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Alterations in thyroid hormone levels in diabetic patients and their contributions to diabetes-related atherosclerosis

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ABSTRACT

Background

Thyroid hormone levels often fluctuate in patients with type-2 diabetes mellitus (DM). The clinical implications of this fluctuation remain unknown. Previous research has shown that small changes in thyroid hormone levels increase the risk of DM-related complications such as atherosclerosis. Because DM-related atherosclerosis is mediated by oxidative damage, we hypothesized that thyroid hormone levels affect the anti-oxidative functions of high-density lipoprotein (HDL) in DM patients. This alteration in the anti-oxidative properties of HDL is reflected as DM-related atherosclerosis.

Methods

We conducted a case-control study that comprised of fifty DM patients with no prior history of thyroid abnormalities and fifty age-, sex-matched healthy controls. Thyroid hormone levels (free-T3, free-T4) and thyroid stimulating hormone (TSH) levels were evaluated in all participants using enzyme-linked immunosorbent assay. The antioxidant properties of HDL were assessed by measuring the levels of serum paraoxonase-1 (PON-1).

Results

DM patients had significantly higher free-T4 (FT4) compared to healthy controls. Free-T3 (FT3) levels were significantly reduced in DM patients than controls. There were no group-differences in TSH levels. PON-1 levels were significantly reduced in DM patients compared to healthy controls. In addition, PON-1 levels increased with increases in FT4 levels in healthy controls. No such changes were observed in DM patients.

Conclusion

We found that thyroid hormone levels are affected in DM patients. Furthermore, thyroid hormone levels predicted alterations in the anti-oxidative properties of HDL. These findings implicate the importance of thyroid hormone fluctuation in DM patients and their potential contribution to DM-related atherosclerosis by reducing the anti-oxidative properties of HDL. Furthermore, this study highlights the importance to carefully manage thyroid hormone levels in patients with DM.

Keywords: Atherosclerosis, thyroid hormones, DM, HDL

INTRODUCTION

Diabetes mellitus (DM) features a heterogeneous group of glucose metabolic defects resulting in abnormal insulin secretion and/or function that are clinically seen as hyperglycemia [1]. DM infamously falls among the top 10 causes of death in adults worldwide and is estimated that approximately 4 million individuals die from DM-related complications (e.g., coronary artery disease, chronic kidney disease) every year. In addition, health-care costs related to DM and its complications are

alarming. In 2017, global health expenditure on diabetes was estimated to be approximately 727 billion dollars [2]. Such extensive costs are often attributed to investigating and treating DM-related complications [3]. Despite these profound detrimental effects stemming from DM-related complications, its precise pathophysiology is yet to be determined.

One pathologic mechanism that underlies DM-related complications is altered high-density lipoprotein (HDL) function [4]. In healthy individuals, HDL has protective effects against atherosclerosis through its antioxidative and anti-inflammatory properties, and its ability to promote cholesterol metabolism by transporting it back to the liver from the peripheral tissues [5].

Because much of the DM-related complications result from atherosclerosis it is postulated that HDL alterations lead to such complications. For instance, Quebac Cardiovascular study noted that the incidence of cardiovascular disorders, secondary to DM-related atherosclerosis, increased by 13% for every 10% reduction in HDL [6]. Altered HDL function without a change in levels can also lead to DM-related complications. Farbstein and colleagues found that the HDL antioxidative function was altered in patients with DM compared to healthy controls (HCs), but the HDL levels were similar between the two groups [7]. Together these studies suggest that altered HDL function might contribute to DM-related complications.

Thyroid hormone abnormalities are often observed in patients with DM [8]. Schroner and colleagues found the prevalence of subclinical and overt hypothyroidism was almost 24% in patients with DM [9]. Epidemiological studies have demonstrated increased cardiovascular events and mortality in individuals with thyroid hormone fluctuations [9]. Slight changes in thyroid hormone levels in healthy individuals with normal thyroid function are associated with atherosclerotic changes in blood vessels, peripheral fat deposition, and alterations in lipid profile [9]. The effects of thyroid hormone fluctuations on DM-related atherosclerosis remain unknown.

