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Evaluation of Dry Fasting Effect during Ramadan on Protein Glycation in Saudi Individuals

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The non-enzymatic protein glycation alters protein function and contributes to many biochemical disorders. The accumulation of advanced glycation end-products (AGEs) is associated with the complications of diabetes, kidney disease, metabolic disorders and degenerative diseases.
Objectives: This study was designed to measure the level of some glycated proteins such as glycated insulin and glycated hemoglobin in Saudi volunteers before and after dry fasting in Ramadan.
Methods: Blood samples were collected twice; first sample (before) and second sample (after) dry fasting. The study included 45 Saudi male volunteers, whose ages ranged between23 and 52 years They were divided into four groups: healthy, type II diabetic patients, smokers, and obese individuals. Plasma glucose level and HbA1c were measured immediately using (Beckman coulter). Assessment of insulin resistance was determined using the equation of homeostasis model for

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insulin resistance (HOMA-IR). Plasma insulin level and glycated insulin % were assayed by TSQ Vantage Triple Stage Quadruple LC/MS Mass Spectrometer from Thermo Scientific.

Results: Level of HbA1c, HOMA IR and glycated insulin in healthy, type II diabetic patients, smokers, and obese individuals showed significant decrease in the second sample. The level of HbA1c showed a significant decrease (*p-value <0.0001*) after dry fasting (5.69 ± 1.55 %), (6.14 ± 1.77%). The level of HOMA IR showed significant decrease (*p-value <0.0001*) after dry fasting (1.97 ± 1), (2.46 ± 1.28). The level of glycated insulin showed significant decrease (*p-value <0.0001*) after dry fasting (6.25 ± 2%), (7.71 ± 2.48%).

Conclusion: Glucose, HbA1c, HOMA IR, and glycated insulin levels showed a significant decrease after dry fasting. Reduced protein glycation and formation of advanced glycation end products, can improve the clinical course of many medical disorders. More researches are needed to understand the molecular mechanisms behind glycation-induced clinical disorders and diseases, as well as to correlate these mechanisms to the diagnosis and prevention of lifestyle-related diseases.

Keywords: Protein glycation; glycated insulin; maillard reaction; dry fasting; insulin resistance.

1. INTRODUCTION

Non-enzymatic binding of sugar to proteins, also known as the Maillard reaction, has gained the interest of scientists for a long period. This condensation of a reducing sugar, such as glucose, with a protein is referred to as "glycation." It was renamed in honor of the pioneer in this field in 1912 [1]. Maillard reactions were discovered and documented for the first time by John E. Hodge in 1955 [2], as glycation relates to health, it was first examined in the food science and preparation field. When HbA1c was discovered to be a ketoamine (abyproduct of glycation) in 1977, researchers began to support the idea of in vivo protein glycation in diabetes research [3-5]. “The non-enzymatic posttranslational modification of carbonyl groups of reducing sugars and free amino groups of proteins is known as advanced glycation end products (AGEs). There are two types of AGEs: endogenous and exogenous AGEs. The endogenous type, develops as the body ages, while the exogenous type is produced during food processing” [1]. Adverse effects on tissue function have been linked to increased AGE formation. It interferes with enzymes and macromolecular activity, as well as binding to leukocyte surface RAGE receptors, both of which cause oxidative stress and inflammation [2]. Hyperglycemia is the outcome of reduced insulin signaling and/or insulin secretion, which are the underlying cause and risk factor of type 2 diabetes mellitus (T2DM). Diabetes complications such as macro- and microvascular issues become more likely when glycemic control is compromised. It has been found in numerous researches that advanced glycation end products (AGEs) are linked to various health issues. AGEs and their precursor molecules are also elevated in smokers and obese people. Researchers estimated that between 25 and 75 milligrams of AGEs are consumed daily, with some of that amount being absorbed by the digestive system [3]. When compared to the average adult's daily intake, eating foods high in AGE can raise daily AGE intake by 25%. Fasting during the holy month of Ramadan is regarded as a unique example of intermittent fasting. Dry fasting during Ramadan is one of the required acts of Muslims worldwide. During Ramadan, Muslims abstain from eating, drinking, and smoking from dawn until dusk [3]. For example, the frequency of large meals is reduced from three to two times per 24 hours, sleep patterns are altered such that overnight sleep is shifted to the daytime, and physical activity is enhanced during prayer. Smoking frequency is reduced owing to a smoking prohibition during the daytime [2]. Body weight and metabolic processes are expected to be affected by lifestyle changes lasting a month, including lipid profiles, inflammation mediators, and insulin sensitivity [3]. Numerous physiological and psychological changes take place throughout Ramadan. When proteins are exposed to glucose for an extended period of time due to changes in glucose tolerance and slower protein turnover with age, AGE buildup is one of these processes [1]. Ramadan fasting may limit the development of intermediate glycation products and collagen fluorescence, which is a measure of AGE content, and hence prevent the cross-linking of protein glycation [3]. “Even though non-enzymatic glycation of proteins like insulin and hemoglobin HbA1c is a well-known phenomenon associated with hyperglycemia in both type 1 and type 2 diabetes, the effects of such glycation on receptor proteins like the IR have yet to be studied” [4]. “It is estimated that two extra

