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Full Length Research Paper

Effect of Ramadan fasting on glucose, glycosylated haemoglobin, insulin, lipids and proteinous concentrations in women with non-insulin dependent diabetes mellitus

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The objective of this study was to investigate the effect of Ramadan fasting on body mass index (BMI) and on certain biochemical parameters of serum in women patients with non-insulin dependent diabetes mellitus. Sixty-six subjects from 3 regions located in the west of Algeria participated in this study. All participating patients were studied a week before Ramadan and at the third week of Ramadan fasting. No statistically significant fluctuations were noted in BMI either during Ramadan or in non-fasting days. However, the rates of glycosylated haemoglobin (HbA1c) decreased slightly ($P < 0.05$) during the last week of the month of Ramadan among the diabetic patients. Also, the glucose levels were significantly ($p < 0.05$) higher in the serum subjects of patients during the fasting period when compared to the level before Ramadan. This could be due to the significant decrease ($P < 0.05$) of insulin levels in patients during the fasting period. The rates of HDL cholesterol recorded in the blood among patients rose significantly ($p < 0.05$) during the Ramadan than during the non-fasting period. The statistically significant increase in HDL-cholesterol explains clearly the beneficial effect of Ramadan fasting on diabetic's serum lipids. Moreover, serum total cholesterol, triglyceride, LDL cholesterol and VLDL cholesterol levels decreased significantly ($p < 0.05$) during the third week of Ramadan than non-fasting day. As for proteinous compounds (protein, creatin and urea), their plasmatic rates all increased substantially ($p < 0.05$) in patients during the fasting period of the month of Ramadan.

Key words: Fasting, ramadan, type 2 diabetes.

INTRODUCTION

Ramadan is the ninth month of the lunar calendar observed each year by more than one billion muslims worldwide, during which all healthy adult muslims abstain from eating, drinking, smoking and having sexual intercourse from sunrise to sunset. Sick or traveling people, as well as breast-feeding or menstruating women, are temporarily exempted from complying with this obligation.

After the condition that precluded fasting resolves, exempted individuals have to compensate for the number of days they did not fast, even though the Ramadan month is over. The time of observance differs each year because it is a lunar calendar and as such, fasting from dawn to sunset occurs at a period that varies with the geographical site and the season. In summer months and northern latitudes, the fast could last up to eighteen hours or more. During Ramadan, muslims typically eat two meals each day. The first is taken immediately following the evening call to prayer after sunset and the other is

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typically eaten just before the commencement of the daily fast.

Research that tried to investigate the positive effect of fasting as well as its innocuousness has shown very supportive evidence especially in the treatment of a number of metabolic disorders, such as diabetes (type 2) which result from two interdependent anomalies. The first of which is the resistance to insulin where a lesser sensitivity is displayed by the relevant cells (adipose tissue, liver and muscles) and the second is a lesser hormonal secretion by the pancreas in response to a high glucose rate in the blood (Rodier, 2001).

Despite numerous studies evidencing the beneficial attributes of fasting, its use for medicinal purposes remains controversial. Some believe it is a dangerous and unhealthy practice whereas others advance that this physiological rest is essential to a good healthy life style. Broadly, fasting provides a moment of respite to the organism wherein different organs can recover, a reason that can prop up this practice in the case of certain illness of metabolic nature. Also, we acknowledge its benefits in purifying the body from toxins and functions that were disturbed by over-eating, malnutrition or a bad intake of nutriments. Furthermore it is an efficient way of preventing certain diseases and assure a better hormonal balance (Afifi, 1997; Fakhrazadeh et al., 2003)

Based on current knowledge, majority of studies on type 2 diabetes (DNID) in relation to the fasting Ramadan month were done on sick male subjects with less work been undertaken on females (Azizi and Rasouli, 1987; Laajam, 1990; Al Hader et al., 1994; Athar and Habib, 1994; Dehgan et al., 1994; Ewis and Afifi, 1997; Al Nakhi et al., 1997; Bougerra et al., 1997; Klocker et al., 1997; Khatib, 1997; Uysal et al., 1997; Sulimani et al.,1999). The purpose of this study was to follow the effect of the fasting Ramadan month on the variations of the blood glucose and certain serum lipids in women type 2 diabetic patients treated with Biguanides with age range of 45-53 years and BMI higher than 27 kg/m^2.

