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Clinical and In-Silico Analysis of Thyroid Dysfunction and Type 2 Diabetes Risk in Non-Diabetic Iraqi Patients with Subclinical Hypothyroidism

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Abstract

Thyroid dysfunction and type 2 diabetes mellitus (T2DM) are interconnected endocrine disorders, yet their causal relationship remains unclear. This study investigates the impact of subclinical hypothyroidism (SCH) on the risk of developing insulin resistance, prediabetes, and T2DM in non-diabetic Iraqi subjects. A total of 300 SCH patients and 200 healthy controls were assessed for fasting blood glucose (FBG), fasting insulin (FI), glycosylated hemoglobin (HbA1c), thyroid-stimulating hormone (TSH), thyroxine (T4), and triiodothyronine (T3) at baseline and after 16 months without treatment. Insulin resistance was evaluated using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). The interaction between thyroid hormones and the insulin receptor tyrosine kinase was investigated using molecular docking methodologies. Results showed that 49 % of SCH patients developed clinical hypothyroidism, while all participants exhibited insulin resistance by the follow-up period. After the follow-up period, 73 % of the patients developed prediabetes, and 27 % progressed to T2DM. Molecular docking analyses revealed direct interactions between T_3, T_4, and the insulin receptor, suggesting a modulatory role of thyroid hormones in insulin signaling. It is concluded that untreated SCH significantly increases the risk of metabolic disorders, supporting thyroid dysfunction as a causative factor in diabetes development. Early detection and management of SCH could be critical in preventing T2DM onset.

Keywords: Diabetes mellitus, Insulin resistance, T2DM, Hypothyroidism, Thyroid dysfunction, SCH

Introduction

Studies have revealed a bi-directional impact of diabetes and thyroid diseases on each other; both illnesses frequently exist side by side in individuals [1,2]. Thyroid dysfunction (TD), which is commonly characterized as an unusual thyroid function analysis result, is more prevalent in individuals with type 2 diabetes (T2DM) than in those without diabetes and can negatively affect metabolic management [3,4]. T2DM risk increases in people with hypothyroidism and those with lower free thyroxine (FT_4) levels in the reference range [5]. Thyroid hormones can directly affect insulin release. Hypothyroidism, the most common form of TD in diabetic individuals, may cause a reduction in production of insulin. Hyperthyroidism causes an increase in beta-cell reactivity to catecholamines or glucose, as well as an increase in insulin clearance [6]. Furthermore, both hypothyroidism and hyperthyroidism can impact insulin metabolism, resulting in insulin resistance [7]. Diabetes, on the other hand, can affect thyroid function by altering thyroid stimulating hormone (TSH) levels in the hypothalamus and disrupting T_4 to T_3 transformation in peripheral tissues [4]. Long-term combination of TD and T2DM may exacerbate the morbidity and mortality associated with

diabetes [4]. Decreased amounts of thyroid hormones, even in the normal range, have been linked to diabetes problems, including acute complications like diabetic ketosis (DK) or diabetic ketoacidosis (DKA) [8] and chronic issues such diabetic nephropathy (DN) [9] and diabetic retinopathy (DR) [10]. Hypothyroidism was linked to a higher risk of DR and chronic renal disease [11]. The link between T2DM and TD is complicated and has not been thoroughly understood [12].

Thyroid hormone (TH) has long been shown to influence glucose homeostasis. TH has been linked to pancreatic β-cell formation and glucose metabolism in many organs, including the liver, gastrointestinal tract, pancreas, adipose tissue, skeletal muscles, and central nervous system. Many studies have linked TH action to pancreatic β-cell growth and function [13,14]. Triiodothyronine (T_3) promotes β-cell proliferation from birth to the 1st week of life. Furthermore, T_3 boosts glucose-stimulated insulin release. TH influences insulin release and glucose absorption by several mechanisms in the gastrointestinal tract, liver, skeletal muscles, and adipose tissue. TH improves glucose absorption by increasing gastrointestinal motility [15]. It boosts hepatic glucose output by increasing GLUT2 expression in the liver, which accelerates endogenous glucose synthesis via gluconeogenesis and glycogenolysis. T_3 boosts hepatic gluconeogenesis by raising the activity of phosphoenolpyruvate carboxykinase (PEPCK), an enzyme that promotes gluconeogenesis [16,17]. Moreover, increased glycogenolysis and gluconeogenesis generate hyperinsulinemia and glucose intolerance, which leads to peripheral insulin resistance [15]. TH promotes lipolysis in adipose tissue, leading to a rise in free fatty acid, which accelerates hepatic gluconeogenesis [18]. TH provokes pancreatic β-cells to secrete insulin and α-cells to produce glucagon [15]. Hyperthyroidism boosts glucose transporter type 4 (GLUT4) gene expression and glucose absorption in skeletal muscles [15]. T_3 can regulate glucose production via a sympathetic channel connecting the hypothalamus paraventricular nucleus (PVN) to the liver. T_3 in the hypothalamic PVN promotes hepatic glucose synthesis, regardless of glucoregulatory hormones [19].

Thyroid problems are among the most prevalent endocrine disorders on the globe. Thyroid illnesses are often classified into 2 categories: Hyperthyroidism and hypothyroidism [20]. Moreover, subclinical hypothyroidism (SCH), also known as moderate thyroid failure, is identified when peripheral thyroid hormone amounts are within normal values but blood TSH are slightly increased. This disorder affects 3 - 8 % of the general population. It is more frequent in women than in males, and the incidence rises with age. The most significant consequence of SCH is the increased risk of progressing to clinical hypothyroidism. SCH is also described as a serum thyroid-stimulating hormone (TSH) level beyond the maximum limit of acceptable levels range, despite normal serum free thyroxine [21,22].

