

Protective Effect of Inulin and the Integrity of the Microvasculature in Diabetes Mellitus

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Type 2 diabetic microangiopathy affects every organ in the body and can lead to serious incapacitating complications. VLDL and apo C1 are two of the main biochemical abnormalities which start and propagate this condition. Inulin fructans prebiotic effect on the colonic flora enhance the bifidogenic strains. These predominate over the pathogenic strains which encourage lipidogenesis, thus reducing hyperlipidemia. Our aim is to find out the possible effect of inulin ingestion on the metabolism of VLDL and apo C1 and their role in the pathogenesis of diabetic angiopathy. Twenty eight obese type 2 diabetic female patients were subjected to this study. Each patient ingested 4 grams of inulin daily for 3 weeks. Their fasting serum level of VLDL and apo C1 were estimated before and after the period of inulin ingestion. There was a significant decrease in fasting level of serum VLDL and apo C1 after inulin ingestion period. In conclusion inulin can be given as a protective and as an add on therapy for type 2 diabetic patients. It reduces two of the main culprits which start and propagate the pathologic pathway of diabetic microangiopathy. This cuts short the other offenders (small HDL, small dense LDL and the small VLDL remnants).

Keywords: Inulin, Type 2 diabetes, Triglycerides, VLDL, Apo C 1, Microvasculopathy.

Diabetic microvascular disease is usually associated with complications in the various body organs. On the long run this can lead to damage,

deterioration and eventually end organ failure in the different body systems. Diabetic microvasculopathy has long been recognized to predominate in type 1

rather than in type 2 diabetics^{1,2}. However, several studies reported that diabetic arteriosclerosis is as common in type 2 as in type 1 diabetic patients^{3,4}.

Hyperglycemia which is a key feature of both type 1 and type 2 diabetes,⁵ is not the only determining factor behind the vascular complications. Trials for the tight control of hyperglycemia, reduced the progression of diabetic microvascular disease. Despite this fact, the morbidity and complications of diabetic arteriosclerosis are still rising^{1,2,6,7}.

Beside hyperglycemia, several factors participate in the pathogenesis and development of diabetic microvasculopathy. These include oxidative stress, systemic (metabolic) inflammation, protein synthesis in the extracellular matrix together with thickening of the capillary basement membrane⁸.

Moreover, very low density lipoprotein (VLDL) is also an important and outstanding determinant in the pathogenesis and progression of the microvascular disease in diabetes mellitus. VLDL is considered a major and independent participant in this regard^{9,10}. Also, it has been observed that agents which act through peroxisome proliferator activated receptor alpha (PPAR α) to lower serum triglycerides (TG) which are carried mainly by VLDL have a beneficial therapeutic effect on diabetic nephropathy¹¹.

Again, apolipoprotein C1 (apo C1) is an important component of the surface protein monolayer which envelops the lipid core of VLDL and high density lipoprotein (HDL) particles. These latter two lipoproteins are the main carriers of apo C1 which is secreted by the liver¹². In human plasma, apo C1 acts as a physiological regulator of the cholesteryl ester transfer protein (CETP) activity. It regulates the transfer of cholesteryl esters between the different lipoproteins, mainly VLDL, HDL and low density lipoprotein (LDL)¹². In normolipidemic persons, apo C1 regulates the actions of HDLs with CETP^{13,14}. In hypertriglyceridemic subjects however, apo C1 acts in an angiopathogenic manner¹⁵.

Ingestion of the oligosaccharide inulin fructans has been known to decrease the synthesis of triglycerides^{16,17}. This oligosaccharide escapes digestion in the upper gastro-intestinal tract. In the colon, inulin is consumed by the bifidogenic bacteria, these latter supercedes the pathogenic strains which encourage lipogenesis^{18,19}.

The aim of the present study is to find out the possible effect of inulin ingestion on the metabolism of VLDL and apo C1 and their role in the pathogenesis of diabetic angiopathy. Reviewing the literature and to the best of our knowledge; this study has not been tried on human subjects before.