MATERIALS AND METHODS

Patients

The trial population comprised muslim women with type 2 diabetes (according to WHO criteria) that practiced Ramadan fasting (World Health Organization and Expert Committee on Diabetes, 1985). An explanatory session was given by the investigators in both French and Arabic languages and written consent was received from each willing participant. Sixty-six patients treated with Biguanides from three Regions (Mostaganem, Mascara and Relizane) located in the West of Algeria participated in this study. Their ages ranged from 45-53 years (mean ± SD = 48.73 ± 2.22) and had a BMI of 27.40 ± 03.08 kg/m^2 (mean ± SD). Patients were excluded from the trial if they had cardiac disease (congestive heart failure, angina pectoris, previous myocardial infarction), impaired kidney or liver function, severe uncontrolled hypertension, severe diabetic complications, or had received therapy with insulin or other investigational drugs that reduces lipidic catabolism such as oral contraception and corticosteroids within the past six months. The study protocol was approved by the ethical committee hospitals of each region.

Study design

The study was performed in October 2003, which coincided with the holy month of Ramadan. All participating patients were studied a week before Ramadan and at the third week of Ramadan fasting. 10 ml of blood sample was withdrawn from 12 h fasting subjects by venipuncture into dry tubes for the pre-fasting period. To respect the 10-12 h fast in the pre-Ramadan period, the blood sampling during Ramadan must be done at 1300 h. Samples were allowed to clot and the serum centrifuged, divided into aliquots and stored at -18°C until analyzed. On both test days, glucose, glycosylated haemoglobin (HbA1c), insulin, triglycerides, high-density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), proteins, creatinin and urea were measured from each of the sixty-six blood samples. During Ramadan, the subjects slept uninterruptedly from 0200 to 0800 h and their average sleep time was thus 1 h shorter during Ramadan than it had been during the control period. Daily working hours in Ramadan are from 0900 to 1500 h; but on the non-fasting days, work starts at 0800 h and finishes at 1700 h.

Measurements

Anthropometric measurements were performed on subjects in light clothing and without shoes. Height was measured to the nearest 0.5 cm and body weight was measured on a level balance calibrated daily and recorded to the nearest 0.1 kg. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared (Tchobrousky and Guy-Grand, 1997). Blood samples were drawn after at least 12 h of fasting for determination of serum levels of glucose, total cholesterol (Tot-c), high-density lipoprotein cholesterol (HDL-c) and triglycerides (TG), proteins (P), creatinin (C) and urea by enzymatic methods using Merck reagent kits and Elan 2000 autoanalyser. High density lipoprotein cholesterol (HDL-c) was measured in the supernatant after precipitation of apolipoprotein B-containing lipoproteins with dextran sulfate and magnesium chloride. LDL cholesterol was calculated with the Friedewald formula [LDL-c = Tot-C-(TG/5 + HDL-c)] when triglycerides concentrations were <5.0 mmol/L. Subjects with triglycerides levels >5.0 mmol/L were excluded (Friedewald et. al., 1972). Procedure for the separation and measurement of the percentage of HbAlc glucosylated haemoglobin in blood samples, in which a) haemolysate is obtained from blood sample; b) the said haemolysate is used to impregnate a weak cationic exchange resin; c) a first buffer solution is passed through the resin to effect the selective elution of the glucosylated haemoglobins HbAla and HbAlb; d) a second buffer solution is passed through the resin to effect the selective elution of the HbAlc glucosylated haemoglobin; and e) if required, the HbA1c content of the collected eluate is analysed and compared with the total haemoglobin present in the haemolysate (Schnek and Schroeder, 1961). To remove antibody-bound insulin, plasma was prepared immediately after venipuncture, mixed with an equal volume of 30% polyetheylene glycol and centrifuged immediately (Hanning et. al., 1985). Plasma free insulin was measured by radioimmuno assay (Soeldner and Slone, 1965). Trained interviewers administered a structured questionnaire to collect information about age, medication and the consumed diet.

Statistical methods

Data were analyzed using the StatBox 6 and were expressed as mean and standard deviation (SD). Parametric values were compared with one way ANOVA and student's t test. The level ($p < 0.05$) was considered as the cut-off value for significance.

Table 1. Change in body mass index, serum glucose and glycosylated haemoglobin (HbA1c) in type 2 diabetic patients.

