

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/



Hemoglobin A1c and blood pressure in type2 diabetic patients



Ahmed Talib Yassen Aldossary¹, Abdullah Ali Mohammed², Ahmed Jasim Nawfal^{3*} Alaa Hashim Alqatab⁴

¹Department of Physiology and medical physics, College of Medicine - University of Anbar, Iraq. ²Department of chemistry and biochemistry, College of Medicine-University of Fallujah, Iraq. ³Department of Physiology and medical physics, College of Medicine - University of Fallujah, Iraq. ⁴Department of Biochemistry, Department of Nursing - College Of Al Safwa University, Karbla, Iraq.

Abstract

Glycated hemoglobin is a strong marker for diabetic control measurements, reflecting average blood glucose levels over the past 3-4 months. This test is affected by many factors and diseases. The objective of this study is to assess the value of glycated hemoglobin in type 2 diabetic mellitus. Most diabetic patients have raised hemoglobin A1c, that is considered to be an important factor in the control of blood sugar levels and the pharmacological efficacy of medicinal products which will never be affected by latest diets as well as treatments. The findings showed that the majority of patients avoided a diabetic control HbA1c level test. High blood pressure (systolic or diastolic) was identified as a significant risk to patients. The mean value of glycated hemoglobin was 8.76%. Hemoglobin A1c > 7% Patients with had markedly increased fasting glucose levels compared to those with HbA1c < 7%. Identifying the level of HbA1c is easier.

Key word: Diabetes mellitus, HbA1c, hypertension, metabolic syndrome.

1. Introduction

The gold standard for assessing long-term glycemic function and exposure to medical care in diabetic patients is glycated hemoglobin. Diabetes mellitus (DM) is described as an increase in blood glucose levels in response to changes in insulin action or development [1, 2]. Hypertension and diabetes are two of the most common major diseases in the world, and they often coexist. Oxidative stress, obesity, and insulin resistance are all essential pathogenesis pathways in both disorders, with insulin resistance being prevalent in prediabetes and diabetes [3, 4].

Many of the acute and chronic complications of diabetes, including type 2 diabetes, can be delayed if blood sugar levels are kept under control [5]. Proper blood sugar and HbA1C regulation play an important role in lowering the delayed complications of diabetes; on the other hand, decreased self-care is linked to a rise in disease complications [6, 7]. Glycated hemoglobin is the product of glucose glycation and hemoglobin amidogen reactions in non-enzymatic catalysis, and it can be used as a key indicator of glycol

metabolism. Most diabetic patients have raised HbA1c, which is identified to be an affected index for the assessment of glycosalated ability and pharmacological properties of medicinal products which will not be affected by medication and recent lifestyle [8, 9].

Khaw et al., The risk of cardiovascular disease in patients with diabetes increased by 10 % to 20% if HbA1c exceeds 1% [10]. A randomized clinical trial of HbA1c in patients with type 2 diabetes and its correlation coefficient with hypertension and coronary heart disease was used. Recent medical data have raised the possibility of variance in glycated hemoglobin [11-14] and blood pressure (BP) [11-13] patients with type 2 diabetes independently predict macrovascular complications and/or cause mortality. However, no research was conducted at the same time to investigate the combined risks involved with fluctuation in HbA1c and systolic BP (SBP). In addition, the variation in effects between HbA1c variability and SBP variability on the frequency of cardiovascular disease (CVD) associated with mean SBP and HbA1c values has scarcely been noticed [18, 19].

*Corresponding author e-mail: <u>dr.physiologist med@uofallujah.edu.iq</u>.; (Ahmed Jasim Nawfal).