In this study, we hypothesized that thyroid hormone level fluctuations in patients with DM contributes to atherosclerosis by altering HDL antioxidative properties. We assessed thyroid hormone levels in patients with DM, with no coexisting thyroid disorders, and healthy controls, and quantified the antioxidative function of HDL by measuring paraoxonase-1 (PON-1) activity.

We found that thyroid hormone levels were significantly altered in patients with DM compared to HC and the thyroid hormone-antioxidative function relationship was altered in DM compared to HC. Together, these data suggest that thyroid hormone fluctuation in DM alters HDL antioxidative function, contributes to atherosclerosis, and thereby resulting in cardiovascular complications.

MATERIALS AND METHODS

Participants

Fifty patients with DM were recruited as referrals from diabetology clinic at Kilpauk Medical College and Hospital. Fifty age- and sex-matched healthy controls (HC) were recruited from Health-Checkup Clinic at Kilpauk Medical College and Hospital. All HCs were screened for undiagnosed DM using random blood glucose (RBG). Those with RBG of < 200mg/dl, had no classic symptoms of hyperglycemia, and were not on any hypoglycemic medications were included as HCs. DM inclusion criteria included (1) male or female patients between the age of 30 and 80 irrespective of blood pressure status, with (2) a confirmed diagnosis of type-2 DM, who previously had fasting plasma glucose levels (FPG) of more than 126 mg/dL at more than two subsequent occasions and (3) were receiving oral hypoglycemic agents. Exclusion criteria included (1) patients with known thyroid disorders, (2) pregnant or nursing mother, (3) history of cardiopulmonary or renal illness.

Data Collection

The study was approved by Kilpauk Medical College Institutional Review Board. Informed consent was obtained from all patients. Demographic data and brief medical history were obtained from all participants. Using a sterile technique, 5 ml of fasting venous blood was collected in a tube through venipuncture of the antecubital vein.

Biochemical analysis

Samples were centrifuged within 1 hour and serum was transferred to separate labeled vials and they were transferred to −80° freezers.

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Plasma glucose (hexokinase method) was measured on a Hitachi 912 autoanalyzer (Hitachi, Mannheim, Germany) using kits supplied by Roche Diagnostics (Mannheim). Serum high-density lipoprotein cholesterol (direct method; polyethylene glycol-pretreated enzymes) were measured using the Hitachi 912 autoanalyzer.

The levels of thyroid stimulating hormone (TSH), free thyroxine (FT4), and free triiodothyronine (FT3) were measured by chemiiluminesence microparticle Immunoassay (CMIA) on Abbott Architect fully automated immunoassay analyzer. AXSYM System (Abbott Laboratories, Abbott Park, USA) PON1 was estimated spectrophotometrically by the method described elsewhere with modifications10. Briefly, the assay mixture consists of 500 µl of 2.2 mmol/l paraoxon substrate in 0.1 mol/l Tris-HCl buffer, pH 8.0 containing 2 mmol/l CaCl2 and 50 µl of fresh serum. After mixing the contents, kinetic measurements were taken immediately at every minute for five minutes, at 405 nm at 25°C. First absorbance reading is taken as 0-minute reading and subsequent absorbance readings were taken as one-minute to four-minute readings. Corrected absorbance readings were obtained by subtracting 1 minute reading with 0 minute reading, likewise, the latter minute reading was subtracted with the previous minute readings. The mean absorbance was calculated. Mean absorbance was used to determine PON1 activity, and standard graph plotted using 1 mM *P*-Nitrophenol. PON 1 activity was expressed in international units (IU). One IU was defined as 1 µmol of p-nitrophenol formed/min/L at 25°C.

Statistical analysis

All analyses were performed using SPSS (version 24.0). Independent-sample *t*-tests were performed to assess mean differences between the 2 groups, HCs and DM. For all tests, there were no outliers, as assessed by a boxplot. The data were normally distributed, as assessed by Shapiro-Wilk's test of normality. There were homogeneities of variance, as assessed by Levene's test. The relationship between paraoxonase-1 and HDL were assessed with Pearson correlational analysis.

RESULTS

The average age of the DM cohort was 50.98 (12) years, and the average age of HC cohort was 50.78 (12) years with no significant difference between the two groups. DM group had 58% males and HC group had 62% males. As expected, patients with DM had a significantly higher random blood glucose level compared to HC, *t(49)=8.094, p<0.0001*, Fig. 1.

