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# High-Level Circulating Total Calcium and Low Phosphate as Predictors of Insulin Resistance among Non-Diabetic Taxi-Motorbike Drivers Living and Working in Cotonou, Benin

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## Abstract

Insulin resistance (IR) is a well-recognized marker of increased cardiovascular diseases (CVDs) and type 2 diabetes (T2D) risk. Therefore, screening for IR predictors would help reduce the likelihood of progression from early stage of IR to T2D or CVDs. However, the knowledge of association between IR and circulating total calcium (CTCa) and phosphate levels among non-diabetic patients in Benin is lacking. We investigated whether CTCa and phosphate levels within the normal ranges are associated with IR risk among taxi-motorbike drivers (TMDs) living and working in Cotonou. We evaluated 134 non-diabetic TMDs (aged 22 - 59 years) based on CTCa, phosphate, glucose, fasting insulin, and IR levels. IR was assessed using the homeostatic model assessment-insulin resistance (HOMA-IR). IR was defined as the 75$^{th}$ percentile of HOMA-IR value. Cardiometabolic factors were analyzed by tertiles of CTCa and phosphate levels (low, middle, and high groups). Logistic regression models evaluated the relationships between IR and CTCa and phosphate levels. Our results showed that participants with high CTCa levels had the highest prevalence of IR, elevated total cholesterol and high-density lipoprotein cholesterol. In a fully adjusted model, the odd ratio (OR) of having IR comparing the highest (>2.50 mmol/L) to the lowest CTCa levels (<2.43 mmol/L) was 6.62 ($p = 0.003$). In contrast, IR level decreased as the phosphate levels increased. The OR for IR comparing the highest (>1.23 mmol/L) and the lowest levels (<1.10 mmol/L) of phosphate was 0.28 ($p = 0.037$). In conclusion, our study demonstrates that elevated CTCa and low phosphate levels are significant predictors of IR in non-diabetic patients. Continuous monitoring of these markers may help identify earlier individuals

at greatest IR risk.

## Keywords

Blood Glucose, Insulin Resistance, Circulating Total Calcium, Phosphate, Taxi-Motorbike Drivers

## 1. Introduction

Insulin resistance (IR) is defined as a decreased sensitivity of the body cells to normal insulin concentrations [1]. The pathogenesis of IR is complex and involves several etiological factors, such as hyperinsulinemia, hyperlipidemia and adipokines (*i.e.*, hormone released from adipocytes), all of which crosstalk with insulin signaling pathways [2]. IR is recognized as the cornerstone of the metabolic syndrome (MetS), which is a clustering of metabolic risk factors including hyperglycemia, obesity, dyslipidemia, and hypertension. Insulin resistant patients had an increased risk of developing type 2 diabetes (T2D) and cardiovascular diseases (CVDs) [3]. Importantly, the burden of T2D is rising globally and even in Africa [4] [5]. Worldwide, diabetes is the ninth leading cause of mortality, with over 1 million deaths in 2017 [4]. Moreover, the estimated number of adults with diabetes in 2030 is projected to be 366 million, and that of hypertension will be 1.56 billion in 2025 [6]. Therefore, preventive strategies are needed to reduce the burden of non-communicable diseases (NCDs), including T2D. It is well known that IR precedes many years before the clinical diagnosis of diabetes in healthy patients [7] [8]. As such, IR may be considered as a critical target for primary prevention of cardiometabolic diseases. Screening for IR predictors would be valuable to reduce the likelihood of progression from early stage of IR to T2D or CVDs.

Previous studies have revealed that circulating total calcium (CTCa) levels (*i.e.*, 1% of the total body calcium), are associated with increased fasting plasma glucose and IR [9] [10] [11], which are characteristic features of prediabetic states. Consistently, cross-sectional and longitudinal studies demonstrated that elevated CTCa levels were associated with increased risk of developing MetS, T2D, as well as CVDs mortality [12] [13] [14], similarly to reports on hyperparathyroidism, an endocrine disorder characterized by elevated calcium levels [15]. Conversely, epidemiologic studies have shown that phosphate concentrations were negatively correlated with both blood glucose and IR levels [11] [16]. However, the knowledge of IR predictors is lacking among populations of Benin, although hyperinsulinemia rate is very high (e.g., 82%, [17]) with an overall IR prevalence ranging between 17.7% and 25.5% [18] [19] [20]. To the best of our knowledge, the influence of CTCa and phosphate levels on IR has never been explored in populations of Benin.

We anticipated that imbalance in CTCa or phosphate levels may predict IR risk

among normoglycemic patients. Therefore, we conducted this cross-sectional study to examine whether plasma CTCa and phosphate levels within the normal ranges are associated with the risk of developing IR in non-diabetic taxi-motorbike drivers (TMDs) living and working in Cotonou city [21] [22].