Parameters	Pre-Ramandan	Ramandan	P -value
Body mass index (Kg/m^2)	27.395 ± 03.082	27.568 ± 03.546	0.36636
Glucose level (g/L)	01.847 ± 00.200	01.983 ± 00.171	0.00002
HbA1c (%)	09.520 ± 03.272	08.748 ± 03.251	0.00078

Values were expressed as mean± SD.

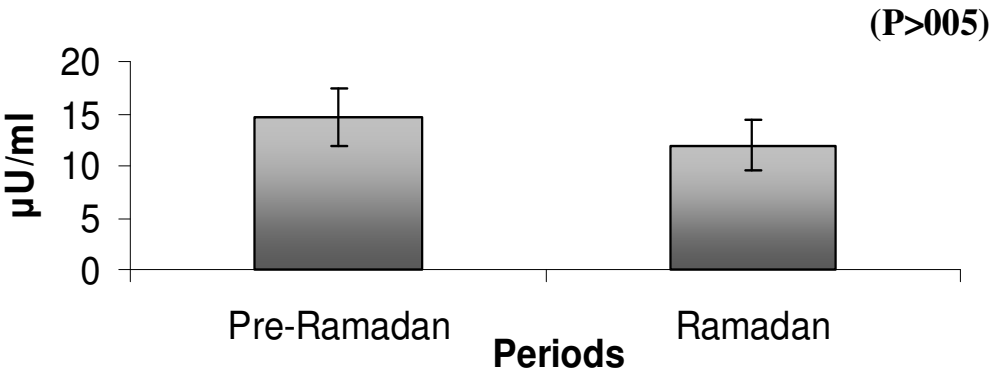

Figure 1. Changes in insulin level in type 2 diabetic patients during the pre-Ramadan and Ramadan period.

RESULTS

Body mass index (BMI), blood glucose and glycosylated haemoglobin (HbA1c)

A total of sixty-six patients with type 2 diabetes, aged 48.73 ± 2.22 years (mean ± SD) were randomized and received a medical treatment in Biguanides. The BMI, measured one week before fasting and third week of Ramadan fasting did not change significantly (mean values ± SD = 27.40 ± 03.55 vs. 27.57 ± 03.08 Kg/m^2). Concerning the glucose levels in the blood, the average values recorded were significantly ($p < 0.05$) higher in the serum of subjects during the fasting period (01.98 ± 00.17 [mean ± SD] g/L) when compared to the level before Ramadan (01.85 ± 00.20 [mean ± SD] g/L). By comparison at the period before the Ramadan, the rates of glycosylated haemoglobin (HbA1c) decreased slightly at $P < 0.05$ from 9.52 to 8.74% among the diabetic patients during the last week of the month of Ramadan. This is shown in Table 1.

Insulin

In general, during Ramadan the average content of plasma insulin among diabetic women were significantly lowered than during the non-fasting period at $P < 0.05$. The values obtained were 14.62 ± 02.79 vs. 11.97 ± 02.48 µU/ml (mean values ± SD) (Figure 1).

Lipidic measurements

Values of HDL cholesterol in the blood were statistically higher ($P < 0.05$) in Ramadan (00.67 ± 00.29] g/L) than the first period before the Ramadan (00.56 ± 00.17 g/L). Moreover, serum total cholesterol, triglyceride, LDL cholesterol and VLDL cholesterol levels decreased remarkably ($P < 0.05$) during the third week of Ramadan than during the non-fasting days (01.84 ± 00.47, 01.49 ± 00.21, 00.91 ± 00.37 and 00.30 ± 00.04 vs 02.10 ± 00.47, 01.77 ± 0.30, 01.22 ± 00.53 and 00.36 ± 00.06 g/L) (Table 2).

Proteinous measurements

During Ramadan, the average content of plasma proteins among diabetic patients were remarkably higher than during the non-fasting period ($P < 0.05$); (mean values ± SD = 80.34 ± 04.75 vs. 73.80 ± 03.25 g/L). Plasma creatin content were insignificantly higher in the second period (that is, Ramadan) with a value of (9.51 ± 1.23 mg/L) compared to (8.47 ± 1.35 mg/L) in the first period before fasting. Finally, urea was statistically ($P < 0.05$) more important during fasting than non fasting period; (0.29 ± 00.03 vs. 0.26g/L ± 00.08 µU/ml) (Table 3).

