Letters in Drug Design & Discovery, 2022, *19*, 888-896

RESEARCH ARTICLE

Thiamine: A Natural Peroxisome Proliferator-Activated Receptor Gamma (PPAR-γ) Activator

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ARTICLE HISTORY

Received: June 18, 2021
Revised: October 12, 2021
Accepted: December 08, 2021

DOI:
10.2174/1570180819666220127121403

Abstract: ***Background*:** There has been increasing evidence of the correlation between thiamine deficiency and type 2 diabetes (T2D). T2D is a condition in which an individual's insulin sensitivity is highly compromised. Peroxisome proliferator-activated receptor gamma (PPAR-γ) is a ligand-activated transcription factor etiologically relevant to T2D. We hypothesized that thiamine could be a PPAR-γ ligand and thus activate PPAR-γ and ameliorate T2D.

***Objective*:** This study aims to establish thiamine as a PPAR-γ ligand *via* molecular docking and dynamics simulations (MDS) and thiamine's ability to induce adipogenesis while upregulating PPAR-γ and AP-2 genes using *in vitro* assays.

***Methods*:** Thiamine/PPAR-γ binding was studied using Schrödinger's Glide. The bound complex was simulated in the OPLS 2005 force field using Desmond. 3T3-L1 preadipocyte cells were differentiated in the presence of thiamine and rosiglitazone and stained with Oil Red O. Nuclear protein from the differentiated cells was used to study the binding of the PPAR-γ response element (PPRE) using an ELISA-based assay. mRNA from differentiated cells was used to study the expression of genes using quantitative RT-PCR.

***Results*:** *In silico* docking shows that thiamine binds with PPAR-γ. MDS indicate that the interactions between thiamine and PPAR-γ are stable over a significant period. Thiamine induces the differentiation of 3T3-L1 preadipocytes in a dose-dependent manner and enhances the PPRE-binding activity of PPAR-γ. Thiamine treatment significantly increases the mRNA levels of PPAR-γ and AP-2 genes.

***Conclusion*:** Our results show that thiamine is a PPAR-γ ligand. Animal studies and clinical trials are required to corroborate the results obtained.

Keywords: PPAR-γ, thiamine, type 2 diabetes, molecular dynamics simulations, adipocytes differentiation, natural ligand.

1. INTRODUCTION

Type 2 diabetes (T2D) is becoming a pressing problem for developing countries. A 2019 study estimated that the number of T2D patients will increase by 51% worldwide and that nearly 11% of the human population will be affected by this disease by 2045 [1]. Causal agents of T2D include age [2], diet [3], genetics [4], and lifestyle [5]. Triggers for T2D, including depression, sedentary behavior [6], consumption of sweetened beverages [7], and intake of fast food rich in lipids [8], are increasing due to the urban lifestyle and peer pressure. Maintaining a healthy lifestyle, good eating habits, and regular exercise can prevent T2D [9].

Thiamine, vitamin B_1, is commonly found in cereals such as rice [10]. All animals have to acquire thiamine through their diet since they cannot synthesize it on their own [11]. Enzymes important for carbohydrate metabolism have thiamine as a cofactor, and thiamine deficiency was found to be a risk factor for T2D [12, 13]. Administration of thiamine along with other nutritional supplements has been shown to lessen T2D markers such as blood glucose level [14]. Mutations in the thiamine transporter gene *SLC19A2* were shown to cause T2D [15, 16]. A number of researchers have reported that thiamine could cure diabetic symptoms [17-21]. Thiamine deficiency is common among people consuming alcohol [22]. Thiamine deficiency leads to several downstream clinical syndromes and finally causes diabetic coma [23]. The molecular pathway behind the relationship between thiamine deficiency and T2D is largely unknown. Researchers implicate various molecules that may play a vital role in this pathway. In this study, we show for the first time that thia-

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1570-1808/22 $65.00+.00

mine is a peroxisome proliferator-activated receptor gamma (PPAR-γ) ligand that plays a central role in T2D.

PPAR-γ is a ligand-activated transcription factor that plays a major role in glucose and lipid metabolism and insulin signaling [24]. Despite its important role in T2D, its physiological ligands have not yet been identified so far [25]. At present, benfotiamine, a highly bioavailable form of thiamine, is known to cure T2D [26]. Since thiamine directly activates PPAR-γ, we propose that T2D patients take thiamine in sufficient quantities to cure the disease. Clinical trials in patients are needed to prove our findings.

2. MATERIALS AND METHODS

2.1. Molecular Docking

The crystal structure of human PPAR-γ was downloaded from the Protein Data Bank (PDB ID: 2PRG) [27]. The downloaded 3D structure was prepared using the Protein Preparation Wizard tool provided in Schrodinger Maestro 2012 [28]. Briefly, bond orders were corrected, hydrogen atoms were added, missing side chains were filled, missing loops were filled, and waters beyond 5Å from protein atoms were deleted. Finally, the protein charge was neutralized (pH=7.0), and appropriate pKa values for side chains, mainly histidine, were assigned and minimized in an OPLS 2005 force field [29]. Rosiglitazone (positive control) and thiamine pyrophosphate were downloaded from the PubChem website and prepared for docking by using the LigPrep tool available in Schrodinger Maestro 2012. The steps include minimization in the OPLS 2005 force field, desalting, and generation of possible states at pH 7.0 ± 2 followed by the production of stereoisomers of all possible conformations (maximum = 32). The nonpolar atoms of the receptor were scaled with Van der Waals' radii of 1 at a partial charge cutoff of 0.25. A grid box was then constructed around the centroid of the ligand present in the crystal structure. Atoms with a partial charge cutoff of 0.25 were scaled to a Van der Waal's radius of 0.8 for ligand atoms. Xtra-precision (XP) docking with default settings was used to dock PPAR-γ with various conformations of thiamine and rosiglitazone. The thiamine/PPAR-γ complex with a high Glide-XP score, dock score, and hydrogen bond interactions similar to the crystal structure was selected for molecular dynamics simulations (MDS). Ligplot+ tool from EMBL-EBI was used for visualizing the molecular interactions between PPAR-γ and thiamine in the docked complex [30]. PyMol was used to superimpose ligands inside ligand-binding domain of PPAR-γ.

