

Diabetogenic Effects of *Parthinium hystrophorous* Induced Allergic Rhinitis

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Abstract: Diabetes mellitus (DM) is known to be associated with the cytokines secreted by Th1 cells, while allergic rhinitis (AR) is mainly regulated by Th2 cytokines. According to Th1/Th2 paradigm, there is an inverse relationship between Th1 and Th2 cytokines and resultantly, both the aforesaid diseases are also inversely correlated. On the other hand, numbers of clinical reports suggest every possible correlation between DM and AR including positive, negative and neutral. However, till to date, no experimental report available, suggesting the changes in glucose homeostasis of animal model(s) of allergic rhinitis, if any. Therefore, in the present study we have observed the changes in glucose homeostasis of the animals bearing AR induced by *Parthinium hystrophorous* (PH).

The condition of AR was induced by intranasal installations of the acetone extract of PH. At the end of experimentation, various parameters for AR and DM were evaluated.

A significant increase was observed in total leukocytes in nasal fluid, serum glucose, thyroxine, dyslipidemia and activity of α -amylase, pancreatic lipid peroxidation, serum and pancreatic nitrite with a concomitant reduction in serum calcium, triiodothyronine, hepatic glycogen and activity of phosphoglucomutase. However, serum insulin, TSH, pancreatic calcium and hepatic glucokinase increased non-significantly. Immune cells infiltration and increased intra alveolar space were observed in lungs tissue, while alterations were also observed in pancreas of AR treated animals.

The induction of AR led to the diabetogenic changes to rats *via* exerting multifaceted metabolic defects in the biochemical machinery regulating glucose homeostasis.

Keywords: Allergic rhinitis, diabetes mellitus, glucokinase, phosphoglucomutase.

INTRODUCTION

Allergic rhinitis (AR) is a clinical condition associated with various features such as congestion, nasal discharge, palatal itch, sneezing, running nose, swelling in nasal mucosa, respiratory problem etc. [1]. However, it is biochemically associated with the increased production of Th2 cytokines and the infiltration of immune cells in the nasal secretion, nasal mucosa and respiratory tract [1-3].

Number of reports suggesting an inverse relation between type 1 diabetes mellitus (DM) and atopic diseases including AR, asthma and eczema [4-7]. Because it is being hypothesized that Th1 and Th2 cells reciprocally counteract each other and therefore, it has been speculated earlier that the prevalence of Th2-mediated diseases is lower in patients suffering with Th1-mediated disease [4, 8, 9]. On the other hand, many recent studies suggest either no association or positive association of AR with DM [8-11]. In fact, AR is known to increase the risk of atherosclerosis, which is also known to induce diabetes mellitus [12-14]. It has also been suggested that the traditional Th1/Th2 theory is oversimplification of the complexity of immune response and therefore, cannot explain various contradictory clinical

outcomes [8]. Moreover, Th1/Th2 theory in context to diabetes and allergic rhinitis suggest the inverse relation between type-1DM and AR, but does not explain the relation between type-2 DM and AR.

Interestingly, no experimental study being reported describing the changes in the glucose homeostasis during allergic rhinitis induced by allergen(s). As there is no experimental report available addressing changes in glucose homeostasis during AR, the present study is an attempt towards revealing the biochemical changes in *Parthinium hystrophorous* extract (PH) induced allergic rhinitis in rats. It would be imperative to mention that in India, parthinium grown abundantly and cause various allergic responses to human population such as asthma, hay fever and dermatitis etc. [15, 16]. In fact, the Indian varieties contain more amount of a sesquiterpene lactone; parthenin, which is mainly responsible for allergic responses [17, 18].

The major parameters considered in this study are total leukocytes count in nasal discharge, histological analysis of lung tissue to establish allergic rhinitis. To understand the diabetogenic changes, if any, we have considered serum glucose, insulin, thyroid hormones (T_3 & T_4), thyroid stimulating hormone (TSH), lipid profile including total cholesterol, triglycerides, LDL-C, VLDL-C and HDL-C, activity of α -amylase and hepatic glucokinase and glucose-6-phosphatase; serum and pancreatic calcium, nitrite and lipid peroxidation.