MATERIAL AND METHODS

Subjects

Twenty eight obese, type 2 diabetic women were recruited from the municipal hospitals of Cairo, Egypt. The age range was 40-65 years.

Inclusion criteria

obese (body mass index > 30), type 2 diabetic females, middle aged or above, hypertensive or not and not under antilipidemic drugs.

Exclusion criteria

Hormonal therapy or contraceptive pills. Endocrine disorders or other metabolic diseases.

Malignancy or organ failure (lung, heart, liver or Kidney). Local or systemic infection including chest, urinary tract, gastro-intestinal tract or skin infection (local or extensive).

Ethical committee approval

The present work has been approved by the ethical committee of the National Research Centre (NRC), Cairo, Egypt. Certificate number 15011.

Consent

All patients signed consent for participation.

METHODS

Inulin fructans type prebiotic was given to the patients as an add on therapy to their conventional antidiabetic treatment. Four grams of inulin were given with milk to each patient daily; 2 grams in the morning and 2 grams in the evening for twenty one consecutive days. This dose was chosen empirically on the assumption that a small amount of inulin can exert a bifidogenic effect²⁰.

Inulin specifications: Inulin A.R (C₆H₁₀O₅) N ALPHA-CHEMIKA Mumbai. 400002 (INDIA) An ISO: 9001: 2000 Certified company.

All patients were subjected for the following before and after the period of inulin intake.

A- Full history. B- Thorough clinical examination. C- Laboratory investigations:

Laboratory investigations

1. Estimation of fasting serum very low density lipoprotein (VLDL). This was done by dividing fasting serum triglycerides in mg/dl by 5 using Friedewald equation for calculating VLDL and LDL²¹.

Fasting serum triglycerides were estimated spectrophotometrically after Fossati (Fossati, 1982). Kit used from Centronic Germany²².

2. Estimation of fasting serum apolipoprotein C1 (apo C1) was by ELISA method after Westerber *et al* 2007²³.

Kit used: “Human Apolipoprotein C-1 ELISA Kit” was used from Assaypro LLC 3400 Harry’s Truman Blvd st. Chales, MO 63301.

Statistical methods

Data were coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software

version 22.0, IBM Corp. Chicago, USA, 2013.

Descriptive statistics were done for quantitative data as minimum and maximum of the range as well as mean± standard deviation (SD) for quantitative parametric data. Inferential analysis of quantitative variables using paired t-test in cases of two dependent groups with parametric data was performed. The level of significance was taken at P value < 0.05.

RESULTS

Mean fasting serum level of very low density lipoprotein (VLDL) dropped significantly after inulin ingestion from 46.7±21.1 mg/dl to 39.6±18.8 mg/dl with P 0.005, table (1) and figure (1).

Mean fasting serum apolipoprotein C1 (apo C1) dropped significantly after inulin ingestion from 0.44±0.2 ug/ml to 0.29±0.09 ug/ml with P < 0.001 table (1) and figure (2).

Table 1. Shows the values of VLDL and apo C 1 before and after inulin ingestion period

| | Before | | After | | ^Change | | #P |
|----------------|-----------|-----------|-----------|-----------|--------------------|------------|---------|
| | Mean±SD | Range | Mean±SD | Range | Median (IQR) | Range | |
| VLDL (mg/dL) | 46.7±21.1 | 12.4–86.8 | 39.6±18.8 | 16.0–78.4 | -8.7 (-12.3–-3.3) | -25.6–43.6 | 0.005* |
| APOC I (µg/mL) | 0.44±0.20 | 0.14–0.72 | 0.29±0.09 | 0.10–0.49 | -0.15 (-0.19–0.02) | -0.44–0.16 | <0.001* |

N=28, ^Negative values indicate reduction, #P-value of paired t-test, *Significant

