## Effect of Nateglinide on C-Peptide level in Fasting Elderly Diabetic Patients: A Placebo-Controlled Cross Over Study

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**Abstract:** Nateglinide is suggested to have a glucose-dependent insulinotropic effect. We recently compared serum insulin levels in fasting and non-fasting elderly diabetic patients following nateglinide administration and did not find a significant difference. Insulin, but not c-peptide is subjected to a significant first pass hepatic metabolism. Given the inclusion of 2 patients with liver dysfunction in the study, we estimated serum c-peptide levels in the same samples to further investigate this finding. Eight elderly diabetics underwent a fixed dose single blinded, placebo controlled, cross-over study. They received either nateglinide 120 mg or a placebo, both under fasting and non-fasting states. Serum c-peptide levels were measured at 30 minutes intervals for four hours, following drug or placebo administration. None of the eight patients developed hypoglycemia under the non-fasting state and only one patient developed mild hypoglycemia (66 mg/dl) under the fasting state in response to nateglinide. Area under the serum c-peptide levels and serum glucose levels, in either of the 4 days. The present results are possibly explained by the lower mean fasting serum glucose levels, which would lead to the relatively lower insulin secretory effect of nateglinide and thus the insignificant difference in insulin secretion between the fasting and the fed state.

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**Key words:** Nateglinide, fasting, c-peptide, elderly

#### **1. Introduction**

Progression of type 2 diabetes is characterized by progressive loss of early insulin secretion, leading to postprandial hyperglycemia (van den Oever et al., 2010). Proinsulin is secreted by the beta cells of the pancreas and is then enzymatically cleaved into Cpeptide and insulin. The two peptides are secreted in an equimolar proportion into the portal circulation (Rubenstein et al., 1969; Horwitz et al., 1975), and can therefore be used to assess endogenous production of insulin. 50-60% of secreted insulin is extracted by the liver during the first pass, in the portal circulation (Polonsky and Rubenstein, 1984). C-peptide, on the other hand, is not degraded by the liver (Polonsky et al., 1983). The kidney is the main organ for the degradation of C-peptide by endopeptidases. Furthermore, the half-life of C peptide in circulation is 2-5 times longer than insulin (Regeur et al., 1978). The use of both insulin and C-peptide data provides a more complete picture of insulin secretion (Cobelli and Pacini, 1988). The ultrasensitive assay revealed that C-peptide production persists for decades after disease onset and remains functionally responsive (Wang et al., 2013).

Nateglinide (an insulin secretory agent), is a very rapid and most short-acting insulin-releasing agent (Standl and Fuchtenbusch, 2003). It is particularly appropriate for the control of postprandial hyperglycemia and allows a more flexible lifestyle and the possibility to skip a meal without the risk of hypoglycemia that would be experienced with glibenclamide therapy. Conversely, an additional meal can be incorporated into the meal plan, preceded by an extra dose without worsening glycemic control (Hanefeld *et al.*, 2000).

Nateglinide showed a glucose-dependent insulinotropic effect *in-vitro* (Hu *et al.*, 2001), suggesting a low risk of hypoglycemia, even if it was administered without meals. Elderly patients (Posner *et al.*, 1993), especially those with poor memory (Perkins *et al.*, 1999) may skip meals and are therefore more prone to hypoglycemic comas. This makes nateglinide a preferable choice in management of type 2 diabetes in elderly patients.

We recently showed that repaglinide did not induce hypoglycemia in fasting elderly diabetic patients. Serum insulin levels were significantly different between the fasting and non-fasting states. On the other hand, nateglinide failed to show this significant difference in serum insulin levels between the fasting and non-fasting states, though hypoglycemia was not reported under the fasting state (Murad *et al.*, 2013). Given the hepatic metabolism of insulin and the inclusion of patients with liver dysfunction, confirmation of the nateglinide effect on insulin secretion in fasting state is warranted. The present study estimated serum c-peptide levels in the same samples to further assess the effect of nateglinide on endogenous insulin secretion under the fasting state. Correlation between serum c-peptide and serum glucose levels was also tested.

## 2. Methods:

## Study protocol

The protocol of the study was approved by the King Abdulaziz University Research Ethics Committee (KAU-REC) and Research Ethics Committee of the faculty of medicine, Ain Shams University, Cairo, Egypt. The investigation was carried out in accordance with the Declaration of Helsinki. Written informed consents were obtained from all participants.