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MATERIALS AND METHODS

Chemicals

All the chemicals and enzymes were purchased from Sigma-aldrich Pvt. Ltd., USA except 2-thiobarbituric acid, which was purchased from Hi-Media Laboratories, Pvt. Ltd., Mumbai. Kits for the estimation of glucose, lipid profile, calcium, α -amylase activity were purchased from Span diagnostics Pvt. Ltd., Surat, India, while ELISA kit for the estimation of insulin, T₃, T₄ and TSH were purchased from Erba diagnostics, Mannheim, Germany.

Preparation of Pollen Extract of *Parthenium Hysterophorus* (PH)

The plants were collected from the nearby local area. The plants were shadowed dried for three days and then the pollens were collected selectively. The collected pollens were grinded and the powder was used to prepare the acetone extract.

Briefly, the 50 gm of powder was dissolved into the 500 ml of absolute acetone and kept on stirring for 4 hrs at room temperature. After the incubation of stirring process, the supernatant was centrifuged at 5000 rpm for 5 min and filtered to exclude gritty particles, if any. The extract was dried overnight by simply evaporating the solvent. The dose of 250 mg of dried extract was dissolved in the 1 ml of acetone and the 50 μ l was used for the nasal instillations in each nostril of the rats, as reported earlier for other pollen extracts [1, 19, 20].

Animals

Healthy colony bred Wistar albino male rats (*Ratus norvegicus*) (150 $\pm$ 10 g) were maintained in polypropylene cages (43 x 27 x 25 cm with floor area of 165.85 cm²/animal). Seven animals were placed in each cage and were maintained under constant temperature (23 $\pm$ 2^oC) and photo- schedule (14h light: 10h dark). They were provided with commercial rodent feed comprised of protein, 20-22%; oil, 3.5%; crude fiber, 4%; ash, 6%; calcium, 1%; phosphorous, 0.5%; lysine, 1.2% and methionine, 0.9% and metabolic energy 3000 KCal (Gold Mohur feeds Ltd, New Delhi, India) *ad libitum* and had free access to drinking water. Standard ethical guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA; registration No. 779), Ministry of Environment and Forests, Government of India and Institutional Ethical Committee for Handling and Maintenance for Experimental Animals were followed.

Experimental Design

Animals were divided into two groups of seven each, where group I (ctrl) served as control and administered nasal instillations of 50 μ l of acetone in each nostril for five consecutive days. While in group II, 50 μ l of the PH was administered in each nostril for five consecutive days. The animals received an intranasal dose at day 15 after the last administration to boost the immune reaction. The rats were challenged at day 22, 23, 24, 25 and 26 for five consecutive days with the same solution. After the 24 hrs of last

administration, on day 27, the animals were sacrificed by cervical dislocation followed by decapitation under the influence of mild chloroform anesthesia [1].

Nasal Discharge, Serum and Tissue Preparation

Nasal discharge was collected by washing the nostrils with 1 ml of PBS in both the groups of animals. Blood from each animal was collected and serum was isolated for the estimation of different biochemical and hormonal parameters. After exsanguinations liver, pancreas and lung tissues were removed quickly, freed from blood clots, weighed and processed for the estimation of protein, hepatic gluco-regulatory enzymes and histological processing.

Biochemical Studies

Estimations of serum glucose, total cholesterol, HDL-C, triglycerides, LDL-C and α -amylase activity were measured using diagnostic kits by following the instructions provided by the manufacturer. However, concentration of VLDL-C was calculated using the formula provided by Friedwald *et al.* [21]. Total hepatic glycogen contents were estimated using anthrone reagent according to the procedure of Carroll *et al.* [22]. The pancreatic lipid peroxidation (LPO) was estimated following the routine protocol of our laboratory [14]. Pancreatic calcium and nitrite concentrations were measured by the methods described by Barnett *et al.* [23] and Vimal and Devaki [24], respectively.

Enzyme Activity Assays

Hepatic Glucose-6-Phosphatase

It is an enzyme, which hydrolyzes glucose-6-phosphate into glucose and also releases one phosphate group free and subsequently glucose release from the cells. In this assay, hepatic homogenate samples were used to determine the enzyme activity by the estimation of inorganic phosphorous released during the reaction. Determination of hepatic glucose-6-phosphatase activity was carried out by following the protocol of Baginski *et al.* [25].