Receive Date: 17 May 2021, Revise Date: 27 May 2021, Accept Date: 07 June 2021

DOI: 10.21608/ejchem.2021.76541.3748

2. Methodology

One hundred male patients with type 2 DM were assigned to AL-Yarmouk Teaching Hospital in Baghdad, Iraq. The results indicated that the percentage of patients was excluded by the HbA1c percent test for their diabetic control evaluation. Hypertension (systolic or diastolic) has been evidenced in large numbers of patients. The mean value of glycosylated hemoglobin was 8.76%. Patients with HbA1c > 7 percent had significant high fasting plasma glucose compared to those with HbA1c < 7 percent. Patients with associated diseases such as hypertension, ischaemic heart disease, bronchial asthma, rheumatoid arthritis have not been excluded from the study. The diagnosis of type 2DM was made by physicians in the past. Every patient was asked about the social history of alcohol intake or smoking. Medical history included diseases (hypertension, rheumatoid anemia, bronchial asthma) or drugs (warfarin, acetylsalicylic acid, alpha-methyldopa, vitamins, tramadol, simvastatin) that interfered with wit. The mean blood pressure is calculated using the following equation: mean arterial pressure = diastolic pressure + 1/3 [systolic pressure-diastolic pressure]. Fasting serum glucose An enzymatic colorimetric test based on the reaction of Trinder. Glycated hemoglobin (HbA1c %), a fast ionexchange resin separation method, is used for this measurement. The principle of this measurement is to lyse the whole blood-EDTA with lysing reagents containing detergent and borate ions. The base of the Labile Schiffs was removed and the hemolysate was mixed with the resin exchange which allowed the non-glycated haemoglobin (HbA0) to bind the resin. Then another resin is used to remove the HbA0 bound resin from the supernatant containing HbA1c. The percentage of glycated hemoglobin is determined by recording spectrophotometrically the absorbance of glycated hemoglobin and total hemoglobin at 415 nm with reference to standard hemoglobin data.

Statistical analysis

The results are expressed as number, percentage, range, median, mean + SE. The data are analyzed using Student t test (one and two tailed, unpaired), Chi squared test, simple correlation test with regression equation, taking p < 0.05 as the lowest limit of significant.

3. Results

The characteristics of the study were shown in Table 1. The median age of patients was 59 years, the duration of type 2 DM ranged from 2 months to 38 years (n=98) and the median time was 8 years. At the time of the study, two patients were diagnosed, i.e. new cases. The family history of type 2 DM was found in 56 percent of the subjects, including first and second grade relatives. Twothirds of patients [64%] admitted micro-and / or macro-vascular complications of type 2 DM to the study; half of them [i.e. Twenty-two patients had more than one complication]. Social history revealed that 25 per cent of patients were currently active smokers and 10 per cent were drinking alcohol. Table 2 showed conditions that interacted with type 2 DM at HbA1c%. Sixty-two patients had a history of diseases other than type 2 DM; thirty of them complained of more than one disease. In this study, hereditary or acquired anemia has not been reported. Hypertension was the leading associated disease found in 34 patients, while the other disease contributed 19 %. Due to long duration of diabetes as well as the associated complications, it is expected to find high percent of polypharmacy prescriptions in this study. Acetylsalicylic acid, a drug that interferes with HbA1c % measurement was indicated for 73 patients; 43 of them used it in combination with other drugs. None of patients was prescribed to him any pharmaceutical preparations related to opioids or their derivatives. Statins [hydroxyl-methyl glutyrate Co-enzyme inhibitors] were prescribed in polypharmacy prescriptions rather than monotherapy. Forty-three patients received more than one drug that interferes with HbA1c % measurement.

HEMOGLODIN THE FIND BLOOD I RESSORE IN 111 E2 DIABETIC I THEATS

Table 1	l c	haract	terist	ics	of	pati	ients	

_				
Mean ±	Median			
SE	(Min—			
	Max)			
	59 (32-80)			
	3.6.12			
SE	(Min—			
8 87 <i>1</i> ±	Max) 8 (0.166-38)			
	8 (0.100-38)			
7.117				
e 2 (No.)				
, , ,				
43				
02				
11				
(No.)				
47				
08				
20				
25				
10				
	SE 57.2 ± 10.01 Mean ± SE 8.874 ± 7.447 e 2 (No.) 43 02 11 (No.) 47 08 20 25			

The results are presented as mean \pm SE, median (minimum value-maximum value), and absolute numbers.