glycation sites, the N-terminus glycine of the A chain or the B chain's one and only lysine 29, are affected by prolonged incubation at one of these sites. The N-terminus of the B chain contains phenylalanine 1, valine 2, and glutamine 4, while the B chain's lysine 29 and the A chain's glycine 1 and glutamine 5 are located in the A chain. Other studies have shown that insulin contains six glucose-binding sites" [5]. "Tyrosine 14 and histidine in the A chain are two of the three additional binding sites found across the A and B chains, each involving one or more tyrosine residues. Copper ions can glycate insulin's histidine, according to Cheng and Kawakishi" [6]. "There is a lower affinity for these glucose-binding sites for D-galactose, D-mannose, and 2-deoxy-D-glucose. Insulin can be glycated by fructose too. As a result of the discovery that insulin can be rapidly glycated, as well as because the insulin receptor (IR) shares a number of insulin-like regions" [7]. "Insulin glycation has the potential to alter its properties, thereby altering its ability to function. After just 5 to 10 minutes, the pancreas produces, and stores insulin, and glycation has been shown to occur in insulin and pro-insulin during this time period" [8]. "The first glycation of insulin by glucose occurs at phenylalanine 1 at the N-terminus of the insulin B chain. With lengthier incubations, an average of two glycations occur per insulin at one of two additional glycation sites: the A chain's N-terminus glycine-1 or the B chain's lone lysine 29.The rapidity with which insulin is glycated and the preference for glycation of specific sites is consistent with other research that has shown the existence of six glucose binding sites on insulin, including one involving the phenylalanine 1, valine 2, and glutamine 4 at the N-terminus of the B chain, another involving the lysine 29 of the B chain, and a third involving the glycine 1 and glutamine 5 of the A chain" [7]. Cheng and Kawakishi revealed that "in the presence of copper ions, the histidine on insulin may be glycated. D-galactose, D-mannose, and 2-deoxy-D-glucose bind to these glucose binding sites with less affinity" [6]. Using the documented link between protein glycation and dry fasting, we anticipated that the negative energy balance imposed by fasting might alter AGE levels in the bloodstream. Saudi subjects were studied before and after dry fasting to determine levels of glycated proteins like (Glycated Insulin and HbA1c) in the current study. The novelty of this study is that, it provides platform for more evaluation in the future, of the health benefits of dry fasting during Ramadan. Glycated protein and anthropometric measurements, age, and way of life were examined for any probable relationship (smoking, food habits, sleep disturbance and physical activity). Protein glycation and Advance glycation end products have been linked to a wide range of clinical conditions [4]. It was investigated whether dry fasting might have an influence on protein glycation, which could lead to new recommendations for modifying one's lifestyle.

2. MATERIALS AND METHODS

This study was conducted at Clinical Biochemistry department, King Abdelaziz University and King Fahd Medical Research Center in Riyadh Saudi Arabia, during the period from April 2020 to June 2022. A number of 45 Saudi male volunteers were recruited, whose ages ranged between 23 and 52 years with a mean value of 33.92 ± 8.1years. They were classified into four groups; healthy control (N=12), smokers (N=11), obese with BMI > 30 (N=11), and T2DM (N=11).