DISCUSSION

During the month of Ramadan fasting, muslims world

Table 2. Changes in lipid levels in type 2 diabetic patients.

Lipid level (g/L)	Pre-Ramandan	Ramandan	P -value
Total cholesterol	02.101 ± 00.473	01.836 ± 00.473	0.00004
Triglyceride	01.769 ± 00.304	01.489 ± 00.213	0.00001
HDL cholesterol	00.557 ± 00.165	00.67 1 ± 00.285	0.00015
LDL cholesterol	01.219 ± 00.527	00.906 ± 00.374	0.00003
VLDL cholesterol	00.356 ± 00.060	00.298 ± 00.043	0.00001

Values were expressed as mean±SD. HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very-low-density lipoprotein.

Table 3. Change in proteinous parameters in type 2 diabetic patients.

Proteinous parameters level (g/L)	Pre-Ramandan	Ramandan	P -value
Proteins	73.801 ± 03.251	80.340 ± 04.750	0.00650
Creatinin	08.471 ± 01.230	09.510 ± 01.230	0.26000
Urea	00.255 ± 00.031	00.291 ± 00.031	0.00325

Values were expressed as mean± SD.

wide consume two meals between sunset and dawn and there is no restriction on the amount or type of food eaten at night (Laajam, 1990; Salman et al., 1992). People may alter their sleeping habits and smoking is limited to night time only. Furthermore, most diabetics reduce their daily activities during this period out of fear of hypoglycemia (Laajam, 1990; Ewis and Afifi, 1997). These factors may certainly explain not only a lack of weight loss, but also a weight gain in such patients. It appeared that in the study conducted, the women with non-insulin dependent diabetes mellitus (NIDM, Type 2 Diabetes) did not present a weight loss during the Ramadan fasting, their body mass index (BMI) levels remains relatively high and maintained fairly close than before fasting period. A review of literature showed controversy about BMI changes in diabetics of male sex during Ramadan. In one group of studies, patients had an increase in their BMI (Rashed, 1992; Klocker et al., 1997). In another group, there were no changes (Laajam, 1990; Ewis and Afifi, 1997; Uysal et al., 1997; Sulimani et al., 1999) while still others reported decrease in BMI (Azizi and Rasouli, 1987; Mafauzy et al., 1990; Athar and Habib, 1994; El Ati et al., 1995; Al Nakhi et al., 1997; Khatib, 1997). According to El Ati et al. (1995), the reduction in the insulin concentration during fasting could be an adaptive mechanism with the safeguarding of the standard weight. In addition, it is well established that the treatments with the Biguanides are often prescribed to the type 2 diabetes showing an overload of weight (Ralph and DeFronzo, 1999).

Blood glucose and glycosylated haemoglobin (HbA1c)

In respect to most cases, no episode of acute complications (hypoglycemic or hyperglycemic types) occured in patients under medical treatment with the Biguanides and only a few cases of biochemical hypoglycemia with-out clinical hazards has been reported. The present results suggest the possibility that increased blood glu-cose levels at the third week of Ramadan may be related to increased gluconeogenesis. These results are in line with those of (Nagra and Giliani, 1991) who reported a 10% increase in glucose level towards the end of Ramadan in adult's males and attributed it to gluconeo-genesis. The glycemia levels noted during the fasting un-doubtedly results from hepatic overproduction of glucose following an activation of the gluconeogenesis in order to compensate for the fall in glycogenolysis (Meyer et al., 1998). Other studies had shown significant decrease in blood glucose towards the end of the fasting month and it was explained by the subjects' intake of hypocaloric meals (Malhotra et al., 1989). The findings are also contrary to those announced by Muazzam and Khalique (1959), Angel and Schwartz (1975) and Iraki et al. (1997) who found non significant change in blood glucose during this period. These differences may be attributed generally to the amount or type of food consumed, changes in body weight, regularity of taking medications and decreased physical activities during the fast (Gwinup et al., 1963; Koren et al., 1987). From these studies, one may assume that during the fasting days which follow a rather large meal taken before dawn (Sahur), the stores of glycogen, along with some degrees of gluconeogenesis maintain serum glucose within normal limits. From the period before the fasting to Ramadan period, the results showed a significant reduction ($P > 0.05$) of the rate of glycosylated haemoglobin (HbA1c) among diabetic patients treated with Biguanides. Several studies had shown that serum HbA1c values (Chandalia et al., 1987; Athar and Habib,

