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A Novel Earwax Method to Measure Acute and Chronic Glucose Levels

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Abstract

Increased chronic glucose is associated with pandemic diseases. To date, there is not a practical, as well as accurate sample for reflecting that level. We measured earwax glucose in 37 controls. They provided standard serum samples, Glycated Haemoglobin (HbA_{1c}) and earwax samples on two time-points, one month a part. The specimens measured baseline fasting glucose, a follow-up postprandial glucose level and a between sample chronic glucose, calculated using the average level on the two occasions. The baseline earwax sample was obtained using a clinical method and the follow-up using a novel self-sampling earwax device. The earwax analytic time was significantly faster using the novel device in comparison to the clinical use of the syringe. Earwax accurately reflected glucose at both assessments with stronger correlations than HbA_{1c}. Follow-up postprandial concentrations were more significant than their respective fasting baseline concentrations, reflecting differences in fasting and postprandial glycaemia and more efficient standardisation at follow up. Earwax demonstrated to be more predictable than HbA_{1c} in reflecting systemic fasting, postprandial and long-term glucose levels and immune by confounders. Earwax glucose was approximately 60% more predictable than HbA_{1c} in reflecting glycaemia over a month. The self-sampling device provided a sample that might accurately reflect chronic glycaemia.

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1. Introduction

Chronic diseases account for the largest cause of deaths globally (71%), and diabetes is the fourth among them[1]. Diabetes and other metabolic disorders are characterised by a sustained increased in glucose levels. Current measurements from "short-term" glucose specimens, such as serum, have significant limitations in the assessment of the average concentration of glucose level. This is because glucose levels can vary greatly during the day. Furthermore, day-to-day hassles during periods of stress [2], smoking [3], high blood pressure [4], Body Mass Index (BMI) [5] and physical activity [6] can affect glucose levels.

Several glucose measurements, such as fasting and postprandial glucose levels have been standardised, aiming to provide a predictable level of glucose concentration. However, taking these tests is often demanding for patients. Furthermore, these tests still do not accurately reflect the average concentration of glucose, which is the required level to monitor the glycaemic profile in metabolic disorders [7]. Indeed, these levels are usually found either below the mean, such as those seen when Fasting Serum Glucose (FSG) samples are taken or below that average when Postprandial Serum Glucose (PSG) ones are used [8].

Glycated haemoglobin (HbA_{1c}) is a form of haemoglobin that shows a positive correlation with both FSG and PSG. Currently, HbA_{1c} is the most commonly used specimen to represent long-term glucose concentrations [9], [10]. People without disease show weaker associations between HbA_{1c} and fasting or postprandial glucose levels when compared to diabetic patients [11]. This undermines the test's ability to screen hyperglycaemic level amongst the general population [2]. Fasting glucose levels show stronger associations with HbA_{1c} than postprandial glucose levels when measured in healthy people and in diabetic patients with poor glycaemic control [9]. This means that HbA_{1c} could be found within a normal range in less severe diabetic patients who frequently have dietary transgressions. This diminishes HbA_{1c} capacity to tightly monitor the mean glucose levels in glucose intolerance and mild diabetes. A

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more effective method for measuring the average concentration of glucose level should equally weight its postprandial and fasting levels across the day.

Currently, HbA_{1c} is used for measuring the preceding three months average plasma glucose concentration. However, this glycated protein is more greatly weighted (75%) towards plasma glucose concentrations of the previous month [12]. It is important to note that HbA_{1c} does not provide predictable information about the glycaemic level over periods lesser than a month, such as those following changes (in weeks) after the prescription of hypoglycaemic drugs [13]–[15]. It also has some additional limitations. HbA_{1c} is not a precise method, as its levels can be affected by biological variables, such as age [2] or by common illnesses, such as anaemia [16] or several hemoglobinopathies (up to 7%) [17]. Even working long hours cause higher HbA_{1c} levels [18]. Additionally, HbA_{1c} is an expensive and commonly unavailable lab test in some developing countries [19]. Ultimately, HbA_{1c} is an indirect approximation of the mean glucose level, given that it is the protein, not the sugar directly that is being measured. Some authors even doubt about HbA_{1c} validity as a diagnostic test for diabetes mellitus and glucose intolerance, due to its aforementioned limitations [2].

