Lipids profile in type II Diabetes mellitus during Ramadan Fasting

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Abstract

A prospective study of (25) patients suffer from type II diabetes mellitus (age 45 – 62 year)
was carried out to determine the biochemical changes of serum cholesterol , HDL – cholesterol ,
LDL cholesterol , triglycedrides , LP (a) and Hba,C.The Comprison analysis of pre–fasting with
fasting and post – fasting samples did not reveal any statistically significant changes in the value
of the lipid profiles . Aslight change in serum albumine was noticed ,but otherwise ther was no
change in other biochemical values , with marked decrease and increase in the weights of the
patients(2).

تغيرات الدهون لدى مرضى السكري (النوع الثاني) خلال شهر رمضان

ضياء فاضل الشيخلي

المستخلص

دراسة تحليلة لـ (25) مريض مصاب بالنوع الثاني لمرض السكري تراوحت اعمارهم بين (25-62) سنة لمتابعة تأثير
صيام شهر رمضان على عناصر الدهون (الكولسترول) الدهون الثلاثية ، الكولسترول الجيد والخبيث وكذلك الهيموغلبين (أ
و ج) . اجريت مقارنة لتلك المواد الكيميائية قبل وبعد صيام شهر رمضان (لمدة 29 يوم)حيث اضهرت الدراسة عدم وجرد
تغيرات جوهرية في اجزاء الدهون ، كما اظهرت الدراسة حصول تغيرات طفيفة في اجزاء الاليومين مع ملاحظة تغيرات في
اوزان المرضى (زيادة ونقصان).

Introduction

Many reports have been published on the variable effects of fasting in Ramadan on serum lipid parameters (1 , 3).The month of Ramadan contains (29-30 days) , the experience of fasting is intended to teach Muslims self . discipline and self restraint and remind them of the impoverished . Muslims observing the fast are required to abstain not only from eating and drinking ,but also from consuming oral medication , intravenous nutrition fluids , (9).Diet control is an important factor in the treatment of type II diabetes (4) fasting in Ramadan is a duty laid down on all adult Muslim with only a few. exceptions.There are a few papers on the effect of Ramadan fasting on lipid parameters and most studies reports change on limited number of lipids sub . fraction (7 ,8).A medline search for the last 10 years did not show any data on APOA-1 ,B in type $\prod$ diabetics during fasting (a) most diabetics reduce their daily activities in fear of hypoglycemia (11) . these factors may result in not only alack of weight loss , but also a weight gain in such patients (10 ,11) .

Methods and analysis

Twenty five patients with type II diabetes were recruited to the study and they agreed to give three fasting samples of blood.The first sample was taken one month prior to Ramadan fasting , the second during the last week of fasting while the third one after one month of Ramadan . The patients were instructed to have their *Suhoor* (last meal) early and to be fasted 12 hours before they gave blood samples. All samples were analyzed immediately after collection .
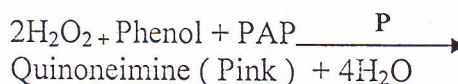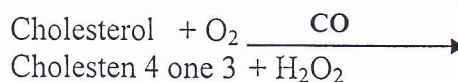
The analysis was as follows
Cholesterol

Enzymatic method: described by Allain and al.(13), which reaction scheme is as follows :

$$Cholesterol\ esters \xrightarrow{\ CE\ } Cholesterol + free\ fatty\ acids$$

$$Cholesterol\ + O_2 \xrightarrow{\ CO\ } Cholesten\ 4\ one\ 3 + H_2O_2$$

$$2H_2O_2 + Phenol + PAP \xrightarrow{\ P\ } Quinoneimine\ (\ Pink\ ) + 4H_2O$$

Reagents Composition:

Vial R1   Buffer

Phosphate buffer	100 mmol / L
Chloro – 4 – phenol	5 mmol / L
Sodium Chlolate	2.3 mmol / L
Triton × 100	1.5 mmol / L
Preservative	

Vial R2   Enzymes

Cholesterol oxydase (CO) IU/L	≥ 100
Cholesterol esterase (CE)	$\geq$ 170 IU/L
Peroxydase (POD)	$\geq$ 1200 IU/L
4 - Amino - antipyrine (PAP) mmol/L	0.25
PEG 6000	

Vial R3   Standard
Cholesterol 200 mg/dL (5.17 mmol/L)

Reagents Preparation:

Add promptly the content of vial R2 (Enzymes), into vial R1 (Buffer).

Mix gently until complete dissolution (approximately 2 minutes).

