand-receptor complexes' structural analysis was carried out via DSV tool.

RESULTS AND DISCUSSION

### Models preparations and molecular docking

The crystal model of ghrelin hormone was modified by the construction of its C-terminal in addition to the amino acid substitution at position 3 of its N-terminal part from D to S and this step was carried out via PyMol tool (Figure 1).
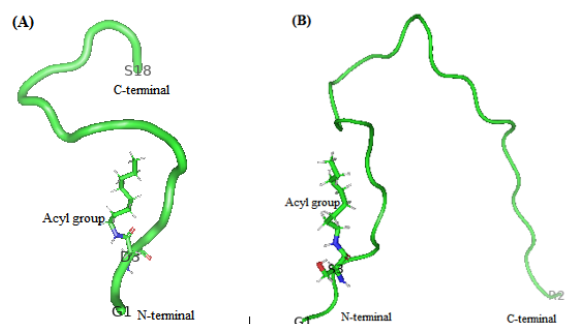
Figure 1: The crystal structure of ghrelin before and after structural modification via PyMol tool. (A) Represented the original model obtained from PDB server while (B) represented the final model after amino acid substitution at position 3 and C-terminal construction.
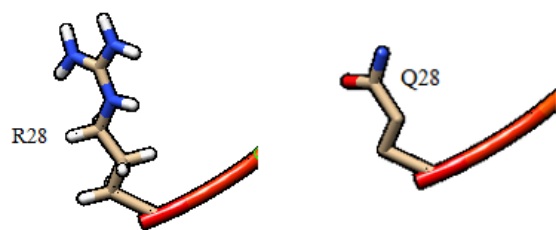
Figure 2: Wildtype and mutant ligand C-terminal at position 28 as showed via Chimera tool. R28 represented the wildtype C-terminal while Q28 for the mutant model C-terminal.
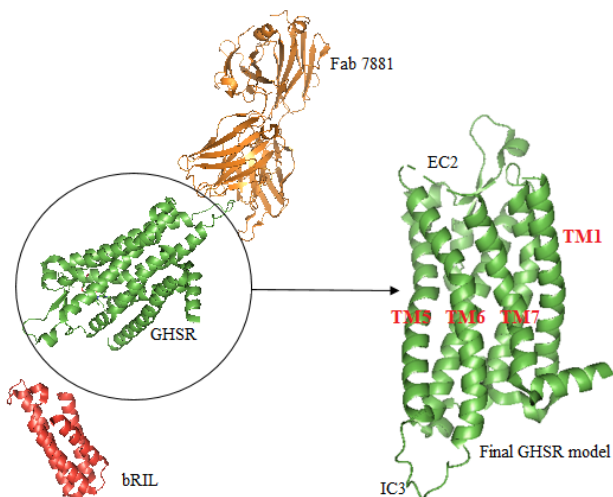
Figure 3: The crystal structure of the GHSR-1a model 6ko5, stability ligands bRIL and Fab 7881 have been eleminated in order to prepare the model for molecular docking with the two ligands: full length ghrelin and R51Q mutant models.

Consequently, the following step was the construction of the mutant model of the modified ghrelin model by amino acid substitution R to Q at position 28 of the ligand C-terminal (Figure 2).

Both arginine and glutamine are differ in their size and charge, R is a polar positive charged amino acid while Q is an uncharged residue. This amino acid substitution did not result in a conformational change in the final structure; this point was in agreement with a prior investigation.[26]

In the GHSR model preparation, thermostability ligands have been eliminated from the crystal structure 6ko5 in order to run a molecular docking step with the ligand models (Figure 3)

Furthermore, wildtype and mutant R51Q ligand models have been individually docked to the GHSR model via the reported online docking server, ClusPro. In addition, primary structural analysis have been applied via DSV tool to figure out which of the obtained ligand-receptor complexes revealed a proper ligand entry inside the receptor binding pocket.