All samples, either glycaemic or HbA_{1c} need to be taken from blood. This entails that sampling is expensive, since qualified workers, such as nurses are required. Blood samples may be associated with some side effects, such as bleeding and infection. Nonetheless, the glycaemic level is still the most requested lab test in Primary Health Care Centres in several countries [20], [21]. The glycaemia represents the third largest lab cost for some health systems [21]. HbA_{1c} is also among the most demanded lab test, and it is believed that it is still unrequested [22]. Therefore, there is a need to find not only a more reliable specimen for measuring chronic glucose concentrations over different periods, but also harmless and more economical.

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Few other biological samples may provide that concentration. Earwax may be that specimen. This oily secretion is secreted into the auditory ear canal by the apocrine and sebum glands of the ear (the ceruminous glands) [23]. It has been demonstrated that no acute influences can affect the level of this secretion, due to the fact that the ceruminogenous glands are not innervated [24]. Similar to the wax produced by bees a bacteriostatic agent capable of storing sugar products (honey) in honeycombs [25], [26] human wax is also capable of accumulating glucose level over long periods and may be immune to the most common strains of the epidermal flora [27], [28]. Furthermore, earwax can be collected from home, without the need of specific storing or transporting conditions.

Although glucose levels have already been measured using earwax samples elsewhere [29], [30] including in diabetic patients [31], those studies did not investigate if the level found in this monosaccharide represented its long term concentration. The aim of this study was to validate the use of earwax for measuring long-term glucose concentration by using a novel, self-cleaning outer ear device which does not require any technical expertise. We collected two right earwax samples extracted one month apart using two different methods (a conventional clinician administered method and the earwax self-sampling device). At the same time two blood based glycaemic samples were obtained during fasting (baseline) and after the intake of one standardised meal (follow-up) one month apart and also HbA_{1c} samples. We hypothesized that the earwax self-sampling device would be an effective method to measure short and chronic glucose levels and a viable alternative to conventional methods. Based on the weak associations between HbA_{1c} and glucose levels we also expected that the novel device would more efficient in reflecting true glycaemic measures. Moreover we predicted that: 1) In comparison to other clinical methods the earwax self-sampling device would reduce the time needed for extraction and analysis of earwax glycemic concentration (EGC); 2) All follow-up concentrations would be larger than their respective baseline concentration; 3) Based on the notion that ceruminogenous glands are not innervated, we expected that earwax, conversely to HbA_{1c} and glycaemia would not be affected by short and long-term confounders.

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2. Materials and Methods

Participants were recruited from staff and student volunteers of Universidad Catolica del Norte (UCN) in Coquimbo, Chile and from its local catchment area by public and internal advertisements. All participants were assessed by the same clinical researcher (S.E). Tables 1-3 describe the sample of thirty-seven healthy participants in detail. All participants were recruited during a southern hemisphere winter (between 6th of July and 3rd of August, 2018). It has previously been demonstrated that different seasons vary the triglyceride composition of this secretion [32]. Asian people and people with intellectual disabilities were excluded due to their differences in earwax characteristics [32], [33]. Participants required to be free from medical illnesses (e.g. anaemia, diabetes, glucose, lactose intolerance), ear pathologies (e.g. impacted earwax, perforated eardrum), and of any medication at the time of recruitment and in the previous month. Subjects were also excluded if they reported, during the previous month any illicit substance use or were exposed to any severe stressor, according to the DMS-III definition [34]. Since it has previously been found that earwax weight does not significantly differ between ear sides[32] we were able to conduct a prospective case-control, rather than a propective cross-sectional study. Participants were interviewed at baseline (day=1) and a follow-up (day=30). During the baseline assessment a range of demographic, clinical and environmental factors were systematically assessed (see Tables 1-3). These included the frequency and severity of the most common day-to-day environmental disturbances, using the Hassles Scale [35], and more unexpected environmental factors, such as significant life events, using the Recent Life Changes Questionnaire (RLCQ; *Miller & Rahe, 1997)* during the month between both visits. Participants also assessed their stress perception during the last four weeks using the Perceived Stress Scale (PSS; *Cohen, 1994)*. Anthropometric variables, such as weight, height, Body Mass Index (BMI) and waist circumference were also detailed during the final assessment. All psychometric tools were validated in Spanish versions.