All individuals were informed about the objectives of the study with informed consent. A well-set questionnaire was used to gather information concerning; personal, family, and medical history, lifestyle, sleep hours, food habits, weight and height, and calculated BMI. Clinical examination finding, and laboratory investigations were also recorded. Individuals with chronic illness such as liver, renal, cardiovascular disease, chronic pulmonary disorders, and any other endocrine disorder apart from Type II DM were excluded, in addition to those who refused to sign the consent.

2.1 Sample Collection

A volume of 5 ml of venous blood was collected from each volunteer after an overnight fast twice. One sample was taken during the last week of Shaban before dry fasting of Holy Ramadan and, the other one during the first week of Shawal after fasting during Holy Ramadan in sterile tubes containing EDTA as an anticoagulant. Blood HbA1c and glucose were then measured immediately before plasma was separated by centrifugation at 3000 rpm.

2.2 Biochemical Analysis

Plasma glucose level was measured immediately by Hexokinase method (beckman coulter). HbA1c was measured immediately using

(beckman coulter). Plasma insulin level and glycated insulin % were assayed by TSQ Vantage Triple Stage Quadruple LC/MS Mass Spectrometer from Thermo Scientific. Assessment of insulin resistance was determined by using the equation of homeostasis model for insulin resistance (HOMA-IR) (107).

$$\text{HOMA-IR} = \frac{\text{Insulin (Mu/L)} \times \text{Glucose (mg/dl)}}{405}$$

2.3 Glycated Insulin Assay (Reagents)

Assay applied by Triple Stage Quadrupole LC/MS TSQ Vantage LC/MS Mass Spectrometer from (Thermo fisher). Methanol9%, Ethanol99% and Chloroform99 were used in sample preparation are from (Sigma Aldrich). Insulin, Human Recombinant from (Sigma Aldrich). D-glucose, phosphate buffer PH 7.4 and sodium azide were used in preparation of glycated insulin from (KAU, college of medicine, clinical biochemistry department Labs). In LC/MS experiment we used column 50 x 2.1 mm Hypersil Gold 1.9um from (Thermo fisher).

2.4 Preparation of Glycated Insulin Control

In 0.1 M phosphate buffer (PH 7.4), glucose and insulin stock solutions (400 mM and 300 mM, respectively) were prepared. Sugars mixed with insulin were used as a control solution in this experiment. Sodium azide at a concentration of 0.02% was used as a preservative in all 30-day incubations in Eppendorf tubes. Incubated samples were then stored at -20°C until further examination.

2.5 Sample Preparation

100 µL of plasma samples were pipetted into 1.5 mL Eppendorf tube, to which 400 µL methanol was added and vortexes thoroughly, then 100 µL chloroform was added and mixed, then 300 µL H_2O was added and the mixture became cloudy with precipitate and mixed well. The tubes were centrifuged for one minute in 14,000 g. Three layers were seen: a large aqueous layer on top, a circular flake of protein in the interphase, and a smaller chloroform layer at the bottom. The top aqueous layer was removed carefully, trying not to disturb the protein flake, then 400 µL methanol was added and mixed well by vortex. Then the tubes were centrifuged for 5 minutes in 10,000 g, and buffy precipitate at the tube wall appeared. Excess methanol was removed as carefully as possible. Because the pellet was delicate. All methanol was removed except few µL of it left to speed drying. Then, the tubes were dried, and stored at -20^{o}C. Sample were prepared at king Abdulaziz University, college of medicine in clinical biochemistry department lab.

2.6 Mass Spectrometry (LC/ MS)

The MS/MS detector was a TSQ Vantage (Thermo-Fisher) triple quadrupole mass spectrometer with an ESI probe connected to the TLX-4 system. Hypersil Gold 1.9um, 50 x 2.1 mm, was the column used. 0.1 % of formic acid in deionized water was used in buffer A, and acetonitrile acidified with 0.1 % formic acid was used in buffer B. The trapping column was loaded with 20 L (injection volume) of the sample solution and 90 % buffer A at a flow rate of 350 L/min for 3 minutes before the target peptides were injected into it. The gradient began at 10% B, climbed to 90% B in 3 minutes, climbed to 80% B in 0.5 minutes, held at 80% B for 0.5 minutes, and then returned to 90% A for 3.60 minutes. A positive scan using analyst software with the following settings: 3000 volts, 50 atmospheres of sheath gas pressure (arbitrary units), 10 atmospheres of auxiliary's gas pressure, 32-degree Celsius capillary temperature (arbitrary units), 500 atmospheres of vaporizer temperature, 1.5 atmospheres of collision pressure. The peak width of both Q1 and Q3 has been set to 0.7 mp. In the positive ion mobility mode, the mass spectrometer performed a full scan analysis. For our experiments, we used TSQ Vantage at the King Fahd Medical Research Center in Riyadh and at the King Abdulaziz Medical City in Jeddah (Thermo-Fisher).