Hepatic Glucokinase

Glucokinase is an enzyme known to sense the concentration of glucose and subsequently contributes to the insulin secretion. In the present study glucokinase activity was measured following the protocol of Goward *et al.* [26].

Hepatic Phosphoglucomutase

It is an enzyme that converts glucose-1-phosphate into glucose-6-phosphate and vice-versa. The reaction was based on the coupled enzyme method, as suggested earlier [27].

Histological Studies

For histological processing, sections of lungs and pancreas were kept aside in 10% PBS buffered formalin. The tissues were dehydrated in the descending grades of isopropanol, finally cleared in xylene and embedded in molten paraffin wax. Sections were cut at 5 μ m thickness, stained with haematoxylin and eosin and viewed under light

microscope to study histopathological changes [14]. Similar process was used for pancreas and stained with aldehyde fuchsin.

Statistical Analysis

Statistical significance was ascribed at $P < 0.05$ or more. Data are expressed as means $\pm$ S.E.M. and were analyzed by Students't-test by using trial version of Prism 5 software for windows (GraphPad, San Diego, CA).

RESULTS

Induction of Allergic Rhinitis

The results of present study showed a significant increase in the number of total leukocytes in nasal discharge, as compared to the control animals. Similarly, the histological analysis of lung tissue showing immune cells infiltration along with increase in intra-alveolar space being observed in PH treated group further established the condition of AR (Table 1 and Fig. 1).

Changes in Serum Variables

In PH treated animals a significant increase in serum glucose, triglycerides, LDL-C, VLDL-C, T_4 , and the activity of α -amylase enzyme was observed. On the other hand a significant decrease was observed in serum concentrations of total cholesterol, HDL-C, calcium and T_3 . However, a non-significant increase was also observed in nitrite, insulin and TSH concentrations (Table 1).

Changes in Hepatic and Pancreatic Parameters

In response to PH nasal instillations, a significant increase in the pancreatic nitrite concentration and lipid peroxidation (LPO) was observed. Similarly, a significant increase was also observed in hepatic glucose-6-phosphatase activity along with a non significant increase in glucokinase.

There was a significant decrease recorded in the activity of hepatic phosphoglucomutase and glycogen content (Table 2).

Table 1. Effects of *Parthinium hysterophorous* (PH) Induced Allergic Rhinitis on Normoglycemic Rats on Total Leukocytes in Nasal Fluid and Serum Variables

Parameter	Control	PH
Total leukocytes (cells/mm ³)	2200 $\pm$ 24.30	8465 $\pm$ 31.84 ^a
Glucose (mg/dl)	68.089 $\pm$ 4.163	104.04 $\pm$ 3.552 ^a
Insulin (μ IU/ml)	18.75 $\pm$ 2.410	23.65 $\pm$ 0.707
T_3 (ng/ml)	9.45 $\pm$ 1.59	4.23 $\pm$ 0.50 ^b
T_4 (nmol/L)	31.774 $\pm$ 2.11	43.09 $\pm$ 2.27 ^b
TSH (mIU/L)	0.421 $\pm$ 0.0317	0.523 $\pm$ 0.0471
α -amylase (IU/dl)	121.220 $\pm$ 8.746	164.760 $\pm$ 12.854 ^c
Calcium (mg/dl)	10.904 $\pm$ 1.033	6.786 $\pm$ 0.613 ^b
Nitrite (mg/dl)	4.138 $\pm$ 0.44	5.661 $\pm$ 0.62
TC (mg/dl)	118.724 $\pm$ 5.20	91.718 $\pm$ 4.42 ^b
TG (mg/dl)	81.966 $\pm$ 2.12	90.026 $\pm$ 2.19 ^c
LDL-C (mg/dl)	59.024 $\pm$ 5.035	107.623 $\pm$ 7.07 ^a
VLDL-C (mg/dl)	16.392 $\pm$ 0.425	18.005 $\pm$ 0.437 ^c
HDL-C (mg/dl)	24.12 $\pm$ 1.53	17.21 $\pm$ 1.14 ^b