Numerous earlier investigations have found that SCH accounts for a high percentage of thyroid dysfunctions (TD) in diabetic patients [23-27]. Subclinical hyperthyroidism has also been found in such individuals, but less frequently than SCH [26,27]. However, none of the studies showed whether SCH is a causative factor or a consequence of DM. So, the important question of which condition led to the other is still not properly answered. Accordingly, we aimed in this study to investigate the effects of subclinical hypothyroidism on the risk of future development of any forms of diabetes, insulin resistance or glucose intolerance in non-diabetic subjects with normal glycemic status but a compromised thyroid function by measuring levels of FI, FBG, HbA_{1c} and calculating the HOMA-IR index of insulin resistance. Since the subjects involved are non-diabetic with normal glycemic status, this may prove whether thyroid dysfunction is a causative factor or a consequence of DM.

Beyond clinical research, it is vital to perform computational study at a molecular level. A key question is whether thyroid hormones directly interact with the insulin receptor, crucial for glucose regulation-a possibility not well explored. We employed computational modeling (molecular docking) to see if T_3 and T_4 could structurally bind to the receptor's critical signaling domain. Discovering a plausible interaction provides essential mechanistic insight, suggesting a direct pathway, potentially independent of gene changes, for thyroid status to influence insulin receptor activity. This molecular viewpoint significantly supports our clinical findings, bolstering the biological case for thyroid dysfunction as a direct contributor to insulin

resistance and T2DM, and highlighting the value of integrating computational modeling with patient observations.

Materials and methods

Study subjects

This study included a total of 500 subjects. The study subjects were divided into 3 groups, the 1st group (group A) included 300 non-diabetic subjects with subclinical hypothyroidism with an age range of (34 - 44) years old. After 16 months follow up period without any treatments, the same 300 subjects were included within the 2nd group (group B), while the 3rd group (group C) consisted of 200 apparently healthy subjects as a control group with an age range of (35 - 42) years old. Explicit matching was performed on sex, lifestyle, and socioeconomic status between the control and experimental groups. The subjects provided their verbal consent, and the study was approved by the scientific and ethics committees of the College of Biotechnology - Al-Nahrain University and the Branch of Chemistry - Applied Sciences Department - University of Technology (Approval No. 40167, Date: 26 / 2 / 2023).

Exclusion criteria

The following subjects were excluded from the study since their associated conditions may have an effect on the results of the study: subjects with type 1 diabetes mellitus, subjects with any known inflammatory conditions, subjects with liver diseases, subjects with kidney diseases, subjects with pancreatic diseases, subjects with hypertension, subjects with malignant diseases, pregnant subjects, subjects taking T_4 treatment, overweight subjects (body mass index; 25 - 29.9), obese subjects (body mass index $\geq$ 30) and subjects with recent surgeries.

Collection of blood samples

Five milliliters of venous blood were taken from all of the study subjects by vein puncturing using plastic disposable syringes. The blood was transferred into gel and EDTA tubes. Serum samples isolated from gel tubes were used for measurement of FBG, FI, TSH, T_4 and T_3. Whole blood samples were used for HbA_{1c} measurement. The samples collected were analyzed immediately.

Measurement of FBG, FI, HbA_{1c}, TSH, T_4 and T_3

Fasting blood glucose was measured in the sera of all the study subjects using the Humalyzer 2000 semi-automated photometer provided with Glucose liquicolor kit manufactured by Human Diagnostics. HbA_{1c} was measured using Hipro Hurricane Specific Protein Immunoassay Analyzer provided with Hipro® Glycosyclated Hemoglobin kit manufactured by Hipro Biotechnology. Fasting insulin, TSH, T_4 and T_3 were measured in the sera of the study subjects using the fully automated Maglumi 800 Chemiluminescence Immunoassay Analyzer provided with MAGLUMI Insulin (CLIA) and MAGLUMI TSH, T_4 and T_3 (CLIA) kits manufactured by Snibe Diagnostic.

HOMA-IR calculation

HOMA-IR index was calculated using the following formula [28]: HOMA-IR = (FI×FBG)/405. Where FI is the fasting insulin expressed in mIU/L or µIU/mL and FBG is the fasting blood glucose expressed in mg/dL.

Statistical analysis

Biochemical data were analyzed and processed using Microsoft Excel (2019), GraphPad Prism 9 and SPSS (statistical package for social sciences) software, version (25). In order to assess the results of this study, analysis of variance (ANOVA) was used to calculate the mean (average) value, standard deviation (SD), and P-value (probability). Pearson correlations were also calculated. Multiple testing corrections were applied to account for the potential inflation of error due to performing multiple correlation tests. Bonferroni correction and false discovery rate (FDR) test were performed to adjust the *p*-values for the correlations.

Molecular docking

To initiate our investigation, we procured 3-dimensional coordinates for each ligand from the PubChem database https://pubchem.ncbi.nlm.nih.gov/ [29]. Concurrently, the experimentally determined crystallographic structure of the target protein receptor (PDB ID: 1I44) was accessed from the Protein Data Bank https://www.rcsb.org [30]. Subsequently, to rigorously evaluate ligand binding affinity and delineate the intricate mechanisms of interaction with the

receptor, we employed Molecular Operating Environment (MOE) software (version 2022.02) [31] for molecular docking and binding site prediction.

Receptor preparation was meticulously performed to accurately reflect the experimental milieu. This involved retaining solvent atoms to maintain a realistic solvation environment within the binding site. The binding site itself was strategically defined through the imposition of dummy atoms, coupled with a spatial wall constraint. This deliberate constraint served to refine the scope of ligand exploration, focusing computational efforts on the relevant binding pocket. Ligand docking was then executed leveraging MOE's Triangle Matcher algorithm, selected for its efficiency in conformational sampling. Recognizing the inherent flexibility of biomolecular systems, we incorporated Induced Fit refinement to explicitly account for both ligand and receptor adaptability. This iterative refinement process facilitated the generation of 20 distinct conformational poses per ligand. To quantitatively rank these poses based on their predicted stability within the binding pocket, we utilized the GBVI/WSA dG free energy function (ΔG, kcal/mol). The thermodynamically most stable, or top-ranked, poses were then carried forward for in-depth examination. Post-docking analyses to elucidate 3-dimensional interaction profiles, including the generation of interaction fingerprints and detailed pose visualizations alongside energy score assessment, were performed using UCSF Chimera [32], facilitating a comprehensive and multifaceted evaluation of ligand-receptor complexes.