2.7 Statistical Analysis

Data was presented as means ± SE where appropriate. Statistical analysis was carried out using a one-way ANOVA with post hoc test (Dunnett or Student-Newman-Keuls test) and Student's *t* test using the computerized package. Differences were considered significant at $P < 0.05$.

3. RESULTS

This study was designed to measure the level of some glycated proteins such as glycated insulin and glycated hemoglobin in Saudi volunteers before and after dry fasting. In total participants,

we found highly significant differences between all parameter in 1st sample (before dry fasting) and second sample (after Ramadan dry fasting) as described in Table 1. Insulin level revealed a significant decrease (*p-value <0.0001*) after Ramadan dry fasting (7.8 mIU/L± 1.91) compared to before dry fasting (6.7 mIU/L± 1.98). Glucose level showed a significant decrease (*p-value <0.0001*) after Ramadan dry fasting (111.97± 29.9mg/dl) compared to before dry fasting (120.16± 35.57mg/dl). Regarding HbA1c, the obtained results showed a significant decrease (*p-value <0.0001*) after Ramadan dry fasting (5.69 ± 1.55 %) compared to before dry fasting (6.14 ± 1.77%). Looking for HOMA IR, it showed significant decrease (*p-value <0.0001*) after Ramadan dry fasting (1.97 ± 1) compared to before dry fasting (2.46 ± 1.28). Glycated insulin showed significant decrease (*p-value <0.0001*) after Ramadan dry fasting (6.25 ± 2%) compare to before dry fasting (7.71 ± 2.48%) as describe in Table (1).

Table 1. Level of insulin, glucose, HOMA, HbA1c and glycated insulin before and after Ramadan (dry fasting) in total participants

	Pre-fasting	Post-fasting	p-value[§]	% Change
Insulin mIU/L	7.8 (1.98)	6.7 (1.91)	<0.0001	15.4 (7.7)
Glucose mg/dL	120.16 (35.57)	111.97 (29.90)	<0.0001	7.5 (6.4)
HOMA	2.46 (1.28)	1.97 (1.05)	<0.0001	20 (7.9)
HbA1c %	6.14 (1.77)	5.69 (1.55)	<0.0001	6.9 (5.8)
Glycated insulin %	7.71 (2.48)	6.25 (2.00)	<0.0001	18.6 (6.1)

Table 2. Comparison of age, sleep hours and smoking

	Diabetes type II		
	Yes	No	p-value
Age (years)	44.3 (6.20)	30.6 (5.17)	<0.0001
Sleep hours	6.8 (1.07)	6.9 (1.27)	0.70
Smoking	27.3	38.2	0.51

Table 1. Levels of insulin, glucose, HOMA, HbA1c and glycated insulin before and after Ramadan (dry fasting) in diabetics type II and non-diabetics

	T2DM(n=11)			
	Pre-fasting	Post-fasting	*p-value*[§]	% Change Mean (SD)
Insulin mIU/L	10.27 (0.57)	9.15 (0.71)	*0.0001*	10.9 (5.5)
Glucose mg/dL	174 (23.81)	159.63 (16.07)	*0.0003*	8.6(6.4)
HOMA	4.36 (0.80)	3.57 (0.56)	*<0.0001*	17.5 (7.1)
HbA1c%	8.87 (1.31)	8.15 (0.94)	*0.006*	7.6 (6.3)
Glycated insulin %	11.35 (1.32)	9.15 (1.10)	*<0.0001*	19.3 (4.1)
	Non-DM (n=34)			
	Pre-fasting	**Post-fasting**	**p-value§**	**% Change Mean (SD)**
Insulin mIU/L	7.23 (1.68)	6.00 (1.49)	<0.0001	16.9 (7.9)
Glucose mg/dL	102.61 (15.25)	96.56 (10.57)	<0.0001	5.2 (6.9)
HOMA	1.84 (0.62)	1.45 (0.50)	<0.0001	20.8 (8.1)
HbA1c%	5.26 (0.59)	4.90 (0.49)	<0.0001	6.6 (5.7)
Glycated insulin %	6.53 (1.34)	5.32 (1.14)	<0.0001	18.4 (6.7)