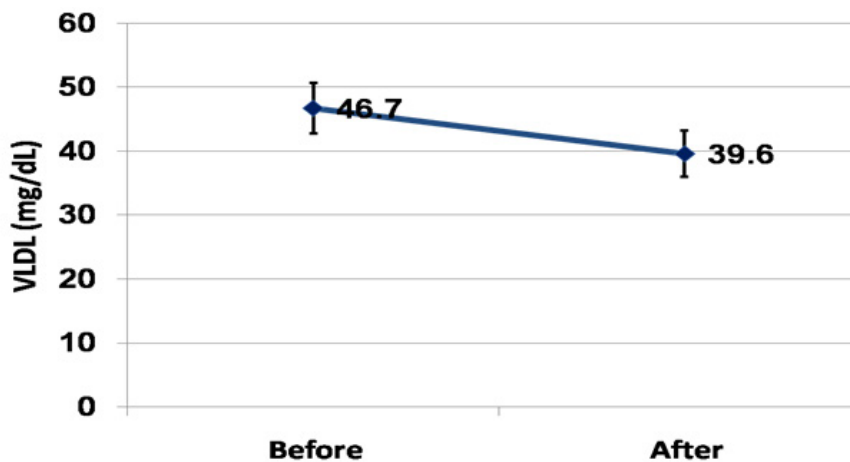


Fig. 1. Shows the significant drop of mean fasting serum VLDL from 46.7 mg/dl to 39.6 mg/dl after the period of inulin ingestion

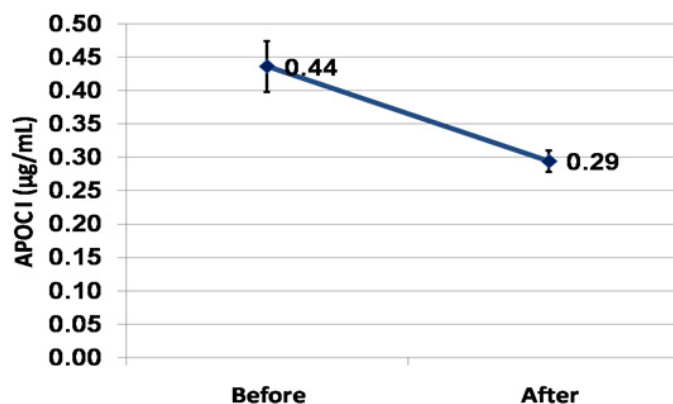


Fig. 2. Shows the significant drop of mean fasting serum apo C1 from 0.44 µg/ml to 0.29 µg/ml after the period of inulin ingestion

DISCUSSION

In diabetes mellitus, pathophysiological disorders start years before manifest pathological changes appear in the microvessels and decades before hyperglycemia is detected²⁴. Unlike the macrovascular changes, atheromatous lesions are not characteristic for microvessels. The indicative changes of diabetes mellitus in microvasculopathy are extracellular matrix deposition, thickening of their walls and the basement membrane of the capillaries⁸. These complications affect almost every organ in the body²⁵.

Diabetes is a leading cause of blindness worldwide. Diabetic retinopathy (DR) is a well known microvascular diabetic complication, which when neglected it progresses to complete loss of vision²⁶. The retina is a part of the central nervous system (CNS). Both of them share anatomical and embryologic features. The presence of DR alerts for searching other vascular CNS complications²⁷. Also, affection of the vasanervorum is among the causes of diabetic neuropathy⁵. Again, diagnosis of DR anticipates the presence or the near occurrence of diabetic nephropathy^{28,29}. This latter starts by glomerular hyperfiltration which progresses to microalbuminuria, macroalbuminuria, renal impairment up to end stage renal failure^{28,29}. Diabetic microangiopathy, also leads to other dreadful complications; Diabetic cardiomyopathy can occur in the presence of patent coronary arteries^{30,31}. However, when the vasa-vasorum are affected, this aggravates the atherosclerosis of the large vessels; the coronaries or elsewhere^{32,33}.

Diabetic microangiopathy leads to special entity of complications termed microamputations³⁴. In the skin microvasculopathy is behind delayed wound healing, gangrenous ulcerations and aggravated skin infections⁵.