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At baseline, in order to collect a standardised amount of earwax secretion at the time of follow-up, the right ear of enrolled participants was cleaned using the Reiner-Alexander syringe to effectively and safely remove any earwax from outer ears [38]. Participants were instructed to avoid using cotton buds or the use of any other cleaning outer ear method during the follow-up period. During the follow-up visit, participants self-cleaned their right ears using a earwax self-sampling device, and the wax collected represented the previous four weeks of earwax secretion according to the manufacturer instructions (www.trears.com).

Morning blood tests were obtained at both baseline and follow up visits. The baseline blood sample was obtained after 8 hours of fasting whereas the follow-up sample was taken 2 hours after consuming a standardised liquid meal, 236 ml of Ensure Avance®. Fasting serum glucose and HbA_{1c} levels were analysed from baseline samples, HbA_{1c} levels and PSG were analysed from the follow-up samples. Chronic glucose level over the preceding one-month period was calculated using the mean between the baseline and the follow-up blood sample of glycaemia. Glucose concentration was extracted from earwax by using the hydrophilic fraction (see Supplementary materials for a detailed description of the methods). On 17th of April, 2017, the local ethics committee of Universidad Católica del Norte, Coquimbo, Chile issued a resolution number 75/2017 in which they approved the conduction of the research. Written informed consent was obtained from all participants. Participants did not receive any financial compensation for taking part in the research.

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Data were checked for normality using the Kolmogorov-Smirnov statistical test and graphics methods. All values were normally distributed (all $p>0.05$). Therefore, we used repeated t-tests for comparing baseline and follow-up levels of all specimens. The long term glucose concentration estimation was calculated using the mean value between FSG and PSG. Linear regression analysis was used to determine the association between different specimens and biological and psychological variables. Pearson correlations (R) were used to determine the association between the baseline and the follow-up EGC with their respective glycaemic sample. Pearson correlations (R) were also used for determining the association between the baseline and the follow-up HbA_{1c} with their respective glycaemic sample. Cohen´s criteria for correlations were used: low when R=0.1-0.3, moderate when R=0.3-0.5 and high when R=0.5-1.0 [39]. The coefficient of determination (R^2) was used for comparing the predictability for measuring different glucose levels between EGC and HbA_{1c}. The level of significance was set at $p\leq0.05$ (two-tailed).

3. Results

The sample consisted of 37 young healthy individuals (mean age 29.9 years), 54.1% women of normal weight, BMI and waist circumference with little exposure to severe hassles or life events (see Tables 1-3 for details).

3.1. Time needed to analyse earwax glucose concentration vs. blood based extimations

The self-sampling device earwax extraction time was considerably faster (04:37 hrs) vs. Reiner-Alexander syringe (12:20 hrs) (see Supplementary material: Table 5 for details).

3.2. Correlation of Earwax Glucose Concentration (EGC) and Glycated Haemoglobin (HbA_{1c}) with glycaemic levels

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Earwax gucose concentration strongly positively correlated with all glycaemic measuraments (all R≥0.62, R^2≥38; p<0.01). HbA_{1c} associations with glycaemic levels exhibited low to moderate correlations across all the measuraments (all R≤0.55, R^2 ≤ 0.30 and 0.10<p<0.01) (Table 4 and Figure 1 in supplementary material for details). The strongest observed HbA_{1c} association was with the mean glycaemic level at baseline (R=0.55, R^2=0.30, p<0.001) and the lowest between follow up HbA_{1c} and mean blood sugar (R=0.35, R^2=0.12 p=0.03) (Table 4 and Figure 1.iii.b and 1.iv.b in supplementary material for details). The lowest correlation between EGC and glycaemic levels was at baseline with the mean blood sugar (R=0.62, R^2= 0.38, p<0.01) and the strongest at follow-up with PSG (R=0.90, R^2= 0.81 p<0.001) (see Table 4 and Figures 1.iii.a and 1.ii.a in supplementary material for details).