To test the hypothesis of whether patients with DM had higher atherosclerosis than HC, we sought to assess group-differences in mean arterial pressure. Patients with more atherosclerotic disease will be expected to have stiffer arteries and thus, higher blood pressure. We observed the patients with DM (M=93.0933, SD=6.6812) had significantly higher mean arterial pressure compared to HC (M=90.0933, SD=6.6147), *t(49)=2.2914, p=0.0262*, Fig. 2. This result suggests that DM patients have higher degree of atherosclerosis compared to HCs.

Next, we sought to assess thyroid hormone alterations in patients with DM. Whereas Free-T3 (FT3) levels were significantly lower in DM (M=3.5364, SD=1.2918) compared to HC (M=3.964, SD=0.6124), *t(49)=2.1445, p=0.0369*, Fig. 3a. Free-T4 (FT4) was significantly higher in DM (M=1.4894, SD=0.1614) compared to HC (M=1.3728, SD=0.1727), *t(49)=3.604*,

p=0.007, Fig. 3b, suggesting that thyroid hormone alterations co-exist in patients with DM. We found no significant group-mean differences in TSH (*p>0.05;* Fig. 3c).

Finally, we sought to assess whether thyroid hormone alterations influenced the antioxidative properties of HDL in patients with DM. We observed no significant differences in HDL levels or PON-1 activity between DM and HC (*p>0.05;* Fig. 4). However, while plotting the relationship between FT4 and PON-1 activity, as shown in Fig. 5a, we found that PON-1 increases with increase in FT4 for HC but no such relationships were seen for HC. This relationship was tested statistically with Pearson's correlations. While there was a significant positive correlation between FT4 and PON-1 for HCs (r=0.4525, p<0.05), there was none for DM (r=1325, p>0.05). Similarly, there was a significant negative correlation between TSH and PON-1 for HCs (r=0.2915, p<0.05; Fig. 5b), there was none for DM (r=0.0374, p>0.05). Together, these results suggest that thyroid hormone-PON-1 relationship in HC is altered in patients with DM.

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DISCUSSION

In the present study, we sought to assess whether thyroid hormone alterations in patients with DM contribute to atherosclerosis. We found that patients with DM exhibited thyroid hormone abnormalities, increased atherosclerosis, and altered relationship between thyroid hormone abnormalities and HDL antioxidative function. These findings suggests that abnormal thyroid hormone levels in DM might promote atherosclerosis by altering antioxidating function of HDL highlighting the importance of closely monitoring and treating thyroid hormone changes in DM patients.

There is a complex interaction between thyroid disorders and diabetes. Previous studies have shown increased incidence of abnormal thyroid hormone levels in diabetic populations [11-15]. For instance, Moghetti and colleagues found that 89% of patients with DM had hypothyroidism and 11% had hyperthyroidism [16]. This observation of high hypothyroidism prevalence among DM patients was also seen independently by other groups [11, 15]. This altered thyroid function in DM patients may be due to alterations in thyrotropin-releasing hormone (TRH) synthesis, TRH release, or reduced peripheral conversion of T4 to T3 [17].

DM also features thyroid hormone fluctuations without overt hyper/hypothyroidism (i.e., euthyroid DM patients). This observation is supported by previous studies that show altered serum T3, TSH, and TSH response to TRH are altered in euthyroid patients with DM [18, 19]. These alterations are prominent in poorly controlled diabetics [18, 19]. We found that patients with DM had lower serum FT3 levels but higher FT4 levels compared to HCs. These results are consistent with those reported by Schlienger JL and colleagues, and reflects impairment in the enzyme 5' monodeiodinase that control peripheral conversion of T4 into T3 [18]. Another plausible mechanism for our observation is that TRH synthesis decreases in DM resulting in reductions in nocturnal TSH increases leading to altered thyroid hormone levels in DM patients [20]. Future studies are required investigating circadian thyroid hormone changes in DM patients.