The primary structural analysis of the models obtained from the docking server showed that only one out of four complexes revealed full molecular interactions between the ligand N-terminal and the receptor (Table 1). Subsequently, this model was selected for further investigation. The same

Table 1: The primary molecular interactions of ligands N-terminal of wildtype and mutant ghrelin models with the receptor.

| Ghrelin/R51Q N-terminal	Wildtype complex	Mutant complex
G1	E124 Q120	E124 Y128
S2	Q120 C198	Q120
S3	R102	R283
F4	F286	R102 F286
L5	F279	F279

Yet, no missing molecular interactions have been observed among the ligand N-terminals of the wildtype and mutant ghrelin models.

steps have been applied in order to obtain a mutant hormone-receptor complex. The mutant hormone model was docked to the receptor using the same online docking server and same active residues. Then the obtained models were submitted into the DSV tool for primary structural analysis as well. Table 1 summarizes the ligand N-terminals of both hormone models with the receptor.

After inserting the selected complex models into a POPC membrane using the CHARMM GUI server, the next step included running MD simulations on the models. The MDs parameters have already been mentioned, and the results, including energy distributions, RMSD, RMSF, Rg, and SASA, were visualized using the QtGrace software, while the total energies were calculated using the prime-mmgbsa tool. In addition, the VMD tool was used to visualize the MDs run and to obtain the complexes final pdb files for the final structural analysis to determine the impact of the R51Q variation on the ligand-receptor binding pattern.

Once the MDs were done and as previously mentioned, trajectories analysis were carried out via QtGrace software. Starting with the investigations of the energy distributions, stable peaks were observed in both wildtype and mutant complexes models (Figure 3).

Moreover, RMSD and RMSF demonstrated that R51Q mutant ghrelin model stabilized the overall complex. In RMSD
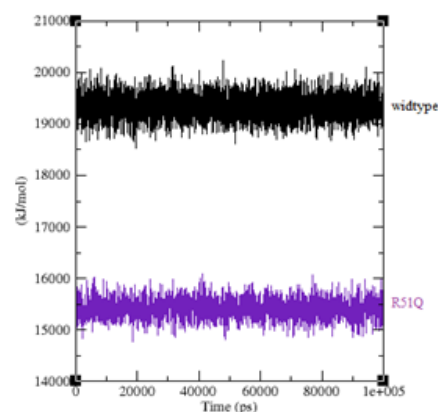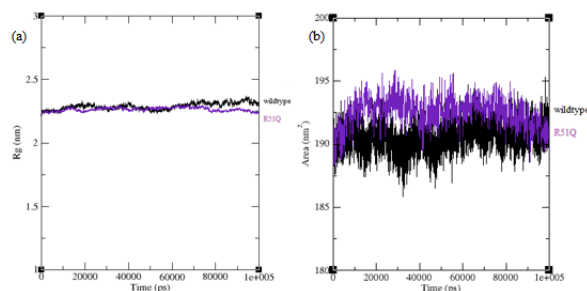

Figure 4: Energy distribution of the wildtype and mutant R51Q complexes verses time plot via QtGrace tool. The *X axis* represented the simulation time in picoseconds (ps) and the *Y axis* represented the energy values in kJ/mol.
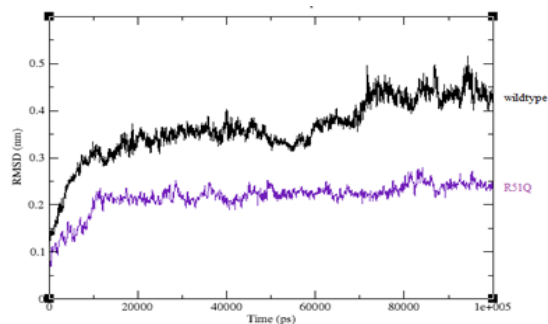
Figure 5: RMSD plots for mutant complexe R51Q after MD simulations 100 nm via GROMACS. The *X axis* represented the simulation time in picoseconds (ps) and the *Y axis* represented the RMSD values in nanometer (nm), each 1 nm equal to 10 Å.
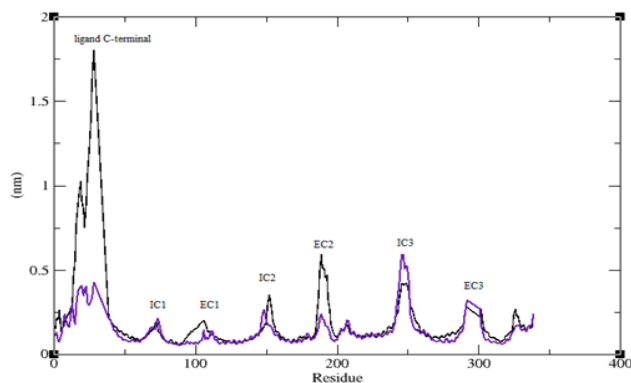

Figure 6: RMSF plot of the R51Q mutant hormone-receptor and wildtype models via QtGrace. The *X axis* represented the number of residues and the *Y axis* represented the RMSF values in nanometer (nm). Black colored plot was belonged to the wildtype complex while the purpul for the mutant complex.