3.3. Accuracy of Earwax Glucose Concentration (EGC) and Glycated Haemoglobin (HbA_{1c}) in measuring chronic glucose concentration

EGC was 59% more accurate in predicting glucose levels than HbA_{1c} for measuring longitudinal (chronic) glucose concentration over the two time points (Follow-up-EGC/Mean glucose level correlation: R=0.84, R^2=0.71; Follow-up- HbA_{1c}/ Mean glucose level correlation R=0.35, R^2=0.12) (see Table 4 and Figure 1.iv.a and 1.iv.b in supplementary material for details).

3.4. Effect of covariates on Earwax Glucose Concentration (EGC) and Glycated Haemoglobin (HbA_{1c}) correlation with glycaemic levels

Earwax samples were not affected by any of the covariates considered (all p>0.05). HbA_{1c} levels were affected by age at follow-up (p<0.01) and tobacco use was negatively associated with FSG (p=0.01) and PSG levels (p=0.02). Increasing level of education were associated with increased HbA_{1c} and PSG levels at follow-up (both p<0.05) (See supplementary Table 6).

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4. Discusssion

In this work we set out to test the validity of earwax for measuring long-term glucose concentration by using a novel self-cleaning outer ear device. The main finding of the study is that by using the earwax self-sampling device, earwax was a more efficient specimen compared to HbA_{1c} in measuring glycaemic levels. Furthermore glucose measuraments were not affected by any of the covariates considered differently than HbA_{1c}. Moreover the novel device proved to be a feasible approach to rapidly extract wax for analysis with substantial time reduction compared to conventional methods.

We found that follow-up samples of glycaemia, HbA_{1c} and EGC were significantly larger in comparison to their respective baseline concentrations. All associations between EGC and cross sectional and longitudinal glycaemic levels showed highly positive correlation coefficients. On the contrary, HbA_{1c} associations with the same short- and long-term glucose levels only exhibited low to moderate correlations. Earwax samples were up to 59% more predictable than HbA_{1c} specimens at reflecting the average glucose concentration over the preceding month period.

Although participants included in this study were healthy, they were exposed to a significant number and hassles and life events when compared with other healthy research samples originating from Chile [40] likely affecting their self reporting of stressful events [37]. However these events were most likely within the remit of stressful jobs or studies, considering that 43.2% of them were undergraduate students or had graduated from University.

The earwax self-sampling device, compared to the Reiner-Alexander syringe significantly reduced the time needed (7:43 hr less) to analyse EGC. The novel device uses a dry method of extraction which bypassed the need to dry samples before analysis, a typical step of conventional water bases methods. The self-sampling earwax device therefore results in a faster and more economical EGC analysis. The earwax self-sampling device processing time is comparable to HbA_{1c} methods, currently the gold-standard for measuring long-term glucose level without inconveniences and associated costs of blood

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letting and significantly faster analysis of glycaemic levels. On the other hand, the novel device is self-sampling device. Therefore, it is a more practical method when it is correctly administrated. One limitations might be the need for compliance with the method

We found stronger associations than found in previous studies that have correlated HbA_{1c} with fasting and postprandial glucose levels among the general population. *Van 't Riet et al.*, (2010) found correlations of only 46% and 33% when fasting plasma glucose and 2hr post-load plasma glucose was correlated with HbA_{1c} in a large sample of controls. It has been shown that HbA_{1c} usually shows increased associations with fasting (71%) and postprandial glucose levels (79%) among diabetic patients, rather than in controls [11]. This improved HbA_{1c} association with the postprandial glycaemic level in diabetic patients is, however, smaller than the follow-up ECG/PSG correlation found here. On the other hand, the HbA_{1c} association with fasting glycaemic level in diabetes was exactly the same that the one that we found here between baseline-EGC and FSG. It may be possible that EGC also shows an improved correlation in this metabolic disorder. Future studies may correlate PSG and FSG with EGC in diabetic patients.