## 2. Patients and Methods

### 2.1. Data Source and Study Participants

We conducted several surveys (between 2004-2018) to investigate the health impacts of air pollution on exposed populations, including TMDs. However, surveillance and research on NCDs are still lacking in TMDs. In the current study, we selected the 2009 survey which offered comprehensive health check-ups, including assessments of cardiometabolic markers among TMDs. This was a retrospective cross-sectional study that analyzed plasma biochemistry data from our 2009 survey. The study population has been described previously [21] [22].

Individuals fulfilling the following criteria were included in the study: male non-smokers without diabetes or CVDs, age ≥ 20 years, and having measurements of variables of interest. Participants having fasting glucose level > 7.0 mmol/L, or without insulin, calcium, phosphate, blood lipids, and uric acid records were excluded. A total of 147 TMDs were assessed for eligibility but 134 met predetermined criteria and were included in the analyses reported in this paper. Written informed consent was obtained from each participant prior to enrolment in the study. The study was evaluated and approved by the Benin Environmental Agency.

### 2.2. Blood Sample Collection and Laboratory Testing

Detailed information about the blood collection and processing has been previously described [20]. All biological analyses were performed by standardized methods within the research Unit NGERE: "Nutrition-Génétique-Exposition aux risques environnementaux", Faculté de Médecine, Nancy, France.

### 2.3. Covariates

Covariates of IR were selected and controlled based on previously published studies [20]. Demographic and clinical information such as age, alcohol intake, height and weight, systolic (SBP) and diastolic blood pressure (DBP) were obtained from each participant through face-to-face interviews by trained doctors. The body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters ($kg/m^2$). Fasting plasma glucose, uric acid, phosphate, CTCa, blood lipids, such as total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were obtained from laboratory analyses, which were performed on a clinical chemistry analyzer (Siemens, Germany). Plasma insulin was determined by radioimmunoassay using a commercial kit (BI INSULIN IRMA, Bio-

rad, Mames la Coquette, France).

### 2.4. Definition of Variables

Fasting insulin level ≥ 15 μU/ml was considered elevated [23]. Hypertension was defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg [24]. Alcohol intake was defined as the average consumption of one or more alcoholic drinks per day. The homeostatic model assessment-insulin resistance (HOMA-IR) was assessed using the formula described by Matthews *et al.* [25]. HOMA-IR = Fasting plasma insulin (μU/ml) × Fasting plasma glucose (mmol/L)/22.5. IR was defined as the 75$^{th}$ percentile of HOMA-IR value [25].

### 2.5. Statistical Analysis

Data are presented as percentage for categorical variables and as mean (± standard deviation, SD) for continuous variables. Survey participants were divided into three groups based on CTCa and phosphate levels: low, middle, and high groups. The cut-off points for CTCa and phosphate were <2.43, 2.44 - 2.50, >2.50 mmol/L and <1.09, 1.10 - 1.23, >1.23 mmol/L, respectively. Demographic and biological characteristics of survey participants were compared by tertiles of CTCa and phosphate using one-way analysis of variance (ANOVA) or chi-square tests as appropriate for the variable. The relationships between IR and CTCa and phosphate levels were examined in three multivariable-adjusted logistic regression models, using backward selection procedure. IR status was set as dependent variable and clinically relevant variables were modeled as predictors. The model 1 adjusted for age and BMI; model 2 additionally adjusted for blood pressure, TC, TG, HDL-C, and LDL-C; and model 3 adjusted for variables in model 2 plus uric acid levels. The low calcium or phosphate level groups were set as the reference groups to calculate the odds ratios (ORs) for IR with the corresponding 95% confidence intervals (CIs) in the middle and high-level groups. Additionally, we analyzed CTCa and phosphate as continuous variables (per SD change). P–values < 0.05 were considered to indicate a statistical significance. Data analysis was performed using IBM SPSS Statistics 20.0 software.

## 3. Results

### 3.1. Univariate Analysis of Demographic and Metabolic Parameters Related to Total Calcium and Phosphate Levels

The means (SD) age, CTCa, and phosphate levels of the survey participants were 39.4 (7.8) years, 2.4 (0.4) mmol/L, and 1.2 (0.2) mmol/L, respectively. Alcohol consumption, elevated fasting insulin (hyperinsulinemia), IR, and hypertension were prevalent in 38.1%, 67.2%, 24.6%, and 47.0% of survey participants, respectively (**Table 1**). The cut-off value for IR (*i.e.*, 75$^{th}$ of HOMA-IR) was 5.9. We also compared demographic and biological characteristics by CTCa and phosphate levels (**Table 1**). Age was inversely associated with CTCa levels (p = 0.032). Participants with high phosphate levels had low concentrations of fasting insulin

and the least HOMA-IR, in comparison with those in the low phosphate levels groups (p = 0.037, p = 0.123, respectively). Participants with high CTCa levels demonstrated marked hyperinsulinemia (p = 0.029), had the highest prevalence of IR (p = 0.014), and elevated TC (p = 0.032) and HDL-C (p = 0.037) levels than did those with low CTCa levels (Table 1).