Add promptly 20 mL vial R1 (Buffer) to the content of vial R2 (Enzymes) .

Vial R2 : If appropriate, use a non-sharp instrument to remove aluminium cap

Manual Assay

Let stand reagent and specimens at room temperature.

Pipette into well identified test	Blank	Standard	Assay
Reagent	1 mL	1 mL	1 mL
Demineralised water	10 µL		
Standard		10 µL	
Specimen			10 µL
Mix. Let stand for 5 minutes at 37°C or 10 minutes at room temperature. Record absorbances at 500 nm (480-520) against reagent blank . Colour is stable for 1 hour.			

Note:Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support .

Calculation:

Calculate the result as follows :

$$\text{Result} = \text{Standard concentration} \times \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}}$$

HDL-Cholesterol (PTA) Precipitant

Principle (14):

This reagent is only for treatments before determination of HDL-Cholesterol with a reagent for total cholesterol.Low density lipoproteins (LDL), very low density (VLDL) and chylomicrons from specimens are precipitated by phosphotungstic acid (PTA) and Magnesium chloride. HDL-Cholesterol obtained in supernatant after centrifugation is then measured with Total Cholesterol reagent (i. e. : CHOLESTEROL CHOD-PAP BIOLABO |REF| 80106).

Reagents Composition :

Vial R1   Precipitant
Phosphotungstic acid (PTA)   13.9 mmol/L
Magnesium chloride   570 mmol/L

Vial R1   Standard
Cholesterol 100 mg/dL (2.58 mmol/L)

Specimen Collection and Handling(6):
Specimens should be collected after 12 h-14 h fasting.
Plasma : collected on EDTA.
Centrifuge and remove plasma from blood cells as soon as possible
(within 3 hours).
Serum : Centrifuge and remove serum from blood cells as soon as
possible (within 3 hours).
Avoid oxalate, fluoride, citrate or heparin.
HDL-Cholesterol in specimen is stable for:
• 1 to 3 days at 2-8°C
• 1 month at - 20°C.

Interferences (15) (16) :
PTA/Mg^{2+} procedure is less sensitive to hyperlipemia than heparin/Mn^{2+} procedure. The procedure is sensitive especially to reaction conditions. It may be affected by temperature, timing in supernatant-precipitate separation. For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

Assay :
With Total BIOLABO Cholesterol CHOD PAP or "Let stand reagents supernatants room temperature .

Pipette in well identified test	Blank	Standard	Assay
Reagent	1 mL	1 mL	1 mL
Distilled water	25 μL		
Standard 100 mg/dL		25 μL	
Supernatant			25 μL
Mix. Let stand for 5 minutes at 37°C or 10 minutes at room temperature. Record absorbances at 500 nm (480-520) against reagent blank. Colour is stable for 1 hour.			

Calculation:
Calculate the result as follows :

$$Result = \frac{Abs\ (Assay)}{Abs\ (Standard)} \times$$

Standard concentration × 1.1

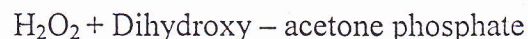Standard remaining undiluted, 1.1 factor takes into account dilution of the specimen during the precipitation step.
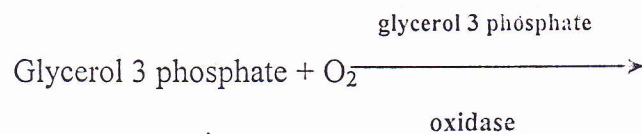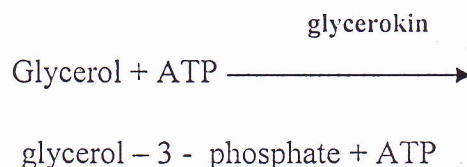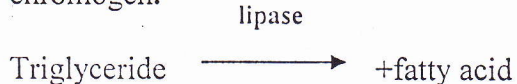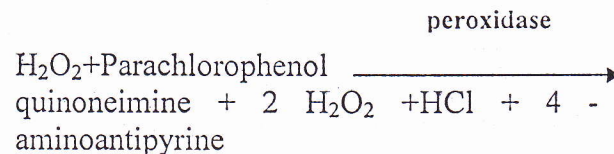
Triglycerides
Enzymatic method:
Principle: (17)

Triglycerides are determined according to the following sequence :
lipase - glycerokinase - glycerol 3 phosphate oxidase - peroxidase - chromogen.