Differences in the period covered by the baseline and the follow-up earwax sample may explain why the correlation between the baseline EGC and FSG ($R=0.71$, $p<0.001$) was much smaller than the association found between the follow-up EGC and PSG ($R=0.90$, $p<0.001$). This might be explained by the fact that only the amount of secreted earwax at the time of follow-up was standardised. Hence, the baseline period covered by the baseline EGC varied among participants and might have been affected by a range of factors. Aside biological differences in fasting and postprandial glycaemic levels, peaks of hyperglycaemia, due to episodes of physical activity or stress before their inclusion in the study might have affected baseline earwax measuraments. This result may reinforces the fact that EGC equally weights episodes of fasting and postprandial glucose levels. Conversely, HbA_{1c} is indeed greatly influenced by FSG than PSG. People spend more time fasting than eating during 24 hrs [9]. We also

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found an increased correlation between HbA_{1c} and FSG (R=0.51) than HbA_{1c} with PSG (R=0.47). The follow-up EGC may be not completely comparable with PSG. We recently showed that the earwax self-sampling device was significantly more efficient than the Reiner-Alexander syringe at removing earwax from healthy outer ears. This suggests that some residual amount of earwax may have been left by the Reiner-Alexander method we used in this study that could have been extracted by the novel device. This would mean that the follow-up earwax sample extracted by the earwax self-sampling device may have also contained some residual earwax, and thus predominantly, but not exclusively, represented the ECC of the last month. The follow-up HbA_{1c} may be also not entirely comparable with PSG. HbA_{1c} is widely used an index of the average level of glucose concentration over the preceding 3 months, although several studies have found that HbA_{1c} is predominantly influenced (75%) by the average concentration of glucose levels of the previous one month [12]. Therefore, both follow-up samples of EGC and HbA_{1c} predominately, but not exclusively represented the mean blood sugar over the last month. Future studies should investigate the same period of glucose concentration, and correlate the mean of blood sugar with a follow-up ECC sample that is obtained after a baseline cleaning procedure that also used the earwax self-sampling device.

EGC was better than HbA_{1c} for reflecting acute levels of glycaemia. All correlations between EGC and FSG and PSG were stronger than the observed coefficients when HbA_{1c} was associated with the same levels of glycaemia. Furthermore, EGC showed the largest difference with HbA_{1c} correlations when EGC was associated with the mean blood sugar studied here. Indeed EGC/mean blood sugar correlation (R) was almost 50% stronger than the HbA_{1c} association with the same mean of glucose level. Furthermore, earwax was approximately 60% more accurate in predicting chronic glucose levels (R^2) than HbA_{1c}. This suggests that earwax is not only better than HbA_{1c} for reflecting acute glucose levels, but also for chronic. Future studies may correlate EGC with mean blood sugar over different periods.

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In relation to confounders, we found, as previous studies [2] that HbA_{1c} levels are affected by age. We also found that HbA_{1c} was also affected by the level of education. It is likely that participants' type of employment may have explained this. It has been shown that jobs which require highly educated workers are also associated with increased working hours [41], which, in turn, are associated with increased HbA_{1c} [18]. We also verified previous results that indicated that smoking decreased FSG and PSG. Earwax, however, was a more stable specimen, since its glucose levels were not affected by any short- or long-term covariate studied here.