Table 2. Mean difference of clinical investigation before and after dry fasting of Ramadan by diabetes status

	Non-diabetic	Diabetic	*p-value*[$]
Insulin mIU/L	16.9 (7.9)	10.9 (5.5)	*0.009*
Glucose mg/ dl	5.2 (6.9)	7.8 (4.1)	*0.13*
Homa	20.8 (8.1)	17.5 (7.1)	*0.21*
HbA1c %	6.6 (5.7)	7.6 (6.3)	*0.68*
Glycated insulin %	18.4 (6.7)	19.3 (4.1)	*0.57*

Table 3. Level of insulin, glucose, HOMA, HbA1c and glycated insulin before and after Ramadan (dry fasting) in Smokers and non-smokers

	Non-smokers (n=29)			
	Pre-fasting	**Post-fasting**	**p-value[§]**	**% Change Mean (SD)**
Insulin mIU/L	7.84 (2.15)	6.56 (2.08)	<0.0001	17.1 (6.9)
Glucose mg/ dl	119.72 (27.26)	112 (30.62)	0.0001	7.3 (6.4)
HOMA	2.45 (1.33)	1.94 (0.20)	<0.0001	21.2 (6.9)
HbA1c %	6.18 (1.75)	5.79 (1.55)	<0.0001	5.8 (5.1)
Glycated insulin %	7.76 (2.49)	6.26 (2.07)	<0.0001	19.2 (6.6)
	Smokers (n=16)			
	Pre-fasting	**Post-fasting**	**p-value[§]**	**% Change Mean (SD)**
Insulin mIU/L	8.20 (1.67)	7.15 (1.55)	<0.0001	12.5 (8.6)
Glucose mg/ dl	120.68 (33.25)	111 (29.50)	0.0001	9.1 (7.2)
HOMA	2.48 (1.23)	2.03 (1.02)	<0.0001	17.8 (9.3)
HbA1c %	6.09 (1.85)	5.5 (1.58)	0.0007	8.9 (5.7)
Glycated insulin %	7.65 (2.53)	6.25 (1.94)	<0.0001	17.5 (5.1)

Table 4. Mean difference of clinical investigation and after dry fasting of Ramadan by smoking status

	Non smokers	**Smokers**	**p-value[$]**
Insulin mIU/L	17.1 (6.9)	12.5 (8.6)	0.08
Glucose mg /dl	4.8 (6.7)	7.7 (5.6)	0.13
HOMA	21.2 (6.9)	17.8 (9.3)	0.21
HbA1c %	5.8 (5.1)	8.9 (5.7)	0.11
Glycated insulin %	19.2 (6.6)	17.5 (5.1)	0.36

Table 5. Level of insulin, glucose, HOMA, HbA1c and glycated before and after Ramadan (dry fasting) according to BMI