Figure 7: (a) The radius of gyration Rg plots for wildtype complex and R51Q model as showed via QtGrace. The X axis represented the simulation time in picoseconds (ps) and the Y axis represented Rg values in nm. **(b)** Solvent accessibility surface area (SASA) plot of R51Q hormone-mutant receptor complexes after 100 ns MD simulations via QtGarce tool. The X axis represented the number of residues and the Y axis represented the SASA values in nanometer square (nm^2).

caculations, stable peaks were observed in the mutant complex compared to that for the wildtype complex (Figure 4).

The unstable RMSD peak for the wildtype model was due to the high flexibility of the ligand C-terminal in addition to the receptor loops as shown via VMD tool. While the mutant ligand exhibited lower flexibilty among the receptor loops, which led
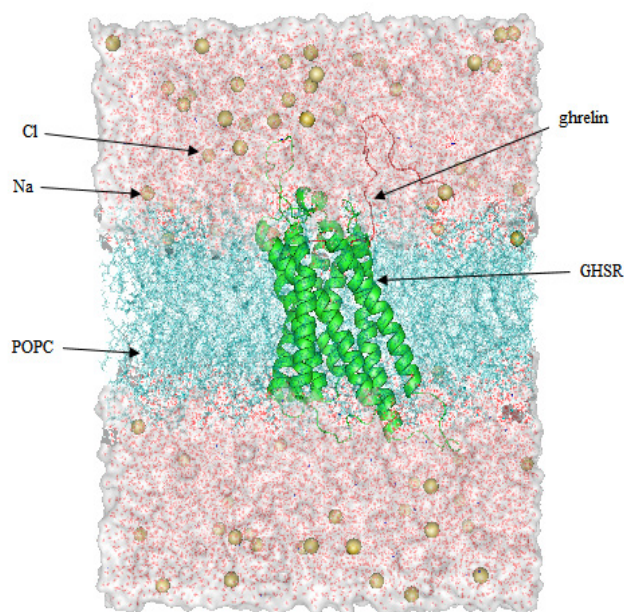
Figure 8: Representation of the wildtype complex (receptor in green and ghrelin model in red) inserted in a 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) membrane patch (in cyan), solvated (grey with red dots) in a 10 × 10 × 10 Å periodic box and neutralized with Na and Cl ions.
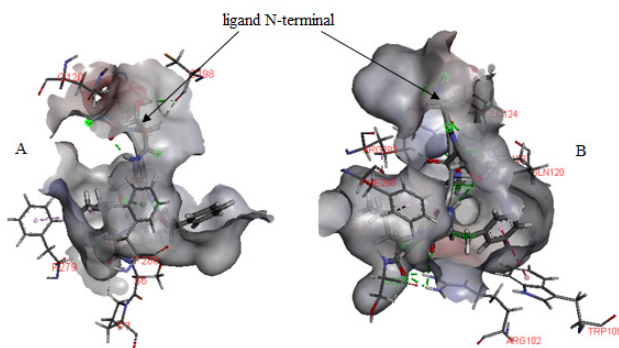
Figure 9: Represntaions figures of the ligand N-terminals interactions with the receptor. (A) for the wildtype complex while (B) for the mutant one. Red labled residues represented the receptor-binding residues.

to increased complex stability as shown when investigating the RMSF plots (Figure 5).

Moreover, Rg investigations showed that both wildtype and mutant complex molecules were stable (Figure 6a). On the other hand, SASA revealed that the mutant complex had lower values (Figure 6b).

In terms of total energy calculations (mmgbsa), the mutant complex was more stable because it exhibited a lower energy value (-1778.8 kJ/mol) than the wildype complex (-139.8 kJ/mol). Low (negative) free energy values indicate that the ligand binds to the receptor spontaneously without expending energy.[27-29]

The last investigation included post MDs structural analysis for both complexes. The pdb files for wildtype and mutant complexes after 100 ns of MD simulations were also

Table 2: The total interactions of variant R51Q-GHSR complex in comparision with wildtype complex.