### 3.2. Predictors of Insulin Resistance in the Study Participants

Table 2 summarizes factors associated with IR in the study participants. Participants in the second and the third tertiles of phosphate levels had a reduced risk

Table 1. Univariate analysis of cardiometabolic factors by plasma calcium and phosphate levels.

| | Study cohort | Phosphate levels (mmol/L) | | | | Total calcium levels (mmol/L) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Low (<1.09) | Middle (1.10 - 1.23) | High (>1.23) | p-Value | Low (<2.43) | Middle (2.44 - 2.50) | High (>2.50) | p-value |
| n (%) | 134 (100.0) | 38 (28.4) | 54 (40.3) | 42 (31.3) | - | 59 (45.0) | 37 (28.2) | 35 (26.7) | - |
| Age, years | 39.4 (7.8) | 39.4 (6.9) | 38.5 (8.2) | 40.2 (8.5) | 0.634 | 40.3 (7.5) | 40.4 (7.9) | 36.4 (7.2) | 0.034 |
| BMI, Kg/m$^2$ | 23.5 (3.9) | 23.6 (4.2) | 23.3 (3.1) | 23.8 (4.1) | 0.84 | 23.4 (3.8) | 23.3 (4.2) | 23.9 (3.5) | 0.756 |
| SBP, mmHg | 134.2 (18.8) | 134.8 (21.3) | 136.3 (18.0) | 131.5 (15.9) | 0.507 | 132.6 (16.7) | 135.8 (20.5) | 135.9 (20.8) | 0.63 |
| DBP, mmHg | 84.7 (13.2) | 85.8 (14.0) | 85.3 (12.9) | 82.9 (12.3) | 0.534 | 84.8 (12.7) | 85.4 (13.2) | 84.0 (14.6) | 0.904 |
| Insulin, µU/ml | 26.6 (20.9) | 32.1 (24.6) | 24.3 (19.0) | 21.7 (15.3) | 0.037 | 21.5 (15.7) | 26.1 (18.2) | 32.2 (23.4) | 0.029 |
| Glucose, mmol/L | 4.2 (0.6) | 4.2 (0.6) | 4.3 (0.7) | 4.1 (0.6) | 0.219 | 4.2 (0.7) | 4.0 (0.7) | 4.4 (0.5) | 0.106 |
| HOMA–IR | 5.1 (4.6) | 6.0 (4.5) | 5.0 (5.6) | 4.1 (3.6) | 0.123 | 4.3 (4.6) | 4.8 (3.6) | 6.4 (5.0) | 0.081 |
| Calcium, mmol/L | 2.4 (0.4) | 2.3 (0.6) | 2.5 (0.1) | 2.5 (0.1) | 0.124 | 2.4 (0.1) | 2.5 (0.02) | 2.6 (0.1) | <0.001 |
| Phosphate, mmol/L | 1.2 (0.2) | 1.0 (0.1) | 1.2 (0.03) | 1.4 (0.1) | <0.001 | 1.2 (0.2) | 1.2 (0.2) | 1.1 (0.2) | 0.401 |
| Uric acid, µmol/L | 350.9 (68.2) | 345.8 (80.8) | 339.6 (67.9) | 367.6 (75.5) | 0.144 | 344.7 (67.3) | 354.3 (70.2) | 361.3 (70.0) | 0.512 |
| TC, mmol/L | 4.3 (1.0) | 4.4 (1.0) | 4.2 (0.9) | 4.2 (0.9) | 0.526 | 4.1 (0.9) | 4.3 (0.9) | 4.6 (1.0) | 0.032 |
| TG, mmol/L | 0.8 (0.4) | 0.8 (0.4) | 0.9 (0.5) | 0.8 (0.3) | 0.767 | 0.8 (0.4) | 0.7 (0.2) | 0.9 (0.4) | 0.255 |
| HDL–C, mmol/L | 1.3 (0.4) | 1.4 (0.5) | 1.3 (0.3) | 1.3 (0.3) | 0.338 | 1.2 (0.4) | 1.4 (0.4) | 1.4 (0.3) | 0.037 |
| LDL–C, mmol/L | 2.6 (0.8) | 2.7 (0.8) | 2.6 (0.8) | 2.6 (0.8) | 0.738 | 2.5 (0.8) | 2.6 (0.8) | 2.8 (0.8) | 0.130 |
| Hypertension, n (%) | 63 (47.0) | 26 (41.3) | 20 (31.7) | 17 (27.0) | 0.541 | 26 (41.9) | 17 (27.4) | 19 (30.6) | 0.619 |
| Alcohol use, n (%) | 51 (38.1) | 21 (41.2) | 13 (25.5) | 17 (33.3) | 0.886 | 23 (45.1) | 16 (31.4) | 12 (23.5) | 0.579 |
| Insulin resistance, n (%) | 33 (24.6) | 19 (57.6) | 6 (18.2) | 8 (24.2) | 0.062 | 8 (25.8) | 9 (29.0) | 14 (45.2) | 0.014 |
| High insulin, n (%) | 90 (67.2) | 42 (46.7) | 26 (28.9) | 22 (24.4) | 0.031 | 32 (36.8) | 29 (33.3) | 26 (29.9) | 0.026 |