$$Triglyceride \xrightarrow{\text{lipase}} +fatty\ acid$$

$$Glycerol + ATP \xrightarrow{\text{glycerokin}}$$

glycerol – 3 - phosphate + ATP

$$Glycerol\ 3\ phosphate + O_2 \xrightarrow[\text{oxidase}]{\text{glycerol 3 phosphate}}$$

$$H_2O_2 + Dihydroxy – acetone\ phosphate$$

The Hydrogen peroxide produced is measured according to a TRINDER type reaction .

$$H_2O_2 + Parachlorophenol \xrightarrow{\text{peroxidase}}$$
quinoneimine + 2 H_2O_2 +HCl + 4 - aminoantipyrine

The intensity of the coloration (quinoneimine) measured is proportional to the triglyceride content of the sample. Presentation and content of the KIT(150tests)

Reagent 1 Standard 1 x 8 ml (liquid)	R1	Glycerol 2.29 mmol/l or 2 g/l of triglycerides (Average MW of triglycerides = 875)	
Reagent 2 Buffer 2 x 90 ml (liquid)	R2	Tris buffer pH 7.6* Parachlorophenol Magnesium	100 mmol/l 2.7 mmol/l 4 mmol/l
Reagent 3 (reconstituted with R2) Enzymes 6 x 25 ml (lyophilized)	R3	Protein base buffer (bovine origin) 4-Aminoantipyrine Lipase Glycerokinase Glycerol-3-phosphate oxidase Peroxidase 0.8 mmol/l	0:4 mmol/l ≥1000 U/l ≥ 200 U/l ≥ 2000 U/l ≥ 200 U/l ATP
1 package insert			

This reagent contains 1% of Tris. A material *safety data sheet is available on request.

Assay :
Wavelength : ————505 nm (492-550 nm)
Zero adjustment : ————reagent blank

	Reagent blank	Standard	Sample
Standard	-	10 μl	-
Sample	-	-	10 μl
Reconstituted Reagent 3	1 ml	1 ml	1 ml
Mix.			

Perform photometry after incubation for:

• 5 minutes at 37°C,

• 10 minutes at 20-25°C.

Interpretation:

Interpretation of the test results should be made taking into consideration the patient's history and, if necessary, the results of any other tests performed.

Calculation :

$$\text{Sample concentration} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times n$$

n = concentration of standard

Conversion Factor:

mmol/l× 0.875 = g/l   g/l×1.14 = mmol/l
mmol/l ×87.5=mg/dl   mg/dl×0.0114= mmol/l

The other biochemical findings were estimated by autoanalyzer and flame photometer .
the medications which were taken by patients are :
a – tenclol 100 mg / day (antihypertensive) .
b – simvastatin 20mg / day (drug lowering lipid) .
c – capotin 50 mg twice daily (vasodilator and antihypertensive) .

Statistical Analysis :

To compare the three groups before, during then after one month fast we use multivariate Analysis of variance .

Results

Table(1)shows the characteristics of the patients studied . most patients were male ranging(55-62) years old while female ranging (45 -58) years old.The hypertensive Patients (7) were on (tenolol 100 mg day)alone and other (6) with (captopril 50 mg two times daily)Others ((4) patients) treated by simvastatin 20 mg/ days as fat lowering agent for more than six month .(16) patients on oral hypoglycemic (Euglicon 5 mg / day) , 6 patients on insulin treatment (20 Iu /day) while only 3 patients on diet restriction .All these medication were continued in the same manner during Ramadan fasting except the change of the time taking these drugs .Table 2 shows the mean serum lipid profile in the three different gropes (25 patients) from which we noticed no valuable change in most parameter during the three period (before ,during and after fasting)While from table 3 we noticed liner function tests were all about normal except mild change in serum albumin (slight increase during fasting and continuing there after) ,other wise there were no statistically significant change .Table 4 shows the change in weights during these three periods ; such that the females undergoing increased in weight while males (7) of theme had gain in weight ,the other (11) patients had decreased in weight .

Table (1) :characteristics of the studied patients.

No.:	25
Sex (m/f) :	18 / 7
Age mean (range) :	54
Diet only	3
Oral hypoglycemic	16
Insulin :	6
Associated CVD :	

IHD :	3
CVA :	3
Renal :	2
Lipid lowering agents :	4
Hypotensive medication :	13

CVD =cardiovascular disease .
CVA = cerebrovascular Accident .

Table (2): Mean Serum Lipid Profile in the 3 Groups.