Table 2 Concomitant illnesses and drugs intake interfered with HbA1c measurements.

variable	No. of patients		
Concomitant illnesses	_		
No illness	38		
Hypertension	15		
Bronchial asthma	04		
Rheumatoid arthritis	09		
Anemia	0		
Hyperlipidemia	4		
≥2 illnesses	30		
Drugs			
No drug intake	21		
Opiates including	0		
tramadol			
Warfarin	1		
Acetyl salicylic acid	31		
Alpha-methyldopa	0		
Vitamins (C and/or E)	3		
Lipid lowering agents	0		
Local injection of	1		
corticosteroids			
≥2 drugs	43		

Blood pressure

The second feature of metabolic syndrome (Diabetes mellitus) was presented in table 3. The mean systolic/diastolic blood pressure was 129.5/85.1 mmHg and the mean blood pressure was 100 mmHg. Blood pressure \geq 130/85 [i.e. the level of metabolic syndrome evidence] was found in 33%.

Table 3. Blood pressure measurements

Table 3. Di	ioou pressure me	easur ements	
Variable	Mean \pm SE	Median	No. of
		(Min—	patients
		Max)	
Blood			
pressure			
(mmHg)			
Systolic	129.50 ± 25.0	130 (74-	
-		182)	
Diastolic	85.1±13.25	80 (65-	
		121)	
Mean	100 ± 15.25	96.3 (74-	
		137.3)	
Systolic		ŕ	52
BP > 130			
Diastolic			39
$BP \ge 85$			
BP			33
<u>≥</u> I30/ <u>></u> 85			

Biochemical monitoring of diabetes mellitus

The median fasting blood glucose and HbA1c % were 167 mmol/l and 8.3% respectively that reflected uncontrolled diabetes (Table 4). Only one patient had normal HbA1c % value. < 5% and 58 out of 75 tested patients had HbA1c $\% \ge 7\%$ i.e. uncontrolled diabetes (Table 4).

Table 4 Biochemical tests for diabetic control.

Biochemical test	Mean ± SE	Median (Min— Max)	No. of patients
Fasting plasma glucose	184.1 ± 65.89	167 (86.5- 400)	100
mg/dL HbA1c (%)	8.765 ± 2.234	8.3 (4.828- 15.1)	75

Normal range of plasma glucose (100-125) mmol/l

Egypt. J. Chem. 64 No. 11 (2021)

Assessment of factors that influenced the diabetic control

The cut-off point of HbA1c (%) to discriminate the controlled from the uncontrolled diabetes is 7%. In this study out of 75 patients practiced HbA1c (%) testing only 17 patients had a value less than 7% (i.e. controlled diabetes) that had the Systolic (124 \pm 28.2) and Diastolic (84.3 \pm 12.9) blood pressure and 58 patients had a value more than 7%, uncontrolled diabetic patients had significant high fasting plasma glucose (188.5 ± 67.2) compared with no significant of Systolic (124 \pm 28.2, 124 \pm 21.5) and Diastolic (84.3 \pm 12.9, 82.7 ± 11.3) blood pressure consecutively [Table 5], the results of the multivariate analyses showed that the independently associated hypertension (Table 1 and 5) were age more than 50 years (57.2 \pm 10.01), and duration of diabetes median time was 8 years (8.874 ± 7.447) .

Table 5 Factors that influenced the diabetic control

control.					
Variable	HbA1c 7% (n=17)	<	HbA1c 7% (n=58)	: >	P value
Systolic blood pressure (mmHg)	124 ± 28	3.2	124 ± 2	1.5	0.978
Diastolic blood pressure (mmHg)	84.3 12.9	±	82.7 11.3	±	0.658
Fasting plasma glucose (mmol/l)	161.2 39.8	±	188.5 67.2	±	0.046

4. Discussion

Patient profiles included in this study showed a wide range of symptoms, health problems of diabetes; consequently, variants and a wide range of biochemical measurements are required in this evaluation. High blood pressure is found to be the second pillar of 33% of metabolic syndrome. Medical data demonstrates that 15 patients were documented for hypertension and 4 patients were diagnosed at the start of the questionnaire, i.e. 14 patients were likely to have higher blood pressure due to insulin resistance. Glycated hemoglobin medical tests are commonly used to evaluate diabetic control rates and are directly linked to average blood glucose levels over a period of 2 - 3 months. Glycated hemoglobin evaluations are

generally described either as a ratio of HbA1c, a common form of glycated hemoglobin or as a proportion of glycated hemoglobin consisting of HbA1c; HbA1c is a good indicator of chronic hyperglycemia that also has a strong correlation with the risk of long-term diabetes complications. In both diabetic and non-diabetic subjects, excessive HbA1c has been identified as an independent predictor for stroke and coronary heart disease [20-22].