	BMI <=25 (n=9)			
	Pre-fasting	**Post-fasting**	**p-value[§]**	**% Change Mean (SD)**
Insulin mIU/L	5.57 (1.23)	4.55 (0.96)	0.004	17.4 (13.1)
Glucose	90.78 (9.16)	90.22 (6.61)	0.80	0.22 (6.8)
HOMA	1.20 (0.25)	0.99 (0.25)	0.003	17.2 (11.5)
HbA1c	4.85 (0.37)	4.46 (0.28)	0.006	7.8 (5.6)
Glycated insulin %	5.39 (0.50)	4.56 (0.56)	<0.0001	15.5 (5.6)
	BMI 25-30 (n=16)			
	Pre-fasting	**Post-fasting**	**p-value[§]**	**% Change Mean (SD)**
Insulin mIU/L	7.48 (1.69)	6.33 (1.76)	<0.0001	16.0 (7.5)
Glucose mg/dl	105.56 (22.47)	100.12 (22.03)	0.007	4.8 (7.1)
HOMA	2 (0.87)	1.63 (0.84)	<0.0001	20.2 (9.1)
HbA1c %	5.44 (1.13)	5.18 (1.08)	0.002	4.6 (5.3)
Gly insulin %	6.73 (1.88)	5.48 (1.42)	<0.0001	18.3 (5.0)
	BMI >30 (n=20)			
	Before dry fasting	**After Ramadan dry fasting**	**p-value[§]**	**% Change Mean (SD)**
Insulin mIU/L	9.44 (1.02)	8.12 (1.11)	<0.0001	14.1 (4.3)
Glucose mg /dl	144.85 (35.46)	131.25 (30.59)	<0.0001	9.1 (3.0)
HOMA	3.40 (1.15)	2.68 (0.94)	<0.0001	21.1 (4.5)
HbA1c %	7.27 (1.90)	6.64 (1.64)	0.0001	8.3 (6.0)
Gly insulin %	9.54 (2.10)	7.65 (1.91)	<0.0001	20.2 (6.8)

Table 6. Mean difference of insulin parameters before and after fasting of Ramadan by BMI status

	BMI <=25	BMI >25-<30	BMI >=30	p-value!	p-value$	p-value§	p-value£
Insulin mIU/L	17.4 (13.1)	16.0 (7.5)	14.1 (4.3)	1.0	0.92	1.0	0.55
Glucose mg /dl	0.22 (6.8)	4.8 (7.1)	9.1 (3.0)	0.15	0.001	0.08	0.0009
Homa	17.2 (11.5)	20.2 (9.1)	21.1 (4.5)	1.0	0.69	1.0	0.48
HbA1c %	7.8 (5.6)	4.6 (5.3)	8.3 (6.0)	0.55	1.0	0.17	0.14
Glycated insulin %	15.5 (5.6)	18.3 (5.0)	20.2 (6.8)	0.81	0.17	1.0	0.15

! Bonferroni multiple-comparison test, comparing normal vs overweight individuals
$ Bonferroni multiple-comparison test comparing normal vs obese individuals
§ Bonferroni multiple-comparison test comparing overweight vs obese
£ One-way ANOVA test

Table 7. Level of insulin, glucose, HOMA, HbA1c and glycated insulin before and after Ramadan (dry fasting) in Healthy individuals

	Pre-fasting	Post-fasting	p-value
Insulin mIU/L	5.58 (1.03)	4.38 (0.75)	<0.0001
Glucose mg/ dl	86.77 (5.81)	86.92 (5.72)	0.92
HOMA	1.19 (0.22)	0.92 (0.14)	<0.0001
HbA1c %	4.74 (0.29)	4.54 (0.27)	0.007
Glycated insulin %	5.30 (0.32)	4.38 (0.21)	<0.0001

According to the gained results, the study participants were classified into four groups: Group I: Healthy control (26.6%): comprised 12 healthy volunteers with normal BMI, non-smoker and non-diabetic, group II: involved 11Smoker participant (24.4%), group III: included 11obese individuals (24.4%) with BMI >30 and group IV, 11type 2 diabetics (24.4%). The results revealed direct correlation between glycated insulin % and HOMA IR, and showed that T2DM patient had higher glycated insulin, HbA1c and HOMA -IR than other groups in first and second sample. Obese individuals showed (9.54%) of glycated insulin in first sample (before dry fasting) and (7.65%) in second sample (after Ramadan dry fasting), and their HOMA was 3.40 before dry fasting and (2.68) after dry fasting. Smokers' group, had glycated insulin (7.65%) before dry fasting and (6.25%) after Ramadan dry fasting and 2.48 HOMA before dry fasting and 2.03 after Ramadan dry fasting. In the healthy individuals, the glycated insulin was (5.30%) in before dry fasting and 4.38% after Ramadan dry fasting and their HOMA was 1.19 before dry fasting and (0.92) after Ramadan dry fasting. In T2DM participants, there was significant relation with age, and no significant association with sleep hours and smoking as describe in Table 2.