In a complementary set of simulations designed to probe protein-protein interactions, specifically between insulin chains A/B (PDB ID: 3I40), and the insulin receptor tyrosine kinase (PDB ID: 1I44), we again utilized MOE 2022.02. For these simulations, the preparation of the receptor structure mirrored our initial approach, preserving all atoms present in the crystallographic data. To guide the placement of insulin chain B, designated as the ligand in this protein-protein docking context, we again employed dummy atoms to demarcate the binding site. Consistent with biological realism, insulin chain B was prepared using an all-atom representation, incorporating hydrogen atoms and refining protonation states to accurately represent physiological conditions.

The protein-protein docking protocol was implemented using MOE's dedicated protein-protein docking algorithm. This algorithm was deliberately configured to prioritize hydrophobic complementarity via Hydrophobic Patch Potential analysis, reflecting the significant role of hydrophobic interactions in protein association. To faithfully model the high specificity observed in biological recognition events, we judiciously constrained both receptor and ligand binding regions to Complementarity-Determining Regions (CDRs), where appropriate given the protein system context. Rigid-body refinement was strategically selected as the initial refinement strategy to preserve the inherent backbone architecture of the interacting partners, while simultaneously enabling side-chain flexibility during subsequent pose optimization. To address potential steric clashes arising from conformational adjustments, side chains were systematically repacked utilizing the MOE rotamer library. Furthermore, electrostatic interactions, which contribute significantly to binding energetics and specificity, were modeled using the Reaction Field potential, incorporating solvation effects to better approximate the physiological environment.

For final energetic evaluation and pose ranking, energy minimization was performed using the Generalized Born Model (GBM). To achieve a balance between computational rigor and efficiency, termination criteria were established, setting a gradient threshold of 0.01 or a maximum of 500 iterations for the minimization process. To ensure the physical plausibility and geometric fidelity of the protein-protein interface, we imposed a pharmacophore restraint characterized by a force constant of 100 kcal/(mol·Å^2) and a radius offset of 0.4 Å. This restraint strategically guided critical residue contacts to adhere to predefined spatial constraints derived from structural or biophysical considerations. Finally, to enable detailed visualization and qualitative analysis of the intermolecular interfaces, docked poses were exported to the DIMPLOT module, enabling the generation of informative 2-dimensional interaction diagrams [33]. Validation of our docking protocol included rigorous assessment of reproducibility via calculating the root-mean-square deviation (RMSD) of the docked insulin chains relative to the experimentally determined crystallographic pose,

thereby bolstering confidence in the robustness and reliability of our computational approach.

Results and discussion

This study involved the investigation of thyroid dysfunction effect on the glycemic status of non-diabetic subjects with subclinical hypothyroidism in order to elucidate whether thyroid dysfunction is a causative factor or a consequence of diabetes mellitus. The levels of FBG, FI, HbA_{1c}, TSH, T_4 and T_3 were measured at the beginning of the study in non-diabetic subjects with subclinical hypothyroidism and their results are represented as group A shown in **Table 1**. After the follow up period, the levels of FBG, FI, HbA_{1c}, TSH, T_4 and T_3 were remeasured in the study subjects and their results are represented by group B in **Table 1**.

Table 1 Levels of FBG, FI, HbA_{1c}, TSH, T_4 and T_3 in the study subjects.

Parameters	Group A Mean ± SD (95 % CI)	Group B Mean ± SD (95 % CI)	Group C Mean ± SD (95 % CI)	*p*-value	Reference range
FBG (mg/dL)	95.0 ± 4.0 (94.54 – 95.46)	163.3 ± 47.3 (157.91 – 168.69)	89.0 ± 5.4 (88.25 – 89.75)	0.000*	70 - 99
FI (µIU/mL)	13.1 ± 1.3 (12.95 – 13.25)	7.3 ± 4.6 (6.78 – 7.82)	18.3 ± 2.5 (17.95 – 18.65)	0.000*	4.03 - 23.46
HbA_{1c} (%)	5.4 ± 0.2 (5.38 – 5.42)	7.7 ± 1.5 (7.53 – 7.87)	5.1 ± 0.3 (5.06 – 5.14)	0.000*	4.5 - 6.5
TSH (µIU/mL)	4.9 ± 0.2 (4.88 – 4.92)	8.2 ± 3.4 (7.81 – 8.59)	2.1 ± 0.7 (2.00 – 2.20)	0.008*	0.3 - 4.5
T_4 (ng/mL)	54.4 ± 13.0 (52.92 – 55.88)	31.4 ± 21.2 (28.99 – 33.81)	82.7 ± 10.7 (81.21 – 84.19)	0.003*	52 - 127
T_3 (ng/mL)	0.88 ± 0.4 (0.83 – 0.93)	0.42 ± 0.21 (0.40 – 0.44)	1.8 ± 0.2 (1.77 – 1.83)	0.05*	0.69 - 2.15

*Significant at the levels of ($p \leq 0.05$). 95 % CI: 95 % confidence intervals. Group A: Patients with SCH (N = 300), Group B: Same patients from group A after the follow-up period (N = 300).

The results show that the subjects with subclinical hypothyroidism had significantly higher levels of FBG compared to the control group while they had a significantly lower level of FI compared to the control group. However, levels of HbA_{1c} were non-significantly elevated in group A subjects compared to the control group. Even though the FBG and FI were significantly different from the control group, they were however, within the normal or reference ranges and all of the subjects with subclinical hypothyroidism were non-diabetic and had normal glycemic status. After the follow-up period, the subclinical hypothyroidism, previously diagnosed in the 300 non-diabetic subjects (group A) advanced to clinical hypothyroidism in 49 % (148 out of 300 subjects, see **Table 2** of the subjects as indicated by the results of TSH, T_4 and T_3 of (group B) in **Table 1**.

It can also be seen that FBG and HbA_{1c} were significantly elevated in the group B subjects compared to both group A and the control group, while levels of FI were significantly lower in group B subjects compared to both group A and the control group.