As one of the most common cardiovascular diseases, hypertension has become a major social and public health concern around the world. The blood pressure result in table 5 is non-significant, but higher HbA1c levels increased the risk of hypertension and systolic hypertension in non-diabetic adults, but not diastolic hypertension. Furthermore, the increased risk of hypertension was increased by the upregulated HbA1c in combination with abdominal obesity and a family history of hypertension [23, 24].

More than two-thirds of type 2 diabetes patients have hypertension, which is often caused by impaired glucose metabolism [25, 26]. It has been suggested that the presence of hyperglycemia is linked to the onset of hypertension. Insulin resistance (IR), hyperinsulinemia, and the excitatory symptoms of hyperglycemia may be the root causes of hypertension [27, 28]. HbA1c has been linked to defects in pancreatic-cell function and the degree of insulin resistance as a reliable predictor of long-term glycemia.[29-31].

The two tests do not calculate the same thing; the average blood glucose of 150 mmol/l is 7 percent HbA1c and 8.8 percent hemoglobin glycosylated. Higher fasting plasma glucose levels, rather than HbA1c, were found to be a strong predictor of future hypertension. Higher fasting plasma glucose levels, but not HbA1c levels, as shown in table 5 with HbA1c 7% (161.2 39.8), are independent risk factors for hypertension development. Hyperglycemia, along with age, obesity, and prehypertension, helped to identify people who were at a higher risk of developing hypertension [32-34]. In addition to having a close relationship with diabetes, HbA1c was also associated with macrovascular disorders such as hypertension and coronary heart disease [35-37]. Diabetes control disorder, measured at HbA1c level, may be associated with hypnosis.

5. Conclusion

We found a positive association between elevated HbA1c at baseline and incident hypertension, suggesting that hyperglycemia may play a role in the development of hypertension even in individuals with no prior history of diabetes. In particular, pre-diabetic HbA1c and 7.0% HbA1c among those diagnosed with diabetes (i.e. those with poorer glucose control) were independently associated with incident hypertension. Combined with evidence that HbA1c is a marker of long-term cardiovascular risk, our results suggest that individuals with elevated HbA1c, even in the absence of diabetes, are at increased risk of hypertension and should be targeted cardiovascular risk factor management hypertension prevention strategies.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient (s) has/have given his/her/their consent for clinical information to be reported in the journal. The patients understand that their names and initials will not be published, and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Funding/Support

There is no funding/support.

References

- 1. Martinez M, Santamarina J, Pavesi A, et al. Glycemic variability and cardiovascular disease in patients with type 2 diabetes. BMJ Open Diab Res Care;9:e002032 (2021).
- 2. A.Uazman, O. Asghar, A. Chazli, and A. M. Rayaz, "Aspectos gerais do diabetes mellitus," *Manual de Neurologia Clínica*, vol. 126, no. 15, pp. 211–222, (2014).
- 3. Si, Y., Wang, A., Yang, Y. et al. Fasting Blood Glucose and 2-h Postprandial Blood Glucose Predict Hypertension: A Report from the REACTION Study. Diabetes Ther 12, 1117–1128 (2021).
- S. Carvalho, A. A. de Almeida, A. F. Borges, and D. Vannucci Campos, "Treatments for diabetes mellitus type II: new perspectives regarding the possible