Values are expressed as mean (standard deviation) or n (%). BMI, body mass index, SBP, systolic blood pressure, DBP, diastolic blood pressure, HOMA-IR, homeostatic model assessment-insulin resistance, TC, total cholesterol, TG, triglycerides, HDL-C, high density lipoprotein cholesterol, LDL-C, low-density lipoprotein cholesterol.

Table 2. Factors independently associated with insulin resistance in the study population.

| | Model 1[a] | | Model 2[b] | | Model 3[c] | |
|---|---|---|---|---|---|---|
| | OR (95% CI) | p-value | OR (95% CI) | p-value | OR (95% CI) | p-value |
| **Phosphate levels, mmol/L** | | | | | | |
| Low (< 1.09 mmol/L) | 1.00 (Reference) | - | 1.00 (Reference) | - | 1.00 (Reference) | - |
| Middle (1.10 - 1.23 mmol/L) | 0.25 (0.10 - 0.84) | 0.025 | 0.25 (0.10 - 0.87) | 0.030 | 0.27 (0.10 - 0.93) | 0.038 |
| High (>1.23 mmol/L) | 0.41 (0.14 - 1.21) | 0.107 | 0.33 (0.11 - 1.04) | 0.058 | 0.28 (0.10 - 0.92) | 0.037 |
| p for trend | 0.055 | - | 0.047 | - | 0.039 | - |
| Per SD increase in phosphorus | 0.55 (0.35 - 0.86) | 0.009 | 0.53 (0.34 - 0.83) | 0.006 | 0.48 (0.30 - 0.78) | 0.003 |
| **Calcium levels, mmol/L** | | | | | | |
| Low (<2.43 mmpl/L) | 1.00 (Reference) | - | 1.00 (Reference) | - | 1.00 (Reference) | - |
| Middle (2.44 - 2.50 mmol/L) | 2.13 (0.67 - 6.82) | 0.203 | 2.27 (0.70 - 7.43) | 0.174 | 2.10 (0.63 - 6.98) | 0.229 |
| High (>2.50 mmol/L) | 4.68 (1.55 - 14.15) | 0.006 | 6.58 (2.00 - 21.59) | 0.002 | 6.62 (1.94 - 22.58) | 0.003 |
| p for trend | 0.024 | - | 0.008 | - | 0.010 | - |
| BMI (>24.28 Kg/m$^2$) | 5.54 (1.81 - 17.00) | 0.006 | 6.29 (1.93 - 20.54) | 0.002 | 5.45 (1.66 - 17.93) | 0.005 |

Insulin resistance was considered as dependent variable and other biological parameters were set as independent variables. BMI, body index mass, CI, confidence interval, OR, odd ratio, SD, standard deviation. [a]Adjusted for age and BMI. [b]Adjusted for age, BMI, blood pressure, total cholesterol, triglycerides, high density lipoprotein, and low–density lipoprotein. [c]Adjusted for all variables in model 2, plus uric acid levels.

of IR compared to those in the first tertile. In contrast, an increase in the CTCa levels (> 2.50 mmol/L) was related to an increased risk of IR in TMDs.

In the fully adjusted model (*i.e.*, model 3), the OR for developing IR comparing the middle and high phosphate levels to the lowest levels were 0.27 (95% CI: 0.10 - 0.93, p = 0.038) and 0.28 (95% CI: 0.10 - 0.92, p = 0.037), respectively. This inverse association between plasma phosphate levels and IR remained robust when phosphate was analyzed as a continuous variable (per SD increase). The OR for IR comparing the middle and high CTCa levels to the lowest CTCa levels were 2.10 (95% CI: 0.63 - 6.98, p = 0.229) and 6.62 (95% CI: 1.94 - 22.58, p = 0.003), respectively. Additionally, elevated BMI (> 24.28 Kg/m$^2$) was independently associated with an increased IR risk in our study population (OR = 5.45, 95% CI: 1.66 - 17.93, p = 0.005, Table 2). Age, blood pressure or blood lipids indicated no significant associations with IR.