Ghrelin/ R51Q	Receptor in Wildtype model			Receptor binding residue in R51Q model		
	Binding residue	Type of interaction	Distance in Å	Binding residue	Type of interaction	Distance in Å
G1	D99	Electrostatic	1.60	E124	Electrostatic	1.96
	D99	C-H bond	2.82	Q120	H-bond	2.99
	Q120	H-bond	1.89	R283	H-bond	2.92
S2	C198	H-bond	2.18	Q120	H-bond	1.90
	C198	C-H bond	2.74	R283	C-H bond	2.39
S3	F286	C-H bond	2.15	S123	H-bond	2.08
				R283	H-bond	2.49
F4	N305	H-bond	2.29	W109	Hydrophobic	4.89
				R102	H-bond	2.86
L5	F279	hydrophobic	5.30	R283	Hydrophobic	5.38
				R102	H-bond	2.87
				F286	hydrophobic	5.15
S6	-		-	R102	H-bond	2.18
				N305	H-bond	2.50
				S301	C-H bond	3.0
P7	P278	C-H bond	2.69	N305	C-H bond	3.03
	C304	Hydrophobic	5.25	P292	C-H bond	2.67
				F286	hydrophobic	5.38
E8	-		-	P292	H-bond	2.12
H9	C304	Electrostatic	4.42	R107	hydrophobic	4.14
	C304	H-bond	2.47			
Q10	K288	C-H bond	3.05	F290	C-H bond	2.69
R11	F290	Electrostatic	4.68	R199	Hydrophobic	4.21
	K288	C-H bond	2.47	R107	H-bond	3.09
	K288	H-bond	1.75	E197	Electrostatic	2.70
				E197	H-bond	2.96
				E197	C-H bond	3.03
R15	-		-	E197	Electrostatic	4.68
				E197	H-bond	1.56
				E197	H-bond	2.12
K20	-		-	D191	Electrostatic	5.59
				D194	Electrostatic	1.72
P21	-		-	Y106	Hydrophobic	4.47
A23	-		-	Q105	H-bond	2.0

obtained using the VMD software and visualized via Pymol tool (Figure 7).

There were no missing interactions between the ligand N-terminus and the receptor model in both complex models. In fact, the N-terminal of the mutated ligand displayed increased interactions with the receptor, resulting in a conformational change in this region (Figure 8).

In addition, the C-terminal region of the wildtype ghrelin model did not exhibit any interactions with the receptor, confirming the observations of a previous study that stated but did not prove that the ghrelin C-terminal region is functionally inactive. In contrast, the C-terminal region of the mutant hormone model made contact with the receptor, specifically with the D191, D197, and E197 of the receptor EC2. The total final interactions between the two complexes are detailed in the following Table 2.

Interestingly, the mutant complex displayed a greater number of molecular interactions between ligand and receptor than the wildtype complex. In addition, the substituted residue Q28 stabilized the ligand C-terminus by being close to the receptor, accounting for the lower RMSD and RMSF values.

In conclusion, the ghrelin gene variation R51Q was previously investigated clinically, and it was found to be associated with metabolic syndrome,[13,30] hypertension,[31] obesity,[32] and gastric cancer.[33] In the current study, it was revealed that this mutation did not result in observable conformational changes in the C-terminal of the ghrelin model hormone, but it did affect the final stability of the complex by generating new molecular interactions between the ligand and receptor models followed by decreased its total energy. Further investigations observed that each wildtype R and mutated Q residue had different pKa and pH values. Arginine (R) had two

pKa values (pKa1: 2.2, and pKa2: 9.0), which is a positively charged, while glutamine (Q) had only one pKa value (2.2) and it belonged to the uncharged carboxamide group. When a high pKa residue (R) replaced a lower pKa one (Q), the ionizable group might become protonated, led to disturb the protonation status, which later led to disturb the electrostatic status of the final protein then afecting its function. Thus, based on our findings, this gene variation R51Q of ghrelin hormone had increased the receptor binding affinity.

ACKNOWLEDGMENT

REFERENCES

1. Raghay K, Akki R, Bensaid D, Errami M. Ghrelin as an anti-inflammatory and protective agent in ischemia/reperfusion injury. Peptides. 2020 Feb 1;124:170226.