2.2. Molecular Dynamics Simulations

MDS were conducted using the Desmond [31] software of Maestro 9.9 GUI (academic version) with the standard OPLS 2005 force field [29]. The thiamine/rosiglitazone-PPAR-γ complexes selected according to the criteria above-mentioned were given as the MDS input directly from the Maestro workspace. The SPC explicit water model [32] that is most suitable for cytosol proteins was used to solvate the complex. The complex was neutralized by adding Na^+ ions. Finally, the topology and force field parameters of the complex were written using the Desmond software. Throughout the simulation, the temperature was kept constant (300 K) using the Nosé–Hoover chain thermostat method [33] with a relaxation time of 1 ps. The Martyna–Tobias–Klein barostat method [34] was used to maintain a constant isotropic pressure of 1 atm with a relaxation time of 2 ps. The smooth particle mesh Ewald method [35] with a tolerance of $1e^{-09}$ was used to study the long-range, and a cutoff method with a cutoff radius of 9 Å was used to study short-range Coulombic interactions. The above-said setup was simulated for 10 ns with a reporting interval of 240 ps.

2.3. 3T3-L1 Differentiation

The preadipose cell line 3T3-L1 at a passage number <20 was obtained from the National Centre for Cell Science (Pune, India). The cells were maintained at 37 °C, 5% CO_2 in 75 cm^2 tissue culture flasks (Jet Biofil, China) at confluency <75% using high-glucose DMEM (Gibco, India) supplemented with 10% calf serum and antibiotic solution. Two days before experiments (−2 days), cells were detached using trypsin–EDTA, seeded into 6-well tissue culture plates (Jet Biofil, China), and grown to 100% confluence. Once plated into 6-well plates, calf serum was replaced by fetal bovine serum. On day 0, the culture medium was discarded without disturbing the cells, and 2 mL of induction medium was added. The induction medium contained insulin, dexamethasone, and PPAR-γ ligands – rosiglitazone (positive control) or thiamine – at different concentrations (0, 10, or 20 μM) and was incubated for two days. After two days (+2 days), the culture medium was again discarded, and 2 mL of medium containing insulin alone was added and incubated for two more days. At +4 days, the insulin medium was discarded, and 2 mL of medium without insulin was added and maintained for another 4 days with a change in medium every two days. At +8 days, cells were examined microscopically for the difference in morphology and stained with Oil Red O.

2.4. Oil Red O Staining

After 8 days of incubation, the culture medium was discarded, and the cells were washed with Dulbecco's phosphate-buffered saline (DPBS). The washed cells were fixed by adding 3.7% formaldehyde solution and incubating for 2 min. After fixation, cells were washed with distilled water, and 1 mL of Oil Red O (Sigma-Aldrich, Bengaluru) was added at a final concentration of 0.08%. The cells were kept in the staining solution for 1 hour, after which the staining solution was discarded, and 1 mL of distilled water was added. The stained cells were immediately photographed before the loss of morphological details.

2.5. PPAR-γ Transcription Factor Assay

In a separate set of experiments, 3T3-L1 cells were differentiated and harvested on day 7. Nuclear protein from the harvested cells was prepared using a nuclear extraction kit. An equal amount of nuclear protein was subjected to an ELISA-based DNA-binding assay using the PPAR-γ Transcription Factor Assay kit (Cayman) according to the manufacturer's instructions [36].

2.6. Quantitative Reverse Transcriptase PCR (qPCR)

The differentiation experiment was conducted again, and the differentiated cells were detached using trypsin–EDTA. The detached cells were stored in RNAlater (Sigma, Bengaluru) at −20 °C until further use. Total RNA was then extracted using Trizol (Sigma, Bengaluru) following the manufacturer's protocol. The OligodT primer (Xcelris, India) and MMLV reverse transcriptase were used to synthesize cDNA according to the suppliers' protocols. Relative quantification of PPAR-γ and AP-2 gene expressions was performed by qPCR using the SYBR Green mix, where glyceraldehyde 3-phosphate dehydrogenase was used as a housekeeping gene. The list of primers used in this study is shown in Table **1**.

Table 1. List of gene-specific primers and sequences.

Gene	Forward Primer	Reverse Primer
PPAR-γ	5'-cgctgatgcactgcctatga	5'-agaggtccacagagctgattcc
AP-2	5'-caaaatgtgtgatgcctttgtg	5'-ctcttcctttggctcatgcc
GAPDH	5'-ctgagtatgtcgtggagtctac	5'-gttggtggtgcaggatgcattg

2.7. Statistical Analysis

Data are presented as mean ± SEM of values obtained from the triplicate experiments unless mentioned otherwise. GraphPad Prism 5.0 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com) was used for statistical analyses. Unpaired *t*-tests were carried out to statistically differentiate between the three doses of thiamine used. The significant difference between the means of different treatments is represented by *, where * = $P < 0.05$ and **= $P < 0.01$.

3. RESULTS

3.1. Molecular Docking and Dynamics Simulation Analyses

Docking results show (Fig. **1**) that thiamine binds to the ligand-binding domain (LBD) of PPAR-γ. Amino acids in the vicinity of thiamine are His449, Tyr473, Ser289, His323, and Tyr327 and have structures identical to the original crystal structure of rosiglitazone, 2PRG (Fig. **1A**). Superimposition of known ligands of PPAR-γ (pioglitazone and rosiglitazone) and thiamine inside its ligand-binding domain shows that many of the binding interactions are shared between the ligands (Fig. **1C**). The docking score of −6.3 Kcal/mol indicates that thiamine binds PPAR-γ with an affinity more or less similar to that of rosiglitazone (Table **2**).

Molecular dynamics simulations show that the interaction between thiamine and PPAR-γ is durable. For instance, Tyr327 hydrogen-bonded with thiamine for 97% of the entire simulation time (Fig. **1B**). Root mean square deviation (RMSD) analysis shows that there is not much deviation for the PPAR-γ C-α or PPAR-γ/thiamine complex with respect to the docked complex throughout the simulation time (Fig. **2A**). The RMSD results of the thiamine complex were comparable to that of the PPAR-γ/rosiglitazone complex (Fig. **2B**). The frequency histogram of RMSD analysis shows that the docked complexes are well equilibrated (Fig. **2C** and **2D**).

Table 2. Docking score and key interacting residues.