After examining the TSH, T_4 and T_3 levels of the subjects in group B after the follow-up period, it was found that of the 300 patients with SCH, 148 subjects (49 %) had developed clinical hypothyroidism (**Table 2**). Patients having a slightly elevated TSH, with T_4 and T_3 within the lower limit of reference range were considered to have SCH, while patients having significantly elevated TSH, with T_4 and T3 significantly lower than the reference range were considered to have clinical hypothyroidism [21,22].

After examining the FBG, FI and HbA_{1c} of the subjects in group B, it was found that 219 subjects (73 %) had developed prediabetes, while 81 subjects (27 %)

had developed T2DM as can be seen in **Table 2**. Patients with a FBG between 100 and 125 mg/dL and HbA_{1c} between 5.7 and 6.4 %, were considered to be prediabetic, while patients with a FBG of ≥ 126 mg/dL and HbA_{1c} ≥ 6.5 % were considered to be diabetic [34]. It is important to note that these subjects were non-diabetic with completely normal glycemic control and status at the beginning of the study, this may prove the diabetogenic effect of thyroid dysfunction.

The results also indicate that of the 152 subjects with SCH, 129 subjects (85 %) developed prediabetes while the rest 23 subjects (15 %) developed T2DM. Moreover, of the 148, 90 subjects (61 %) developed prediabetes, with the rest 58 subjects (39 %) developing T2DM. This shows that the majority of T2DM cases (58 out of 81) were developed originally in patients with clinical hypothyroidism rather than SCH.

Table 2 The number, percentage and distribution of the study subjects according to the different conditions involved in the study.

Study subjects	SCH* N (%)	Clinical hypothyroidism* N (%)	Prediabetes** N (%)	T2DM** N (%)
Group A	300 (100)	0 (0)	0 (0)	0 (0)
Group B	152 (51)	148 (49)	219 (73)	81 (27)
Group C	0 (0)	0 (0)	0 (0)	0 (0)
Distribution of prediabetes and diabetes cases within group B subjects				
Group B	**Prediabetes** N (%)**		**T2DM** N (%)**	
SCH* (N = 152)	129 (85 %)		23 (15 %)	
Clinical hypothyroidism* (N = 148)	90 (61 %)		58 (39 %)	

*SCH (slightly elevated TSH, with T_4 and T_3 within the lower limit of reference range, clinical hypothyroidism (significantly elevated TSH, with T_4 and T_3 significantly lower than the reference range) [21,22].
**Prediabetes (FBG: 100 – 125 mg/dL, HbA_{1c}: 5.7 – 6.4 %), T2DM (FBG: ≥ 126 mg/dL, HbA_{1c} ≥ 6.5 %) [34].

Table 3 Number and percentage of insulin resistant subjects estimated based on HOMA-IR index.

HOMA-IR index					
Study subjects	**HOMA-IR (Mean ± SD)**	**HOMA-IR Range values**	***p*-value**	**IR N (%)**	**Normal N (%)**
Group A (N = 300)	2.2 ± 0.4	1.8 – 2.6	0.000*	57 (19)	243 (81)
Group B (N = 300)	2.9 ± 0.6	2.3 – 3.5		300 (100)	0 (0)
Group C (N = 200)	1.1 ± 0.2	0.9 – 1.3		0 (0)	200 (100)

*Significant at the levels of ($p \leq 0.05$). IR; insulin resistance, N; number of subjects, IR: HOMA-IR > 1.9 [35].

HOMA-IR index was calculated using the formula described by Matthews *et al.* [28]: [FI (μIU/mL) X FBG (mg/dL)]/ 405. Bonora *et al.* [35] verified the HOMA-IR index using the hyperinsulinemic-euglycemic clamp approach, revealing a substantial correlation (r = 0.820, $p < 0.0001$). As a result, HOMA-IR is accepted as a viable approach for assessing peripheral insulin sensitivity in epidemiologic investigations. The results

of the HOMA-IR index shown in **Table 3** suggest that 19 % of the non-diabetic subjects with subclinical hypothyroidism (group A) had insulin resistance at the beginning of the study. After the follow period, all of the study subjects (100 %) developed insulin resistance (group B), and the levels of the HOMA-IR increased considerably in those 19 % of the subjects who had insulin resistance at the beginning of the study.

The cases of new-onset insulin resistance found in the study subjects developed after the follow-up period suggest that thyroid dysfunction (specifically, SCH which later developed into clinical hypothyroidism) is responsible for the development of new-onset insulin resistance. This discovery was consistent with prior research, which found that SCH may cause insulin resistance by decreasing the rate of insulin-stimulated glucose transfer caused by a GLUT 2 gene translocation. Furthermore, research found that in hypothyroidism, lowered insulin clearance by the kidneys reduced the physiological demand for insulin. Furthermore, anorectic conditions may lead to decreased insulin production in hypothyroidism. Moreover, insulin resistance has also been associated with hypothyroidism in a number of preclinical and *in vitro* studies [36], which found that peripheral muscles become less responsive to insulin under hypothyroid circumstances. Dysregulated leptin metabolism has been proposed as a possible cause of such illness [37]. Furthermore, some writers have shown a strong link between insulin resistance and hypothyroidism [38].

The bad glycemic control discovered in the findings of the current investigation, shown by the elevated amounts of FBG and HbA_{1c} seen in group B participants, could at least to some extent in be induced by the clinical hypothyroidism condition formed in the patients, since hypothyroidism has been reported to be characterized by impaired glucose absorption from the GI tract, protracted peripheral accumulation, gluconeogenesis, reduced hepatic glucose synthesis, and reduced disposal [39]. Levels of FBG, HbA_{1c} and FI were significantly correlated with thyroid hormones as can be seen by the pearson correlation calculations in **Table 4**.

Table 4 The correlation between TSH, T_3 and T_4 with the biochemical parameters in the patients group.