## Subjects

Eight Egyptian Type 2 diabetic elderly subjects ( $\geq 60$  years) completed the study. Three of the eight subjects were previously treated with metformin and five were newly diagnosed. Exclusion criteria included the current use of insulin secretagogues, fasting blood glucose above 270 mg/dl, late complications of diabetes or severe concurrent disease. Given the frequency of comorbidities in elderly, enrolled patients included those with stable comorbidities that did not represent a contraindication to nateglinide. Baseline characteristics of the study group are shown in Table (1).

# Study design

This study was a fixed dose, single blinded, placebo controlled, cross-over study. All patients underwent comprehensive geriatric assessment and laboratory evaluation before the beginning of the study. Metformin was not administered on the mornings of the study. Nateglinide or placebo was administered on non-consecutive days. Each was administered on two days (one on the fasting protocol and the other on the non-fasting protocol). On the testing days, patients were admitted to the Geriatrics and Gerontology Department at Ain Shams University Hospital. Two baseline venous samples were collected at 30 min interval, starting at 8:30am. Immediately after the second baseline sample, patients received nateglinide 120 mg (Starlidine, International Drug Agency for Pharmaceutical Industries, Port Said, Egypt) or a placebo (Alexandria Company for Pharmaceuticals and Chemical Industries, Egypt). An ordinary Egyptian breakfast composed of bran mixed

bread and brown beans (500 kcal, 55% carbohydrate, 30% fat, and 15% protein) was administered. Venous samples were collected at 30 min intervals (starting 30 min after meal) for the next 4 hours. Patients were closely observed all through the study period for any symptoms suggestive of hypoglycemia. For the fasting protocol, the same procedure was performed except for meal serving. In order to avoid any possible risk of hypoglycemia, capillary blood glucose was checked every 30 min or as needed during the fasting protocol. The study was stopped if glucose level dropped to 60 mg/dl and hypoglycemia was corrected. Sampling was also stopped if the patient presented with symptoms of hypoglycemia and requested termination of the study, even if blood glucose level was above 60 mg/dl. Blood samples were centrifuged and serum was stored at -20°C, till analysis. Serum C-peptide concentrations were estimated in each sample. Serum insulin and glucose were estimated in the same samples in a previous study (Murad et al., 2013).

# Serum C-peptide assay

Samples were assayed for C-peptide at the Clinical Pathology Department at Ain Shams University Hospital, Cairo. Serum C-peptide was measured using enzyme-linked immunosorbent assay (Accu Bind ELISA Microwells, Monobind Inc, Lake Forest, CA). The assay has < 1% cross-reactivity with proinsulin, with no detectable reaction with insulin or glucagon. The detection range was 0.2-10 ng/ml. Hemolyzed samples were not included in analysis. Serum glucose levels were previously measured using a glucose oxidase method (Murad *et al.*, 2013).

# Statistical analysis

The sample size of eight patients was estimated based on the data provided by (Rudovich et al., 2004) to detect a 50% difference between c-peptide levels in the fasting and non-fasting state following nateglinide administration, with a power of 80% at the 5% level of significance. Data were expressed as mean  $\pm$  S.E.M. Significant differences between groups of data were assessed using the paired Student's t-test. Significant difference was assumed if p < 0.05. Area under the curve (AUC) was calculated using graphpad prism (version 3.02). The mean of the two baseline samples was used for comparison with the other time points. Baseline levels on different days were compared using one way ANOVA. There were 7 missing values (2%), 5 samples due to hemolysis and two samples because study was terminated for one patient in one day after 3 h. Missing values were substituted by imputed data (calculated by the expectation-maximization method (EM), SPSS version 17.0, SSPS Inc, Chicago, IL, USA). Pearson Correlation was used to estimate the correlation between serum glucose and serum cpeptide levels.

#### 3. Results

### Effect on Serum C-peptide Level

Baseline serum c-peptide levels were not significantly different on different days (p > 0.05, one way ANOVA).

Nateglinide, administered before breakfast, induced a significant increase in serum c-peptide level, at 1.5, 3.5 and 4 h following breakfast, compared to the baseline level (p < 0.05; paired Student's t test; Figure 1a). In the fasting state, a significant difference compared to baseline serum c-peptide levels was seen at 0.5 and 1.5 h following nateglinide administration (p < 0.05; paired Student's t test; Figure 1a). Comparing each time point to its corresponding one in the non-fasting state showed no significant difference between serum c-peptide level in the fasting and the non-fasting states except at the 3.5 h time point following nateglinide administration (p < 0.05; paired Student's t test; Figure 1a). The area under the time concentration curve (AUC<sub>BL-4h</sub>) was not significantly different between the fasting and non-fasting state (p > 0.05; paired Student's t test; Figure 1b).