Compound	Docking Score (Kcal/mol)	Amino Acids Interacting with the Ligand
Thiamine	–6.3	His449, Tyr473, Ser289, His323, and Tyr327
Rosiglitazone	–7.0	His449, Tyr473, Ser289, His323, and Tyr327

3.2. 3T3-L1 Differentiation and Oil Red O Staining

The addition of thiamine at a final concentration of 20 μM was well tolerated by 3T3-L1 cells. Upon microscopic examination, differentiation was observed after 6 days of induction in groups treated with 10 and 20 μM thiamine and 10 μM rosiglitazone but not in the 0 μM thiamine group. The same was observed when the cells after 8 days of induction were stained with Oil Red O (Fig. **3A**-**D**). ImageJ analysis of the photographs of Oil Red O-stained cells showed that thiamine (10 and 20 μM) treatment significantly increased the induction of 3T3-L1 cells and that the enhanced induction by 20 μM thiamine is comparable to that of that 10 μM rosiglitazone (Fig. **3E**).

3.3. PPAR-γ Transcription Factor Assay

Activated PPAR-γ present in the nuclear extract of the differentiated cells bound to the PPAR response element (PPRE), which resulted in higher absorbance. As evident from (Fig. **4**), the activation of PPAR-γ is significantly higher by the thiamine (10 and 20 μM) treatment group than by the untreated group. The positive control, rosiglitazone, also significantly increased PPAR-γ activation (Fig. **4**).

3.4. Quantitative Reverse Transcriptase PCR (qPCR)

Activation of PPAR-γ by its ligands increases the expression of its receptor and subsequently its target genes. In order to investigate the effect of thiamine on the expression of mRNA of PPAR-γ and AP-2, 3T3-L1 preadipocytes were treated with thiamine (10 and 20 μM) or rosiglitazone (10 μM), and total RNA was analyzed by qPCR. Thiamine treatment dose-dependently upregulated the expression of both PPAR-γ and AP-2 genes ($P < 0.01$) compared to the untreated group (0 μM). Rosiglitazone also significantly increased the expression of PPAR-γ and AP-2 genes (Fig. **5A** and **B**).

4. DISCUSSION

T2D is a debilitating disease with an increasing prevalence worldwide, especially among adolescents and young adults [37]. In developed countries, a patient with diabetes spends about US$140 to US$2990 on an average per year [38]. PPAR-γ is one of the best targets for the amelioration of T2D. Activation of PPAR-γ was shown to cure T2D and its allied comorbidities [39]. However, drugs that activate

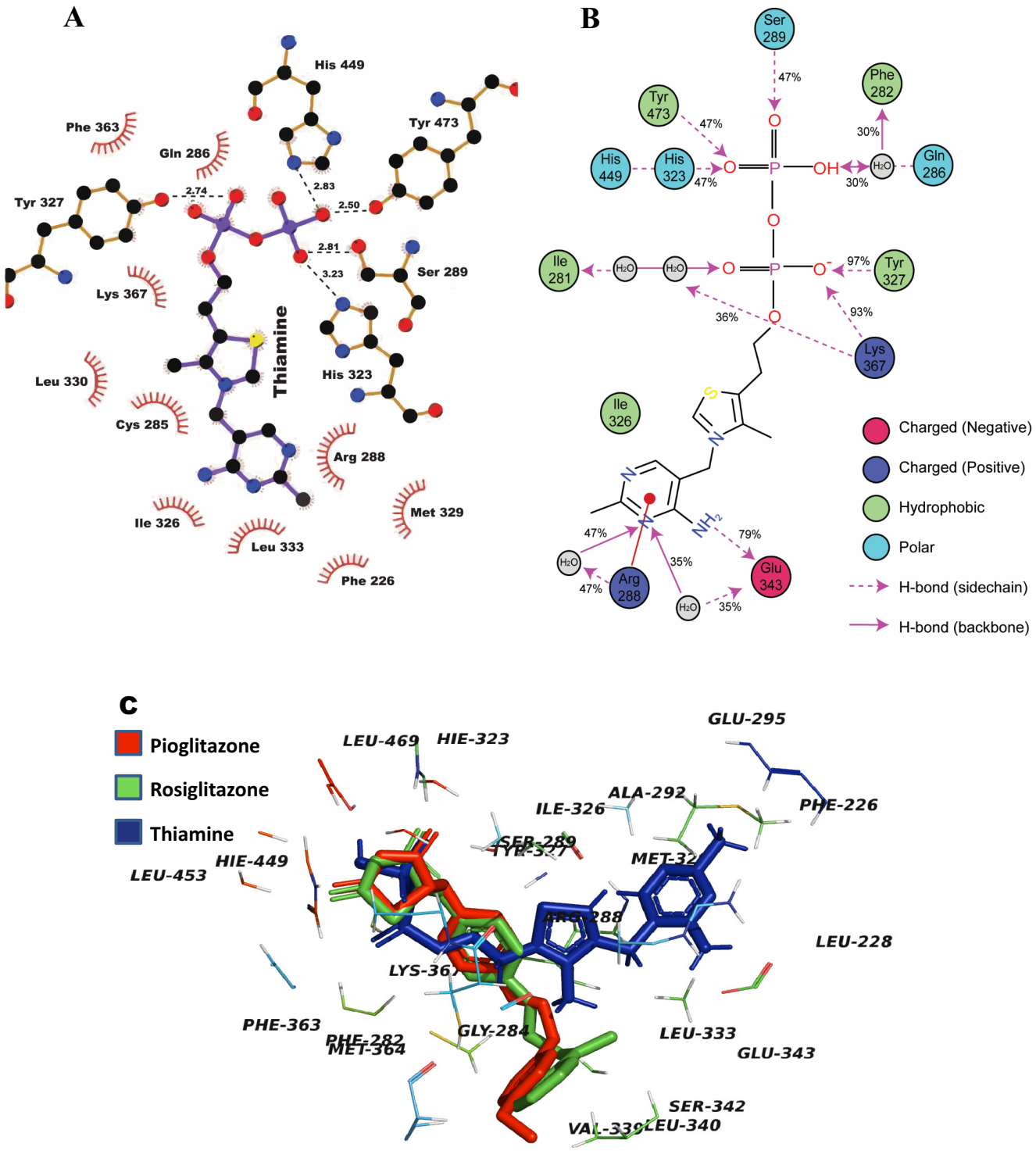

Fig. (1). A. Docked structure of thiamine inside the binding pocket of PPAR-γ. Thiamine is docked with the crystal structure of PPAR-γ obtained from PDB (id: 2PRG) using the procedure described in the Methods section. Hydrogen bonds formed between amino acid side chains and thiamine are shown as dashed lines, and thiamine is shown as balls and sticks. Other amino acids in the ligand-binding pocket and in proximity to the ligand are shown in red. Interatomic distance in Å is also labeled. **B**. Protein–ligand interactions during molecular dynamics simulations under the OPLS 2005 force field. Thiamine is depicted as a stick model. Amino acids interacting with thiamine are shown. Hydrogen bonds are shown as arrows. Retention of hydrogen bonds (as % time period) during the simulated time period is labeled. **C**. Superimposed structures of the ligands, pioglitazone, rosiglitazone, and thiamine inside PPAR-γ ligand-binding domain. The ligands were shown as sticks, and nearby amino acids in the ligand-binding domain were shown as wires. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