1994; Belkhadir et al., 1994; Sulimani, 1997; Sulimani et al.,1999; Farad Bensenouciet et al., 2002; Azizi and Siahkolah, 2003; Yarahmadi et al., 2003; Sari et al., 2004) did not change during Ramadan fasting. Other studies suggested that HbA1c levels decreased during this month (Mafauzy et al., 1990; Sulimani et al., 1999; Gustaviani et al., 2004). The reduction in the rate of glycosylated haemoglobin (HbA1c) is a proof justifying the beneficial effect of the medicinal treatment taken during the Ramadan fasting. The glycosylated haemoglobin results from a slow and irreversible nonenzymatic fixation of glucose (or other oses) on haemoglobin during the lifespan of the red globules of blood. This denomination definitively replaced the unsuitable term of glycosyled haemoglobin; the glycosylation is an enzymatic mechanism of proteinic biosynthesis (ANAES, 2000). The HbA 1c is henceforth recognized as the only reference dosage use to ascertain balance of diet consumed by the diabetics, of therapeutic measurements to undertake and the necessity in certain situations to modify the treatment to reach the objectives recommended among the patients (Drouin et al., 1999; ANAES, 2000). The rate of HbA1c is directly proportional to the concentration of glucose in blood. It is independent of the daily variations of the glycaemia and is not affected by physical exercise, fasting or recent ingestion of sugar. Its dosage among patients does not require 12 h of fasting (ANAES, 2000). At the second period of the month of Ramadan, a rather significant reduction in the rates of HbA1c which thus testify to the beneficial action of the medicinal treatment undertaken by the patients particularly during the period of fasting was recorded. Each point gained on the reduction of HbA1c results in an unquestionable clinical benefit, as suggested by majority of preliminary studies (ANAES, 2000). The D.C.C.T (diabetes control and complications trial) advances the same ideas in which a reduction in HbA1c remarkably decreases the risks of specific complications among the diabetic patients (Drouin et al., 1999). Also, the UKDPS (UKPDS, 1998) has noted clearly the clinical benefit which can bring back significantly a plasmatic reduction of 1% of HbA1c in terms of reduction in the risk of micro and macro vascular complications among the type 2.diabetics

Lipidic measurements

As seen in this study, LDL-cholesterol was lower in the serum of 21 day Ramadan compared to levels before Ramadan. Also, HDL-cholesterol levels were higher in the serum subjects at the end Ramadan compared to levels before Ramadan. These results were in agreement with those reported by certain authors on healthy persons (Leake and Rankin, 1990; Maislos et al., 1993; Aldouni et al., 1998; Temizhan et al., 2000; Toda and Morimoto, 2000). Few studies have reported increases in high density lipoprotein (HDL) cholesterol in diabetics during Ramadan (Dehgan et al., 1994; Khatib, 1997; Uysal et al., 1997). One report indicated an increase in low density lipoprotein (LDL) cholesterol and decrease in HDL-cholesterol (Bougerra et al., 1997). These variations certainly result from the differences in the food habits and the composition of meal consumed by the studied populations. Recent data reported by Lacono and Dougherty (1991) have shown that ingestion of poly unsaturated fatty acid (PUFA) in fat diet reduces LDL–cholesterol without affecting HDL-cholesterol, when the PUFA percentage is not higher than 11% of total energy intake. It appears that the increase of HDL-c values during the third week of fasting coincides with the reduction of blood VLDL-c levels. The increase in the hydrolysis of hepatic VLDL-c undoubtedly favored the synthesis of the HDL-c at the diabetics (Basdevant, 1994). The previous data concerning HDL-cholesterol explain clearly the beneficial effect of Ramadan fasting on serum lipids of diabetics.