TSH *vs* Parameters	**Correlation (r)**	***p-value***
FBG	0.757	0.011*
FI	−0.505	0.004*
HbA_{1c}	0.736	0.000*
T_4	−0.929	0.000*
T_3	−0.859	0.000*
T_4 *vs* Parameters	**Correlation (r)**	***p-value***
FBG	−0.667	0.009*
FI	0.889	0.001*
HbA_{1c}	−0.657	0.000*
TSH	−0.929	0.000*
T_3	0.866	0.000*
T_3 *vs* Parameters	**Correlation (r)**	***p-value***
FBG	−0.573	0.042*
FI	0.761	0.010*
HbA_{1c}	−0.563	0.001*
TSH	−0.859	0.000*
T_4	0.866	0.000*

*Correlation is significant at the level of ($p \leq 0.05$), (r): Pearson correlation.

Thyroid hormones exert a direct influence on insulin levels and secretion. This was statistically proven in this study by the pearson correlations found in **Table 4**. The low levels of FI found in group B subjects of this study (**Table 1**) could also partially be explained by the low levels of thyroid hormones resulting from the conditions of SCH and hypothyroidism. A prior study found that hypothyroidism decreased insulin production via beta cells, but hyperthyroidism improved beta-cell reactivity to catecholamine or glucose due to increased beta-cell mass [40]. All of these changes happen as a consequence of alternations in thyroid hormones, which raise the likelihood of developing T2DM and can give rise to diabetic complications or aggravate diabetes symptoms [41].

Diabetes and thyroid illness are induced by endocrine malfunction, and they have been shown to have a reciprocal influence. Variations in thyroid hormone levels, even those within the normal range, might precipitate the onset of type 2 diabetes mellitus (T2DM), especially in patients with prediabetes. Thyroid abnormalities can aggravate type 2 diabetes, whereas diabetes can increase thyroid dysfunction. Insulin resistance has been shown to have a significant influence in both T2DM and thyroid dysfunction. Consequently, failure to diagnose low thyroid hormone levels in diabetes and insulin resistance in both illnesses can lead to poor patient care [41].

The bulk of past research has linked hypo/SCH to type 2 diabetes. One meta-analysis research revealed support for a long-term connection between hypothyroidism and FT_4 in the reference range and the risk of T2DM, but not for hyperthyroidism or TSH in the reference range [42]. Another study of a number of research indicated that subclinical hypothyroidism or hypothyroidism is the most prevalent kind of TD in diabetes individuals [12,43-47]. However, none of the previous studies have established whether thyroid dysfunction is causative factor or a consequence of DM, in other words none of the studies answered the important question of which condition leads to which.

The results of the present study, however, suggest that thyroid dysfunction represented by subclinical hypothyroidism which later developed into clinical hypothyroidism in about half of the study subjects, is responsible for the insulin resistance and low insulin levels found in the subjects which ultimately led to the development of the conditions of prediabetes and T2DM in previously non-diabetic subjects. However, this does not rule out that DM could also impact thyroid hormones and possibly lead to TD, but we can safely suggest with confidence that in the sample of Iraqi subjects involved in this study, untreated TD represented by subclinical and clinical hypothyroidism was responsible for the development of new-onset insulin resistance, prediabetes and T2DM. Our findings prove that thyroid dysfunction is a serious risk factor for the future development of T2DM.

To support these findings, we performed molecular docking methods to investigate the direct interaction of T_4 and T_3 hormones with the insulin receptor. Our initial investigations into the molecular interactions between the novel ligands (T_3 and T_4) and the insulin receptor tyrosine kinase (PDB ID: 1144) unveiled distinct and informative binding characteristics. Utilizing 2D interaction diagrams and insightful 3D binding pose visualizations (as depicted in **Figures 1** and **2**), our analyses elucidated demonstrably different binding mechanisms by which these ligands stabilized the ligand-receptor complexes. Notably, Ligand T_4 exhibited a robust binding mode, characterized by a calculated binding free energy (ΔG) of -7.11 kcal/mol. T_4 achieved stable anchorage within the active site by orchestrating a network of strong hydrogen bonds. Specifically, we observed key hydrogen bonds with the side chain amide of GLN 20, at a distance of 3.68 Å, and the backbone carbonyl of LEU 18, at 3.14 Å. Furthermore, T_4 engaged in a weaker, yet discernible, hydrogen bond interaction with ASN 153, measured at 4.27 Å. This multifaceted hydrogen bonding network underscored a well-defined and energetically favorable binding orientation for T_4 within the receptor pocket.

Conversely, Ligand T_3, while exhibiting comparable binding free energy ($\Delta G = –7.12$ kcal/mol), adopted a noticeably distinct binding strategy. Our analysis revealed that T_3 secured its position primarily through a triad of hydrogen bonds. These interactions involved the side chain amid MET 95 (3.68 Å), the backbone carbonyl of MET 155 (3.26 Å), and the backbone carbonyl of GLY 165 (3.24 Å). Intriguingly, T_3 also demonstrated an additional stabilizing feature not observed in T_4's binding mode: a π-hydrogen interaction with the side chain of VAL 26 (4.24 Å). This

unique π-hydrogen interaction suggests an alternative and potentially significant contribution to T_3's binding affinity, representing a clear divergence from the interaction profile of T_4 (refer to **Table 7**, **Figures 1** and **2** for comprehensive details).

To quantitatively assess the thermodynamic stability of these interactions, we employed the GBVI/WSA dG scoring function, the outcomes of which are summarized in **Table 7**. Interestingly, while T_3 presented with a marginally superior binding affinity (ΔG = –7.12 kcal/mol) and also displayed lower conformational variability, as indicated by a lower RMSD of 1.77 Å, Ligand T_4 paradoxically demonstrated a superior total interaction energy (E = –40.61 kcal/mol vs. –36.09 kcal/mol for T_3). This apparent discrepancy suggests that T_4 achieves enhanced overall stabilization through the accumulation of numerous weaker, yet collectively significant, interactions. This occurs despite its slightly higher RMSD value (3.83 Å), potentially indicative of greater conformational flexibility in its binding mode.