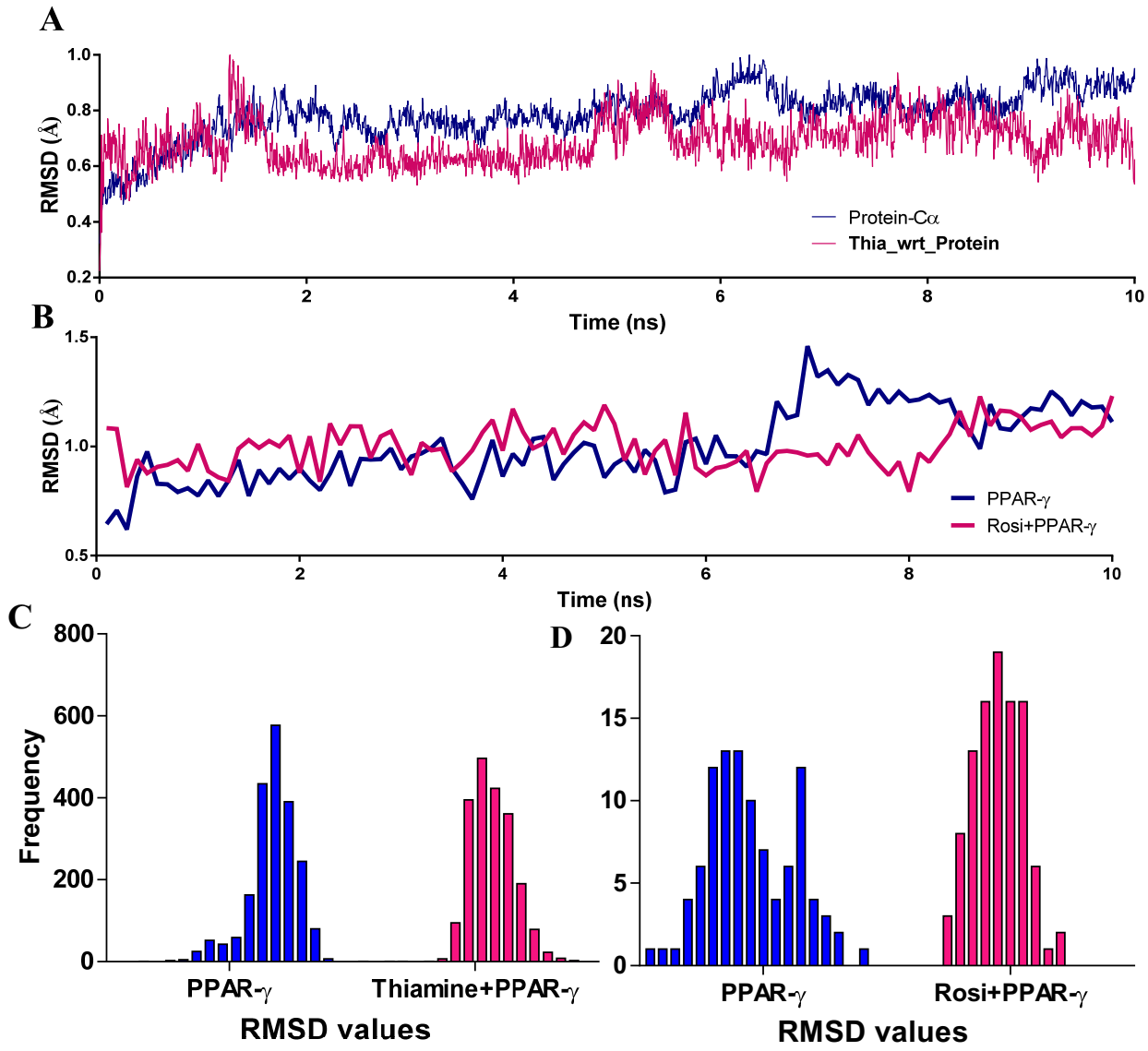

Fig. (2). A and **B.** RMSD of PPAR-γ and the thiamine/rosiglitazone (Rosi) - PPAR-γ complex calculated by molecular dynamics simulations. The docked structures were neutralized, dissolved in water, and simulated for 10 ns (nanoseconds) in the OPLS 2005 force field and other parameters mentioned in the Methods section using Desmond. **C** and **D.** Frequency distribution of RMSD shows that the complex is highly equilibrated. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

PPAR-γ, especially the thiazolidinedione (TZD) class of drugs, were shown to cause numerous side effects [39]. Hence, it is essential to find PPAR-γ activators with limited side effects [25].

Thiamine deficiency is highly prevalent in T2D patients [40] and is one of the major risk factors in T2D [12]. It also causes various T2D symptoms, such as hyperglycemia, oxidative stress, and endothelial dysfunction, and its replenishment decreased these symptoms [13]. Thiamine-supplemented food reduced glycated hemoglobin and fasting glucose levels in Black South African women [41]. A gradual increase in thiamine deficiency was noted during T2D progression [42]. Hence, we hypothesized that thiamine is a PPAR-γ and can be used to treat T2D. Analogs of thiamine such as benfotiamine are already being used as T2D drugs [43].

Our docking and 3T3-L1 cell differentiation assay results show that thiamine binds to PPAR-γ and induces adipogenesis of 3T3-L1 cells. Thiamine treatment activates PPAR-γ as evidenced from our transcription factor assay, which in turn upregulates genes such as AP-2. Our study is corroborated by the results of Feng *et al*. [44], which showed the PPAR-γ-mediated protective effect of thiamine in cardiac beriberi. Analysis of the crystal structures of PPAR-γ LBD shows that His323 plays a vital role in PPAR-γ/TZD interaction [45]. Our docking and dynamics simulation studies show that thiamine interacts with the critical His323 residue of PPAR-γ. It has been found that an acidic head group (such as the car-

bonyl group of TZD) is essential for PPAR-γ activation. We used thiamine in its pyrophosphate (acidic) form for docking studies since this is its physiological form. The acidic head groups of most PPAR-γ ligands interact with the polar amino acids Gln286, Ser289, His323, Tyr327, Lys367, His449, and Tyr473 present in the LBD of PPAR-γ [46]. In this study, molecular dynamics simulation shows that thiamine interacted with all the above-mentioned amino acids for a significant duration. At present, the limitations of molecular dynamics simulation are the time required for the process and approximation of molecular interactions by the force fields [47]. We have successfully used docking studies to find endogenous PPAR-α ligand [48]. Molecular dynamics is also routinely used for finding PPAR ligands [49]. Hu *et al*. [50] showed that thiamine acts as a suppressor of PPAR-γ in bone marrow-derived macrophages. However, in this study, we show that thiamine indeed activates PPAR-γ and induces adipogenesis. Rival *et al*. [51] showed that partial agonists of PPAR-γ did not upregulate AP-2 mRNA, but that full agonists can upregulate it. Since the present study shows the upregulation of AP-2 mRNA by thiamine, it is likely that thiamine is a full agonist of PPAR-γ.