Biochemical parameters	One month pre-fast	End of Ramadan fast	One month after the end of fast	P value
T. cholesterol	5.6 ± 1.1	5.8 ±1.3	5.9 ±1.4	0.6
Triglycerides	1.84 ±0.98	2.02 ±1.1	1.8 ±0.8	0.7
HDL-C	1.2 ±0.35	1.2 ±0.3	1.2 ±0.4	0.9
LDL-C	3.5 ± 1.2	3.69 ±1.1	3.8 ±1.2	0.6
LP(a)	37.9 ±24.8	35.5 ±22.8	31.3 ±22.1	0.6

All values are in mmol/L except LP(a) is expressed in mg/dL values are mean ± SD

Table(3): Mean Serum biochemistry values in the 3 groups.

Biochemical parameters .	One month pre-fast	End of Ramadan fast	One month after the end of fast	P value
FBS	10 ±4.2	12.0 ±5.2	9.8 ±4.3	0.1
HbA,C	8.6 ±2.1	8.2 ±1.6	7.9 ± 2.1-	0.5
Urea	5.76 ±1.9	6.0 ±2.1	8.4 ±2.1	0.2
Creatinine	84.4 ± 27.2	88.9 ±25.8	88.8 ±34.2	0.7
Total protein	75.4 ±3.7	73.9 ±3.9	75.6 ±4.4	0.2
Albumin	138.7 ±3.2	40 ±4.3	41.9 ±3.6	0.00*
Calcium	2.39 ±0.1	2.4 ±0.1	2.4 ±0.1	.9
Phosphorous	1.2 ±0.1	1.3 ±0.1	1.31 ±2.1	0.1
Total Bilirubin	9.5 ± 6.4	8.7 ±7.2	9.1 ±5.3	0.8
ALT	32.0 ±24.5	28.6 ±9.6	33.9 ±24.8	0.6
ALKP	100.8 ±31.3	94.9 ± 32.5	95.4 ±25.0	0.6

* P - value significant
all values are in mmol/L except HbA,C was expressed in percentage .

Table(4):The weight change during the three periods .

	Pre fast	During fast	After fast
Female (7) Weight range in kg (63 – 88) Average : 75.5 kg		(64.3 - 89.6) Average : 76.9 kg	(62.8 -87.5) Average : 75.2 kg
a - (7)	**Male :** (56-75) kg Average : 65.5 kg	(57.8-77) kg Average : 67.4 kg .	(57- 74.1) kg Average : 65.5
b- (11)	(58 -72) kg Average : 65 kg	(57.5-70) Average : 63.7 kg	(57.7 – 71.8) Average : 64.8 kg

Discussion

In the present study 25 diabetic patients fulfilled the study design, three blood samples were taken from each patient for analysis. Dietary habits are known to vary from one Muslim Country to another and such changes in dietary habits may be responsible for the changes in the daily caloric intake observed in Ramadan fasting. For example a decrease of daily energy intake during Ramadan has been reported: in Indian Muslim (5).Thus the variation in total caloric consumption may be responsible for the discrepancy in the observed effect of fasting on serum lipid parameters in different studies reported previously from different Muslim Countries. Adlouni et al (6) reported a fall in serum LDL-cholesterol and triglyceride level which was associated with loss of body weight in fasting.A recent study by Mahboob et al (7) showed a favorable effect of

fasting on lipid profiles of 35 male normal and hyperlipidemic volunteers.There was significant decrease in serum total cholesterol, triglyceride and LDL-C together with a marked increase in HDL-C.This positive effect was more obvious in hyperlipidemic volunteers who reduced their mean daily energy intake by more than 500 kcal/day.Similar findings were reported also by latif(8). On the other hand, some other reports (9) indicated an increase in the total cholesterol, TG, and LDL-C during fasting. This effect was attributed to a high consumption of carbohydrates and fats during the month of Ramadan. As a result, physical activities are reduced during the fasting period.People usually restrict their activities during the daytime and spend a long time at night a wake, eating, taking and watching television. The lack of significant effect of fasting on lipid profiles in our study is attributed

probably to the above factors (10) .The change in serum albumin was slightly increased during fasting and continued there after. This could be secondary to a high meat intake. P- Value was significant only when the pre-fast samples were compared with post-fasting samples.Further study, .however is required to confirm this finding by measuring different food constituents during Ramadan .From this study we noticed the variation in weights (11) .Such that the average change is 1.4 Kg. gain in the females while 1.9 Kg. gain in 7 male patients and 1.3 Kg. decrease in 11 male patients .Further study , however is required to confirm this finding by measuring different food constituents during Ramadan .

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