The mean serum FT4 level in our study was higher in the DM population compared to HC. This result is consistent with the study Udiong CEJ and colleagues that showed higher T4 and lower TSH in patients with DM compared to patients without DM [15]. We found group equivalence in TSH between DM and HCs. One such mechanism for this observation is that altered thyroid hormone levels might be influenced by oral hypoglycemic drugs taken by patients with DM. For example, previous studies have demonstrated that insulin, an anabolic hormone, enhances the levels of FT4 while it suppresses the levels of T3 by inhibiting the hepatic conversion of T4 to T3 [15]. On the other hand, certain oral hypoglycemic agents are also known to suppress the levels of FT4 while causing raised levels of TSH [15]. Another mechanism reported by Suzuki and colleagues is linked to the presence of Thyroid Hormone Binding Inhibitor (THBI), an inhibitor of extrathyroidal conversion enzyme of T4 to T3, and dysfunction of the hypothalamus-hypophyseal thyroid-axis [21].

In healthy individuals, HDL cholesterol feature four key properties: (1) cholesterol efflux and reverse cholesterol transport, (2) anti-oxidative capability, (3) anti-inflammatory ability, and (4) the ability to increase vascular nitric oxide production resulting in vasodilation [5]. Because DM-related atherosclerosis is mediated by oxidative damage, in this study we sought to focus on the anti-oxidative function of HDL [22]. Many apolipoproteins and enzymes have been integral for the antioxidant function of HDL [23]. One such enzyme is paraoxonase-1 (PON-1) that we used to assess the anti-oxidative function of HDL.

In our study, there was no correlation between FT3 and the PON-1 activity in both patients with DM and healthy controls. In healthy controls, the FT4 positively correlated with the PON-1 activity and the TSH negatively correlated with the PON-1 activity whereas this correlation was absent in DM patients. The lack of correlation in patients with DM might be due to the altered thyroid hormone level which in turn impairs HDL function. This impaired HDL function increases the risk of DM-related atherosclerosis as seen as increased MAP in DM compared to HCs.

The lack of correlation can also be partially explained by alterations in cholesterol ester transfer protein (CETP), a protein that transfers cholesterol from HDL to very-low density lipoprotein (VLDL) in DM

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[24]. For instance, in a study by Tan and colleagues, they showed increases CETP activity with increases in FT4 in DM patients [25]. This enhanced CETP activity in DM might lead to enrichment of HDL with triglycerides and reduce the cholesterol content in HDL [26, 27]. This alteration of HDL particle lipid composition results in apoprotein A-I (apoA-I) destabilization and shedding from HDL [28, 29]. This lack of apoA-I may contribute to impaired PON-1 activity and thereby contributing to decreased HDL antioxidative function in patients with DM [30].

In conclusion, in this study we have shown that thyroid hormone alterations are consequential in patients with DM albeit thyroid levels being within normal limits. Such alterations affect HDL anti-oxidative function thereby contributing to atherosclerosis in DM patients. Therefore, monitoring and treating thyroid hormone levels might be crucial in DM patients to prevent atherosclerotic complications.

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FIGURE LEGENDS:

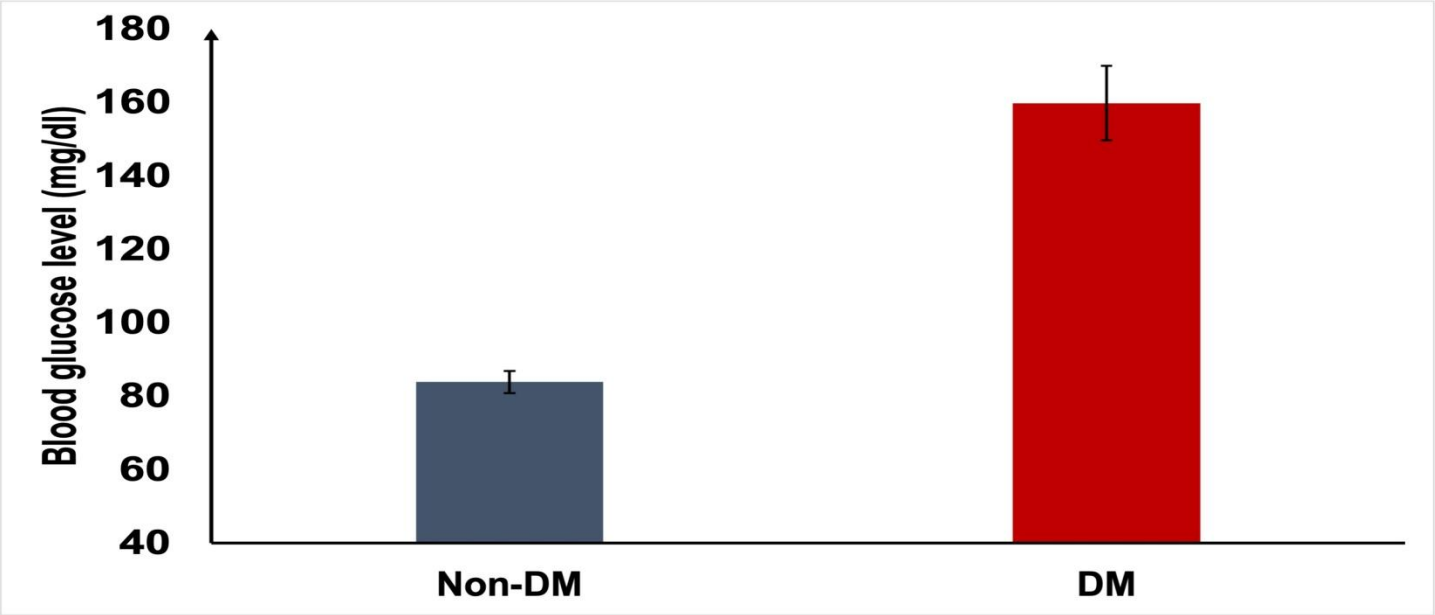

Fig. 1: Bar graph represents mean random blood glucose levels for diabetes mellitus (DM; red) and healthy controls (HC; blue)