2. Schalla MA, Stengel A. Effects of microbiome changes on endocrine ghrelin signaling–A systematic review. Peptides. 2020 Nov 1;133:170388.

3. Shiimura Y, Horita S, Hamamoto A, Asada H, Hirata K, Tanaka M, Mori K, Uemura T, Kobayashi T, Iwata S, Kojima M. Structure of an antagonist-bound ghrelin receptor reveals possible ghrelin recognition mode. Nature Communications. 2020 Aug 19;11(1):4160.

4. Acosta A. Control of food intake. Yamada's Textbook of Gastroenterology. 2022 Apr 15:478-86.

5. Nogueiras R. Mechanisms In Endocrinology: The gut–brain axis: regulating energy balance independent of food intake. European Journal of Endocrinology. 2021 Sep;185(3):R75-91.

6. Sovetkina A, Nadir R, Fung JN, Nadjarpour A, Beddoe B. The physiological role of ghrelin in the regulation of energy and glucose homeostasis. Cureus. 2020 May 3;12(5).

7. Lee J, Kim MH, Kim H. Anti-Oxidant and Anti-Inflammatory Effects of Astaxanthin on Gastrointestinal Diseases. International Journal of Molecular Sciences. 2022 Dec 7;23(24):15471.

8. de la Nuez Veulens A, Rodríguez Fernández RE, Álvarez Ginarte YM, Montero Cabrera LA. In silico strategy for detailing the binding modes of a novel family of peptides proven as ghrelin receptor agonists. Journal of Molecular Modeling. 2020 Nov;26:1-4.

9. Cornejo MP, Castrogiovanni D, Schiöth HB, Reynaldo M, Marie J, Fehrentz JA, Perello M. Growth hormone secretagogue receptor signalling affects high-fat intake independently of plasma levels of ghrelin and LEAP 2, in a 4-day binge eating model. Journal of neuroendocrinology. 2019 Oct;31(10):e12785.

10. Tegshee B, Kondo K, Soejima S, Muguruma K, Tsuboi M, Kajiura K, Kawakami Y, Kawakita N, Toba H, Yoshida M, Takizawa H. GHSR methylation-dependent expression of a variant ligand and receptor of the ghrelin system induces thymoma tumorigenesis. Oncology Letters. 2021 Nov 1;22(5):1-0.

11. Abizaid A, Hougland JL. Ghrelin signaling: GOAT and GHS-R1a take a LEAP in complexity. Trends in Endocrinology & Metabolism. 2020 Feb 1;31(2):107-17.

12. Ferré G, Louet M, Saurel O, Delort B, Czaplicki G, M'kadmi C, Damian M, Renault P, Cantel S, Gavara L, Demange P. Structure and dynamics of G protein-coupled receptor–bound ghrelin reveal the critical role of the octanoyl chain. Proceedings

of the National Academy of Sciences. 2019 Aug 27;116(35): 17525-30.

13. Martins LT, Koczicki L, de Souza Klingenfus A, Wischral LM, Kubo KS, Frigeri HR. Molecular Studies on Preproghrelin Gene: Signal Peptide, Ghrelin Coding Region and Variants–A Brief ReportFocusing on rs34911341 Polymorphism. Avicenna Journal of Medical Biochemistry. 2017 Dec 6;5(2):50-3.

14. Wajnrajch MP, Ten IS, Gertner JM, Leibel RL. Genomic organization of the human ghrelin gene. International Journal on Disability and Human Development. 2000 Oct 1;1(4):231-4.

15. Ukkola O, Ravussin E, Jacobson P, Snyder EE, Chagnon M, Sjöström L, Bouchard C. Rapid communications: Mutations in the preproghrelin/ghrelin gene associated with obesity in humans. The Journal of Clinical Endocrinology & Metabolism. 2001 Aug 1;86(8):3996-9.

16. Malik JS, Yennurajalingam S. Prokinetics and ghrelin for the management of cancer cachexia syndrome. Ann Palliat Med. 2019 Jan 1;8(1):80-5.

17. Giorgioni G, Del Bello F, Quaglia W, Botticelli L, Cifani C, Micioni Di Bonaventura E, Micioni Di Bonaventura MV, Piergentili A. Advances in the development of nonpeptide small molecules targeting ghrelin receptor. Journal of Medicinal Chemistry. 2022 Feb 14;65(4):3098-118.