As for placebo, it showed a significant increase in serum c-peptide level, on the non-fasting day at the 1 and 2 h time points, compared to the baseline level (p < 0.05; paired Student's t test; Figure 1c). On the fasting day, however, there was no significant increase in serum c-peptide level, compared to baseline levels at any of the eight time points following nateglinide administration. Comparing each time point to its corresponding one in the non-fasting state showed a significant difference between serum c-peptide level in the fasting and the non-fasting states all through the sampling period with the exception of the 1.5 and 3 h time points (p< 0.05 for time points 0.5, 2, 2.5, 3.5, 4h

time points and $p < 0.01$ for the 1 h time point; paired								
Student's	t	test;	Figure	1c).	This	resulted	in	a
significant difference in AUC <sub>BL-4h</sub> ( $p = 0.011$ ; paired								
Student's	t te	st; Fig	ure 1d).					

# Correlation between serum c-peptide and serum glucose levels

As shown earlier (Murad et al., 2013), meal resulted in an elevation of serum glucose level after placebo administration. Nateglinide (120 mg) administered before breakfast prevented this elevation in serum glucose level. Nateglinide induced one mild hypoglycemic event in the fasting state. The patient experienced symptoms suggestive of hypoglycemia in the form of tachycardia, sweating and drowsiness, three hours after nateglinide administration. His serum glucose level was 66 mg/dl. The patient requested the termination of the study on that day. Serum glucose level was slightly, but significantly lower than level, 3 h following baseline nateglinide administration in the fasting state (24.4  $\pm$  17.3 mg/dl; p = 0.005; paired Student's t test).

Given the renal excretion of c-peptide, results of the single patient with renal impairment were excluded and correlation between c-peptide levels and glucose levels was examined. There was no significant correlation between c-peptide levels and glucose levels in the nateglinide group (r = 0.96, p = 0.4 in the fasting state and r = 0.086, p = 0.49 in the non-fasting state; Pearson correlation; figure 2a).

There was no significant correlation between cpeptide levels and glucose levels in placebo group, either (r = 0.69, p = 0.54 in the fasting state and r = 0.073, p = 0.58 in the non-fasting state; Pearson correlation; figure 2b).

Characteristic	
Gender (female/male)	6/2
*Age	$63.1 \pm 5.3$ years
* Body mass index	$35 \pm 4.1 \text{ Kg/m}^2$
*Known duration of diabetes	$1.9 \pm 2.5$ years
*HbA1c	$6.1\pm0.8$ %
*ALT	$27.1 \pm 16.6 \text{ IU/l}$
*AST	$31.2 \pm 19.8$ IU/1
*Serum creatinine	$0.86 \pm 0.83 \text{ mg/dl}$
Co-morbidities (N)	
Hypertension	2 patients (on enalapril)
Ischemic heart disease	1 patient (on nitroglycerin and aspirin)
Renal impairment	1 patient (S.Cr. 2.9 mg/dl)
Chronic liver disease	1 patient with Hepatitis C virus (AST 60 IU/l, ALT 77 IU/l);
(AST, ALT)	1 patient with Portal hypertension (AST 21 U/l, ALT 29 U/l)

	Table	۱.	Baseline	characteristics	of	the study group
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\* Data are presented as means  $\pm$  S.D.



**Figure 1.**Mean serum c-peptide concentrations ( $\pm$  SEM) and the area under the time c-peptide concentration curve (AUC<sub>BL-4h</sub>) after treatments with 120 mg nateglinide (a&b) and placebo (c&d) in the fasting and non-fasting state. For serum c-peptide concentrations, \* indicates p < 0.05; compared to baseline values; # indicates p < 0.05 compared to the corresponding time point of the fasting protocol. For area under the time insulin concentration curve, \* indicates p < 0.05 compared to the non-fasting state; paired Student's t test.



**Figure 2.**Relationship between serum c-peptide and serum glucose levels in elderly diabetic patients following (a) nateglinide (120 mg) and (b) placebo in the fasting and non-fasting states. There was no significant correlation in either of the four groups; Pearson correlation.