Subsequent cluster analysis, performed on the 20 generated poses per ligand, brought to light a dominant binding mode for T_4, encompassing a notable 75 % of the sampled population. This pronounced conformational convergence, coupled with T_4's robust overall interaction energy, strongly positions T_4 as a particularly promising scaffold for further optimization efforts. This is noteworthy even considering its higher RMSD, which in this context may be interpreted not as instability, but rather as an indication of inherent flexibility or the potential to explore alternative, yet still productive, binding modes.

In the latter phase of our study, we turned our attention to the protein-protein docking of the native insulin chains (A and B) with the insulin receptor tyrosine kinase (PDB ID: 1144). This analysis revealed high-affinity interactions of profound importance to the physiological function of this receptor system, as visually represented in **Figures 3** and **4**. Our findings distinctly demonstrated that Insulin chain B exhibits superior binding characteristics, achieving an impressive interaction energy of –54.62 kcal/mol (as detailed in **Table 7**). This exceptionally robust stabilization, as anticipated, is driven by a complex interplay of hydrogen bonds and hydrophobic interactions operating at the interface between insulin and its receptor, highlighting the critical nature of these interactions for biological function.

Table 7 Energy score for each ligand against the target protein.

Compound	Protein	Ligand	Receptor	Interaction	Distance (A°)	E (Kcal/Mol)	S (energy score)	RMSD
T3	1I44	I 2	O MET 95 (A)	H-donor	3.68	–1.2	–7.1234469	1.7741928
		N 9	SD MET 155 (A)	H-donor	3.26	–1.6		
		N 9	O GLY165 (A)	H-donor	3.24	–3.5		
		6-ring	CG1 VAL 26 (A)	pi-H	4.24	–0.7		
T4		I 2	O GLN 20 (A)	H-donor	3.68	–0.6	–7.1134357	3.8325155
		I 3	OD1 ASN 153 (A)	H-donor	4.27	–0.7		
		N 8	O LEU 18 (A)	H-donor	3.14	–5.7		
Insulin A							–51.401295	1.3886175
Insulin B							–54.620449	0.52960116

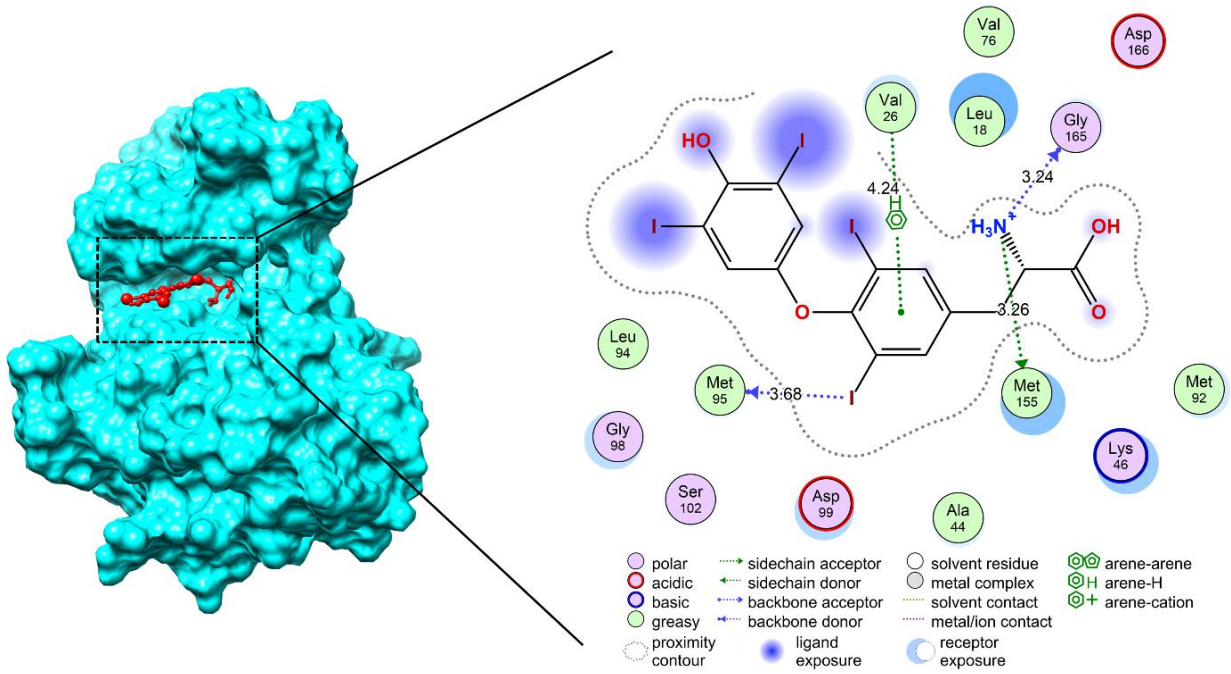

Figure 1 Binding pose and interaction profile of ligand T_3 with the insulin receptor tyrosine kinase (PDB ID: 1I44).