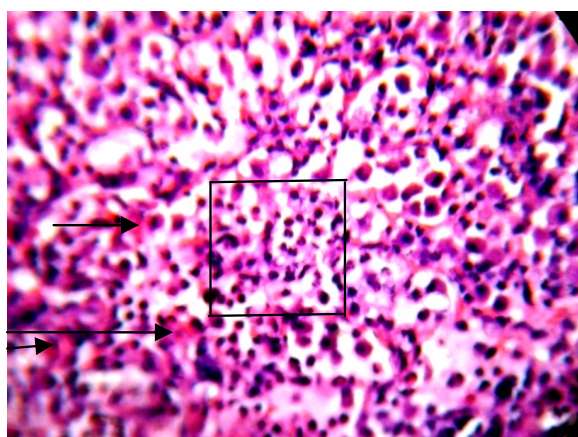
Data are means $\pm$ S.E.M. (n=7); ^a, $P < 0.001$; ^b, $P < 0.01$ and ^c, $P < 0.05$ as compared to the respective control values.

Changes in Pancreatic Histology

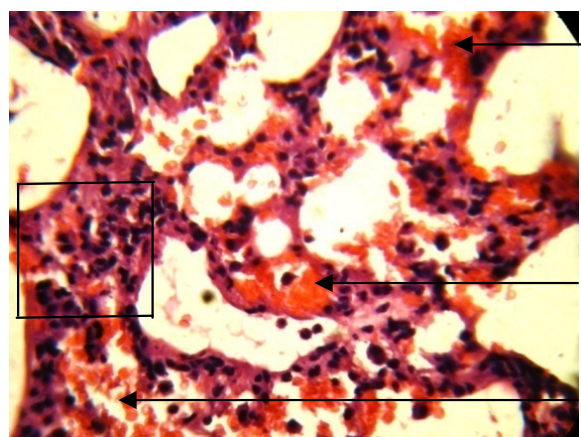
Histological analyses of pancreatic tissue revealed the alterations in the tissue architecture suggesting necrotic/apoptotic phenotype of the islets in PH treated animals (Fig. 2).

DISCUSSION

A significant increase in leukocytes infiltration in the nasal fluid, while increased macrophages and eosinophils in



A.



B.

Fig. (1). H & E staining of lung tissues showing the increase in intra-alveolar space (showed in white) and the infiltration of immune cells. (A) Control group showing the normal architecture of the alveoli and macrophages infiltration (black), while arrow showing the presence of eosinophils in the animals administered with acetone nasal instillation. (B) Increase in the intra-alveolar space and the increased infiltration of macrophages (black) and eosinophils (red in color showed with arrows). Magnification X 200.

lung tissue consistently revealed the condition of allergic rhinitis (AR) by pollen extract of PH, which is in accordance with the earlier reports on AR [2, 3, 15, 18]. However, other allergens are also reported in the literature, but we have given the preference to the pollen extract of *Parthenium hysterophorus* due to its relevance in Indian context, as it is abundantly present in India and USA, but Indian varieties are rich in major allergen parthenin [15-18]. It would be imperative to mention here that parthenin mainly soluble in acetone and that was the reason to consider acetone extract in the present study.

Table 2. Effects of *Parthenium hysterophorus* (PH) Induced Allergic Rhinitis on Normoglycemic Animals on Tissue Variables

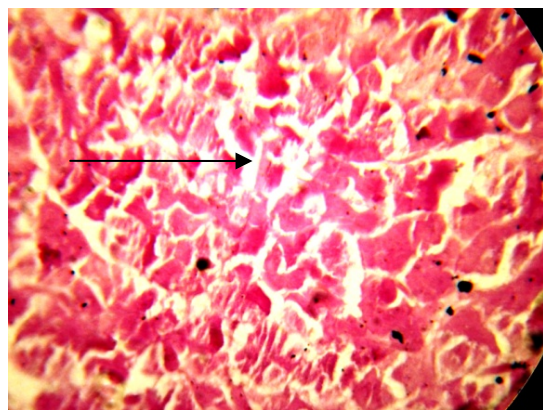
Parameter	Control	PH
Glucokinase activity (Units/mg protein)	0.0310 ± 0.002	0.0347 ± 0.003
Glucose-6-phosphatase (μM of Pi liberated/min/mg protein)	3.130 ± 0.068	3.861 ± 0.042 ^a
Phosphoglucumutase (mU/mg protein)	7.24 ± 0.467	5.52 ± 0.312 ^b
Glycogen (mg/100 gm)	113.24 ± 3.27	102.24 ± 2.68 ^c
Pancreatic nitrite (mg/dl)	1.159 ± 0.108	1.685 ± 0.095 ^b
Pancreatic calcium (mg/dl)	1.539 ± 0.140	1.716 ± 0.117
Pancreatic LPO (nM TBARS/mg protein/hr)	0.433 ± 0.032	0.685 ± 0.041 ^a

Data are means ± S.E.M. (n=7); ^a, $P < 0.001$; ^b, $P < 0.01$ and ^c, $P < 0.05$ as compared to the respective control values.