In normolipidemic persons apo C1 acts as a normal physiologic regulator of lipid metabolism^{13,14}. In hypertriglyceridemic subjects, apo C1 acts in a pathological manner¹⁵. Hypertriglyceridemia and excess VLDL are features of type 2 diabetes mellitus. Elevated VLDL together with its integral moiety of apo C1 are key factors in the pathological sequences of type 2 diabetic vasculopathy. Apo C1 plays a critical metabolic role in this regard. It affects in an abnormal manner the metabolism of both VLDL, HDL and the redistribution of their triglycerides and cholesteryl ester content. Apo C1 stimulates VLDL hepatic synthesis³⁵. Also, it inhibits the lipoprotein lipase which hydrolyses VLDL^{36,37}. At the same time it inhibits the recognition of VLDL by their cellular receptors; It hinders the ligand (apo E) present on the surface of VLDL particles from binding to their receptors in the liver. This delays the catabolism of these particles and retards their clearance from the circulation^{38,39}. The net result of the above pathological steps is the accumulation of VLDL in the blood stream. These accumulating VLDL particles interact with HDL and LDL in a pathological manner¹⁵.

Concerning HDL, the accumulating apo C1 (integral to the abundant VLDL particles), readily dissociate from VLDL and rapidly associate with HDL particles. Being a strong inhibitor of

CETP, apo C1 reduces the capacity of CETP in the HDL for transferring its cholesteryl esters (CE) to VLDL in exchange of the latter's triglycerides^{15,40}. This results in "deranged triglycerides (TG)/ high density lipoprotein (HDL) axis"^{41,42}. The net result is the accumulation of this CE in the HDL turning it into an abnormally functioning particle. LDL particles are subjected to a similar mechanism like that to which HDL particles are exposed^{41,42}.

The prolonged stay of VLDL particles in the circulation (as a result of the defective and delayed catabolism); delivers excess of their TG content to HDL and LDL. Upon reaching the liver, TG rich HDL & LDL particles are exposed to the hepatic lipoprotein lipase enzyme (LPL) which hydrolyses their TG content converting them to small HDL particles and small dense atherogenic LDL particles. The first are lost in urine while the latter easily percolate through the vascular endothelium, become readily oxidized and engulfed by the macrophages. This starts and perpetuates the damaging inflammatory process in the vessel wall⁴³.

After the transfer of their TG to both LDL and HDL, the donor VLDL particles lose size. When the small size VLDL particles enter the different vascular beds, their TGs are hydrolyzed by the lipoprotein lipase enzyme in the (liver, skeletal muscles, myocardium and adipose tissue). The new small particles are termed VLDL remnants. These are proinflammatory as they behave like small LDL particles.

This study examined the effect of inulin intake on serum level of very low density lipoprotein (VLDL) and apolipoprotein C1 (apo C1). Inulin ingestion significantly reduced both VLDL and apo C1. These two compounds are heavily implicated in the pathogenesis of diabetic microangiopathy^{9,10,15}.

CONCLUSIONS

In conclusion, inulin may help to reverse the pathogenic process of diabetic microangiopathy by lowering both VLDL and apo C1. Lower levels of these two compounds, in turn, decrease the proinflammatory small dense LDL particles and the proinflammatory small VLDL remnants. Also, by improving the deranged TG/ HDL axis, inulin reduces the production of the ineffective small

HDL particles which are lost in urine. Thus inulin which is a safe and edible fiber, can be given to diabetic subjects as both a protective and as an add on therapy for diabetic microangiopathy.

This study should be extended on a wider scale including a greater number of patients and a longer duration of inulin intake. At the same time end points should be incorporated in the study including number of sessions of laser photocoagulation in patients with diabetic retinopathy. Also serum creatinin, estimated glomerular filtration rate (eGFR) and urinary albumin in patients with diabetic nephropathy. All these should be determined before and after inulin intake period

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