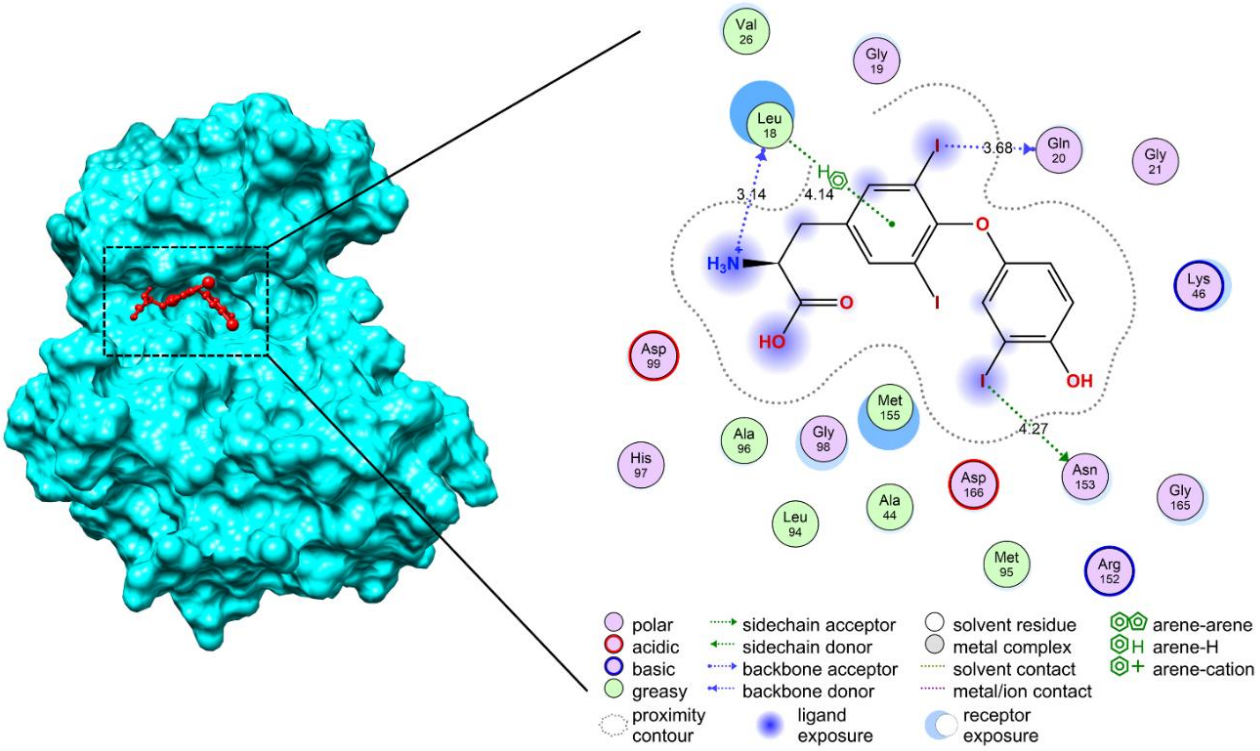

Figure 2 Binding pose and interaction profile of ligand T_4 with the insulin receptor tyrosine kinase (PDB ID: 1I44).

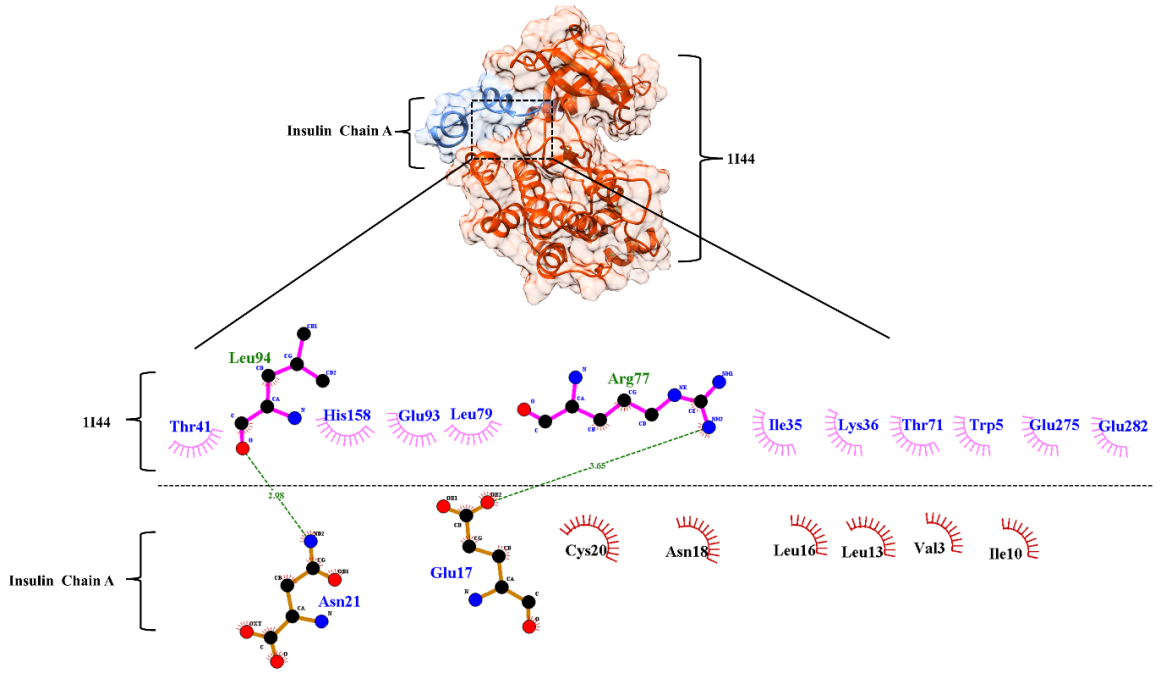

Figure 3 Binding pose and interaction profile of insulin chain A with the insulin receptor tyrosine kinase (PDB ID: 1I44).