Two samples may appear to be a small number to reflect the average glucose concentration over one month, especially when considering that glucose is a substance with a reactive profile of secretion. Indeed, some studies have used the area under the curve formula using several time points of glucose samples across the day for estimating the average concentration in this sugar [42]. The mean between fasting and postprandial glycaemic levels, however, has proven to be an predictable index for reflecting the average concentration of glycaemia. In fact, this index is also used with diabetic patients. *Svendson* and co-workers found that the average glucose level derived from approximately 2 to 300 blood measurements from 18 Type 1 diabetes patients correlated almost perfectly (R=0.96) with HbA_{1c} [43]. *Ozmen et al* found that the mean plasma glycaemic level derived from fasting and postprandial plasma glucose levels also correlates strongly with HbA_{1c} in Type 2 diabetic patients [44]. Recently, the mean between postprandial and fasting glycaemic levels was also used for monitor treatment in women with gestational diabetes mellitus [45]. The mean blood sugar between FSG and PSG may be even more valid and reliable for estimating the average glucose concentration among healthy people. Controls present less variability in their 24hr glucose levels compared to diabetic patients [46]. Nonetheless, it may be more accurate to say that the estimated glucose mean of this study was obtained from longitudinal values, rather than the chronic glucose level. The Realtime Continuous Glucose Monitoring (RT-CGM) was lately developed with the aim of providing a glucose reading and trend every five minutes for up to seven days. Although RT-CGM may be a useful educational and motivational tool, diabetes self-

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management that includes the use of RT-CGM is likely to be more time-consuming for patients and force them to focus on different aspects of diabetes. Twice-daily self monitoring of blood glucose is still required to calibrate the RT-CGM device and to inform treatment decisions in those using prandial insulin. Discrepancies between finger-stick blood glucose and sensor values may distress patients. Furthermore, high and low glucose threshold alarms may be disturbing'. It has been reported that these devices produce a large amount of information that patients do not how to handle it[47], [48].

A Randomised Clinical Trial (RCT) study design may be another way to test the hypothesis that earwax glucose is more predictable than HbA_{1c} for measuring chronic glucose levels especially if several time points are considered. Inter-individual differences related to participants' abilities to absorb different meal components may have also an effect on their glucose levels [49]. Some studies use the glucose tolerance test after the intake of 75 gr of glucose, rather than postprandial levels after the intake of one standardised meal. We used *Ensure*, as standardised meals contain glucose and several other nutrients, which may have different absorption rates, affecting, PSG levels. Moreover, the PSG test that we used has been widely used in several other research projects [50], [51] This is because, in comparison to normal meals, *Ensure* is easier to absorb due to its liquid characteristics. Furthermore, we excluded any participants with food allergies, such as lactose intolerance, which may have altered the absorption rates of some nutrients. With regards to the differences between plasma and serum, some studies report that plasma glucose is higher than serum glucose whereas other studies found no difference [52]. The measurement of glucose in serum is not recommended for the diagnosis of diabetes [53]. We did not use FSG or PSG to make any diagnosis, we recruited a sample of healthy participants to investigate their glucose levels using different specimens.

5. Conclusion

Earwax showed to be more predictable than HbA_{1c} at reflecting acute and chronic glucose levels in healthy people. Earwax was also a more stable specimen since it was not affected by any confounders.

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Future larger validation longitudinal studies could correlate a higher number of fasting and postprandial plasma glucose samples with EGC and consider randomisation to confirm the superiority of earwax methods. The earwax self-sampling device proved to be an effective method to measure EGC and may be utilised in diabetes and other metabolic disorders. EGC using the novel device may be a harmless, economic and a suitable test for measuring long-term glucose concentrations.

6. Patent

The earwax self-sampling device and method are patented (PCT/IB2018/060470).

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Authords contribution

Conceptualization, A.H.V; methodology, A.H.V and L.O.; software, A.H.V ;validation, A.H.V, and J.B; formal analysis, A.H.V, L.O and R.S investigation, A.H.V and J.B.; resources, A.H.V, A.H.Y and J.B.; writing—original draft A.H.V, D.A and A.H.Y preparation, A.H.V, L.O and L.A; writing—review and editing, A.H.V, A.H.Y, D.A and A.H.Y and L.A.; supervision, A.H.Y, A.H.V and D.A; project administration, A.H.V, L.O and S.E; funding acquisition, A.H.V and J.B. All authors have read and agreed to the published version of the manuscript.

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preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Conflicts of Interest

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Abbreviations:

HbA_{1c}: Glycated Haemoglobin; FSG: Fasting Serum Glucose; PSG: Postprandial Serum Glucose; EGC: Glycemic concentration; UCN: Universidad Catolica del Norte; BMI: Body Mass Index

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