Serum total cholesterol and triglyceride levels decreased significantly ($P< 0.05$) towards the end of the study. Most patients with NIDDM showed no change or slight decrease in concentration of total cholesterol and triglyceride (Al Hader et al., 1994; Dehgan et al., 1994; Al Nakhi et al., 1997; Bougerra et al., 1997; Ewis and Afifi, 1997; Klocker et al., 1997; Khatib, 1997; Uysal et al., 1997). Concerning the healthy subjects, it seems that total cholesterol levels increased during Ramadan fasting whilst triglyceride levels were unaffected (Fedail et al., 1982). Other studies (Gwinup et al., 1963; Irwin and Feeley, 1967; Gumaa et al., 1978; Fedail et al., 1982; Shokry MI, 1986; El-Hazmi et al., 1987) have reported increased levels of serum cholesterol or triglycerides levels at the end of Ramadan fasting. In all, changes in blood lipids observed seem to be variable and depend probably on the quality and quantity of food consumption and the degree of weight changes. It has been recently shown that consumption of low fat diet induces a decrease in serum cholesterol concentration only when accompanied by body weight loss (Lichtenstein, 1994). In fact, some studies have suggested a correlation between weight lost and decrease in total cholesterol levels (Sakr, 1975). In addition, the increase in the glucose levels during the fasting undoubtedly reduced the catabolism and the significant mobilization of the triglycerides from peripheric tissues to plasmatic circulation. These results may be also explained by different food habits of the studied populations and the type of food consumed during Ramadan as there is a tendency towards increased intake of carbohydrate and fat.

Proteinous measurements

Comparing the benchmark period (non fasting period), a more significant level of proteins in the serum was found during Ramadan among patients treated with Biguanides. This can be the result of over-expression of certain hor-

mones during prolonged fasting periods, such as glucagon which can stimulate a higher mobilization of some constituents of peripheral tissues such as amino acids (Jensen et al., 1998). This amino acid surge is usually caused by the need to compensate through glucogenesis the deficit in glucose in the peripheral tissues (Herman et al., 2000). According to Herman et al. (2000) protein levels have the same patterns as BMI and thus muscular mass. This has been confirmed by the results gotten from this study where patients following Biguanides treatment have a higher values of BMI particularly during the Ramadan fasting.

Concerning urea, the average values during the entire course of the experiment were within the range found in a healthy person (0.15 to 0.5 g/l). Albeit during fasting, urea rates were significantly higher among Biguanides users. This might be the cause of the slight dehydration due to abstinence from drinking during fasting period which led to a fall in diuresis and a consequent rise in urea in the blood (Bonneau, 2001). Also ammoniac discharge following desamination and disamidation of certain amino acids used in the process of neoglucogenesis is the primary cause of an intense synthesis of hepatic urea (Nagra et al., 1998). There was a clear rise in creatinin during Ramadan, among Biguanides users. This can be explained by an absent in protein mobilization and/or low renal clearance. Lacour (1992) indicated that creatinin does not depend only on glomerular filtration but also on certain food substances rich in this element such as meat product whose consumption probably increases during this month.

Insulin

The insulin rates decreased significantly ($P < 0.05$) in diabetic women during the Ramadan compared to the period before the fasting. These results were in agreement with those advanced by Sadr et al. (2001) and Al arouj et al. (2005). In contrast, Bagraic et al. (1994) suggested that plasma insulin and C-peptide levels do not change during the Ramadan fasting. According to a recent study, these two variables were reported not to have changed in women but to decrease in men (Yarahmadi et al., 2003). When bloods samples are taken at 4 h interval, the results showed that plasma insulin and C-peptide level increased at 08:00 h and decrease later in the day (Khatib and Shafagoj, 2004). Insulin resistance decrease in both genders during Ramadan, but this change was significant only in men (Yarahmadi et al., 2003).

The reduction in the insulin secretion is probably the most important phenomenon endocrine function during the food fasting. Its weak production accompanied by a notable secretion of glucagons during the month of Ramadan are undoubtedly the principal elements allowing the activation of lipolysis, the start up of gluconeogenesis and muscular proteolysis (Barrè et al., 2001; Basdevant et al., 2007). Thus, during the prolonged fasting, the maintenance of a certain plasmatic insulin production undoubtedly avoids the exaggerated manifesttation of certain metabolic reactions such as lipolysis and cetogenesis (Salti et al., 2004).

Conclusions

In conclusion, it appears that the holy month of Ramadan fasting was not harmful to the health of diabetic women studied. It however showed a beneficial effect on the evolution of glycosylated haemoglobin and certain studied lipidic parameters such as HDL-c and LDL-c. It would be extremely interesting to know if the Ramadan fasting affects the atherogenic lipoproteinic particles (LP B, LP B:E, LP B:CIII, among others), particularly in obese diabetic patients.

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