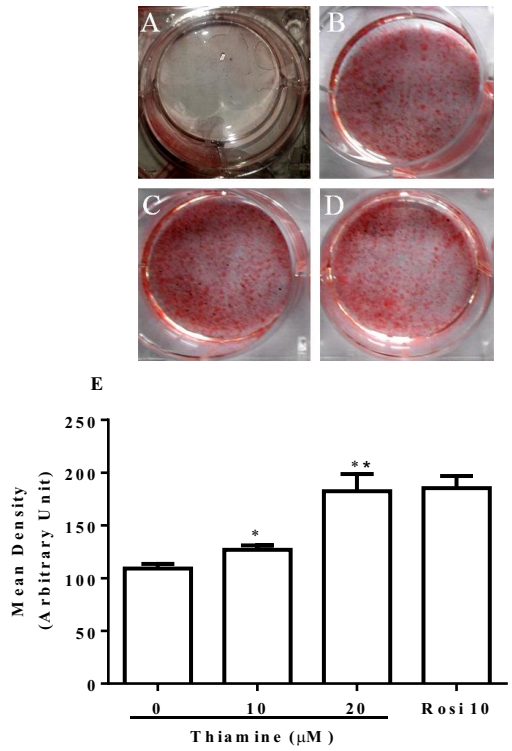

Fig. (3). Oil Red O-stained 3T3-L1 differentiation assay in the presence of thiamine 0 μM (**A**), 10 μM (**C**), and 20 μM (**D**) and in the presence of rosiglitazone (Rosi) 10 μM (**B**). Confluent 3T3-L1 cells were treated with PPAR-γ ligands and were maintained in an adipocyte differentiation medium as per procedures given in the Methods section. The differentiated cells were stained with Oil Red O and photographed. Photographs were then analyzed using the ImageJ software, and density values were plotted (**E**). Each column represents the mean of values obtained from experiments conducted in triplicates. **P* < 0.05 and ***P* < 0.01 *vs*. control (0 μM). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

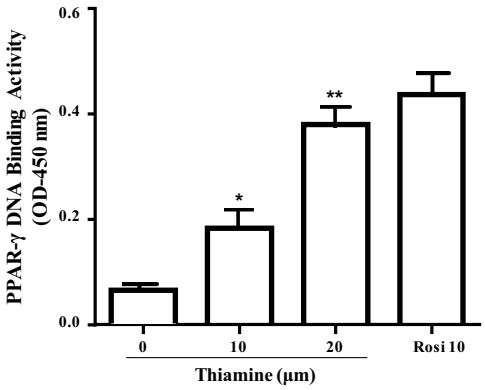

Fig. (4). PPAR-γ DNA binding activity. After the induction of 3T3-L1 with thiamine and rosiglitazone (Rosi), cells were harvested, and nuclear protein was extracted using a kit. PPAR-γ DNA binding activity was then measured using an ELISA-based PPAR-γ transcription factor assay kit. **P* < 0.05 and ***P* < 0.01 *vs*. control (0 μM).

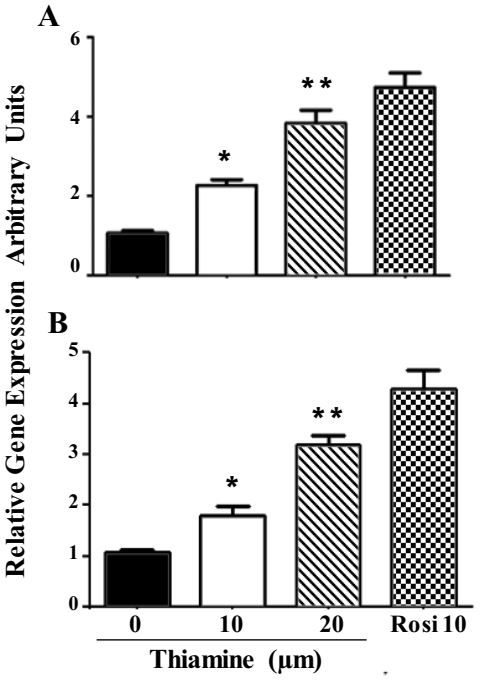

Fig. (5). Relative expression of mRNA (A) PPAR-γ and (B) AP-2. Total RNA was extracted from the differentiated 3T3-L1 cells, and mRNA was selectively converted to cDNA and quantified by qPCR. Each column represents the mean of values obtained from experiments conducted in triplicates. **P* < 0.05 and ***P* < 0.01 *vs*. control (0 μM).

CONCLUSION

In this study, we show for the first time that thiamine could activate PPAR-γ *in vitro*. However, more studies involving animal models are required to prove thiamine as a natural PPAR-γ ligand. Bioavailability and solubility problems, if any, could be resolved by synthesizing analogs such as benfotiamine. In addition, the binding of thiamine with other PPAR subtypes (α and δ) needs to be studied.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All data are available for public view after the publication of the manuscript. They will be available in the corresponding author's ResearchGate profile (https://www.researchgate.net/profile/Venkata-Narala).

FUNDING

This work was supported by the Science and Engineering Research Board (SERB) (EMR/2017/000973), Department of Science and Technology and Council of Scientific and Industrial Research (CSIR), Government of India (37(1488)/11 EMR-II).