## 4. Discussion

In the current study, we examined whether CTCa and phosphate levels within the normal ranges associate with IR risk among non-diabetic TMDs living and working in Cotonou. Our results showed that fasting insulin, glucose levels as well as IR level were higher in patients with elevated CTCa than those with low calcium levels. We applied three multivariable-adjusted logistic regression models and checked whether this association ascribed to CTCa was not driven by

potential confounders, such as age, BMI, blood lipids or uric acid levels. Interestingly, logistic regression models indicated that CTCa concentrations > 2.50 mmol/L (10.0 mg/dL) were associated with elevated IR risk in TMDs. For example, in the fully adjusted analysis (*i.e.*, model 3), we found that the OR decreased by 10% when comparing the highest versus the lowest CTCa levels, but the association remains statistically significant (OR = 6.62, p = 0.003). This suggests that none of the confounders assessed in this study impacted the link between IR and CTCa levels. This result strengthened findings of previous studies that investigated the association between IR and circulating calcium levels [10] [11]. A cross-sectional study on 1182 healthy subjects from Canada found that subjects with high serum calcium levels had the highest concentration of glucose and IR [10]. Similarly, Yamaguchi *et al.* examined 480 T2D patients and reported that elevated serum calcium level was strongly associated with fasting glucose and HOMA-IR levels [26].

Furthermore, prospective studies demonstrated that elevated total calcium levels and albumin-corrected calcium were associated with increased risk of incident MetS and diabetes [12] [27] [28]. Consistently, a prospective study by Lorenzo *et al.* demonstrated that individuals with calcium level ≥ 2.38 mmol/l (9.5 mg/dl) were at increased risk of developing T2D [13].

This study findings also revealed an inverse association between plasma phosphate levels and IR. We found that low plasma phosphate levels were associated with elevated fasting insulin levels and a higher prevalence of IR, similarly to reports of previous studies [16]. Accordingly, logistic regression models indicated that individuals with plasma phosphate > 1.10 mmol/L had a reduced risk of having IR than those in the low phosphate level group. This association remains robust after adjustment for relevant confounders. This finding was consistent with reports by Haap *et al.* who demonstrated that low serum phosphate levels were associated with elevated blood glucose and a decreased insulin sensitivity [16]. Similarly, a cross-sectional study on 1701 healthy patients reported an inverse association between serum phosphate and HOMA-IR [11]. Furthermore, a growing body of evidence suggests an inverse association between phosphate levels and risk of MetS [29] [30].

The molecular mechanisms explaining the association of elevated CTCa and low phosphate levels with IR are not fully understood. However, it is speculated that elevated calcium levels could decrease glucose uptake by reducing insulin receptor activity or the number of glucose transporters in adipocytes and muscles cells [28] [31]. It is also suggested that glucose-mediated insulin secretion from pancreatic $\beta$-cells requires calcium-dependent enzymes and calcium channels [10] [32] [33]. Furthermore, compelling evidences revealed that disturbed calcium homeostasis could lead to functional deterioration of pancreatic $\beta$-cells (*i.e.*, production and secretion of insulin) [10] [34]. Additionally, phosphate is required for ATP generation, and it is postulated that low phosphate levels could compromise the phosphorylation of carbohydrate intermediates in the glucose metabolism pathway [35]. This subsequently leads to development of IR.

Our study findings also demonstrated that higher BMI (>24.3 kg/m$^2$) was associated with an increased IR risk, similarly to what was reported in previous studies [36]. Epidemiologic studies associated higher CTCa with overweight/obesity, high blood pressure, and higher TC [37] [38]. In our study, patients with elevated CTCa levels had higher TC and HDL-C levels. However, TC and HDL-C together with blood pressure, TG and LDL-C did not predict IR on logistic regression analyses, probably because of our study design, which included only normoglycemic patients.

Our study had several limitations. This was a cross-sectional study and longitudinal changes in total plasma calcium or phosphate levels are unavailable. Therefore, causality cannot be directly established. Additionally, the sample size was relatively small and participants could only be divided into three groups. This limits generalizability of our findings. Moreover, calcium homeostasis is regulated by vitamin D levels, parathyroid hormone, and ionized calcium. However, no information was available, and we could not evaluate the influence of vitamin D, parathyroid hormone, and ionized calcium on the relationship between plasma calcium and IR risk. Despite its limitations, this study strengthened data of previous studies conducted in other countries. Additionally, TMDs that have been the focus of this study was an understudied and at-risk population. Further, this study adds additional flows to existing literature on IR predictors within Benin. Plasma calcium and phosphate concentrations are easy to measure and cost effective. As such they could be used as potential screening markers to detect IR in the pre-clinical phase. In this regard, reported data may help policy makers when planning and implementing preventive interventions.