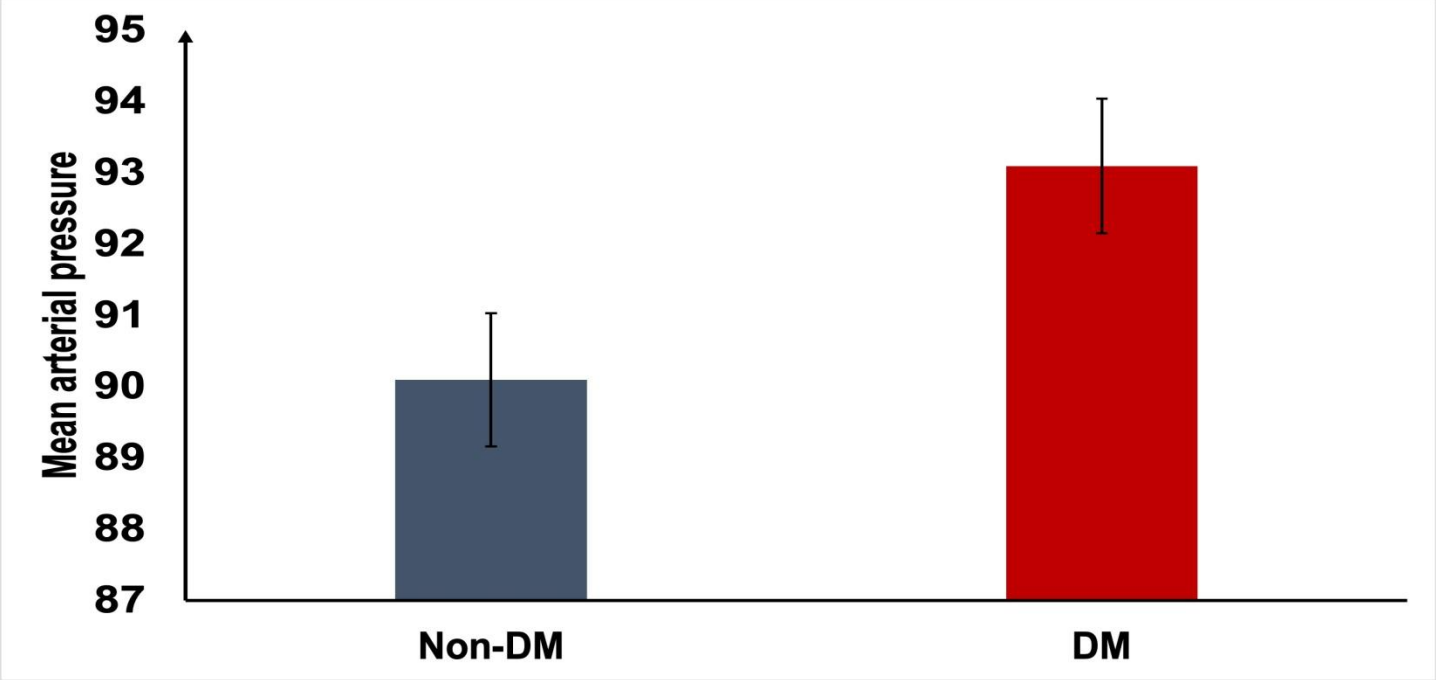

Fig. 2: Bar graph represents average mean arterial pressure for diabetes mellitus (DM; red) and healthy controls (HC; blue)

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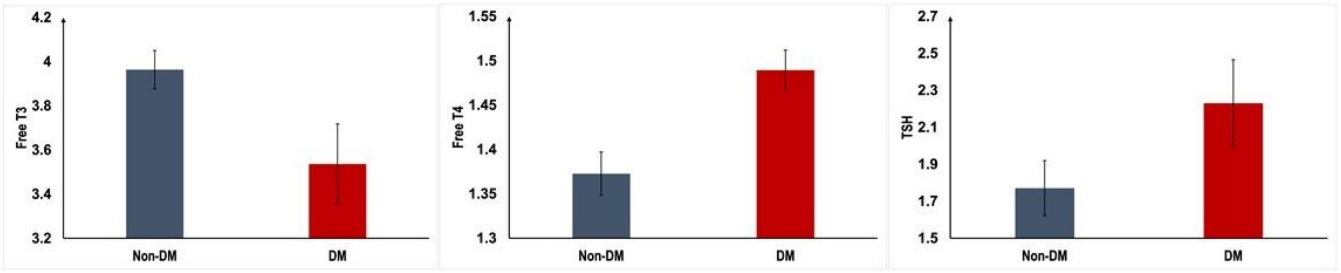

Fig. 3a Fig. 3b Fig. 3c

Fig. 3a: Bar graph represents mean Free-T3 levels for diabetes mellitus (DM; red) and healthy controls (HC; blue)

Fig. 3b: Bar graph represents mean Free-T4 levels for diabetes mellitus (DM; red) and healthy controls (HC; blue)

Fig. 3c: Bar graph represents mean TSH levels for diabetes mellitus (DM; red) and healthy controls (HC; blue)