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Saeedi, P.; Petersohn, I.; Salpea, P.; Malanda, B.; Karuranga, S.; Unwin, N.; Colagiuri, S.; Guariguata, L.; Motala, A.A.; Ogurtsova, K.; Shaw, J.E.; Bright, D.; Williams, R. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res. Clin. Pract.,* **2019**, *157*, 107843. http://dx.doi.org/10.1016/j.diabres.2019.107843

[2] Frasca, D.; Blomberg, B.B.; Paganelli, R. Aging, obesity, and inflammatory age-related diseases. *Front. Immunol.,* **2017**, *8*, 1745. http://dx.doi.org/10.3389/fimmu.2017.01745 PMID: 29270179

[3] Medina-Remón, A.; Kirwan, R.; Lamuela-Raventós, R.M.; Estruch, R. Dietary patterns and the risk of obesity, type 2 diabetes mellitus, cardiovascular diseases, asthma, and neurodegenerative diseases. *Crit. Rev. Food Sci. Nutr.,* **2018**, *58*(2), 262-296. http://dx.doi.org/10.1080/10408398.2016.1158690 PMID: 27127938

[4] Jia, X.; Yang, Y.; Chen, Y.; Xia, Z.; Zhang, W.; Feng, Y.; Li, Y.; Tan, J.; Xu, C.; Zhang, Q.; Deng, H.; Shi, X. Multivariate analysis of genome-wide data to identify potential pleiotropic genes for type 2 diabetes, obesity and coronary artery disease using MetaCCA. *Int. J. Cardiol.,* **2019**, *283*, 144-150. http://dx.doi.org/10.1016/j.ijcard.2018.10.102 PMID: 30459114

[5] Kolb, H.; Martin, S. Environmental/lifestyle factors in the pathogenesis and prevention of type 2 diabetes. *BMC Med.,* **2017**, *15*(1), 131. http://dx.doi.org/10.1186/s12916-017-0901-x PMID: 28720102

[6] Gulley, L.D.; Shomaker, L.B. Depression in youth-onset Type 2 Diabetes. *Curr. Diab. Rep.,* **2020**, *20*(10), 51. http://dx.doi.org/10.1007/s11892-020-01334-8 PMID: 32857299

[7] Manios, Y.; Lambrinou, C-P.; Mavrogianni, C.; Cardon, G.; Lindström, J.; Iotova, V.; Tankova, T.; Rurik, I.; Stappen, V.V.; Kivelä, J.; Mateo-Gallego, R.; Moreno, L.A.; Makrilakis, K.; Androutsos, O. Lifestyle changes observed among adults participating in a family- and community-based intervention for diabetes prevention in Europe: The 1st year results of the feel4diabetes-study. *Nutrients,* **2020**, *12*(7), 1949. http://dx.doi.org/10.3390/nu12071949 PMID: 32629949

[8] Gibbons, C.; Blundell, J.; Tetens Hoff, S.; Dahl, K.; Bauer, R.; Baekdal, T. Effects of oral semaglutide on energy intake, food preference, appetite, control of eating and body weight in subjects with type 2 diabetes. *Diabetes Obes. Metab.,* **2021**, *23*(2), 581-588. http://dx.doi.org/10.1111/dom.14255 PMID: 33184979

[9] MacDonald, C.S.; Nielsen, S.M.; Bjørner, J.; Johansen, M.Y.; Christensen, R.; Vaag, A.; Lieberman, D.E.; Pedersen, B.K.; Langberg, H.; Ried-Larsen, M.; Midtgaard, J. One-year intensive lifestyle intervention and improvements in health-related quality of life and mental health in persons with type 2 diabetes: A secondary analysis of the U-TURN randomized controlled trial. *BMJ Open Diabetes Res. Care,* **2021**, *9*(1), e001840. http://dx.doi.org/10.1136/bmjdrc-2020-001840 PMID: 33441418

[10] Carpenter, K.J. The discovery of thiamin. *Ann. Nutr. Metab.,* **2012**, *61*(3), 219-223. http://dx.doi.org/10.1159/000343109 PMID: 23183292

[11] Fitzpatrick, T.B.; Thore, S. Complex behavior: From cannibalism to suicide in the vitamin B1 biosynthesis world. *Curr. Opin. Struct. Biol.,* **2014**, *29*, 34-43. http://dx.doi.org/10.1016/j.sbi.2014.08.014 PMID: 25260119

[12] Eshak, E.S.; Arafa, A.E. Thiamine deficiency and cardiovascular disorders. *Nutr. Metab. Cardiovasc. Dis.,* **2018**, *28*(10), 965-972. http://dx.doi.org/10.1016/j.numecd.2018.06.013 PMID: 30143411

[13] Page, G.L.; Laight, D.; Cummings, M.H. Thiamine deficiency in diabetes mellitus and the impact of thiamine replacement on glucose metabolism and vascular disease. *Int. J. Clin. Pract.,* **2011**, *65*(6), 684-690. http://dx.doi.org/10.1111/j.1742-1241.2011.02680.x PMID: 21564442

[14] Karkabounas, S.; Papadopoulos, N.; Anastasiadou, C.; Gubili, C.; Peschos, D.; Daskalou, T.; Fikioris, N.; Simos, Y.V.; Kontargiris, E.; Gianakopoulos, X.; Ragos, V.; Chatzidimitriou, M. Effects of α-lipoic acid, carnosine, and thiamine supplementation in obese patients with type 2 diabetes mellitus: A randomized, double-blind study. *J. Med. Food,* **2018**, *21*(12), 1197-1203. http://dx.doi.org/10.1089/jmf.2018.0007 PMID: 30311825

[15] Beltramo, E.; Mazzeo, A.; Lopatina, T.; Trento, M.; Porta, M. Thiamine transporter 2 is involved in high glucose-induced damage and altered thiamine availability in cell models of diabetic retinopathy. *Diabetes Vasc. Dis. Res.,* **2020**, *17*(1), 1479164119878427.

[16] Jungtrakoon, P.; Shirakawa, J.; Buranasupkajorn, P.; Gupta, M.K.; De Jesus, D.F.; Pezzolesi, M.G.; Panya, A.; Hastings, T.; Chanprasert, C.; Mendonca, C.; Kulkarni, R.N.; Doria, A. Loss-of-function mutation in thiamine transporter 1 in a family with autosomal dominant diabetes. *Diabetes,* **2019**, *68*(5), 1084-1093. http://dx.doi.org/10.2337/db17-0821 PMID: 30833467

[17] Beltramo, E.; Berrone, E.; Tarallo, S.; Porta, M. Effects of thiamine and benfotiamine on intracellular glucose metabolism and relevance in the prevention of diabetic complications. *Acta Diabetol.,* **2008**, *45*(3), 131-141. http://dx.doi.org/10.1007/s00592-008-0042-y PMID: 18581039

[18] Malecka, S.A.; Poprawski, K.; Bilski, B. Prophylactic and therapeutic application of thiamine (vitamin B1)-a new point of view. *Wiadomosci lekarskie (Warsaw, Poland: 1960),* **2006**, *59*(5-6), 383-387.