- role of calcium and cAMP interaction," *European Journal of Pharmacology*, vol. 830, pp. 9–16, (2018).
- 5. R. A. Pamungkas and K. Chamroonsawasdi, "Self-management based coaching program to improve diabetes mellitus self-management practice and metabolic markers among uncontrolled type 2 diabetes mellitus in Indonesia: a quasi-experimental study," Diabetes & Metabolic Syndrome: Clinical Research & Reviews, vol. 14, no. 1, pp. 53–61, (2020).
- Q. Y. Qiu, B. L. Zhang, M. Z. Zhang et al., "Combined influence of insulin resistance and inflammatory biomarkers on type 2 diabetes: a population-based prospective cohort study of inner Mongolians in China," *Biomedical and Environmental Sciences*, vol. 31, no. 4, pp. 300–305 (2018).
- 7. Y. Lin, S. Ye, Y. He, S. Li, Y. Chen, and Z. Zhai, "Short-term insulin intensive therapy decreases MCP-1 and NF-κB expression of peripheral blood monocyte and the serum MCP-1 concentration in newlydiagnosed type 2 diabetics," *Archives of Endocrinology and Metabolism*, vol. 62, pp. 212–220 (2018).
- 8. Nanayakkara, N., Curtis, A.J., Heritier, S. et al. Impact of age at type 2 diabetes mellitus diagnosis on mortality and vascular complications: systematic review and meta-analyses. Diabetologia 64, 275–287 (2021).
- 9. Kim KS, Kim SK, Lee YK, Park SW, Cho YW. Diagnostic value of glycated haemoglobin HbA(1c) for the early detection of diabetes in high risk subjects. *Diabet Med.*; 25(8):997–1000 (2008).
- 10. Khaw KT, Warcham N. Glycated hemoglobin as a marker of cardiovascular risk. *Curr Opin Lipidol*.;17 (6):637–643 (2006).
- 11. Ma WY, Li HY, Pei D, et al. Variability in hemoglobin A1c predicts all-cause mortality in patients with type 2 diabetes. *J Diabetes Complications*; 26:296–300 (2012).
- 12. Bouchi R, Babazono T, Mugishima M, et al. Fluctuations in HbA1c are associated with a higher incidence of cardiovascular disease in Japanese patients with type 2

- - diabetes. J Diabetes Investig; 3:148–55 (2012).
 - 13. Takao T, Matsuyama Y, Yanagisawa H, et al. Association between HbA1c variability and mortality in patients with type 2 diabetes. *J Diabetes Complications*; 28:494–9 (2014).
 - 14. Hirakawa Y, Arima H, Zoungas S, et al. Impact of visit-to-visit glycemic variability on the risks of macrovascular and microvascular events and all-cause mortality in type 2 diabetes: the ADVANCE trial. *Diabetes Care*; 37:2359– 65 (2014).
 - 15. Hsieh YT, Tu ST, Cho TJ, et al. Visit-tovisit variability in blood pressure strongly predicts all-cause mortality in patients with type 2 diabetes: a 5.5-year prospective analysis. *Eur J Clin Invest*; 42:245–53 (2012).
 - 16. Hata J, Arima H, Rothwell PM, et al. Effects of visit-to-visit variability in systolic blood pressure on macrovascular and microvascular complications in patients with type 2 diabetes mellitus: the ADVANCE trial. Circulation;128:1325–34 (2013).
 - 17. Takao T, Kimura K, Suka M, et al. Relationships between the risk of cardiovascular disease in type 2 diabetes patients and both visit-to-visit variability and time-to-effect differences in blood pressure. *J Diabetes Complications*, 29:699–706 (2015).
 - 18. Li, Y.-T.; Wang, Y.; Hu, X.-J.; Chen, J.-H.; Li, Y.-Y.; Zhong, Q.-Y.; Cheng, H.; Mohammed, B.H.; Liang, X.-L.; Hernandez, J.; et al. Association between Systolic Blood Pressure and Diabetic Retinopathy in Both Hypertensive and Normotensive Patients with Type 2 Diabetes: Risk Factors and Healthcare Implications. Healthcare, 9, 580, (2021).
 - 19. Nga TTT, Blizzard CL, Khue LN, Le Van Ngoc T, Bao TQ, Otahal P, Nelson MR, Magnussen CG, Van Tan B, Srikanth V, Thuy AB, Son HT, Hai PN, Mai TH, Callisaya M, Gall S. The Interdependence of Blood Pressure and Glucose in Vietnam. High Blood Press Cardiovasc Prev. Mar;28(2):141-150, (2021).
 - Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c Test in Diagnosis and Prognosis of