4. DISCUSSION

Non-enzymatic glycation of protein occurs by a reaction between the aldehyde group of a reducing sugar in the open chain form and the free amino groups of the protein molecule. The resulting labile Schiff's base can subsequently undergo molecular rearrangements to form a more stable Amadori product [9], which after additional dehydration reactions, and further molecular rearrangements, can lead to the formation of AGEs [10]. Our results revealed that, there was significant increase in glycated insulin and HbA1c directly with HOMA IR in T2DM group compared with all study groups. HbA1c has the potential to reflect the history of mean insulin sensitivity over the preceding weeks or months, and it serves as a marker of insulin sensitivity in those who have a normal glucose tolerance and can be used as a diagnostic tool for the early detection of insulin resistance [11].

For the past few years, significant efforts have been made to evaluate the direct role of non-enzymatic glycation reaction in the development of diabetic complications [12]. Glycated insulin has been measured in the pancreas of various animal models of type 2 diabetes and in both isolated islets and clonal β-cells exposed to

elevated glucose concentrations in tissue culture [13]. The observations in animal models have been confirmed and revealed that~9% insulin in type 2 diabetic subjects circulates in glycated form [14]. In previous study they found that measurement of glycated insulin in the plasma of patients with type 2 diabetes, using the euglycemic clamp technique, a pure and chemically characterized glycated insulin has been shown to exhibit a ~70% decrease in glucose-lowering action in normal humans compared with physiological concentrations of native insulin [15]. These observations support our hypothesis of glycated insulin in glucose toxicity and impairment of insulin action in type 2 diabetes.

Several studies in human subjects suggested potential links of AGEs to insulin resistance and oxidative stress. Tan et al (2011) measured AGE levels, associated inflammatory markers, and insulin resistance by the homeostatic model assessment index or HOMA-IR in 207 healthy subjects without diabetes. Serum levels of AGEs correlated in a very statistically significant manner with HOMA-IR in both male and female subjects [16]. Tahara et al (2012) reported on 322 non diabetic Japanese subjects; serum AGE levels correlated with HOMA-IR in these subjects, and after multiple correlation analysis, AGEs, together with waist circumference, the degree of insulin resistance was linked to glycosylated hemoglobin and lipids [17]. Sarkar et al. (2010) investigated the relationship between total carbonyl compounds in serum and HOMA-IR levels in type 2 diabetics and discovered a significantl relationship between insulin resistance and carbonyl levels [18]. Oxidative stress has long been associated with diabetic complications and more recent studies indicate that oxidative stress is also causal in the development of β cell dysfunction and insulin resistance [19]. The results of the present study revealed that, there was significant increase in level of glycated insulin, HbA1c and insulin resistance among obese and smoker groups. High HbA1c levels in obesity and smoking can be used as a screening tool to detect insulin sensitivity and resistance at an early stage. Sayej, W. N et al had reported that reactive glycation products are present in aqueous extracts of tobacco and in tobacco smoke in a form that can rapidly react with proteins to form AGEs [20]. Frati, AC et al found that compared to non-smokers, smokers had significantly higher levels of fasting glucose, fasting insulin and HOMA-IR, this suggest that, smokers have a high risk of developing an insulin resistance and insulinemia, compared with a matched group of non-smokers [21]. Serum insulin concentrations have been shown to be greater in smokers compared with non-smokers, even when controlling for factors that affect insulin resistance. In addition, after smoking three cigarettes, both non-smokers and smokers experienced an impairment in glucose tolerance and elevated insulin resistance was observed [21]. Eliasson et al found a dose-response relationship between quantity of cigarettes smoked per day and degree of insulin resistance [22].

Advanced glycation occurs under a variety of conditions, including oxidative stress and insulin resistance. Insulin resistance is a condition in which cells fail to respond to the normal actions of insulin. Obesity and smoking have been attributed to increase insulin resistance. Gupta et al. [23] conducted a study on the effect of cigarette smoking on insulin resistance in asymptomatic adults and reported statistically significant increase in serum insulin and HOMA-IR index in smokers, ($p < 0.001$). Suganthy et al. [24] conducted a similar study on obesity and insulin resistance among cigarette smokers and reported impaired insulin response in cigarette smokers. It has been postulated that smoking induced biochemical changes in subjects with obesity makes them vulnerable to insulin resistance and T2DM. Targher et al. [25] reported a link between cigarette smoking and insulin resistance in T2DM and associated dyslipidemia. Insulin resistance in adults has been recognized for decades as a cardinal feature in the development of type 2 diabetes and has been associated with obesity, metabolic syndrome, hypertension, and heart disease [26]. Obesity Research Clinical Practice also repeatedly stated that insulin resistance is significantly related to obesity and cardio metabolic risk [27]. In addition, obesity and smoking are linked to increased oxidative stress. Keaney and colleagues1 [28] reported that smoking, diabetes, and obesity are independently associated with increased oxidative stress in men and women in a large community-based cohort.