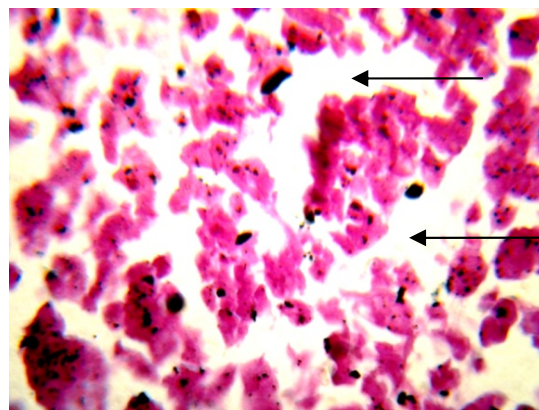
The increase in serum glucose along with marginally increased insulin reveals an initial condition of type 2 diabetes (prediabetes), which is known to be associated with mean/moderate increase in glucose along with the insulin [28]. We tried to assess the possible reasons of the observed diabetic condition by exploring various biochemical parameters and found that AR led to the increase in the serum and pancreatic nitrite concentration, activity of α -amylase and a condition of dyslipidemia. Similarly, the

alterations in thyroid hormones and TSH were also observed. In addition to this, activities of hepatic enzymes regulate glucose metabolism were also altered.

The increased serum nitrite is an indicator of increase in nitric oxide (NO) *in vivo* [29, 30]. This is in agreement with the earlier report suggesting the increased activity of inducible nitric oxide synthase (iNOS) during AR and resultantly the increase in NO [31, 32]. It may be further supported by the significant increase in the pancreatic nitrite concentration, which is an indicator of increased pancreatic NO and is known to induce β -cells apoptosis [33]. However, in response to AR an increase in α -amylase activity, suggesting the increased production of glucose from dietary carbohydrates and contributing to net increase in serum glucose [34]. Similarly, dyslipidemia is also known to be associated with asthma [10] and similar to these observations, present study also showed a condition of dyslipidemia represented by increase in triglycerides, LDL-C and VLDL-C along with a reduction in HDL-C and total cholesterol. This might be the outcome of hypothyroid condition, which is also known to be induced dyslipidemia with the presently observed features, as reported earlier [12, 14, 35, 36]. Interestingly, dyslipidemia is known to be associated with both allergy and diabetes mellitus [10, 12, 14, 35, 36]. It seems that dyslipidemia may be a common link connecting both the mentioned diseases, as observed in the present study. Another interesting finding was the alterations in the serum thyroid hormones to the animals bearing AR. The increased T_4 , TSH with a concomitant decrease in T_3 (metabolically more active form), clearly suggesting a condition of low T_3 syndrome or hypothyroidism [12]. This decrease in T_3 might be the outcome of diminished activity of hepatic 5'deiodinase enzyme, which contributes to the peripheral conversion of $T_4 \rightarrow T_3$, which could not be ruled out in present study (mostly T_3 synthesize by this pathway) [37-39]. However, increase in T_4 is also known to promote insulin secretion, *in vitro* from pancreatic cells, which further consolidated the non-significant increase in serum insulin observed in present study [40]. Another reason for increased insulin could be the non significant increase in the glucokinase activity, which is a sensor of glucose concentration and known to stimulate



A.



B.