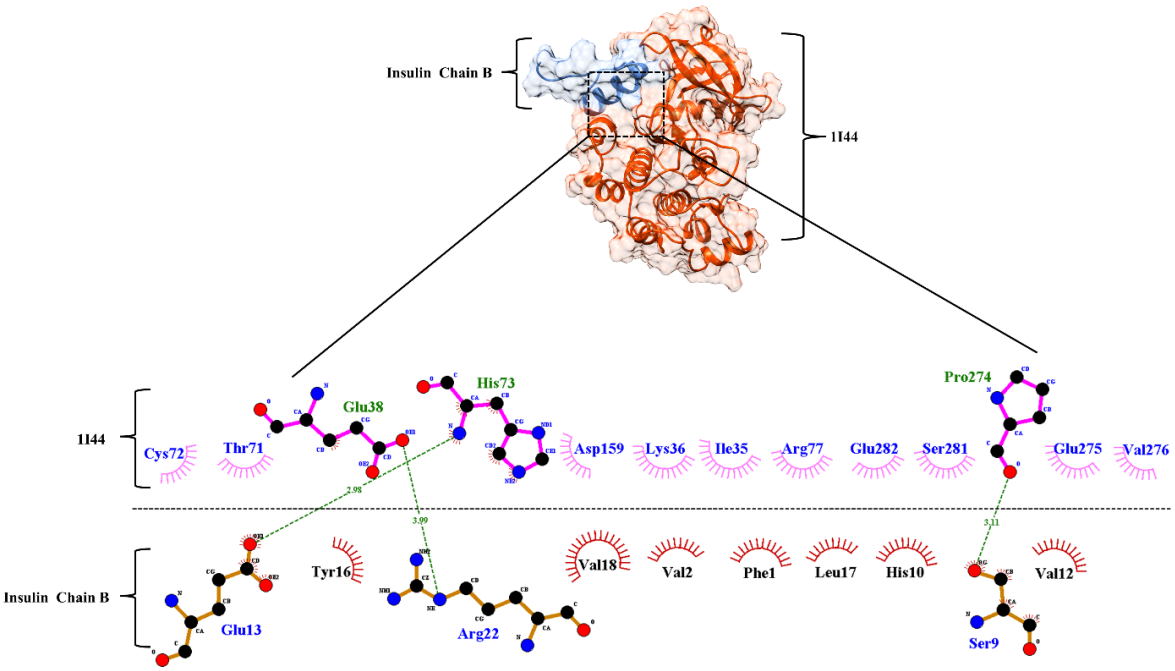

Figure 4 Binding pose and interaction profile of insulin chain B with the insulin receptor tyrosine kinase (PDB ID: 1I44).

Molecular docking of ligands T_3 and T_4 with the insulin receptor tyrosine kinase (PDB ID: 1I44) indicated comparable binding affinity based on similar overall energy scores (S scores around –7.12, **Table 7**). Despite this affinity parity, Ligand T_4 exhibits greater binding stability due to its distinct interaction profile. While both ligands display different conformational deviations (RMSD T_3: 1.77 Å, T_4: 3.83 Å), T_4's higher RMSD is interpreted as indicative of beneficial conformational flexibility rather than instability. T_3's binding is characterized by hydrogen bonds with MET 95, MET 155, GLY 165, and a pi-H interaction with VAL 26 (**Figure 1**, **Table 7**). Conversely, T_4 engages through hydrogen bonds with GLN 20, ASN 153, and LEU 18 (**Figure 2**, **Table 7**). These divergent binding modes, visualized in **Figures 1** and **2**, coupled with the

interpretation of T_4's RMSD as flexibility, suggest that although both ligands bind with similar affinity, T_4 achieves a more stable and adaptable interaction with the receptor.

The implications of these interactions in the context of diabetes development are particularly noteworthy. Thyroid hormones, especially T_3 and T_4, are known to influence glucose metabolism, and their potential interaction with the insulin receptor suggests a possible role in modulating insulin signaling. The practical findings of the present study that sub-clinical and clinical hypothyroidism contribute to insulin resistance, prediabetes and diabetes align with these computational results. Reduced levels of thyroid hormones have been linked to impaired insulin receptor signaling, contributing to diminished glucose uptake and increased insulin resistance [48].

If T_3 and T_4 can bind to the insulin receptor in a manner similar to insulin, they may act as modulators, either enhancing or disrupting normal receptor function [49]. Given that insulin binding exhibited a significantly stronger interaction energy (–54.62 kcal/mol for chain B), it is unlikely that T_3 or T_4 could fully mimic insulin's role [50]. However, their binding affinities suggest they might serve as allosteric modulators or partial agonists rather than direct antagonists. Studies have reported that thyroid hormones can enhance insulin receptor expression and activation, thereby improving insulin sensitivity in euthyroid states, but their deficiency may contribute to insulin resistance [51,52].

Recent research has highlighted the influence of thyroid hormone signaling on the PI3K/Akt pathway, which is critical for glucose homeostasis and insulin action. Hypothyroidism has been linked to reduced activation of this pathway, contributing to impaired glucose metabolism and increased insulin resistance [53]. This aligns with the findings of our study which showed increased insulin resistance in hypothyroid patients, reinforcing the hypothesis that T_3 and T_4 may function as modulators of insulin receptor signaling.

To the best of our knowledge this study represents the 1st attempt to investigate the direct binding interaction of T_3 and T_4 hormones with the insulin receptor tyrosine kinase using molecular docking methodologies. While some studies have suggested crosstalk between thyroid hormones and insulin signaling pathways [48], our results provide computational evidence supporting the hypothesis that T_3 and T_4 could modulate insulin receptor activity. Further experimental validation, including in vitro binding assays and functional studies, is required to confirm these computational predictions.

Given the limited prior research in this area, these findings offer novel insights into the interplay between thyroid function and insulin receptor activity. Future studies should incorporate molecular dynamics simulations and experimental validation to assess ligand stability over time and explore potential allosteric effects that could enhance receptor modulation.

Conclusions

About half of the untreated SCH cases developed into clinical hypothyroidism. SCH and clinical hypothyroidism led to the development of new-onset insulin resistance, prediabetes and T2DM in non-diabetic subjects, proving that TD is a causative factor of DM and a serious risk factor for the future development of T2DM. The docking result investigating the molecular binding dynamics of T_3 and T_4 with the insulin receptor tyrosine kinase, revealed that T_3 and T_4 have comparable yet distinct binding strategies. The interaction of T_3 and T_4 with the insulin receptor suggests that these thyroid hormones may act as modulators rather than direct agonists or antagonists, potentially influencing insulin signaling and glucose metabolism. These computational findings strongly correlate with the observations that hypothyroidism contributes to insulin resistance, prediabetes, and diabetes, suggesting that thyroid dysfunction plays a significant role in metabolic disorders.

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Declaration of Generative AI in Scientific Writing

No AI software or tools were used in the generation or writing of this research manuscript.

CRediT author statement

Mustafa Kahtan Al-Bayaty contributed ti the conceptualization and design of the study, data analysis,

and writing of the original draft. Mohammed Shamil Ali was responsible for data collection, curation, and contributed to the writing—review and editing. Alabbas Abdulkareem Majeed assisted with methodology development and formal analysis. Ragheed Hussam Yousif was responsible for the molecular docking study and analysis part.

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