## 5. Conclusion

Our current study demonstrates that higher CTCa (>2.50 mmol/L) and low phosphate levels (<1.10 mmol/L) are strong predictors of IR, in non-diabetic TMDs. Continuous monitoring for these markers may help identify individuals at greatest risk for IR. However, whether elevated CTCa and low phosphate levels are precursors or consequences of IR requires further studies.

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Médecine, Nancy, France.

## Author's Contribution

All authors contributed to the study design, participated to data acquisition. PHA performed laboratory work, analyzed data, and wrote the manuscript. All authors read and approved the final manuscript.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

## References

[1] Lebovitz, H.E. (2001) Insulin Resistance: Definition and Consequences. *Experimental and Clinical Endocrinology & Diabetes: Official Journal, German Society of Endocrinology and German Diabetes Association*, **109**, S135-S148. https://doi.org/10.1055/s-2001-18576

[2] Beale, E.G. (2013) Insulin Signaling and Insulin Resistance. *Journal of Investigative Medicine: The Official Publication of the American Federation for Clinical Research*, **61**, 11-14. https://doi.org/10.2310/JIM.0b013e3182746f95

[3] Hanley, A.J.G., Wagenknecht, L.E., Norris, J.M., Bryer-Ash, M., Chen, Y.I., Anderson, A.M., *et al.* (2009) Insulin Resistance, Beta Cell Dysfunction and Visceral Adiposity as Predictors of Incident Diabetes: The Insulin Resistance Atherosclerosis Study (IRAS) Family Study. *Diabetologia*, **52**, 2079-2086. https://doi.org/10.1007/s00125-009-1464-y

[4] Khan, M.A.B., Hashim, M.J., King, J.K., Govender, R.D., Mustafa, H. and Al Kaabi, J. (2020) Epidemiology of Type 2 Diabetes—Global Burden of Disease and Forecasted Trends. *Journal of Epidemiology and Global Health*, **10**, 107-111. https://doi.org/10.2991/jegh.k.191028.001

[5] El-Kebbi, I.M., Bidikian, N.H., Hneiny, L. and Nasrallah, M.P. (2021) Epidemiology of Type 2 Diabetes in the Middle East and North Africa: Challenges and Call for Action. *World Journal of Diabetes*, **12**, 1401-1425. https://doi.org/10.4239/wjd.v12.i9.1401

[6] Lago, R.M., Singh, P.P. and Nesto, R.W. (2007) Diabetes and Hypertension. *Nature Clinical Practice Endocrinology & Metabolism*, **3**, 667. https://doi.org/10.1038/ncpendmet0638

[7] Reddy, K.J., Singh, M., Bangit, J.R. and Batsell, R.R. (2010) The Role of Insulin Resistance in the Pathogenesis of Atherosclerotic Cardiovascular Disease: An Updated Review. *Journal of Cardiovascular Medicine* (*Hagerstown, Md*), **11**, 633-647. https://doi.org/10.2459/JCM.0b013e328333645a

[8] Adeva-Andany, M.M., Martínez-Rodríguez, J., González-Lucán, M., Fernández-Fernández, C. and Castro-Quintela, E. (2019) Insulin Resistance Is a Cardiovascular Risk Factor in Humans. *Diabetes & Metabolic Syndrome*, **13**, 1449-1455. https://doi.org/10.1016/j.dsx.2019.02.023

[9] Wu, X., Han, T., Gao, J., Zhang, Y., Zhao, S., Sun, R., *et al.* (2019) Association of Serum Calcium and Insulin Resistance with Hypertension Risk: A Prospective Population-Based Study. *Journal of the American Heart Association*, **8**, e009585. https://doi.org/10.1161/JAHA.118.009585

[10] Sun, G., Vasdev, S., Martin, G.R., Gadag, V. and Zhang, H. (2005) Altered Calcium Homeostasis Is Correlated with Abnormalities of Fasting Serum Glucose, Insulin Resistance, and Beta-Cell Function in the Newfoundland Population. *Diabetes*, **54**, 3336-3339. https://doi.org/10.2337/diabetes.54.11.3336

[11] Akter, S., Eguchi, M., Kochi, T., Kabe, I., Nanri, A. and Mizoue, T. (2020) Association of Serum Calcium and Phosphate Concentrations with Glucose Metabolism Markers: The Furukawa Nutrition and Health Study. *Nutrients*, **12**, E2344. https://doi.org/10.3390/nu12082344

[12] Chou, C.-W., Fang, W.-H., Chen, Y.-Y., Wang, C.-C., Kao, T.-W., Wu, C.-J., *et al.* (2020) Association between Serum Calcium and Risk of Cardiometabolic Disease among Community-Dwelling Adults in Taiwan. *Scientific Reports*, **10**, Article No. 3192. https://doi.org/10.1038/s41598-020-60209-w