Fig. (2). Aldehyde fuchsin staining of pancreatic islets showing changes in the pancreatic islet of PH treated animals (A) Control group showing the normal architecture of the pancreatic islet with normal intercellular spaces (showed by white color). (B) PH treated animals showing the necrotic/apoptotic phenotype of the pancreatic cells with damaged islet and increased intercellular spaces. Magnification X 200.

insulin secretion *via* opening the calcium channels [41, 42]. Thyroid hormones are known to affect the basal metabolic activities and participate both in the glucose production and glucose disposal *via* facilitating the glucose entry into the cells through enhancing the expression of glucose transporter receptors (GLUT) [43]. The overall influence of the thyroid hormones on the glucose homeostasis depends upon the balance of thyroid economy and in the present observations it seems that low T₃ concentration diminished the cellular entry of glucose as well as glucose production, but significant increase in the activity of glucose-6-phosphatase along with the diminished phosphoglucomutase activity led overall increase in glucose production *via* promoting the gluconeogenesis and glycogenolysis [27, 44-46].

The significant increase in the activity of glucose-6-phosphatase, reduction in phosphoglucomutase along with decreased hepatic glycogen clearly demonstrated the biochemical changes towards progression of diabetes mellitus. It is well established that glucose-6-phosphatase hydrolyzes glucose-6-phosphate into glucose and contribute to glucose production [45, 46]. On the other hand phosphoglucomutase participates to glycogen synthesis *via* converting glucose-6-phosphate into glucose-1-phosphate [27], which is also evident by decrease in hepatic glycogen. It is noteworthy that insulin is also known to promote glycogen synthesis [44], but in the present study irrespective of increase in insulin, glycogen found to be decreased, which further confirmed the role of phosphoglucomutase inhibition [27].

In order to reveal the insulin signaling and pancreatic health, we have carried out pancreatic lipid peroxidation (LPO), nitrite and calcium concentrations in pancreatic tissue homogenate and histological analysis of pancreatic islets. Results of these studies revealed increase in LPO, nitrite and a non significant increase in calcium along with the necrotic changes in the pancreatic islets, which further support the progression of diabetogenic changes in the animals bearing PH induced allergic rhinitis. Similarly, decrease in serum calcium is also associated with the diabetes induced renal tubular damage because of poor tubular reabsorption of minerals including calcium.

The increase in LPO; an indicator of oxidative stress and membrane fluidity represent the structural defects and/or apoptotic phenotype of pancreatic cells biochemically [12, 14]. Increase in pancreatic oxidative stress during diabetes mellitus is already well established [12, 14, 34]. This might be the outcome of increased NO radicals concentration in pancreatic islets, as suggested by increased pancreatic nitrite concentration. These results further consolidated the progression of diabetes mellitus, as increase in pancreatic NO *via* increase in iNOS activity during inflammation and diabetes is one of the know pathways leading β -cells apoptosis, while increased iNOS activity is also known for AR [14, 31-33, 47]. A non-significant increase in glucokinase and pancreatic calcium concentration might be the outcome of positive stimuli to promote insulin secretion form pancreatic cells as an adaptation to the increase serum glucose. However, marginally increased insulin could not counteract the overall increase in serum glucose suggesting a condition of insulin resistance [28]. Interestingly, necrotic lesions in the pancreatic islets of AR bearing animals further

suggest the condition of diabetes mellitus. However, necrotic changes in the pancreas were associated with the marginal increase in insulin, which might be the outcome of initial adaptation of the cells by producing and secreting more amount of insulin, as reported for type 2 DM [28].

After reviewing all the observations of present study, it is concluded that *Parthinium hysterophorous* pollen extract induces allergic rhinitis to the animals, which is found to be associated with the progression of diabetes mellitus to the animals. This diabetic condition might be mediated through the pleiotropic defects in the glucose and cholesterol metabolism including increase in glucose production, diminished glucose disposal, hypothyroidism, pancreatic lesions probably thorough overproduction of NO radicals and dyslipidemia.

This is first study which provides initial clues to evident the role of allergic rhinitis in the progression of diabetes mellitus, most probably type 2. However, further studies considering long duration to get significant results and cytokines profiling as well as reversal of diabetogenic condition by anti-allergic therapies to the animals bearing AR is recommended to confirm the direct role of AR in the progression of DM.

ABBREVIATIONS

AR	=	Allergic rhinitis
PH	=	<i>Parthinium hysterophorous</i>
DM	=	Diabetes mellitus
NO	=	Nitric oxide
TG	=	Triglycerides
LDL-C	=	Low density-lipoprotein cholesterol
HDL-C	=	High density-lipoprotein cholesterol
VLDL-C	=	Very low density-lipoprotein cholesterol.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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