18. Audrey Cook BV, DECVIM-CA DA. What's New in the Management of Feline Chronic Kidney Disease.

19. Ma Y, Zhang H, Guo W, Yu L. Potential role of ghrelin in the regulation of inflammation. The FASEB Journal. 2022 Sep;36(9):e22508.

20. Wang Y, Guo S, Zhuang Y, Yun Y, Xu P, He X, Guo J, Yin W, Xu HE, Xie X, Jiang Y. Molecular recognition of an acyl-peptide hormone and activation of ghrelin receptor. Nature Communications. 2021 Aug 20;12(1):5064.

21. Ghani U, Desta I, Jindal A, Khan O, Jones G, Hashemi N, Kotelnikov S, Padhorny D, Vajda S, Kozakov D. Improved docking of protein models by a combination of alphafold2 and cluspro. BioRxiv. 2021 Sep 7:2021-09.

22. Briones R, Blau C, Kutzner C, de Groot BL, Aponte-Santamaría C. GROmaρs: A GROMACS-based toolset to analyze density maps derived from molecular dynamics simulations. Biophysical journal. 2019 Jan 8;116(1):4-11.

23. Shukla R, Tripathi T. Molecular dynamics simulation of protein and protein–ligand complexes. Computer-aided drug design. 2020:133-61.

24. Guterres H, Park SJ, Zhang H, Perone T, Kim J, Im W. CHARMM-GUI high-throughput simulator for efficient evaluation of protein–ligand interactions with different force fields. Protein Science. 2022 Sep;31(9):e4413.

25. Lee J, Patel DS, Ståhle J, Park SJ, Kern NR, Kim S, Lee J, Cheng X, Valvano MA, Holst O, Knirel YA. CHARMM-GUI membrane builder for complex biological membrane simulations with glycolipids and lipoglycans. Journal of chemical theory and computation. 2018 Dec 11;15(1):775-86.

26. Ramachandran G, Kumar M, Selvi Rani D, Annanthapur V, Calambur N, Nallari P, Kaur P. An in silico analysis of troponin I mutations in hypertrophic cardiomyopathy of Indian origin. PLoS One. 2013 Aug 13;8(8):e70704.

27. Limongelli V. Ligand binding free energy and kinetics calculation in 2020. Wiley Interdisciplinary Reviews: Computational Molecular Science. 2020 Jul;10(4):e1455.

28. Rizzi A, Jensen T, Slochower DR, Aldeghi M, Gapsys V, Ntekoumes D, Bosisio S, Papadourakis M, Henriksen NM, De

Groot BL, Cournia Z. The SAMPL6 SAMPLing challenge: assessing the reliability and efficiency of binding free energy calculations. Journal of computer-aided molecular design. 2020 May;34(5):601-33.

29. Lokhande KB, Ballav S, Yadav RS, Swamy KV, Basu S. Probing intermolecular interactions and binding stability of kaempferol, quercetin and resveratrol derivatives with PPAR-γ: docking, molecular dynamics and MM/GBSA approach to reveal potent PPAR-γ agonist against cancer. Journal of Biomolecular Structure and Dynamics. 2022 Feb 2;40(3):971-81.

30. Mora M, Adam V, Palomera E, Blesa S, Diaz G, Buquet X, Serra-Prat M, Martín-Escudero JC, Palanca A, Chaves JF, Puig-Domingo M. Ghrelin gene variants influence on metabolic syndrome components in aged Spanish population. PloS one. 2015 Sep 16;10(9):e0136931.

31. Berthold HK, Giannakidou E, Krone W, Trégouët DA, Gouni-Berthold I. Influence of ghrelin gene polymorphisms on hypertension and atherosclerotic disease. Hypertension Research. 2010 Feb;33(2):155-60.

32. Al-Nbaheen MS. Relationship between single nucleotide polymorphism studies in ghrelin gene with obesity subjects. Journal of King Saud University-Science. 2023 Jan 1;35(1):102393.

33. Zhang X, Zhai L, Rong C, Qin X, Li S. Association of ghrelin gene polymorphisms and serum ghrelin levels with the risk of hepatitis B virus-related liver diseases in a Chinese population. PloS one. 2015 Nov 23;10(11):e0143069.