- Diabetic Patients. Biomark Insights. Jul 3;11:95-104. (2016).
- 21. Eyth E, Naik R. Hemoglobin A1C. [Updated 2020 Apr 30]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Jan (2021).
- 22. Nofri Rahmadika, Adi Imam Tjahjadi, and Rovina Ruslami. Performance of Point-of-Care Testing Compared with the Standard Laboratory Diagnostic Test in the Measurement of HbA1c in Indonesian Diabetic and Nondiabetic Subjects. Journal of Diabetes Research, Volume 2020, 6 pages, (2020).
- 23. Fisher NDL, Curfman G. Hypertension-A Public Health Challenge of Global Proportions [J]. JAMA, ,17,1757–9, (2018).
- Song, J., Wei, N., Zhao, Y. et al. Elevated glycosylated hemoglobin levels and their interactive effects on hypertension risk in nondiabetic Chinese population: a crosssectional survey. BMC Cardiovasc Disord 20, 218 (2020).
- Ferrannini E, Cushman WC. Diabetes and hypertension: the bad companions [J]. Lancet: 380:601–10, (2012).
- 26. Lin Qiu, Weihua Wang, Rina Sa, Feng Liu, "Prevalence and Risk Factors of Hypertension, Diabetes, and Dyslipidemia among Adults in Northwest China", International Journal of Hypertension, vol. 2021, Article ID 5528007, 10 pages, (2021).
- 27. Wijkman, M.O., Claggett, B., Diaz, R. et al. Blood pressure and mortality in patients with type 2 diabetes and a recent coronary event in the ELIXA trial. Cardiovasc Diabetol 19, 175 (2020).
- 28. Chehregosha H, Khamseh ME, Malek M, Hosseinpanah F, Ismail-Beigi F. A view beyond HbA1c: role of continuous glucose monitoring [J]. Diabetes Ther. 10 (3):853–63, (2019).
- 29. Boursier G, Sultan A, Molinari N, Maimoun L, Boegner C, Picandet M, et al. Triglycerides and glycated hemoglobin for screening insulin resistance in obese patients [J]. Clin Biochem. 53:8–12, (2018).
- 30. Mi, J., Song, J., Zhao, Y. et al. Association of hemoglobin glycation index and its interaction with obesity/family history of hypertension on hypertension risk: a

112.10 02.02.1, 11.0 11.2 22.002 11.2001.2 11.12 21.22.10 11.12.10

- community-based cross-sectional survey. BMC Cardiovasc Disord 20, 477 (2020).
- 31. Chudleigh RA, Bain S. Cardiovascular safety of liraglutide for the treatment of type 2 diabetes. Expert Opin Drug Saf. 16:627–35, (2017).
- 32. Heianza, Y., Arase, Y., Kodama, S., Hsieh, S. D., Tsuji, H., Saito, K., Hara, S., & Sone, H. Fasting glucose and HbA1c levels as risk factors for the development of hypertension in Japanese individuals: Toranomon hospital health management center study 16 (TOPICS 16). Journal of human hypertension, 29(4), 254–259 (2015).
- 33. Si Y, Wang A, Yang Y, Liu H, Gu S, Mu Y, Lyu Z. Fasting Blood Glucose and 2-h Postprandial Blood Glucose Predict Hypertension: A Report from the REACTION Study. Diabetes Ther. Apr;12 (4):1117-1128 (2021).
- 34. Zhang X, Yang Z, Zhang M, Zhu Y, Zhao Z, Huang Z, Li C, Zhou M, Farmer AJ, Tang J, Wang L. Independent effects of 2hPG, FPG and HbA1c on cardiovascular risk: Analysis of a nationally representative sample from China. Diabetes Res Clin Pract. Mar;173:108672 (2021).
- 35. Balducci S, Zanuso S, Cardelli P, Salerno G, Fallucca S, Nicolucci A, et al. Supervised exercise training counterbalances the adverse effects of insulin therapy in overweight/obese subjects with type 2 diabetes. Diabetes Care.35 (1):39–41 (2012).
- 36. Mansour A. A. Prevalence and control of hypertension in iraqi diabetic patients: a prospective cohort study. The open cardiovascular medicine journal, 6, 68–71 (2012).
- 37. Bianchi C, Miccoli R, Trombetta M, Giorgino F, Frontoni S, Faloia E, et al. Elevated 1-hour postload plasma glucose levels identify subjects with normal glucose tolerance but impaired β-cell function, insulin resistance and worse cardiovascular risk profile:the GENFIEV study. J Clin Endocrinol Metab. 98(5):2100–2105 (2013).