The results of our second sample revealed that, there was significant decrease in the level of glycated insulin and HbA1c, and insulin resistance in all study groups. In line with our result, a previous study, demonstrated a significant decrease in HbA1c level during

Ramadan dry fasting in diabetic patients. (Kenichi I washige 2004) found that the urine level of AGEs in patients with Rheumatoid Arthritis was significantly reduced by calorie restriction. Two homogenous trials involving long-term (6–16 weeks) caloric restriction showed that low-AGE diets reduced circulating AGEs concentrations in adults with type 2 diabetes [29]. In addition, Permal Deo et al showed significant reduction in AGEs values in overweight but otherwise healthy adults when subjected to 52 weeks of energy restricted diet. Long-term (16 weeks) dietary AGE restriction significantly reduced circulating AGEs concentration in healthy adults [30].

As mentioned, insulin resistance and oxidative stress are linked to the protein glycation. Several studies found that, intermittent fasting and caloric restriction, or alternate-day feeding, had been shown to increase resistance to toxicity and stress and to prolong maximum life span in experimental animals and humans [31]. Guntari Prasetya et al demonstrated that intermittent fasting during Ramadan may lead to concomitant beneficial effects by improving insulin sensitivity and reducing insulin resistance [32], they also found that weight loss, a reduction in fat mass, and a reduction in waist circumference can all increase insulin sensitivity. Therefore, the significant reduction in body weight, fat mass, and waist circumference demonstrated in these studies might be the factors that reduce insulin resistance in participants during Ramadan fasting [33]. Other studies have demonstrated that fasting, even in the absence of fat loss, has resulted in a reduction of leptin levels and an increase of adiponectin, which results in improvements of insulin resistance. It has long been known that fasting can reduce body weight and increase metabolic health [34]. A study by Larson-Meyer et al. showed that 25% calorie reduction via either diet alone or diet in conjunction with exercise led to improvements in insulin sensitivity and reduction in β-cell sensitivity in overweight, glucose-tolerant individual [35]. In addition, Fasting and caloric restriction have been associated with reduced incidence of chronic diseases and cancers. These effects have been attributed to reduced oxidative stress [36]. Faris MA et al suggested that the mechanism is that Ramadan fasting results in oxidative stress reduction, and therefore, lower level of reactive oxygen species [37]. J NutrMetab; they found that a short-term fasting study (7 days) showed a significant reduction in levels of urinary 15FIP among females. (194) Ibrahim et al suggested that Ramadan fasting results in oxidative stress reduction, and therefore, lower level of reactive oxygen species [38].

It is clear from these observations that the concentration of insulin circulating in the glycated form in diabetics, obese and smokers is significant compared with control subjects. Considerable data supports the role of glycation of insulin as a feature of beta-cell dysfunction and insulin resistance. However, individual variations of glycated insulin in subjects from the various groups possibly reflect the collection of a single fasted sample together with possible individual differences in beta cell secretory activity, diet history and lifestyle.

5. CONCLUSION

Glucose, HbA1c, HOMA IR, and glycated insulin levels showed a significant decrease after dry fasting in addition, fasting can decrease accumulation of AGEs by reducing attachment of sugar or sugar derivative to a protein that involves in non-enzymatic glycation via enhance proteolysis and decreases protein synthesis. Reduced protein glycation and formation of advanced glycation end products, can improve the clinical course of many medical disorders. More researches are needed to understand the molecular mechanisms behind glycation-induced clinical disorders and diseases, as well as to correlate these mechanisms to the diagnosis and prevention of lifestyle-related diseases.

CONSENT

As per international standard or university standard, Participants' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethical approval for this study was obtained from King Fahad Medical city. (IRB Number H-01-R-012).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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