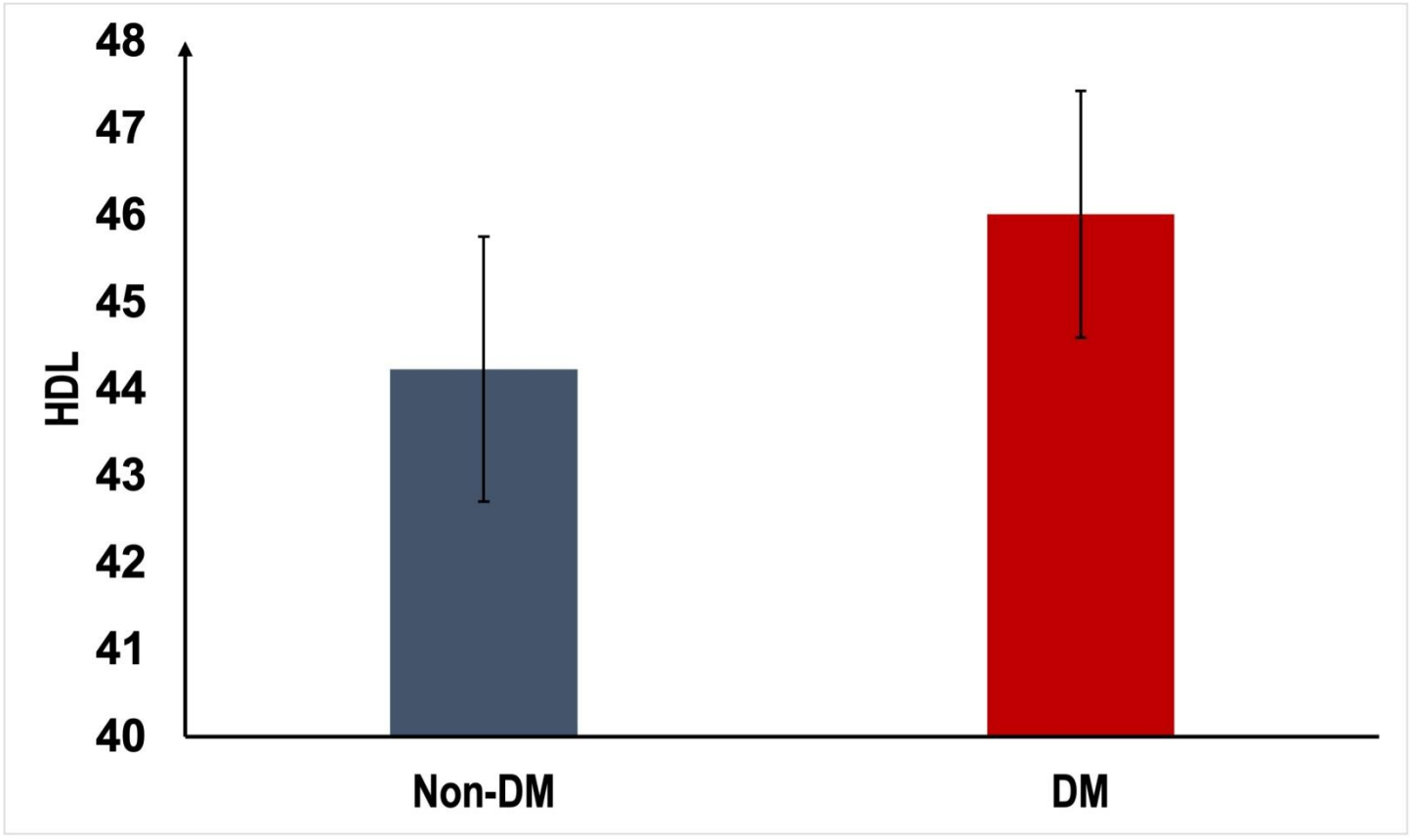

Fig. 4: Bar graph represents mean HDL levels for diabetes mellitus (DM; red) and healthy controls (HC; blue)

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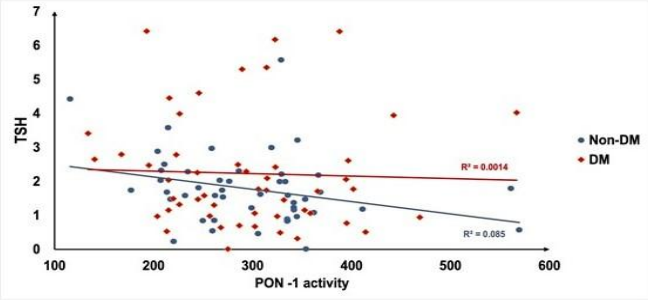

Fig. 5a

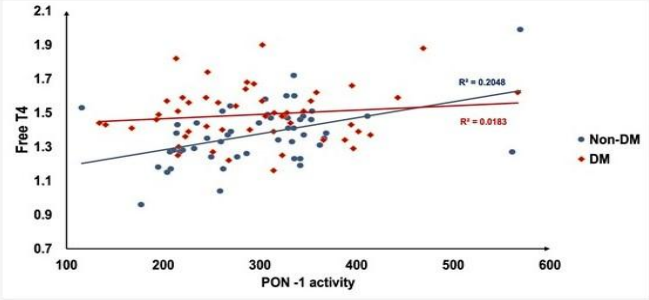

Fig. 5b

Fig. 5a: Linear regression plot showing associations between PON-1 activity and Free-T4 for DM (red) and HCs (blue)

Fig. 5b: Linear regression plot showing associations between PON-1 activity and TSH for DM (red) and HCs (blue)

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