[19] Rabbani, N.; Thornalley, P.J. Emerging role of thiamine therapy for prevention and treatment of early-stage diabetic nephropathy. *Diabetes Obes. Metab.,* **2011**, *13*(7), 577-583. http://dx.doi.org/10.1111/j.1463-1326.2011.01384.x PMID: 21342411

[20] Karachalias, N.; Babaei-Jadidi, R.; Kupich, C.; Ahmed, N.; Thornalley, P.J. High-dose thiamine therapy counters dyslipidemia and

advanced glycation of plasma protein in streptozotocin-induced diabetic rats. *Ann. N. Y. Acad. Sci.,* **2005**, *1043*, 777-783. http://dx.doi.org/10.1196/annals.1333.090 PMID: 16037305

[21] Thornalley, P.J. The potential role of thiamine. The potential role of thiamine (vitamin B1) in diabetic complications. *Curr. Diabetes Rev.,* **2005**, *1*(3), 287-298. http://dx.doi.org/10.2174/157339905774574383 PMID: 18220605

[22] Hoyumpa, A.M., Jr. Mechanisms of thiamin deficiency in chronic alcoholism. *Am. J. Clin. Nutr.,* **1980**, *33*(12), 2750-2761. http://dx.doi.org/10.1093/ajcn/33.12.2750 PMID: 6254354

[23] Vieregge, P.; Stuhlmann, W. Diabetic coma and Wernicke-Korsakoff syndrome. On the clinical significance of acquired thiamine deficiency. *Fortschr. Neurol. Psychiatr.,* **1987**, *55*(4), 130-139. http://dx.doi.org/10.1055/s-2007-1001815 PMID: 3596452

[24] Bermúdez, V.; Finol, F.; Parra, N.; Parra, M.; Pérez, A.; Peñaranda, L.; Vílchez, D.; Rojas, J.; Arráiz, N.; Velasco, M. PPAR-gamma agonists and their role in type 2 diabetes mellitus management. *Am. J. Ther.,* **2010**, *17*(3), 274-283. http://dx.doi.org/10.1097/MJT.0b013e3181c08081 PMID: 20216208

[25] Subramani, P.A.; Reddy, M.C.; Narala, V.R. The need for physiologically relevant peroxisome proliferator-activated receptor-gamma (PPAR-γ) ligands. *Endocr. Metab. Immune Disord. Drug Targets,* **2013**, *13*(2), 175-183. http://dx.doi.org/10.2174/18715303113139990003 PMID: 23713695

[26] Balakumar, P.; Rohilla, A.; Krishan, P.; Solairaj, P.; Thangathirupathi, A. The multifaceted therapeutic potential of benfotiamine. *Pharmacol. Res.,* **2010**, *61*(6), 482-488. http://dx.doi.org/10.1016/j.phrs.2010.02.008 PMID: 20188835

[27] Nolte, R.T.; Wisely, G.B.; Westin, S.; Cobb, J.E.; Lambert, M.H.; Kurokawa, R.; Rosenfeld, M.G.; Willson, T.M.; Glass, C.K.; Milburn, M.V. Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-gamma. *Nature,* **1998**, *395*(6698), 137-143. http://dx.doi.org/10.1038/25931 PMID: 9744270

[28] Sastry, G.M.; Adzhigirey, M.; Day, T.; Annabhimoju, R.; Sherman, W. Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. *J. Comput. Aided Mol. Des.,* **2013**, *27*(3), 221-234. http://dx.doi.org/10.1007/s10822-013-9644-8 PMID: 23579614

[29] Banks, J.L.; Beard, H.S.; Cao, Y.; Cho, A.E.; Damm, W.; Farid, R.; Felts, A.K.; Halgren, T.A.; Mainz, D.T.; Maple, J.R.; Murphy, R.; Philipp, D.M.; Repasky, M.P.; Zhang, L.Y.; Berne, B.J.; Friesner, R.A.; Gallicchio, E.; Levy, R.M. Integrated Modeling Program, Applied Chemical Theory (IMPACT). *J. Comput. Chem.,* **2005**, *26*(16), 1752-1780. http://dx.doi.org/10.1002/jcc.20292 PMID: 16211539

[30] Laskowski, R.A.; Swindells, M.B. LigPlot+: Multiple ligand-protein interaction diagrams for drug discovery. *J. Chem. Inform. and Modeling,* **2011,** *51* (10), 2778-2786.

[31] Bowers, K.J.; Chow, E.; Xu, H.; Dror, R.O.; Eastwood, M.P.; Gregersen, B.A.; Klepeis, J.L.; Kolossvary, I.; Moraes, M.A.; Sacerdoti, F.D.; Salmon, J.K.; Shan, Y.; Shaw, D.E. Scalable algorithms for molecular dynamics simulations on commodity clusters. In: *Proceedings of the 2006 ACM/IEEE Conference on Supercomputing,* 11-17 Nov. 2006 Tampa, Florida http://dx.doi.org/10.1145/1188455.1188544

[32] Berendsen, H.J.C.; Postma, J.P.M.; van Gunsteren, W.F.; Hermans, J. Interaction Models for Water in Relation to Protein Hydration. In: *Intermolecular Forces*; Pullman, B., Ed.; Springer: The Netherlands, **1981**; Vol. 14, pp. 331-342. http://dx.doi.org/10.1007/978-94-015-7658-1_21

[33] Evans, D.J.; Holian, B.L. The Nose–Hoover thermostat. *J. Chem. Phys.,* **1985**, *83*(8), 4069-4074. http://dx.doi.org/10.1063/1.449071

[34] Martyna, G.J.; Tobias, D.J.; Klein, M.L. Constant pressure molecular dynamics algorithms. *J. Chem. Phys.,* **1994**, *101*(5), 4177-4189. http://dx.doi.org/10.1063/1.467468

[35] Essmann, U.; Perera, L.; Berkowitz, M.L.; Darden, T.; Lee, H.; Pedersen, L.G. A smooth particle mesh Ewald method. *J. Chem. Phys.,* **1995**, *103*(19), 8577-8593. http://dx.doi.org/10.1063/1.470117