#### 4. Discussion

Dependence of the insulin-secretory effects of antidiabetic drugs on serum glucose level represents a relative protection against hypoglycemia. Experimental studies on perfused rat pancreas showed that nateglinide increases the sensitivity of insulin secretion to glucose (Morimoto *et al.*, 1998). Furthermore, increasing glucose concentration in  $\beta$ cell electrophysiological studies decreased the concentration of nateglinide that inhibit  $\beta$ -cell K<sup>+</sup><sub>ATP</sub> current (Hu and Wang, 1998). Nateglinide also inhibits dipeptidyl peptidase-IV and increases the bioavailability of glucose-dependent insulinotropic polypeptides (incretins). This may contribute to the glucose dependent nature of its insulinotropic effects (McKillop et al., 2009).

The failure of nateglinide to show this significant difference in serum insulin levels between the fasting and non-fasting states in our recent study (Murad et al., 2013), requires further investigations. As mentioned earlier, 50-60% of secreted insulin is extracted by the liver during the first pass, in the portal circulation (Polonsky and Rubenstein, 1984). Furthermore, an increase in hepatic extraction of insulin after glucose administration has been reported (Kaden et al., 1973; Jaspan and Polonsky, 1982). Hepatic extraction of insulin is also affected by portal insulin levels (Jaspan and Polonsky, 1982). Given our study (Murad et al., 2013) estimated insulin levels in peripheral blood to compare between fasting and fed state, confirming results using a different test is warranted. C-peptide, being mainly degraded by the kidneys and having a longer half-life than insulin (Regeur et al., 1978) represents a plausible option. It provides a means to evaluate endogenous insulin secretion even in the presence of circulating insulin antibody (Kuzuya et al., 1978).

In the present study, nateglinide administration did not induce a significant difference between serum c-peptide levels in the fasting and non-fasting states. In contrast to our results, nateglinide administered in the fasting state in a clinical study resulted in a minimal insulin secretion as compared to the nonfasting state (Keilson et al., 2000). However, the mean fasting serum glucose concentration in the Keilson study was 243 mg/dl. In our study, the mean fasting serum glucose concentration was 130 mg/dl. Given that nateglinide is more effective when glucose concentrations are the highest (Keilson et al., 2000), this may explain the relatively lower insulin secretory effect of nateglinide in our study and therefore explains the insignificant difference in insulin secretion between the fasting and the fed state. However, in the present study, there was a significant difference between serum c-peptide levels in the fasting and non-fasting states following placebo administration, suggesting that nateglinide did increase insulin secretion in the fasting state. In line with this, serum glucose level was slightly but significantly lower than baseline level, 3 h following nateglinide administration in the fasting state (24.4  $\pm$ 17.3 mg/dl) with even one mild hypoglycemic event. Interestingly, in the Keilson study, nateglinide 120 mg did decrease serum glucose level in the fasting state > 30 mg/dl, at 3 and 4 hours following drug administration. However, statistical analysis was not reported on that finding. Moreover, studying the correlation between c-peptide levels and glucose levels after nateglinide administration did not show a significant correlation.

Given the renal degradation of c-peptide and the report of reduction in urinary C-peptide excretion despite elevated plasma C-peptide concentrations in moderate renal disease (Kuzuya *et al.*, 1978), we excluded the data of the single patient with renal impairment during performing the correlation between c-peptide and glucose levels. This reduction in sample size should not affect the power of the study given that sample size calculation for nateglinide based on the data provided by Keilson *et al.* (2000) is four patients only. Exclusion of this single patient did not affect the results of the study (data not shown).

The dose of nateglinide used here (120 mg) is reported as the maximally effective dose for lowering glucose without the occurrence of hypoglycemic events (Levy *et al.*, 1995; Keilson *et al.*, 2000). Though higher doses were used in both studies (up to 240 mg), the maximum meal time dose of nateglinide according to the prescribing information is 120 mg (Starlix®: US prescribing information). The use of this dose may explain in part the absence of any major hypoglycemic events, in spite of the insignificant difference in c-peptide levels between the fasting and fed state.

### Conclusion

At the maximal mealtime dose, nateglinide did not cause hypoglycemia in elderly patients. Under fasting states, however, caution is required.

## Limitations of the study

A generic formulation of nateglinide has been used. In view of the fact that the reference listed drug (Starlix®) is not available in Egypt or Saudi Arabia, we used the formulation available for clinical use in our countries. Missing values is another limitation; however as mentioned above, they were substituted by EM method and were only 2% of the samples.

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