[13] Lorenzo, C., Hanley, A.J., Rewers, M.J. and Haffner, S.M. (2014) Calcium and Phosphate Concentrations and Future Development of Type 2 Diabetes: The Insulin Resistance Atherosclerosis Study. *Diabetologia*, **57**, 1366-1374. https://doi.org/10.1007/s00125-014-3241-9

[14] Walsh, J.P., Divitini, M.L. and Knuiman, M.W. (2013) Plasma Calcium as a Predictor of Cardiovascular Disease in a Community-Based Cohort. *Clinical Endocrinology*, **78**, 852-857. https://doi.org/10.1111/cen.12081

[15] Kumari, P., Arya, A.K., Pal, R., Sood, A., Dahiya, D., Mukherjee, S., *et al.* (2022) Comparison of Profile of Primary Hyperparathyroidism with and without Type 2 Diabetes Mellitus: Retrospective Analysis from the Indian Primary Hyperparathyroidism Registry. *Endocrine Practice: Official Journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists*, **28**, 96-101. https://doi.org/10.1016/j.eprac.2021.09.012

[16] Haap, M., Heller, E., Thamer, C., Tschritter, O., Stefan, N. and Fritsche, A. (2006) Association of Serum Phosphate Levels with Glucose Tolerance, Insulin Sensitivity and Insulin Secretion in Non-Diabetic Subjects. *European Journal of Clinical Nutrition*, **60**, 734-739. https://doi.org/10.1038/sj.ejcn.1602375

[17] Awede, B., Lawani, S., Adehan, G., Akakpo, J., Dossou, E., Amoussou-Guenou, M., *et al.* (2017) Glucose Tolerance in Non-Diabetic Adult Subjects of an Urban West-African Population. *Nigerian Journal of Physiological Sciences: Official Publication of the Physiological Society of Nigeria*, **32**, 159-164.

[18] Sossa, C., Delisle, H., Agueh, V., Makoutodé, M. and Fayomi, B. (2013) Insulin Resistance Status and Four-Year Changes in Other Cardiometabolic Risk Factors in West-African Adults: The Benin Study. *European Journal of Preventive Cardiology*, **20**, 1042-1050. https://doi.org/10.1177/2047487312460214

[19] Mabchour, A.E., Delisle, H., Vilgrain, C., Larco, P. and Sodjinou, R. (2016) Abdominal Obesity and Other Cardiometabolic Risk Biomarkers: Influence of Socioeconomic Status and Lifestyle on Two African-Origin Population Groups, Cotonou (Benin) and Port-au-Prince (Haiti). *The Pan African Medical Journal*, **24**, Article No. 306.

[20] Avogbe, P.H. and Sanni, A. (2022) Hyperuricemia and Associated Cardiometabolic Factors: A Cross-Sectional Study on Taxi-Motorbike Drivers Working in Cotonou, Benin. *Avicenna Journal of Medical Biochemistry*. http://ajmb.umsha.ac.ir/InPress

[21] Avogbe, P.H., Ayi-Fanou, L., Autrup, H., Loft, S., Fayomi, B., Sanni, A., *et al.* (2005) Ultrafine Particulate Matter and High-Level Benzene Urban Air Pollution in Relation to Oxidative DNA Damage. *Carcinogenesis*, **26**, 613-620. https://doi.org/10.1093/carcin/bgh353

[22] Ayi-Fanou, L., Avogbe, P.H., Fayomi, B., Keith, G., Hountondji, C., Creppy, E.E., *et*

*al*. (2011) DNA-Adducts in Subjects Exposed to Urban Air Pollution by Benzene and Polycyclic Aromatic Hydrocarbons (PAHs) in Cotonou, Benin. *Environmental Toxicology*, **26**, 93-102. https://doi.org/10.1002/tox.20533

[23] Ten, S. and Maclaren, N. (2004) Insulin Resistance Syndrome in Children. *The Journal of Clinical Endocrinology and Metabolism*, **89**, 2526-2539. https://doi.org/10.1210/jc.2004-0276

[24] Joint Committee for Guideline Revision (2019) 2018 Chinese Guidelines for Prevention and Treatment of Hypertension—A Report of the Revision Committee of Chinese Guidelines for Prevention and Treatment of Hypertension. *Journal of Geriatric Cardiology*: *JGC*, **16**, 182-241.