[36] Panati, K.; Subramani, P.A.; Reddy, M.M.; Derangula, M.; Arva Tatireddigari, V.R.R.; Kolliputi, N.; Narala, V.R. The nitrated fatty acid, 10-nitrooleate inhibits the neutrophil chemotaxis *via* peroxisome proliferator-activated receptor gamma in CLP-induced sepsis in mice. *Int. Immunopharmacol.,* **2019**, *72*, 159-165. http://dx.doi.org/10.1016/j.intimp.2019.04.001 PMID: 30981081

[37] Lascar, N.; Brown, J.; Pattison, H.; Barnett, A.H.; Bailey, C.J.; Bellary, S. Type 2 diabetes in adolescents and young adults. *Lancet Diabetes Endocrinol.,* **2018**, *6*(1), 69-80. http://dx.doi.org/10.1016/S2213-8587(17)30186-9 PMID: 28847479

[38] Ramzan, S.; Timmins, P.; Hasan, S.S.; Babar, Z.U. Cost analysis of type 2 diabetes mellitus treatment in economically developed countries. *Expert Rev. Pharmacoecon. Outcomes Res.,* **2019**, *19*(1), 5-14. http://dx.doi.org/10.1080/14737167.2018.1513790 PMID: 30146917

[39] Artasensi, A.; Pedretti, A.; Vistoli, G.; Fumagalli, L. Type 2 Diabetes Mellitus: A review of multi-target drugs. *Molecules,* **2020**, *25*(8), E1987. http://dx.doi.org/10.3390/molecules25081987 PMID: 32340373

[40] Nix, W.A.; Zirwes, R.; Bangert, V.; Kaiser, R.P.; Schilling, M.; Hostalek, U.; Obeid, R. Vitamin B status in patients with type 2 diabetes mellitus with and without incipient nephropathy. *Diabetes Res. Clin. Pract.,* **2015**, *107*(1), 157-165. http://dx.doi.org/10.1016/j.diabres.2014.09.058 PMID: 25458341

[41] Chikowore, T.; Pisa, P.T.; van Zyl, T.; Feskens, E.J.; Wentzel-Viljoen, E.; Conradie, K.R. Nutrient patterns associated with fasting glucose and glycated haemoglobin levels in a black South African population. *Nutrients,* **2017**, *9*(1), E9. http://dx.doi.org/10.3390/nu9010009 PMID: 28106816

[42] Cinici, E.; Dilekmen, N.; Senol, O.; Arpalı, E.; Cinici, O.; Tanas, S. Blood thiamine pyrophosphate concentration and its correlation with the stage of diabetic retinopathy. *Int. Ophthalmol.,* **2020**, *40*(12), 3279-3284. http://dx.doi.org/10.1007/s10792-020-01513-2 PMID: 32715366

[43] Ziegler, D.; Papanas, N.; Schnell, O.; Nguyen, B.D.T.; Nguyen, K.T.; Kulkantrakorn, K.; Deerochanawong, C. Current concepts in the management of diabetic polyneuropathy. *J. Diabetes Investig.,* **2021**, *12*(4), 464-475. PMID: 32918837

[44] Feng, C.; Li, D.; Chen, M.; Jiang, L.; Liu, X.; Li, Q.; Geng, C.; Sun, X.; Yang, G.; Zhang, L.; Yao, X. Citreoviridin induces myocardial apoptosis through PPAR-γ-mTORC2-mediated autophagic pathway and the protective effect of thiamine and selenium. *Chem. Biol. Interact.,* **2019**, *311*, 108795. http://dx.doi.org/10.1016/j.cbi.2019.108795 PMID: 31419397

[45] Xu, H.E.; Lambert, M.H.; Montana, V.G.; Plunket, K.D.; Moore, L.B.; Collins, J.L.; Oplinger, J.A.; Kliewer, S.A.; Gampe, R.T., Jr; McKee, D.D.; Moore, J.T.; Willson, T.M. Structural determinants of ligand binding selectivity between the peroxisome proliferator-activated receptors. *Proc. Natl. Acad. Sci. USA,* **2001**, *98*(24), 13919-13924. http://dx.doi.org/10.1073/pnas.241410198 PMID: 11698662

[46] Gim, H.J.; Choi, Y.S.; Li, H.; Kim, Y.J.; Ryu, J.H.; Jeon, R. Identification of a novel PPAR-γ agonist through a scaffold tuning approach. *Int. J. Mol. Sci.,* **2018**, *19*(10), E3032. http://dx.doi.org/10.3390/ijms19103032 PMID: 30287791

[47] Durrant, J.D.; McCammon, J.A. Molecular dynamics simulations and drug discovery. *BMC Biol.,* **2011**, *9*(1), 71. http://dx.doi.org/10.1186/1741-7007-9-71 PMID: 22035460

[48] Narala, V.R.; Adapala, R.K.; Suresh, M.V.; Brock, T.G.; Peters-Golden, M.; Reddy, R.C. Leukotriene B4 is a physiologically relevant endogenous peroxisome proliferator-activated receptor-alpha agonist. *J. Biol. Chem.,* **2010,** *285* (29), 22067-22074.

[49] Sullivan, H-J.; Wang, X.; Nogle, S.; Liao, S.; Wu, C. To probe full and partial activation of human peroxisome proliferator-activated receptors by pan-agonist chiglitazar using molecular dynamics simulations. *PPAR Res.,* **2020**, *2020*, 5314187. http://dx.doi.org/10.1155/2020/5314187 PMID: 32308671

[50] Hu, S.; He, W.; Du, X.; Huang, Y.; Fu, Y.; Yang, Y.; Hu, C.; Li, S.; Wang, Q.; Wen, Q.; Zhou, X.; Zhou, C.; Zhong, X-P.; Ma, L.

Vitamin B1 helps to limit *Mycobacterium tuberculosis* growth *via* regulating innate immunity in a peroxisome proliferator-activated receptor-γ-dependent manner. *Front. Immunol.,* **1778**, *2018*, 9. PMID: 30166982

[51] Rival, Y.; Stennevin, A.; Puech, L.; Rouquette, A.; Cathala, C.; Lestienne, F.; Dupont-Passelaigue, E.; Patoiseau, J.F.; Wurch, T.; Junquéro, D. Human adipocyte fatty acid-binding protein (aP2) gene promoter-driven reporter assay discriminates nonlipogenic peroxisome proliferator-activated receptor gamma ligands. *J. Pharmacol. Exp. Ther.,* **2004**, *311*(2), 467-475. http://dx.doi.org/10.1124/jpet.104.068254 PMID: 15273253