[25] Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F. and Turner, R.C. (1985) Homeostasis Model Assessment: Insulin Resistance and Beta-Cell Function from Fasting Plasma Glucose and Insulin Concentrations in Man. *Diabetologia*, **28**, 412-419. https://doi.org/10.1007/BF00280883

[26] Yamaguchi, T., Kanazawa, I., Takaoka, S. and Sugimoto, T. (2011) Serum Calcium Is Positively Correlated with Fasting Plasma Glucose and Insulin Resistance, Independent of Parathyroid Hormone, in Male Patients with Type 2 Diabetes Mellitus. *Metabolism*: *Clinical and Experimental*, **60**, 1334-1339. https://doi.org/10.1016/j.metabol.2011.02.003

[27] Sing, C.W., Cheng, V.K.F., Ho, D.K.C., Kung, A.W.C., Cheung, B.M.Y., Wong, I.C.K., *et al*. (2016) Serum Calcium and Incident Diabetes: An Observational Study and Meta-Analysis. *Osteoporosis International*: *A Journal Established as Result of Cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*, **27**, 1747-1754. https://doi.org/10.1007/s00198-015-3444-z

[28] Rooney, M.R., Pankow, J.S., Sibley, S.D., Selvin, E., Reis, J.P., Michos, E.D., *et al*. (2016) Serumcalcium and Incident Type 2 Diabetes: The Atherosclerosis Risk in Communities (ARIC) Study. *The American Journal of Clinical Nutrition*, **104**, 1023-1029. https://doi.org/10.3945/ajcn.115.130021

[29] Osadnik, K., Osadnik, T., Delijewski, M., Lejawa, M., Fronczek, M., Reguła, R., *et al*. (2020) Calcium and Phosphate Levels Are among Other Factors Associated with Metabolic Syndrome in Patients with Normal Weight. *Diabetes*, *Metabolic Syndrome and Obesity*: *Targets and Therapy*, **13**, 1281-1288. https://doi.org/10.2147/DMSO.S232497

[30] Shimodaira, M., Okaniwa, S. and Nakayama, T. (2017) Reduced Serum Phosphorus Levels Were Associated with Metabolic Syndrome in Men but Not in Women: A Cross-Sectional Study among the Japanese Population. *Annals of Nutrition & Metabolism*, **71**, 150-156. https://doi.org/10.1159/000480354

[31] Ojuka, E.O. (2004) Role of Calcium and AMP Kinase in the Regulation of Mitochondrial Biogenesis and GLUT4 Levels in Muscle. *The Proceedings of the Nutrition Society*, **63**, 275-278. https://doi.org/10.1079/PNS2004339

[32] Norling, L.L., Colca, J.R., Kelly, P.T., McDaniel, M.L. and Landt, M. (1994) Activation of Calcium and Calmodulin Dependent Protein Kinase II during Stimulation of Insulin Secretion. *Cell Calcium*, **16**, 137-150. https://doi.org/10.1016/0143-4160(94)90008-6

[33] Tabuchi, H., Yamamoto, H., Matsumoto, K., Ebihara, K., Takeuchi, Y., Fukunaga, K., *et al*. (2000) Regulation of Insulin Secretion by Overexpression of $Ca^{2+}$/Calmodulin-Dependent Protein Kinase II in Insulinoma MIN6 Cells. *Endocrinology*, **141**, 2350-2360. https://doi.org/10.1210/endo.141.7.7553

[34] Rorsman, P., Braun, M. and Zhang, Q. (2012) Regulation of Calcium in Pancreatic $\alpha$- and $\beta$-Cells in Health and Disease. *Cell Calcium*, **51**, 300-308. https://doi.org/10.1016/j.ceca.2011.11.006

[35] Xie, W., Tran, T.L., Finegood, D.T. and van de Werve, G. (2000) Dietary P(i) Deprivation in Rats Affects Liver cAMP, Glycogen, Key Steps of Gluconeogenesis and Glucose Production. *The Biochemical Journal*, **352**, 227-232. https://doi.org/10.1042/bj3520227

[36] Jurkovičová, J., Hirošová, K., Vondrová, D., Samohýl, M., Štefániková, Z., Filová, A., *et al*. (2021) The Prevalence of Insulin Resistance and the Associated Risk Factors in a Sample of 14-18-Year-Old Slovak Adolescents. *International Journal of Environmental Research and Public Health*, **18**, 909. https://doi.org/10.3390/ijerph18030909

[37] Lind, L., Jakobsson, S., Lithell, H., Wengle, B. and Ljunghall, S. (1988) Relation of Serum Calcium Concentration to Metabolic Risk Factors for Cardiovascular Disease. *BMJ* (*Clinical Research Ed*), **297**, 960-963. https://doi.org/10.1136/bmj.297.6654.960

[38] Ren, X.-H., Yao, Y.-S., He, L.-P., Jin, Y.-L., Chang, W.-W., Li, J., *et al*. (2013) Overweight and Obesity Associated with Increased Total Serum Calcium Level: Comparison of Cross-Sectional Data in the Health Screening for Teaching Faculty. *Biological Trace Element Research*, **156**, 74-78. https://doi.org/10.1007/s12011-013-9856-8